MICRO BIOTEC 19

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BOOK OF ABSTRACTS

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Plenary Presentations

Plenary I

Microbial diversity – a key to success in industry

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In our societies pursuit to substitute petroleum as carbon and energy source, industrial microbiology is one jigsaw piece. Microbial conversion of alternative carbon sources, such as plant derived sugars or glycerol into fuels or chemicals of higher value is a promising approach. Many papers are published about a plethora of ideas, which carbon source to convert into what chemical, however very rarely we hear about success stories making it really to the market. The economic constraints for success are harsh and interestingly these constraints are directly translating into constraints for the bioprocess. Ideally, high product titers with very high yield are obtained in a very short time and the final culture broth should be appropriate for easy purification of the desired compound (proper pH value and as few contaminants as possible...) With such constraints we are moving very far from natural habitats of microorganisms, which already gives a hint, why many processes do not work as envisioned.
Nevertheless, I advocate that nature already solved most of our problems and that proper understanding of nature and its incredible diversity is a helpful basis for the industrial microbiologist. Understanding includes biochemistry and physiology, but also morphology and here come the challenges: while some pet organisms are well known (but far from understood), the majority of microorganisms is not even culturable as yet. Furthermore, current university curricula and fashion driven science politics are characterized by a lack of basic skills of microbiology. In my talk I want to point out some small examples how we can exploit our knowledge and how we can apply our research to utilize the wealth of solutions which nature provides us with. I hope to convey appreciation that studying microbiology is worthwhile, understanding diversity is valuable and preserving diversity is of utmost importance.

Plenary II

SABANA project: demonstrating the application of microalgae in agriculture and aquaculture

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Although microalgae have been proposed for multiple applications, today only direct human related applications are commercial, mainly due to the high production cost and low scale of microalgae production facilities. Market analysis demonstrates that to apply microalgae in other fields such as agriculture and aquaculture large facilities capable to produce low cost biomass are required, for that the coupling with wastewater treatment is mandatory. Thus, SABANA project aims to develop a complete biorefinery obtaining microalgae related products for agriculture and aquaculture reusing nutrients from effluents at scale up to 5 ha. After two years two complete R&D (0.5 ha) and PRODUCTION (1.5 ha) facilities have been completed. The reliability of large scale low-cost raceway reactors has been validated, the reactors being operated in full recirculation mode to save water and nutrients. Harvesting of the biomass has been optimized by combining a pre-concentration and dewatering step using ultrafiltration membrane and nozzle separator. Concerning cell disruption, it has been confirmed that the utilization of high pressure homogenizers allows to obtain enough cell disruption for further extraction processes, minimizing energy consumption. Extracts obtained from the biomass sludge demonstrate relevant effects both as biostimulant and biopesticide. Concerning biostimulant effect both Auxin-like and Cytokinin-like activities were found, in some cases increases in the plant growth performance larger than 200% being measured. Concerning biopesticides, results demonstrate the inhibition of growth of five of the most relevant fungal pathogens, up to 60%. These effects have been demonstrated at small scale, in experiments performed at laboratory, but they have been also confirmed at real field conditions. Concerning aquaculture, although the biochemical composition of the microalgae biomass is highly valuable, the digestibility of the biomass is largely dependent of the strain. To improve the digestibility of the biomass it is recommendable to perform a previous cell disruption, also by high pressure homogenization. Fish trials performed demonstrate that the major advantage of incorporating microalgae biomass is not related with the improvement of growth but with the enhancement of health of fishes as probiotic. First end products are being evaluated, prior to market evaluation by companies. Thus, SABANA is expected to conclude with real commercial processes demonstrated at commercial level.

Plenary III

Exploration of Microbial Diversity: from the microbiome to the global Virome

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Plenary IV

Fungal-based biorefinery

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Filamentous fungi have an inevitable role in nature, and without them the world look different. The fungi can assimilate a variety of materials including carbohydrates, lignin, fat etc. and produce a variety of enzymes and metabolites such as carboxylic acids and alcohols, while the fungal biomass can also be used as feed or food. Therefore, these fungi can be used to develop biorefineries, in order to consume residuals and wastes (food wastes, industrial organic wastes, forestry wastes or agricultural residuals) and develop food, feed, metabolites, enzymes and/or biopolymers. Our research group is working with filamentous fungi since 1999 and are developing fungal-based biorefineries. In this presentation, the role of fungi in assimilating a variety of wastes and residuals and producing ethanol, enzymes, bioplastics, fish feed, and even human food will be explained. The fungal used were mainly from Ascomycetes and Zygomycetes classes.

Plenary V

Science and Society: From Flint Michigan to Washington DC

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Once a booming industrial city, in 2014, a bankrupt Flint, Michigan reduced expenses by switching its water source from Lake Huron to the Flint River. Omission of anti-corrosive chemicals by the local water utility was soon followed by reports of red, foul water, lead contamination, and outbreaks of Legionnaires' disease. In 2016, the Michigan Department of Health & Human Services recruited an interdisciplinary research team to investigate whether changes in the municipal water system increased risk of this water-associated infection. Our research findings on the 2014-15 Legionnaires' disease outbreaks will be discussed in the context of the social, political, and legal upheaval of the Flint Water Crisis. Responses by the media, the Michigan electorate, and the National Academies of Sciences, Engineering, and Medicine will also be highlighted.

The Flint public health crisis also draws attention to the role of scientists in society. One opportunity for individuals to advance science, health care, education, and public policy is through our professional organizations. Using the American Society for Microbiology as an example, how researchers, teachers, and clinical microbiologists can work together for the greater good will be discussed.

Keynote Presentations

Lanthipeptides: a nature´s toolbox with a phlethora of biotechnological applications

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Natural products are the most successful source of drug leads and continue to provide greater structural diversity than combinatorial chemistry. Having access to this chemical diversity is the major challenge.
Lanthipeptides are ribosomally synthesized and post-translationally modified peptides (RiPPs) which have a wide range of biological activities. The post-translation modifications installed are unique to each lanthipeptide and are responsible for their activities and stability. Lantibiotics, lanthipeptides with antimicrobial activity, are interesting and promising drug candidates for application as alternatives to the traditional antimicrobial therapeutics. Currently, several clinical and preclinical trials exploit this potential. In addition, lanthipeptides biosynthetic machinery holds high biotechnological application for in vivo and in vitro bioengineering approaches.

Eco-evolutionary dynamics during *Escherichia coli* colonization of the mouse gut

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Bacterial laboratory experiments where evolution is followed in real time have allowed us to test theoretical predictions on microbial adaptation and to unravel how pervasive the spread of beneficial mutations can be when they face novel environments. Much less is known about bacterial real time evolution in more natural environments, such as that comprising the gut microbiota. The pace and pattern of evolutionary change during the life of a health mammal is currently unknown. We have been following the emergence of new strains in commensal E. coli when it colonizes the gut of laboratory mice (in vivo experimental evolution). These semi-controlled experiments haven revealed that rapid evolutionary change occurs, which is marked by strong effect mutations, evolution of mutator clones and high rates of horizontal gene transfer.

Discovery of new natural products chemistry, enzymology and bioactivity from the LEGE culture collection

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Over many millions of years, nature has perfected its chemistry. This is evident from the intricate structures of small molecule natural products that have been driven by the need to generate potent bioactivities. It is also evident in enzyme structure function, which enables such complex molecules to be generated. In a near future, we should be able to grasp nature's solutions to interact with biological targets, but also its way of doing chemistry, which tends to be more efficient, less polluting and overall more sustainable. To do so, it is important to maintain an elevated rate of discovery of new natural products and their biosynthetic pathways. In my talk, I'll present examples of novel secondary metabolites and unprecedented enzymatic reactivity coming from the cyanobacteria hosted at the LEGE culture collection (CIIMAR, Univ. Porto).

Functional and taxonomic signatures of the octocoral microbiome in health and disease

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The sustainability of coral reefs bears fundamental implications to the functioning of coastal ecosystems and the biogeochemistry of our planet. Contrary to tropical hard corals (Class Hexacorallia), in temperate octocorals (soft corals, Class Octocorallia), the functional relationship between host health and its symbiotic consortium is yet to be unveiled. Here, we employ comparative metagenomics to uncover the distinct functional and phylogenetic features of the microbiomes of healthy and necrotic octocorals (*Eunicella* and *Leptogorgia* species), seawater and sediments. While the healthy octocoral microbiome was distinguished by the presence of Endozoicomonadaceae and other Oceanospirillales and Alteromonadales phylotypes, a pronounced increase of Flavobacteriaceae and Alphaproteobacteria phylotypes, originating from seawater, was observed in necrotic tissue. Increased abundances of eukaryotic-like repeat motifs (ankyrin and WD40) important in host-symbiont recognition, exonucleases for DNA repair, and restriction endonucleases and CRISPR/Cas proteins involved in viral defence characterised the healthy octocoral microbiome. Congruently, higher viral loads were observed in seawater and necrotic octocorals than in healthy octocorals. Higher abundances of genes encoding for heat-shock proteins, inorganic ion transport and iron storage further contributed to distinguish between healthy and diseased octocoral microbiomes. Conversely, enrichments in genes encoding for spermidine synthesis, the type VI secretory pathway, and bacterial chemotaxis highlighted the opportunistic nature of the microbiome of necrotic host tissue. The augmentation of arginase and nitric oxide reductase genes in necrotic tissues furthermore points towards a sophisticated mechanism used by opportunists to silence the host's immune response. We predicted 462 BGCs across the data, with seawater microbiomes dominated by terpene BGCs while the necrotic octocoral microbiome possessed an unprecedented diversity of non-ribosomal peptide synthetase (NRPS) along with bacteriocin and homoserine lactone BGCs. Healthy octocoral microbiomes were instead characterized by ribosomal peptides, NRPS and type I polyketide synthase BGCs. Genome-resolved metagenomics showed evidence for genome reduction among octocoral-specific, unclassified gammaproteobacterial symbionts in healthy octocorals, while genomes from opportunistic Flavobacteriaceae and Rhodobacteraceae were prevalently assembled from necrotic tissue metagenomes. In conclusion, anti-viral defence, micronutrient acquisition, heat-stress response mechanisms and amenability to host-microbe gene transfer are signatures of the healthy octocoral microbiome. The identified functions may represent beneficial holobiont traits that can guide future octocoral microbiome manipulations.

Exploring microorganisms and bioresources diversity for biopolymer production

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Large amounts of wastes generated worldwide poses several environmental problems. On the other hand, some of these wastes are potential resources of high-value chemicals and materials. A more efficient and sustainable use of resources must be envisaged aiming at simultaneously reduction and valorisation of residues.
Biopolymers can be used in a wide range of applications. The replacement of the synthetic polymers by biodegradable biopolymers is hindered by the highest market price of the latter. Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers that can be synthesized by several microorganisms and internally accumulated as carbon and energy reserves. The use of agro industrial wastes as feedstocks, as well as novel process operation strategies for PHA production may contribute for the reduction of the polymer final price.
The production of microbial biopolymers by using renewable resources and less energy intensive approaches contribute to lower the process operational costs. In this presentation, sustainable processes for PHA at lab and pilot scale will be presented.

Understanding the impact of pneumococcal conjugate vaccines through multiple approaches based on colonization studies

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Streptococcus pneumoniae (also known as the pneumococcus) is a leading cause of several infectious diseases worldwide such as otitis media, pneumonia, bacteremia and meningitis. This human pathogen colonizes asymptomatically the nasopharynx. Children attending day-care centers are the major reservoirs of pneumococci contributing significantly to its transmission in the community. The polysaccharide capsule represents the major virulence factor of this pathobiont. To date, 98 capsular types (serotypes) have been described. In 2001, a seven-valent pneumococcal conjugate vaccine (PCV7, targeting seven serotypes) became available in Portugal through the private market. In 2010, PCV7 was replaced by PCV13 (a 13-valent vaccine targeting the same seroytpes of PCV7 plus six others). In July 2015, PCV13 was introduced in the National Immunization Plan.
For several years, we have been studying pneumococcal colonization in children attending day-care centers in Portugal. I will discuss how we are using such studies to understand pneumococcal biology and how it is affected by use of PCVs.

Culture Collections and Their role in the Preservation of Biological Resources

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Culture collections are Core facilities that support the scientific community through different activities that include preservation of biological material, exchange of strains worldwide, biotechnology processes and education, among others. In this context, culture collections as Biological Resource Centers also play a key role in the description of new prokaryotic species as repositories of reference strains (type strains) that are then made available to the scientific community for research purposes.

Since 2001, the description of new species represented by viable cultures must include the designation of a type strain and a viable culture of that strain must be deposited in at least two publicly accessible culture collections in different countries from which subcultures must be available (Parker et al., 2019). Currently more than 16,000 prokaryotic type strains representing validly described species are preserved in more than 130 culture collections (Wu et al., 2018). These numbers reflect the excellent collaboration between the collections holding the type strains, the International Committee on Systematics of Prokaryotes (ICSP), taxonomists describing new species and journal editors (e.g. IJSEM). This collaboration effort has led to the implementation of a mutually beneficial system, whereby the information and biological material that one needs to carry out further systematic research or identify organisms is widely available and accessible (Tindall 2008).

Implementation of the Nagoya protocol in 2014, on Access and Benefit Sharing has raised new challenges to access microbial resources, including type strains. The situation has resulted in limited access to type material because several Nagoya protocol signatory countries have the policy to tightly control all access and distribution of microbial resources (including type strains). Such a restrictive policy is in direct conflict with the need to make these reference strains available worldwide without any restrictions for scientific research (Overmann & Hartman Scholz, 2017). Furthermore, the latest topic of debate under the Nagoya Protocol umbrella is whether digital sequence information should be in the scope of the protocol and how it should be ruled, putting at risk the fundamentals of open science and open data access.

Finally, the recent proposal to use genomic sequences as type material (Whitman, 2016) poses yet, another challenge that will involve several players, including culture collections, the Nagoya Protocol and the scientific community.

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Active Learning to Improve Student Learning and Faculty Engagement in the Undergraduate Science Classroom

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Active learning in the classroom has been repeatedly demonstrated to increase student learning and performance. However, the transition of many science classrooms into active learning spaces has been slowed by concerns that class time spent on activities must replace necessary coverage of content. The perceived conflict between content and active classroom learning can be resolved with the application of undergraduate curriculum guidelines developed by professional societies, and of repeated measures of student knowledge throughout the learning process. An additional barrier to the application of active learning is science instructors lack of familiarity, or discomfort, with classroom techniques demonstrated to improve student learning. A growing number of resources are available to guide instructors in the development of active learning skills and a diverse collection of 'classroom-ready' activities can quickly transform a science classroom into an active learning space. Gradual transition to active learning is as valuable as complete transformation since even small amounts of time with active learning have been demonstrated to benefit students.

Microbiology for all: the International Microorganism Day

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The Microbiology community celebrated, in 2019, the 3rd edition of the International Microorganism Day (IMD). This event was launched in Portugal, in 2017, by the Portuguese Society of Microbiology, in partnership with Ordem dos Biólogos e Agência Ciência Viva, with the high sponsorship from the UNESCO National Commission. The Federation of the European Microbiology Societies (FEMS) internationally supports the Day together with an extensive network of microbiology scientific societies and associations and individual microbiologists around the world. Celebrated on September 17, the Day marks the date of the launch of the basis of Microbiology upon reaching the microscopic life, when Anton van Leeuwenhoek - a businessman with no fortune or academic degrees - in 1683 sent a letter to the Royal Society of London in which he described the first observation of microorganisms. The goal of the IMD is to promote Microbiology as a vast area of professional activity and career and to raise awareness among young people and society in general on the essential role played by an invisible multitude of very different living beings in the Life Sciences, in our health, in the environment and sustainable development, in the quality of life, as well as the potential of microorganisms as efficient and versatile cell factories in Biotechnology

The official site of the IMD (http://internationalmicroorganismday.wordpress.com/), together with the logo, the mascots, and the IMD social networks (posting material for the day with the hashtag #internationalmicroorganismday; following updates on Facebook: fb.me/IntMicroDay; Twitter: https://twitter.com/IntMicroDay and Instagram:https://www.instagram.com/internationalmicroorganismday/) allowed us to gather all those involved in the celebrations in Portugal, Europe and this year also in Brasil and Latin America, creating an identity and group spirit and providing continuous and updated information, especially closer to the date. Twitter was the most popular, with 85.3K views during the 80-day period leading up to the celebrations, compared to 28.7K views over the same period in 2018. It is not possible to know the number of people, including pre-school and high-school students, involved in the celebrations of the Day worldwide, visiting exhibitions, attending lectures, brewing and tasting beer, discussing topics of scientific, *social* or practical *relevance,* discovering the invisible world of microorganisms

The purpose of this presentation is to promote the IMD and to give you an idea and the feeling of former celebrations.

Nanotechnology in the food industry: ‘plenty of room’ to innovate

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Nanotechnology has been presented as one of the most promising technologies in different key areas of nowadays. The nanocomposites for aeronautics and the new nano-delivery systems for health showed several advantages in different applications as well as different levels of maturity. In the food area, the use of nanotechnology is new and few applications can be found in our current day-life; antimicrobial nanoparticles for food packaging is one of the examples. Among researchers and some technology companies, the opinion is that nanotechnology can help the food industry in several applications and bring advantages not only for the industry but also for consumers in general. However, only few companies are focusing their research and development on nanotechnology-based products, which can be justified by the cost, regulatory aspects and the consumer behavior.
During this presentation some of the possible applications of nanotechnology in food are presented and discussed, mainly focused on three main food trends, such as personalization, sustainability and urbanization, and how is expected that nanotechnology will influence the future of food.

Enzyme design for polyester synthesis

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Although plastics (fossil fuel derived polymers) are indispensable for our current lifestyle, they are causing too much damage to the environment. If current plastic waste disposal trends continue, in 2050 around 12,000 MMt of waste will be present in landfills or the natural environment (1). It is thus urgent to find more sustainable alternatives. We are studying enzymatic synthesis as a way to obtain sustainable polyesters. We will present our recent Quantum Mechanics/Molecular Mechanics Molecular Dynamics simulation studies about the catalytic mechanisms for polycaprolactone (co)polymers synthesis and hydrolysis (2---4) by the wild type enzymes *Archaeoglobus fulgidus* carboxylesterase (AfEST) and *Candida antarctica* lipase B (CALB) and variants.

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Phages/biofilm interaction: strategies to improve phage efficacy against infectious biofilms

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The complex heterogeneous structure of biofilms confers to bacteria an important survival strategy. Biofilms are frequently involved in many chronic infections in consequence of their low susceptibility to antibiotics as well as resistance to host defences. The increasing need of novel and effective treatments to target these complex structures has led to a growing interest on bacteriophages (phages) as a strategy for biofilm control and prevention. Theoretically, the close proximity of cells within the biofilm structure could enhance phage-host interaction and facilitate phage infection. Conversely, the biofilm structure and composition as well as the physiological state of the biofilm cells may be an obstacle to phage infection. Nonetheless, phages have developed mechanisms to overcome biofilm barriers in a natural evolutionary prey-predator model. A thorough characterisation of biofilm/phage interaction and the identification of the weak aspects of biofilms and the strong features of phages are thus important to develop efficient phage-based biofilm control strategies. Phages can be used alone, as a cocktail to broaden the spectra of activity, or in combination with other antimicrobials to improve their efficacy. In this presentation studies involving the use of phages for the treatment or prevention of bacterial biofilms will be summarized, highlighting the biofilm features that can be tackled with phages or combined therapy approaches.

Oral Presentations

OP1. Combination of geochemical methods and high-throughput sequencing to better understand the distribution and impact of single and multi-metal pollutions at the soil aggregate scale

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Trace metals, especially in mixtures, can disturb and modify the competitive balance between microorganism species in soils, which in turn induce modifications of biogeochemical cycles (C, N, P...). Soils are spatially heterogeneous environments structured in aggregates of different sizes. Similarly to microorganisms, metals present heterogeneous and specific distribution patterns among these micro-aggregates. The objective of this work was to better understand the spatialized impact of heavy metals (Cu-Cd-Cr) on soil microbial communities, according to their respective distribution within the different soil microhabitats, and the temporal evolution of microbial communities structure in response to the multi-metal pollution. By applying a high-throughput sequencing approach we studied the restructuring action of a mixed Cu-Cd-Cr contamination on the microbial community of a grassland-soil at the microaggregate-scale. The soil was contaminated with equitoxic concentrations of Cu, Cd and Cr, alone or mixed and regularly physically fractionated (2-64 days). The bulk soil and the size-fractions were analyzed for mass balance, free, soluble and total metals contents and soil properties (pH, Eh, OC and N contents). Microbial biomass, and bacterial density/diversity were measured (16SrDNA rtPCR and Miseq sequencing,). This study provides a new vision of soil bacterial community structure. It opens the way for the detection and identification of rare taxa and potential new bio-indicators of soil health.

I1. Environmental Microbiology and Biotechnology

OP2. Biological synthesis of nanocatalysts for wastewater treatment and energy production

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Metal nanoparticles (MNPs) can be applied in a wide range of fields, including medicine, environmental remediation and catalysis. Due to their extremely small size and large surface, MNPs offer high activity, stability and selectivity, properties that are normally not seen in the bulk form of the corresponding metal. NPs are mainly produced by physicochemical methods, but most of these require the use of toxic organic solvents and/or high-energy input. Biological synthesis of metal nanoparticles (Bio-NPs) is an attractive alternative to the conventional methods. Microorganisms have the ability to perform redox reactions with high specificity under mild temperature and pressure, providing an environment-friendly strategy for the production of a wide range of nanosized materials.
In the present work we explored new applications of Bio-NPs by studying their catalytic activity in the reductive removal of pharmaceutical products (PhP) and biological photoproduction of hydrogen, both of which have been little explored. The results obtained demonstrate the high catalytic activity of Bio-Pt synthetized by the model sulfate-reducing bacteria Desulfovibrio vulgaris in the removal of 17β-estradiol and the antibiotics ciprofloxacin and sulfamethoxazole [1]. Moreover, the estrogenic activity decreased significantly after the reaction, demonstrating that the products formed are less toxic than the parent compound [1]. The excellent performance of Bio-Pt makes it a promising catalyst to treat pharmaceutical wastes. Concerning energy production, a novel strategy was explored to produce H_2 from visible light using Desulfovibrio desulfuricans as biocatalysts and their biologically synthesized metal nanoparticles working as semiconductor. The results obtained demonstrate the high efficiency and potential of the present system to produce H_2 from light using a non-photosynthetic organism.
This work is a proof of concept showing that the biogenic nanoparticles are powerful catalysts to be used in innovative processes for wastewater treatment and energy production.

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I1. Environmental Microbiology and Biotechnology

OP3. Critical metals accumulation by strain *Serratia fonticola* A3_242: unveiling mechanisms of bacterium-metal interactions

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There are rising concerns about the issue of critical high-tech elements such as Gallium (Ga) and Indium (In). It is urgent to look at to secondary and unexplored resources as viable sources of metals. Biotechnological approaches afford possibilities as sustainable metal recovery technologies. In this perspective, the use of native microorganisms or bio-engineered organisms allowing for accumulation and reuse of metals from wastes promoting recycling, minimizing harmful waste and hazard and dissipation.
A selected metal resistant strain, *Serratia fonticola* A3_242, was explored to know its potential as bioaccumulator of Ga/In. This strain was able to accumulate 1.04 ± 0.01 µg Ga/mg protein, 80.4 ± 24 µg In/mg protein and 3.7 ± 1.2 µg Al/mg protein when grown in R-2A broth medium with 0.1 mM of metal for 6 hours. In metal competition assays, in presence of Ga and In simultaneously, strain A3_242 accumulated 27 ± 6 fold more gallium. Moreover, in simultaneous presence of the 3 metals (Ga, In and Al), the strain accumulated approximately 36 ± 8 and 9 ± 2 fold more gallium and aluminum, respectively, than in the single metal assays. The indium accumulation was not notably affected by presence of the other tested metals.
Different approaches were also used to identify and elucidate potential mechanisms involved in bacterial ability to handle with these elements. Proteomic variations analysis of strain A3_242 submitted to Ga/In stress allowed visualization of an overexpressed protein with indium, which was identified by mass spectrometry as a Superoxide Dismutase (SOD). The strain was also subjected to random transposon mutagenesis (Tn5) to determine its genetic determinants responsible for In and Ga resistance. One Ga susceptible mutant (T_12) was recovered and the transposon insertion was identified into a gene coding for a major facilitator superfamily (MFS) protein, a multidrug resistance transporter. The metal accumulation capacity of mutant was affected. It was able to accumulate the double amount of gallium or aluminum but just accumulated 0.7 fold of indium comparatively with the native strain.

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I1. Environmental Microbiology and Biotechnology

OP4. Bioaugmentation of Aerobic Granular Sludge with specialized degrading granules treating 2-fluorophenol wastewater

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The industry growth has been accompanied by an increase in the amount of industrial chemicals being released into the environment. Indigenous microbial communities in wastewater biotreatment processes are not always effective in removing xenobiotics.
This work aimed to evaluate the efficiency of a new bioaugmentation strategy in an aerobic granular sludge sequencing batch reactor (AGS-SBR) system fed with 2-fluorophenol (2-FP). Bioreactor performance in terms of phosphate and ammonium removal, 2-FP degradation and chemical oxygen demand (COD) was evaluated.
The new bioaugmentation strategy consisted in producing granules using extracellular polymeric substances (EPS) extracted from AGS as a carrying matrix and a 2-FP degrading strain, *Rhodococcus* sp. FP1. The produced granules were used for the bioaugmentation of a reactor fed with 2-FP. Shortly after bioaugmentation, the produced granules broke down into smaller fragments inside the bioreactor, but 2-FP degradation occurred. After 8 days of bioaugmentation, 2-FP concentration inside the reactor started to decrease, and stoichiometric fluorine release was observed 35 days later. Phosphate and ammonium removal also improved after bioaugmentation, increasing from 30% to 38% and from 20 to 27%, respectively. Complete ammonium removal was only achieved when 2-FP feeding stopped, and phosphate removal was not recovered during operation time. COD removal also improved after the addition of the produced granules.
The persistence of *Rhodococcus* sp. FP1 in the reactor was followed by qPCR. *Rhodococcus* sp. FP1 was detected 1 day after in the AGS and up to 3 days after bioaugmentation at the effluent. Nevertheless, the 2-FP degradative ability remained thereafter in the granules. Horizontal gene transfer could have happened from the 2-FP degrading strain to indigenous microbiome as some bacteria isolated from the AGS, 3 months after bioaugmentation, were able to degrade 2-FP.
This study presents a promising and feasible bioaugmentation strategy to introduce specialized bacteria into AGS systems treating recalcitrant pollutants in wastewater.

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II2-Molecular Microbiology and Microbial Physiology

OP5. Horizontal gene transfer overrides mutation in *Escherichia coli* colonizing the mammalian gut

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Bacteria evolve by mutation accumulation in laboratory experiments, but tempo and mode of evolution in natural environments are largely unknown. Here, we study the ubiquitous natural process of host colonization by commensal bacteria. We show, by experimental evolution of *Escherichia coli* in the mouse intestine, that the ecology of the gut controls the pace and mode of evolution of a new invading bacterial strain. If a resident *E. coli* strain is present in the gut, the invading strain evolves by rapid horizontal gene transfer (HGT), which precedes and outweighs evolution by accumulation of mutations. HGT is driven by 2 bacteriophages carried by the resident strain, which cause an epidemic phage infection of the invader. These dynamics are followed by subsequent evolution by clonal interference of genetically diverse lineages of phage-carrying (lysogenic) bacteria. We show that the genes uptaken by HGT enhance the metabolism of specific gut carbon sources and provide a fitness advantage to lysogenic invader lineages. We conclude that phage-driven HGT is a key eco-evolutionary driving force of gut colonization-it accelerates evolution and promotes genetic diversity of commensal bacteria.

II2-Molecular Microbiology and Microbial Physiology

OP6. The heme homeostasis in *Staphylococcus aureus* is achieved by a link between the heme biosynthesis and the uptake pathways

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Staphylococcus aureus synthesizes heme de novo through the coproporphyrin-dependent (CPD) pathway, but also captures heme from the host hemoglobin via the Isd heme uptake system. In this work, we show for the first time that the two systems are connected via two key enzymes of the two pathways, namely the heme monoxygenase IsdG of heme uptake system and the coproporphyrin ferrochelatase (CpfC) of the heme biosynthesis pathway. We observed that IsdG has the capacity to impair the ferrochelatase activity of CpfC. This inhibition is linked to a protein-protein interaction that occurs between the IsdG and CpfC proteins, as shown by fluorescence anisotropy and FLIM-FRET. Importantly, it was also observed that the increase of the external heme, as it occurs in host colonization niches, promotes this interaction. The importance of this crosstalk between the two systems relies on the need to avoid intracellular accumulation of heme which is highly toxic for any bacterial cell.
Interestingly, a phylogenetical analysis revealed that IsdG-like proteins are present in organisms with heme biosynthetic pathways more often than in organisms that only contain genes for heme uptake systems. Therefore, we propose that in several bacteria the control of heme homeostasis is done by the heme monoxygenase IsdG protein.

OP7. Microbial extracellular electrically conductive filaments for bioenergy applications: structural and functional insights

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Geobacter bacteria are abundant in diverse natural environments and have the ability to reduce external acceptors by exporting electrons from inside the cells to the exterior via extracellular electron transfer (EET) mechanisms [1]. These mechanisms led to the development of *Geobacter*-based biotechnological applications, including the bioremediation of toxic or radioactive soil minerals and the conversion of organic wastes into electric current in microbial fuel cells (MFC) [2]. The EET chain includes components at the bacterial outer-membrane, inner-membrane, periplasm and electrically conductive filaments that, altogether, allow electron transfer to extracellular acceptors. Recently, cryogenic electron microscopy revealed an unprecedented structure of the *Geobacter sulfurreducens* electrically conductive filaments, which are formed by the polymeric assembling of a hexaheme c-type cytochrome [3]. Each monomer contains six low-spin hemes coordinated by two axial histidines. In the filament, all hemes from the same monomer are coordinated by their own axial histidine except the fifth heme that coordinates to a histidine from a neighboring monomer. This was the first report of an electrically conductive filament formed by a c-type cytochrome polymer in which closely-stacked hemes provide a continuous path for electron flow. The cellular location of these bacterial filaments, running from the entire periplasm through the outer-membrane and expanding to the extracellular space, indicates that they are likely to receive electrons from the most abundant periplasmic cytochromes, a family composed by five triheme c-type cytochromes named PpcA [4]. The atomic level structure determined for a bacterial conductive filament will be presented as well as the biochemical strategies to reveal the mechanisms that trigger EET through the filaments. This information will be further used to the rational design of effective *G. sulfurreducens* strains for improved MFC-based bioenergy applications.

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II2-Molecular Microbiology and Microbial Physiology

OP8. Yeast response and tolerance to acetic acid: focus on cell envelope properties, assessed by AFM

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Acetic acid is a food preservative, a growth inhibitory compound present in Agro-food & forestry residues hydrolysates and a by-product of Saccharomyces cerevisiae metabolism [1]. Together with high concentrations of ethanol and other bioprocess-associated stresses, acetic acid contributes to alcoholic fermentation inhibition or arrest and decreased ethanol productivity [1]. The adaptive genome response to acetic acid stress involves the remodeling of plasma membrane and cell wall, thus reducing acetic acid-diffusion rate through the cell envelope, limiting its reentry following the active efflux of acetate [1, 2]. The ATP-binding cassette (ABC) transporter Pdr18 is a determinant of yeast response and tolerance to acetic acid, mediating ergosterol transport at plasma membrane and alleviating plasma membrane-induced-permeabilization [2].
In this work, the time-course of the alteration of cell wall nanomechanical properties and structural integrity following yeast exposure to acetic acid stress were assessed using Atomic Force Microscopy (AFM) and lyticase sensitivity assays. Cell wall stiffness and resistance to lyticase were found to increase during adaptation to acetic acid stress. Moreover, the expression of PDR18 was found to be required for maximum cell wall stiffness and resistance to lyticase action. Results reinforce the concept that cell wall remodeling and PDR18 expression are important determinants of acetic acid stress response and tolerance.

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III3-Bioeconomy and Sustainable Development

OP9. Polysaccharide-based membranes for donwnstream processing

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This work discusses the production of polysaccharide polymers by bacterial cultures and their use for the production of membranes, aiming their stability under operating conditions and their biodegradability after use. Different techniques for the purification of the biopolymers produced will be presented and discussed critically, namely by dialysis and by dia-nanofiltration.
The protocols for preparation of homogeneous and composite membranes will be discussed as well as the characterisation of the membranes developed, in what concerns their mechanical, structural and transport properties. The use of these membranes in different applications, namely on the processing of organic solvents by pervaporation and on gas permeation and drying using membranes, will be presented and discussed.
A critical assessment of the development of biopolymeric membranes will be presented, aiming to foster the discussion within the scientific community.

OP10. Isolation and potential of non-conventional yeasts for the production of added-value compounds from pectin-rich residues, in particular sugar beet pulp

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The utilization of agrofood residues rich in pectin, namely sugar beet pulp (SBP), are considered interesting substrates for the production of bioethanol and other added-value compounds. However, *Saccharomyces cerevisiae* ferment glucose, mannose and galactose but is not able to catabolize other sugar-monomers present in pectin rich-residues hydrolysates (e.g. galacturonic acid, arabinose and xylose). The exploitation of non-conventional yeast strains for the bioconversion of sugar beet pulp into value products is desired, even though the genetic modifications of *S. cerevisiae* by expressing heterologous pathways are also being attempted.
To isolate yeasts with potential for efficient SBP bioconversion, this fresh residue was suspended in sterile water supplemented with peptone (1%). After two days of incubation at 30ºC, samples were plated in YPD-agar medium with chloramphenicol. The yeast strains isolated were identified by amplification of internal transcribed spacer (ITS) and D1/D2 regions of ribosomal DNA and sequencing data revealed the presence of *Kluyveromyces marxianus*, *Rhodotorula mucilaginosa*, *Cryptococcus laurentii* and *Clavispora lusitaniae*. The thermotolerant *K. marxianus* strains isolated catabolize arabinose and xylose but only the *R. mucilaginosa* strains were able to grow using galacturonic acid as the sole C-source.
The potential of the yeast strains isolated from SPB (*K. marxianus* IST389, R. mucilaginosa IST390) and other yeasts from the IST collection isolated from other agrofood wastewaters or oak wine barrel (*R. mucilaginosa* IST423 and *Meyerozyma guilliermondii* IST369) for SBP bioconversion was examined using the industrial strain *S. cerevisiae* EthanolRed as reference . Yeast cultivation in minimal medium supplemented with individual and mixed sugars and/or acetic acid (present in the hydrolysates due to pectin backbone acetyl groups) and in SPB hydrolysates (obtained from ERANET-IB partner) was performed and sugar utilization and production of ethanol and other metabolites were analysed by HPLC. *K. marxianus* and *M. guilliermondii* produced small amounts of ethanol (0.8% (v/v)) from SBP hydrolysate essentially from glucose, as *S. cerevisiae*, but also metabolized arabinose, xylose and acetic acid and produced arabitol. *R. mucilaginosa* IST390 growth in SBP was characterized by the production of high yields of carotenoids (about 312µg/g dry biomass).

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OP11. Can a traditional delicacy contribute to forest sustainable development and regional bioeconomy?

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Terfezia arenaria is a desert truffle, a seasonal edible hypogeous mycorrhizal fungi, considered a traditional delicacy, highly valued all over the Mediterranean Basin countries. In Portugal, it is traditionally harvested in Alentejo and Beira Baixa regions representing an important income for local economy. In addition to its economic value, the inclusion of *Terfezia* in our diet is also of gastronomic and nutritional interest. As it is highly appreciated, if produced in larger scale and at lower prices, it may be an excellent product to use in the context of ""plant-based meet"" concept. Although several studies have demonstrated the nutritional value of a few Mediterranean desert truffles species, there is no available data regarding *T. arenaria*, which is the species with highest productivity potential in Portugal. In this work we present the first estimates regarding the nutritional value of T. arenaria and its potential productivity in Alentejo, Portugal.

During the spring of 2019, a total of 58 ascocarps of *T. arenaria* were collected in 4 sampling areas of Lavre, Alentejo revealing an average productivity of 19.0 kg/ha. Physical data were collected, referring to fresh weight and ascocarp diameter; and a chemical composition analysis was performed, namely moisture (76%), proteins (13.69 %), lipids (2.20%), raw fibre (10.14 %), carbohydrates (66.69 %), ash (7.28 %) and caloric content (387 kcal). This is the first Portuguese study reporting production analysis and nutritional overview of endogenous *T. arenaria* ascocarps, and is in accordance with results obtained for other desert truffle species, as well as with overall nutritive characteristics of mushrooms.

The (re)introduction of these plant-fungi symbioses are key tools that can act as soil stabilizers and remediators, contributing for a sustainable forest development. Furthermore, these preliminary findings suggest that *T. arenaria*, due to its environmental, nutritional and economic potential production can be part of a multi-objective forest management (especially after fire) to enhance biodiversity, recreation and landscape values that are highly appreciated by users, and a key element for regional bioeconomy and their sustainable development.

OP12. Microbial biocatalysis of CO_2 reduction and H_2 production

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Developing sustainable processes to reduce the levels of CO_2 is one of the most urgent and challenging issues facing our society. Hydrogenation of $CO_2$for the production of useful chemicals such as formate represents a valuable process for CO_2capture. This approach provides not only a mechanism for CO_2sequestration but also a way to produce an interesting H_2 storage material. Thus, it is crucial to find suitable catalysts for this process. We have been studying anaerobic bacteria and their enzymes as biocatalysts for the reversible reduction of $CO_2$to formate and H_2 production from formate. Using whole cell studies we have identified highly active bacteria in H2 production as well as CO_2reduction [1,2]. We have characterized the enzymes involved, a NiFeSe-hydrogenase [3] and W-formate dehydrogenase [4], and produced variants with improved properties [5]. The O_2 stability and robustness of these enzymes has also been exploited in the development of efficient semi-synthetic photo- and electrocatalytic systems [6,7].

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OP13. *Verticillium dahliae* does not significantly change the structure and function of the olive belowground microbial communities but alters their co-occurrence interactions

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Verticillium wilt of olive (VWO), caused by the soilborne fungus *Verticillium dahliae* Kleb, is a relevant disease affecting olive cultivation in many areas where this tree is cultivated. The use of tolerant/resistant cultivars is considered an efficient control tool of the disease. A greenhouse experiment was set up to: (i) describe the root-associated microbial communities of two olive cultivars (cv.) qualified as tolerant (cv. Frantoio) and susceptible (cv. Picual) to *V. dahliae*, and (ii) assess whether the belowground-associated microbiome participate in the tolerance/susceptibility level of these cultivars to VWO. Nursery-produced olive plants were grown in pots containing non-sterile ad hoc prepared soil. After acclimation, a group of plants were inoculated with a defoliating (highly-virulent) representative of *V. dahliae*. A control group of non-inoculated plants was treated just with water. Surface disinfected roots and rhizosphere soil from both groups of plans were sampled at 0, 8, 15, and 30 (four plants per time-point and per cultivar) days after inoculation. DNA and RNA were extracted and sequenced (PCR products of the V3-V4 region of the 16S rRNA gene for bacteria and the ITS2 for fungi) by Illumina MiSeq. Bioinformatic, statistical and network analyses were then performed to analyze the composition, functionality and co-occurrence interactions of the endosphere and rhizosphere microbial communities. The belowground microbial communities of the two cultivars are similar, minor significant differences in diversity and composition of root-associated microbiota were found between olive cultivars regardless they were inoculated or not with the pathogen. Presence of *V. dahliae* caused changes mainly in communities' taxa relative abundance, mostly in the VWO-susceptible cultivar. However, notable differences were found in the communities co-occurrence interactions in response to the pathogen presence. Both cultivars showed changes in the topology of the networks, both at DNA and RNA level, and modifications of the positive/negative edges ratio.
A correlation between microbial networks modifications and susceptibility/tolerance to the pathogen was found. Therefore, changes in the microbial communities interactions may explain, at least partially, the differential VWO susceptibility of the tested olive cultivars.

OP14. Impact of plant genotype and plant habitat in shaping bacterial pathobiome: a comparative study in olive tree

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Plant-inhabiting microorganisms interact directly with each other which effect is being recognized to influence the disease process. However, the role of the host plant and plant habitat in shaping pathobiome composition and their implications for host susceptibility/resistance to a particular disease, are currently unknown. For the elucidation of these questions was chosen as a model system the olive knot (OK) disease, which is caused by *Pseudomonas savastanoi* pv. *savastanoi* (Pss). Thus, both epiphytic and endophytic bacterial community of asymptomatic and OK-symptomatic twigs of olive cultivars of varying susceptibilities to OK disease, were investigated by molecular identification of cultivable isolates. Our results indicate that OK disease is the main driver of the bacterial community causing changes on their diversity, abundance and composition. The microbiota most perturbed was found in the OK-susceptible cultivar and in the endophytic communities. Plant habitat (epiphytes vs. endophytes) also showed an important role in shaping microbial community assemblage, in particular in symptomatic twigs of the OK-susceptible cultivar. Host cultivar had little effect on the bacterial microbial community composition, being its effect mainly observed in bacterial community assemblage of OK-symptomatic twigs. Overall, the pathobiome seems to result from an intricate interaction between Pss, the resident bacteria, and the host. Specific bacterial genera were associated to the presence and absence of OK disease in each cultivar, and their ability to trigger disease should be studied in the future.

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OP15. Sex and age taxonomical and functional differences of Egyptian mongoose gut microbiota

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Gut microbiota has been progressively acknowledged as a fundamental component of mammals' biology. Egyptian mongoose [*Herpestes ichneumon* (Linnaeus, 1758)] is a carnivore species, member of the Herpestidae family, whose distribution in Europe is restricted to the Iberian Peninsula. This species fecal microbiota has been previously studied by our group using culture-dependent methods but never using a culture-independent approach. In this work, we investigated the gut microbiota of 20 Egyptian mongoose specimens sampled in South Portugal, using Single Molecule Real-Time sequencing of the 16S rRNA gene to characterize its gut bacterial microbiota and to investigate sex- and age class-related taxonomical and functional differences. Our results show a core gut bacterial microbiota dominated by Firmicutes, Fusobacteria, Actinobacteria, and Proteobacteria. Four genera were uniquely found in females, and six in males, while eight genera were restricted to adult age class and five to juvenile'. Besides these compositional distinctions, the differential functional profile of this carnivore species was evaluated for the first time. Males showed a significantly higher abundance of amino acid and citrate cycle metabolic pathways, contrasting with females that evidenced a significant overrepresentation of galactose metabolic pathways. Also, specimens from the adult age class showed a significantly higher abundance of cationic antimicrobial peptide resistance pathways, when comparing with juveniles that exhibited a significant overrepresentation of two-component systems associated with antibiotic synthesis, flagellin production, chemotaxis control, and biofilm formation. These functional dissimilarities possibly reflect sex- and age-related differences in mongoose's diet and behavior, supporting the importance of gut microbiome characterization to fully comprehend mammal's ecology.

OP16. Dynamics of the pine tree rhizosphere microbiome in response to *Fusarium circinatum* infection

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Fusarium circinatum causes pine pitch canker, which is an emergent disease affecting *Pinus* forests worldwide, representing both ecological and economic risks. This phytopathogen affects pine trees with varying severity degrees, with Pinus radiata being the most susceptible species. The rhizosphere microbiome plays a fundamental role on plant nutrition, pathogen resistance and other factors related to plant fitness. While plant-pathogen interactions have been reasonably studied, the microbiome response to above-ground infections has been poorly characterized in pine species. In this study we proposed to evaluate the Pinus rhizosphere microbiome changes following *Fusarium circinatum* infection.
Eight months old seedlings, belonging to *Pinus radiata* (highly susceptible to *F. circinatum*) and *Pinus pinea* (highly resistant to *F. circinatum*), were acclimatized in greenhouse conditions for 3 months prior to fungal inoculation. For each species, experiments included a non-inoculated control group (n=8 plants) and a group (n=14 plants) inoculated on the stem with *F. circinatum*. When 50% of the plants in a group displayed evident disease symptoms (apical damp off), 5 plants per treatment were sampled. The rhizospheric soil of each plant was collected for DNA extraction and the 16S rRNA gene V3 region was amplified. The structure of the microbiome was assessed by DGGE (all samples) and next generation sequencing (three selected samples per treatment).
DGGE analysis revealed a shift in the rhizosphere microbiome of infected trees relative to control, being more accentuated in susceptible species. Furthermore, DGGE profile cluster analysis revealed that the profile of infected *P. radiata* trees showed low similarity (60%) with the control group. Regarding *P. pinea*, this value was of 80%. Analysis based on massive parallel sequencing corroborated the aforementioned results, specifying an abundance increase of members of the Kofleriaceae family and an abundance decrease of Oxalobacteriaceae family members. Overall diversity and richness were not significantly affected. Our results demonstrate distinct responses of the rhizosphere microbiome of two *Pinus* species after *F. circinatum* inoculation. Further studies are needed to determine the contribution of microbiome shifts in the disease's development, in order to evaluate possible mitigation measures based on microbiome manipulation.

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OP17. An integrative process for the extraction and polishing of intracellular carotenoids from *Rodhotorula glutinis* yeast using protic ionic liquids

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Carotenoids are important pigments naturally synthesized by plants and microorganisms, which are widely applied in food, cosmetic, and pharmaceutical product formulations. In addition to their use as colouring agents, these pigments have been suggested as additives to prevent health diseases, as for example, cancer, macular degradation and cataracts. Since these naturally pigments are usually obtained in complex raw materials, for example, intracellular environment of microorganisms, adequate downstream processing technologies are required for their recovery at commercial purity requirements. Herein, an alternative, more sustainable, biocompatible, and reusable process for the extraction and polishing of intracellular carotenoids from *Rhodotorula glutinis* yeast cells by using ionic liquids (ILs) was studied. It was evaluated the aptitude of different ammonium-based ILs as a substitute of volatile organic compounds (VOCs). Hexanoate-based ILs were the most efficient in the recovery of intracellular carotenoids with extractions rates sixfold higher than the common volatile organic solvent (DMSO). The economic and environmental sustainability of the process was demonstrated, by integrating the cell-disruption stage with a subsequent three-phase partitioning unit, which allowed the recovery of pure carotenoids as a solid precipitate at the interface. The IL solution was recycled for three consecutive cell-disruption stages with good carotenoid extraction yields. This study shows the potential of the use of ILs in the extraction of biologically active molecules (i.e., carotenoids) at mild and accessible conditions, as alternative to environmentally non-favourable VOCs.

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OP18. Integrated bioprocess approach for the production of xylooligosaccharides

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The demand of prebiotic ingredients has been growing over the years as consumers pay more attention to their health. Xylooligosaccharides (XOS) are considered emergent and competitively priced prebiotics, presenting high potential as food ingredients. As a result, the industry is focused on developing new approaches to improve their production efficiency to meet the increasing demand while reducing costs. Hence, the main purpose of this work was to develop an integrated bioprocess, based on one-step fermentation, for the production of prebiotic XOS, towards the simplification and cost reduction of the process.
The one-step fermentation of 13 agro-residues was done using two *Trichoderma* species. The most promising results were found for *T. reesei* using brewers' spent grain (BSG) as substrate. BSG is an inexpensive and abundant agro-industrial residue that was proven interesting for the production of arabino-xylooligosaccharides (AXOS).
In order to reduce the production time obtained with *T. reesei* (3 d), the *Bacillus subtilis* 3610 wild type (wt) was successfully used to produce AXOS through direct fermentation of BSG, reducing the production time to 12 h. Genetic engineering was used to further optimize the microorganism performance, by cloning the *T. reesei* xylanase gene coupled with a secretion tag into the *B. subtilis* chromosome (*B. subtilis* 3610 clone 2). This strategy led to a yield increase of 33 % comparing to the wt, and 29 % comparing to the *T. reesei*.
B. subtilis 3610 clone 2 was also selected for downscale production of XOS by direct fermentation of commercial beechwood xylan. The maximum production yield, 306 ± 4 mg/g (XOS/xylan), was achieved after 8 h of fermentation operating under one-time impulse fed-batch regimen.
In vitro studies using human fecal inocula were performed to evaluate and compare the potential prebiotic effect of commercial lactulose and the XOS herein produced. The significant increase in the production of short chain fatty acids and CO_2, added to the reduction of pH and ammonia concentration suggest that the XOS hold potential functional properties for human health. The results gathered provide important insights for the development of new integrated strategies for XOS production from agro-residues.

OP19. Nanofiltration technology-based purification of microbial biosurfactants, mannosylerythritol lipids (MELs), as a new downstream route

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Microbial biosurfactants (BS) are promising compounds to replace their chemical relatives due to their lower toxicity and higher biodegradability. Within BS, mannosylerythritol lipids (MELs) are extracellularly secreted glycolipids, known for their low critical micelle concentration and tensioactive properties. Although their wide range of potential applications, MEL scale-up production has been hampered by the lack of sustainable bioprocesses, capable of attaining high titres from cheap renewable raw materials and efficient downstream separation.
MEL have been described to be produced from a variety of microorganisms. *Moesziomyces* spp. (former *Pseudozyma* spp.), a basidiomycetous yeast, such as *M. antarcticus* and *M. aphidis*, are examples of the best MEL producers. Two different strategies, regarding substrate utilization, have been reported for MEL production: (i) the use of vegetable oils, where the higher MEL titres reported are obtained, but MEL contamination with considerable amounts of residual unconsumed lipids [1]; thus increasing MEL losses over downstream separation [2]. (ii) the use of sugar-based substrates, where lower residual lipids are present, but MEL titres obtained are also significant lower; thus, reducing process economical competitiveness.
In this regard, the current study aimed at developing a competitive strategy for MEL purification, using nanofitltration technology to separate MEL from residual lipids. This strategy took advantage of the difference between molecular weights of MEL and lipidic impurities. The results of this study show that using a culture of *M. antarcticus* fed with 40 g/l of glucose and 20 g/l of waste fried oil resulted in a MEL titre of 12.1 g/L and residual lipids of 2 g/L. Further purification of such MEL crude, extracted with ethyl acetate, using an home-made PBI-based membrane allows to recover more than 80% of total MEL with a purity of around 97%, after 6 diavolumes of ethyl acetate.
The downstream strategy here described is an important step forward towards an efficient downstream route for MEL against other lipids (either non-consumed ones from substrate or extracted from yeast cells).

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OP20. Utilization of agro-industrial and forestry residues to produce second-generation bioethanol with *Saccharomyces cerevisiae* (ATCC® 26602TM)

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The use of biomass residues is strongly advocated under European Union (EU) legislation in order to help achieve the climate and energy targets of the EU for 2020 and beyond. The Portuguese Government developed a “National plan for the promotion of biorefineries”, with a strategy until 2030, to promote a sustainable use of renewable resources, and to take advantage of readily available residues. Portugal has a considerable potential for residual biomass (forestry, agricultural, industrial) that can be valorized in a context of biorefineries to produce a wide spectrum of marketable products (food, feed, chemicals, and materials) and energy (fuels, power and/or heat), with environmental, economic and social benefits. Primary sludges from pulp mills, pine residues (such as stumps) and rice straw were identified as significant resources for biorefining, generated by industrial, forestry and agricultural activities, respectively. These residues were applied in simultaneous saccharification and fermentation (SSF) processes to produce second-generation bioethanol using 15 FPU/gCH of cellulase and *Saccharomyces cerevisiae* (ATCC® 26622TM) yeast. A previous treatment was carried out upon pine residues and rice straw to remove inhibitory lignin from their structure. After pretreatment with potassium hydroxide and sodium chlorite, rice straw was composed of 73.3% of cellulose, 17.8% of hemicelluloses and 3.7% of residual lignin. In the following SSF, the straw pulp yielded an ethanol concentration of 53 g/L at 85% conversion and 2.2 g/(L h) productivity. Pine stumps pretreated with steam explosion, organosolv and soda treatments had 95.8% of cellulose and 3.0% of residual lignin. The obtained pine pulps produced up to 79 g/L of ethanol at 1.1 g/(L h) productivity and 97% conversion yield. Primary sludges, composed of 57.0-79.0% of carbohydrates and 4.5-6.7% of residual lignin, were used as received, without previous treatment. The effects of i) the sludge consistency; ii) the supply of different and low-cost yeast additional nutrient; iii) a step-wise scale-up; and iv) the synergistic action of yeast mixed culture (*S. cerevisiae* (ATCC® 26622TM) with *Pichia stipitis* DSMZ 3651) in the SSF efficiency were evaluated. Ethanol concentrations up to 64 g/L were produced, at 60-68% conversion yield and 1.5 g/(L h) productivity.

OP21. Novel capsular depolymerases-based strategy to kill multidrug-resistant pathogenic bacteria

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Multidrug resistant pathogens represent one of the greatest threats to human health of the new millennium. ESKAPE bacterial pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and other *Enterobacteriaceae* species) are the leading group among these so-called superbugs, which rapidly acquire resistances to several (and sometimes all) available antibiotics and cause a variety of nosocomial infections (e.g. bacteraemia and wound infections).
Our research has been leading an innovative approach based on bacteriophage-derived enzymes (called capsular depolymerases) against *A. baumannii* (see video at ref 1). Previously, we found that some bacteriophages (i.e. viruses that specifically infect bacteria) acquired the ability to infect different Acinetobacter hosts through acquisition of different capsular depolymerases (2). These enzymes located at the bacteriophage tails bind and degrade specific bacterial capsules types (2). Recently, recombinantly expressed capsular depolymerases showed to be active in several environment conditions, non-nontoxic to mammalian cells and able to make *A. baumannii* fully susceptible to host complement effect, namely in i) *Galleria mellonella* caterpillar, ii) murine and iii) human serum models (3, 4). A single intraperitoneal injection of depolymerase protect 60% of mice from dead, with significant reduction of pro-inflammatory cytokine profile (4). We show that capsular depolymerases fit the new trend of antimicrobials needed, as they are highly specific, stable and refractory to resistance as they do not kill bacteria per se, instead they remove bacterial surface polysaccharides, diminishing bacterial virulence and exposing them to the host immune system. This innovative antimicrobial approach can be applied to other pathogenic bacteria.

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OP22.Impressive photodynamic inactivation of multidrug-resistant hospital bacterial strains and biofilm growing bacteria by imidazolyl cationic porphyrins

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The worldwide emergence of multidrug-resistant (MDR) bacteria are considered by the World Health Organization (WHO) one of the main causes of mortality by infectious diseases. It has been estimated that more than 80 % of all microbial infections are caused by formation of bacteria biofilms.[1] According to WHO recommendations, an urgent investment in R&D is essential for the development of new antibacterial entities with alternative mechanisms of action, to avoid that around 10 million people will die annually worldwide by 2050.[2,3] Antimicrobial photodynamic therapy is one of the methodologies that has received significant attention, for not being associated with the development of microorganism resistance after treatment.[4] The present work intends to overcome these challenges by the development of new photosensitizers based on cationic imidazolyl moieties with different amphiphilicities, molecular weights and number of charges. Their antimicrobial activity was tested towards a panel of multidrug-resistant hospital bacterial strains collected from patients, either Gram-positive and Gram-negative bacteria, and *S. aureus* biofilms. Total inactivation was found for concentrations as low as 1 μM in planktonic bacteria with irradiation at 415 nm (LED, 1.36 J/cm2). On the other hand, in *S. aureus* biofilm, we observed the size and number of charges effect and an irradiation with 5 J/cm² in the presence of just 5.2 nM of the smaller photosensitizer showed an impressive destruction of the biofilm (7log)[5]. Confocal microscopy images and computational studies support the obtained results. Based on this unprecedented results, Zn(II) complexes of (1,3-dimethylimidazol-2-yl)porphyrinates open new opportunities for PDI of antibiotic-resistant bacteria and biofilms.

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OP23. Characterization, manipulation and antitumor activity of the extracellular carbohydrate polymer from the cyanobacterium *Synechocystis* Δ*sigF* mutante

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Many cyanobacteria produce extracellular polymeric substances (EPS), mainly composed of heteropolysaccharides that can remain associated to the cell or be released into the surrounding environment (RPS). Their particular characteristics make them very attractive for biotechnological applications. Despite the increasing interest on cyanobacterial polymers, the knowledge on the biosynthetic pathways involved in EPS production and export is still limited, hindering the implementation of industrial systems based on these polymers. In this work, we show that the transcription regulator Group 3 sigma factor F (SigF) is involved in the control of EPS production in Synechocystis PCC 6803. The results obtained with a knockout mutant Δ*sigF* indicated that, although growth is significantly impaired, the total carbohydrates content of the culture is 2-fold higher and the production of RPS is 3 to 4-fold higher compared to the wild-type [1]. The process for the isolation of the extracellular carbohydrate polymers from Synechocystis wild-type and ΔsigF cultures was optimized [2], and the polymers were extensively characterized in terms of monosaccharide composition, protein and sulphate contents, rheological properties and molecular weight [3]. Furthermore, the biological activity of these polymers was evaluated on well-established and characterized cancer cell lines [3]. The polymers showed a strong effect towards melanoma, thyroid and ovary cancer cell lines, decreasing cell viability and inducing apoptosis at high rates, via p53 and caspase-3 activation. Manipulation of sulphate content and molecular weight of the polymers is being carried out and the preliminary results showed that both features are important for this antitumor activity. In conclusion, SigF is the first regulatory element associated to RPS production in Synechocystis. Moreover, Synechocystis Δ*sigF* is a promising platform to study/manipulate RPS production and to obtain higher amounts of a biological active polymer. The features associated to this polymer makes it suitable for biomedical applications, namely cancer therapeutics.

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OP24. Bac3Gel: a substrate to recreate lung microbiota for the screening of antimicrobial agents

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Introduction: Cystic fibrosis (CF) mucus exhibits altered chemical and viscoelastic features, limiting its clearance and leading to chronic bacterial infections. Current bacterial culture fails to recreate bacterial communities and microenvironments of lung microbiota. Additionally, it is difficult to induce representative human multi-bacterial infections in animals.
Materials and Methods: Bac3Gel, a three-dimensional hydrogel with a graded structure, was adopted to recreate lung microbiota. Bac3Gel exhibits a polysaccharidic backbone, typical of CF mucus, and mucin. Extensive rheological analyses were carried out to produce Bac3Gel with viscoelastic properties that much those of CF mucus. Additionally, in vitro infections were induced within Bac3Gel by culturing *P. aeruginosa* and *S. aureus*, the prevalent bacteria colonizing the airway CF mucus, either in mono or multi-cultures. Finally, Bac3Gel, infected for 24h with P. aeruginosa, were treated for 24h with three different antibiotics, to which P. aeruginosa is sensitive, and compared in effectiveness to standard bacterial cultures.
Results and Discussion: Bac3Gel exhibits similar viscoelastic properties alterations to those reported for CF sputum, but its viscoelastic properties can be easily changed to match those of physiologic airway and intestinal mucus. Bac3Gel successfully sustains growth of *P. aeruginosa* and *S. aureus* with a bacterial concentration of 109 CFU/mL after 24h of infection. When co-cultured, both *P. aeruginosa* and *S. aureus* co-exist within Bac3Gel, while in planktonic conditions S. aureus was not present. Bacteria resulted more susceptible to antibiotic treatment under planktonic conditions than when cultured within Bac3Gel, where these instead displayed increased antibiotic tolerances even at high antibiotic concentrations (10 MIC). The sensibility difference between Bac3Gel and planktonic cultures confirmed the well-reported mismatch between planktonic conditions and clinical outcomes.1-2
Conclusions: These results indicate that Bac3Gel is a promising substrate to recreate lung microbiota for the screening of antimicrobial agents. The versatile production process of Bac3Gel allows to generate microgradients of viscoelastic properties, nutrients, and gases, which are typical of lung microbiota. Overall, Bac3Gel holds the potential to recreate relevant microbiota environments, including intestinal microbiota, and can be adopted as a universal substrate for bacterial culture.

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OP25. Micoteca da Universidade do Minho – Creating Opportunities for Exploitation and Utilization of Microbial Resources

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Micoteca da Universidade do Minho (MUM) was founded in 1996 with the mission to be a resource centre for fungal biodiversity preservation and information creating solutions for sustainable development and human well-being. It has implemented since 2011 a Quality Management System based on the normative reference ISO 9001. The scope of the certification is centred in the deposit, preservation and supply of well characterised filamentous fungal strains. The main objective of MUM is to maintain and provide quality and authentic fungal strains for biotechnological and life sciences research as well as acting as a centre for knowledge, information and training in the field of mycology. With over 20 years of activity it has achieved several landmarks such as being the driving force for University of Minho host the statutory seat of the pan-European microbial resource research infrastructure (MIRRI). MUM has striving for the implementation of Portuguese microBiological Resource Center Network (Pt-mBRCN) and, more recently, the Portuguese node for MIRRI.
It has currently in its e-catalogue over 800 strains, from 261 different species belonging to 78 genera. MUM fungal collection started with its main focus on *Aspergillus* and *Penicillium* genera from food matrices, however, MUM is a dynamic collection and has widened its holdings according to national and international collaborations and demands adding, throughout the years, environmental and clinical isolates.
With the premise of continual improvement of quality in the services offer by MUM and without losing its vision “A world in which fungal biodiversity is preserved and available for all” the major key-performance indicators of this collection, including some success stories, will be presented and discussed.

OP26. UCCCB - University of Coimbra Bacteria Culture Collection - the new partner in research and industry

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The University of Coimbra Bacteria Culture Collection (UCCCB) is the first Portuguese culture collection dedicated to the diversity of Bacteria, registered and recognized by the World Federation of Culture Collections as the collection nº 1179 (https://www.uc.pt/en/uid/ucccb).
UCCCB's mission is to identify, preserve, characterize and distribute reference bacterial strains and genetic resources while providing customer-oriented services. Moreover, it will preserve, characterize and make available in collaboration with researchers relevant microbial resources for the national and international scientific community, as well as for industry.
UCCCB has the knowledge and the conditions to describe new bacterial taxa (species, genera, families) and has done so over the years. Most of the original strains of the new taxa described by UC researchers are included in this collection (https://www.uc.pt/en/uid/ucccb/catalogue). UCCCB is developing a full database to be available online in a format of e-catalogue constructed following the directives of the WFCC, to be connected with other international Culture Connections.
The collection consists of three sub-collections of bacteria isolated from samples from various environments: i) Environmental Collection includes bacteria isolated from the ocean floor hydrothermal zones, from hospital environments, metal-contaminated environments, river sediments and copper surfaces; Ii) Human Collection consists of bacteria isolated from hospital inpatient samples, in particular, *Helicobacter*, *Pseudomonas aeruginosa* and *Staphylococcus* isolates; and iii) Host-interaction Collection includes plant-endophytic or pathogenic plant-isolated bacteria, frog-isolated bacteria and nematode-isolated bacteria.
The UCCCB has over 20 species with the sequenced genome and the Environmental Microbiology research group of FCTUC that gives technical support to the collection has collaborations with JGI-Joint Genome Institute (USA) under the Community Science Program, ""The Genomic Encyclopaedia of Bacteria and Archaea"" project for bacterial genome sequencing.
Beyond the services of preservation and distribution of microorganisms, the UCCCB has the facilities and equipment needed for, biochemical and molecular characterization including PLFA profiling, lipoquinone analysis and molar %G+C quantification. UCCCB also has expertise and gives scientific support for genetic engineering and modifying synthetic microbial chassis.
The UCCCB wants to contribute by making a bridge between researchers and industry, supporting research in microbiology in the areas of Biology, Biotechnology and Biomedicine, helping the drift to biobased processes.

OP27. Coimbra Collection of Algae (ACOI), a unique biological resource

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The Coimbra Collection of Algae (ACOI) is a biological resource center that provides access to one of the world's largest collections of living microalgae. It is part of the National Infrastructure EMBRC.PT created in 2016 and is included in the World Federation for Culture Collections (WFCC). ACOI aims to promote sustainability and improve the quality of life through microalgae by developing fundamental and applied research and providing high-quality products and services. ACOI develops two main research lines: (1) Biodiversity and conservation of microalgae including cryopreservation, classical and molecular taxonomy, ultrastructure and cytology; (2) Microalgae biotechnology consisting in cultivation methodologies for biomass production at laboratory scale, characterization of metabolites like lipids, polysaccharides, and pigments, determination of extracts bioactivity. It provides around 4,000 strains representative of algae diversity in soil, freshwater and brackish habitats, genomic DNA and algae extracts, to both institutions and industries, for teaching, research or business purposes. ACOI provides knowledge, advice and training on taxonomy, sampling, cultivation and conservation, cryopreservation and microalgae biotechnology. It gives access to facilities including cultivation rooms, well-equipped laboratories and specialized research platforms.

OP28. Cyanobacteria and microalgae collections as sources of valuable bioactive compounds – the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC)

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Cyanobacteria are very diverse organisms in terms of morphology, habitat and ecology and are well known for the diversity of secondary metabolites that they produce either when living isolated or in symbiosis. Among those metabolites, toxins are extensively studied due to the harmful effects they cause on the ecosystems and on human health. Cyanotoxins can have neurotoxic, hepatotoxic, cytotoxic and dermatotoxic properties, being exposure to humans via drinking water, dermal contact during recreation or via food contaminated with the toxins. Apart from producing toxins, and due to their ancestral origin, ecological and biochemical diversity, cyanobacteria are a prolific source of compounds with potential biotechnological applications, namely in the pharmacological field. A wide range of secondary metabolites exhibiting pharmaceutical properties such as antibacterial, antiviral, antifungal, anti-inflammatory and anticancer have been described. Bioactive compounds from cyanobacteria may also have allelopathic activity with potential use to control algal blooms or as antifouling in the marine environment. Cyanobacteria extracts can also prevent the development of some invertebrates and so they can be candidates to develop antifouling agents that are environmentally friendly. The potential of cyanobacteria as source of new bioactive compounds is enormous, with the advantage of being applicable in many different areas of biotechnology, with many industrial applications. To achieve that, culture collections are essential infrastructures as the base for the discovery of new compounds. The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biological resource centre located at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), comprising more than 1000 different cyanobacterial and microalgae strains. LEGE-CC strains were mainly isolated from Portuguese ecosystems (including Madeira and Azores Islands) but also from other countries worldwide (e.g. Australia, Brazil, Colombia, Morocco, Mexico, Dominican Republic, Cape Verde). In this presentation we will highlight the screening efforts that have been doing regarding different applications and activities (e.g. anti-cancer, anti-biofouling, anti-microbial, anti-biofilm, anti-obesogenic and related diseases, cosmetics, food, etc.). LEGE-CC is member of World Federation for Culture Collections (WFCC), European Culture Collections Organisation (ECCO) and it is also part of the Research Infrastructure EMBRC.PT.

OP29. The importance of synergies between researchers and society on a scientific project

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The articulation of the scientific community with society (school community; stakeholders; general public) is essential for a collective learning that enhances an educational structure capable of generating intellectual (knowledge) and social (networks and partnerships) capital. The MARE R&D Center has focused on communicating with the school community throughout the implementation of a structured educational project (SERMARE – Sensibilizar e Educar para uma Sociedade Azul) that contributes to an education system capable of empowering people to be active citizens in protecting the Ocean and developing the Blue Economy.
Capitalizing on this know-how, the project “ReSEt – Restoration of estuarine saltmarshes towards sustainability”, an experimental study in the Mondego estuary aiming the protection and restoration of estuarine marshes and biodiversity, defined three main axis as a priority – ecosystem; biodiversity; society. Here, societal outcomes are considered to be of equal importance and not merely secondary, and the transfer of knowledge and dissemination essential for the project success.
A strategy based on a broad integration of society based on the active participation of citizens in the developed activities was defined, encompassing: a) Workshops – students; teachers; stakeholders; general public; b) Bootcamp ReSEt – practical science course for students; c) Interactive visits to the study site; d) “Despesca ReSEt” – traditional activity in which the general public will participate in a project task.
The effective dissemination at various levels – school, stakeholder and general, of R&D knowledge and innovation will certainly make a decisive contribution to the preservation of ecosystems, either directly by a participatory society, or through the definition of environmental policies and plans of decision makers that rests with policy makers and other stakeholders.

OP30. Use of *Hanseniaspora guilliermondii* and *Hanseniaspora opuntiae* in co-fermentation with *Saccharomyces cerevisiae* to enhance the aromatic profile of craft beer

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Saccharomyces cerevisiae is the most commonly used yeast species in traditional ale beer production, but non-*Saccharomyces* yeasts have the potential to produce beverages with high sensorial complexity, leading to distinct flavours and aromas. Using *Saccharomyces* and non-*Saccharomyces* strains as mixed starters is regarded as an effective strategy to obtain a completely fermented beverage with improved organoleptic profile that meets the demands of the consumers, which only recently began being explored for beer brewing. In this study, 21 non-*Saccharomyces* strains belonging to 14 distinct species were isolated from grapes, wine and beer samples, and further characterised. Preliminary organoleptic evaluation of pure culture wort fermentations carried out by each isolate led to the selection of *Hanseniaspora guilliermondii* and *H. opuntiae*, two species associated with fermentations of wine, cider, and cocoa, but not beer. The potential of simultaneously and sequentially inoculating *H. guilliermondii* IST315 and *H. opuntiae* IST408 with the commercial beer yeast *S. cerevisiae* US-05 for beer bioflavouring was investigated by determining sugar consumption and volatile compound production after 2 weeks of fermentation in 1-L flasks and one month of secondary fermentation inside a bottle. Results indicate that while the amounts of sugars consumed and ethanol produced are unaffected, both *Hanseniaspora* strains significantly impact the volatile composition of beer when co-fermenting with *S. cerevisiae*, reducing the total amount of ethyl esters compared to *S. cerevisiae* pure culture fermentation. Interestingly, both strains increase the concentration of ethyl acetate ('fruity', 'solvent-like' aroma) at least 2-fold in co-fermentation with *S. cerevisiae*, but only *H. guilliermondii* IST315 significantly increases the overall concentration of acetate esters due to the production of 5-times more phenylethyl acetate ('floral', 'honey' aroma). These findings highlight the importance of non-*Saccharomyces* strains in shaping the aromatic composition of beer and suggest a role for *Hanseniaspora* spp. in improving it, as confirmed by an expert sensory panel.

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OP31. Prediction of (thermos)stable ene-reductases from genomes: Asymmetric hydrogenation of (R)-carvone by FOYE-1

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We work on the functional annotation of novel biocatalysts and among those we are interested on ene reductases. Here we present how a thermo- and solvent-stable enzyme can be predicted.
The ene reductase FOYE-1 from *Ferrovum* sp. JA12 belongs to the old yellow enzyme family. These flavoenzymes reduce various α,β unsaturated substrates at the expense of a nicotinamide cofactor, producing chiral molecules. Some accept artificial cofactors such as 1-benzyl-1,4-dihydronicotinamide (BNAH). Besides the typical ene reductase substrates (maleimides) we tested several industrial relevant compounds and among those (S)- and (R)-carvone were most promising. FOYE-1 shows a strong tolerance to organic solvents. Activity could be even increased with 20 vol% of ethanol, acetone and isopropanol. It is stable for up to 25 h in afore mentioned solvents, when stored on ice. An upscaling reaction with the crude extract preparation of FOYE-1 and (R)-carvone as substrate and BNAH as cofactor resulted in a 65 % isolated yield of (2R,5R)-dihydrocarvone.
It can be concluded that FOYE-1 has a high tolerance and stability towards organic solvents even at elevated temperatures. Together with the acceptance for the cost efficient BNAH, it becomes practical. This was predictable from the genome: to some extent!

OP32. Genome-wide responses of *Escherichia coli* to changing RNA polymerase concentrations

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In *Escherichia coli*, DNA supercoiling affects and is affected by genome-wide transcription rates [1]. We studied transcription profiles by RNA sequencing of *E. coli* MG1655 cells in various media, differing in richness, which causes RNA polymerase concentrations to differ, while not affecting growth significantly [2][3][4]. We compared the behaviour of two gene cohorts: i) supercoiling sensitive (SS) genes [5] and, ii) essential (E) genes [6]. We find that the average single-gene responses of these gene cohorts, as measured in fold changes, differ quantitatively (in a statistical sense) and qualitatively. While the responses of SS genes cannot be distinguished from average genome-wide responses, E genes have abnormally weak responses. This is not due to clustering in a small number of polycistronic transcription units. Instead, we show evidence that the cause may be associated with local topological features (obtained from [7]). Namely, E genes have, on average, 15% fewer inputs than expected by chance, which could explain higher stability. In contrast, SS genes only have 9% fewer inputs than expected by chance. Moreover, SS genes with high number of inputs (4 or more) are those exhibiting the higher fold changes and, while constituting only 7% of the cohort, they explain 16% of the mean of the fold changes in this cohort. On the other hand, essential genes with 4 or more inputs are very rare (3% of the cohort) and explain solely 3.5% of the mean of the fold changes in this cohort. We hypothesize that these results provide clues for the abnormally small number of inputs of essential genes. They also assist in better understanding the gene regulatory mechanisms that provide the gene regulatory network of *E. coli* with robustness to perturbations.

OP33. Tackling azole antifungal resistance in *Candida glabrata*: in the crossroad between three complex regulatory pathways

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Infections by *Candida* species are the 4th leading cause of bloodstream nosocomial infections and rank first among fungal infections. Candida glabrata presents high levels of acquired resistance to fluconazole, the most commonly used antifungal. The study of transcription regulatory networks underlying resistance mechanisms is required to develop innovative therapies that circumvent resistance phenotypes.
The PathoYeastract (http://pathoyeastract.org) database was developed for the analysis and prediction of regulatory associations at the gene and genome levels in *Candida* spp. [1]. It also allows the use of comparative genomics tools for the study of cross-species evolution of regulatory networks. This repository is freely available and includes +28,000 unique documented regulatory associations, +100 DNA binding sites and +130 TFs. Using this repository and its analysis tools, we predicted new transcription factors (TFs) possibly involved in the regulation of fluconazole resistance in *C. glabrata*. The TF *Rpn4* was identified as a fluconazole resistance regulator. RNA-seq unveiled the regulon of *Rpn4* comprising 212 genes upon fluconazole stress. The positively regulated targets are enriched in proteasome activity, heme biosynthesis and ergosterol biosynthesis genes, including *ERG11*, encoding the fluconazole target. *Rpn4* was thus found to be required for the maintenance of ergosterol levels upon fluconazole stress, which is associated with a role in cell permeability and control of fluconazole intracellular accumulation. Moreover, the TF *Mar1* was also identified as a regulator of fluconazole resistance. Its regulon during fluconazole stress comprises 337 genes, as determined by RNA-seq. The positively regulated targets contribute for homeostasis of membrane lipid composition. A role for this TF in the incorporation of sphingolipids in the plasma membrane was found, shown to affect membrane permeability and consequently intracellular fluconazole accumulation.
This work provides a comprehensive analysis on the discovery of new antifungal regulatory networks in *C. glabrata*. The results highlight the use of bioinformatics tools at the genome-level to predict and analyze antifungal regulatory networks. The project contributed to unveil new fluconazole resistance mechanisms focused on distinct aspects of plasma membrane homeostasis, thus providing clues to pursue the relevance of such mechanisms in the clinical setting.

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OP34. An “Omics” approach to understand rickettsial pathogenesis

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Approximately 1 billion people are estimated to be at risk of infection with obligate intracellular bacteria. Among these pathogens are different species of *Rickettsia*, causing globally emerging communicable diseases known as rickettsioses, which are expected to increase the burden to public health. The identification of many rickettsial species whose pathogenicity to humans is still uncertain strengthen the need to better understand the pathogenesis of the disease. Although different rickettsial species are responsible for very distinctive clinical onsets, the molecular determinants underlying differences in pathogenicity remain elusive.
We have previously reported that a drastic phenotypic difference between the highly pathogenic (*R. conorii*) and the non-pathogenic (*R. montanensis*) lies in their ability to survive and proliferate within macrophages. These results led to our current working hypothesis that macrophage permissiveness to infection acts as a key mechanism in rickettsial virulence, likely through a “Trojan horse” mechanism. Therefore, in order to gain more insights into the molecular details governing macrophage-*Rickettsia* interactions, we have employed two high-throughput methodologies aiming to profile changes in macrophage transcriptome (RNAseq) and proteome (SWATH-MS/MS) upon infection. Our recent results revealed that, in contrast to *R. montanensis* (non-pathogenic), *R. conorii* (highly pathogenic) substantially reprograms several host signaling pathways towards an M2-like phenotype (anti-inflammatory). Specifically, our data evidenced the sophisticated ability of *R. conorii* to: (1) evade innate immunity by modulating the expression of several anti-inflammatory molecules switching immune signals in THP-1 macrophages into a hyporesponsive state; (2) prolong host cell survival by inducing the expression of several pro-survival genes, thus protecting the replicative niche; (3) manipulate the expression of several gene expression regulators such as transcription factors and non-coding RNAs, altering host transcription programs during infection; (4) metabolically reprogram THP-1 macrophages according to Rickettsia metabolic needs.
Together, these findings provide the first insights into the molecular processes hijacked by a highly pathogenic Rickettsia species to subvert macrophage-mediated killing and thereby establish a replicative niche within phagocytic cells. These results are instrumental to identify specific host signaling pathways manipulated by rickettsial species that can be used for the development of novel targeted therapies for rickettsioses.

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OP35. The *Chlamydia trachomatis* protein IncL targets host cell lipid droplets

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Chlamydia trachomatis is the leading cause of sexually transmitted bacterial diseases. In women, it can cause infertility and ectopic pregnancy. This obligate intracellular human pathogen thrives within a membrane-bound compartment, the inclusion. Chlamydia manipulates host cell trafficking by using bacterial effector proteins delivered into the host cell by a type III secretion system. These effectors include inclusion membrane (Inc) proteins with important roles in *Chlamydia*-host cell interactions. We aimed to identify and characterize *C. trachomatis* Inc proteins subverting host vesicular trafficking and/or showing tropism for eukaryotic organelles. We performed a functional screen and fluorescence microscopy analysis using the yeast *Saccharomyces cerevisiae* ectopically expressing the predicted cytosolic domains of Inc proteins. We identified two Incs causing vacuolar protein sorting defects in yeast. Furthermore, we found one Inc, IncL, showing tropism for lipid droplets (LDs), major organelles for the storage of neutral lipids and which have been shown to be recruited to the *Chlamydia* inclusion during infection. We hypothesize that this Inc might have an important role in this process, as preliminary experiments indicate that the overexpression of IncL by *C. trachomatis* increases the recruitment of LDs to chlamydial inclusions. In addition, using mammalian cells ectopically expressing truncated versions of IncL, we identified a region responsible for targeting the protein to LDs and a region mediating the interaction of IncL with the eukaryotic phosphoserine/threonine binding protein 14-3-3β. We are currently generating a *C. trachomatis* mutant for the gene encoding IncL, in order to understand its biological role during infection.

OP36. Application of nucleic acid mimics in the selection of aptamers against staphylococcal enterotoxins

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Staphylococcal food poisoning (SFP) is a gastrointestinal disease caused by the consumption of contaminated food containing toxins pre-formed by enterotoxigenic *Staphylococcus*, known as staphylococcal enterotoxins (SE). Current detection of these toxins relies on time-consuming antibody-based immunoassays associated with cross-reactivity, low sensitivity, and interference from food matrices. Aptamers are typically single-stranded oligonucleotides (ssDNA or RNA) with a defined three-dimensional shape that bind with high affinity to a target molecule. They have several advantages over antibodies, including robustness, low cost, and no need for animals or cell cultures to be produced. Aptamers are selected by the so-called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) method which consists in the screening of a random oligonucleotide library down to highly specific sequences for the target by the repetition of successive steps of selection, amplification and conditioning. Traditional procedures are limited to DNA/RNA nucleotides, which are naturally susceptible to nuclease degradation. Nucleic acid mimics (NAM) are seen as potential alternative and have the added advantage of unique binding characteristics and improved nuclease resistance. However, the use of NAMs requires more complex SELEX procedures and mutant versions of polymerases that incorporate modified nucleotides. In our project, a SELEX procedure was applied using magnetic beads for target immobilization and further separation of bound from unbound sequences. 2'-deoxy-2'-fluoroarabinonucleotides (FANA) were successfully incorporated in the initial oligonucleotide library construction using the D4K mutant polymerase. Radiolabelling of oligonucleotides was used for monitoring the various SELEX process steps. After performing nine rounds of selection, it has been possible to identify twenty-four potential FANA aptamers for staphylococcal enterotoxin A (SEA). Although the nucleotide comparison shows no consensus sequences between selected oligonucleotides, the analysis of secondary structures reveals enrichment in similarly folding patterns. The results obtained so far reinforce the potential of applying NAMs in SELEX procedures and suggest that, for SEA, selection points to preferential secondary structures which are similar to other DNA aptamers already described for the same toxin. More rounds with more stringent conditions are planned to improve the selection of highly specific sequences. This technological platform might be easily adapted to any food poisoning toxin.

OP37. New ligninolytic enzymes by directed evolution

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Lignin is a heterogeneous aromatic biopolymer that accounts for nearly 30% of the organic carbon on Earth. It is one of the few renewable sources for aromatic chemicals on which the chemical industry so heavily relies. Extraction of higher value from lignin, currently considered a bio-waste, is increasingly recognized as being crucial to the economic viability of integrated biorefineries. Lignin valorization requires the cooperative action of diverse, robust and optimized biocatalysts. We have been focusing in bacterial enzymatic systems that are less explored as compared with those of fungal origin but hold an enormous potential considering the easiness of gene cloning, protein production and the high number of molecular tools available for enzyme engineering towards improved efficiency and robustness required for industrial applications.
Directed evolution through random mutagenesis by error-prone PCR or DNA-shuffling followed by high-throughput screening gave been used to improve the efficiency of the hyperhermophilic bacterial laccase *Aquifex aeolicus* McoA and PpDyP a dye-decolourising peroxidase (DyP) from *Pseudomonas putida* MET94. This approach has led to the identification variants featuring up to 1000-fold enhanced catalytic efficiency for phenolic lignin-related substrates. Biochemical and structural analysis using X-ray, SAXS docking and molecular dynamics simulationsMD of wild type enzymes, hit variants, and single variants constructed using site-directed mutagenesis, unveiled the critical role of acquired mutations from the catalytic, stability and structural viewpoints allowing a multidirectional comparative analysis. These studies identified structural and functional determinants of substrate specificity, track evolutionary trajectories and recognize the dynamics and constraints of enzyme evolution. They opened perspectives for further evolution of these enzymes for improved properties that are major limiting factors in their industrial applications.

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OP38. *Streptococcus pneumoniae* microevolution among children attending a single day-care center

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Background: *Streptococcus pneumoniae* (pneumococcus) is a major community-acquired pathogen that colonizes the nasopharynx asymptomatically. Children attending day-care centers are major reservoirs of pneumococci contributing to their dissemination in the community. We aimed to study pneumococcal evolution during colonization and transmission within a day-care center.
Methods: We revisited a longitudinal study carried out between 1998-1999. During one-year, monthly nasopharyngeal swabs (n=414) were obtained from 47 children attending one of three rooms from the same day-care center. Overall, 259 pneumococci were isolated. A dominant 19F clone (19F-Pn3, n=89) was identified by *Sma*I-PFGE [1]. In the current work, we performed whole genome sequencing (WGS) of all 19F-Pn3 isolates using Illumina NextSeq. Assembly of reads and MLST determination were performed using INNUca v3.1; genomes were annotated using Prokka v1.12. SNP and small indel detection was carried out using Breseq v0.30.0. Gene gain/loss was assessed using get_homologues v05012017, followed by BLAST and manual curation.
Results: 19F-Pn3 strains could be divided in two MLST profiles differing in a single locus: ST177 (n=73) and ST179 (n=16). A prophage was detected in the ST177 isolates. ST177 was disseminated among children from the three rooms studied; ST179 was only detected in one room. ST177 strains were isolated from 27 children (1-7 isolates/child). In total, 84 mutations were detected during the year generating 42 genomic profiles. Twenty-three mutations (27%) were identified in two or more isolates suggesting that they had become fixed; of these, nine were detected in more than one child indicating that transmission had occurred. ST179 strains were isolated from five children (1-6 isolates/child). During the year, 23 mutations were identified generating 16 genomic profiles. Three mutations only were detected in more than one isolate suggesting that most were transient. Mutation rates of ST177 and ST179 were estimated as 1.18x10-5 and 1.56x10-6 mutations/site/year, respectively.
Conclusions: Microevolution of pneumococcus occurs within the day-care center setting and is highly dynamic. While most mutations are transient, a few become fixed and can be promptly disseminated.

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OP39. Lost in transition: Defining the role of exoribonucleases in the shift between exponential and stationary phases

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The transition between exponential and stationary phase is a natural phenomenon for all bacteria and requires a massive readjustment of the bacterial transcriptome. Exoribonucleases are key enzymes in the transition between the two growth phases since they can rapidly degrade the RNA that is no longer necessary. In *Escherichia coli* there are three main exoribonucleases responsible for the RNA degradation: RNase II, RNase R and PNPase. RNase II and RNase R are both hydrolytic enzymes an belong to the same family, on the other hand, PNPase is a phosphorolytic enzyme that can also act as a polymerase. In this work we used RNA-Seq experiments to analyze the transcriptomic differences between the exponential and stationary phases of WT cells and deletion mutants for the different exoribonucleases (Δ*rnb*, Δ*rnr*, Δ*pnp* and Δ*rnb*Δ*rnr*). Overall when comparing the cells from exponential phase with the cells from stationary phase more than 1000 transcripts were differentially expressed, but only 491 core transcripts were common to all strains. There were some differences in the number and transcripts affected depending on the strain, suggesting that exoribonucleases influence the transition between these two growth phases differently. We also compare the effects of the absence of the hydrolytic degradation (RNase II/RNase R double mutant) with the absence of the phosphorolytic degradation (PNPase). It seems that deletion of PNPase leads to a higher transcriptomic change than even the deletion of both RNase II and RNase R. Interestingly, we also found that the RNase II/RNase R double mutant is similar to the RNase R single mutant in exponential phase while in stationary phase it seems to be closer to the RNase II single mutant. This is the first global transcriptomic work comparing the roles of exoribonucleases in the transition between exponential and stationary phase.

OP40. Bacterial metabolites produced under iron limitation attract *Caenorhabditis elegans* and kill pinewood nematode

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The Pine Wilt Disease (PWD) caused by the pathogenic *Bursaphelenchus xylophilus*, the pinewood nematode, is one of the most devastating diseases of forests pine trees in the world, with tremendous ecological, environmental and economic damage. The ecosystem of the *Pinus pinaster* trees was investigated as a source of bacteria producing metabolites affecting this ecosystem: *P. pinaster* trees as target-plant, nematode as disease effector and its insect-vector as shuttle. For example, metals and metal-carrying compounds contribute to the complex tree-ecosystems. This work aimed to detect novel secondary metabolites like metallophores and related molecules produced under iron limitation by PWD-associated bacteria and to test their nematicidal activity on nematodes. After screening 357 bacterial strains from Portugal and USA, two promising metallophore-producing strains *Erwinia* sp. A41C3 and *Rouxiella* sp. Arv20#4.1 were chosen and investigated in more detail. The genomes of these strains were sequenced, analyzed, and by using the webtool antiSMASH, it was possible to identify the gene clusters for secondary metabolite production, especially siderophores. A combinatorial approach of liquid chromatography-coupled tandem mass spectrometry (LC-MS) linked to molecular networking was used to describe these compounds. Siderophores were enriched by HPLC and 30 fractions per strain were obtained. Two major metabolites were detected by HPLC analyses and described. One HPLC fraction of strain Arv20#4.1 showed to be a hydroxamate-type siderophore with higher affinity for chelation of Cu. The HPLC fraction of strain A41C3 with highest metal affinity showed to be a catecholate-type siderophore with higher affinity for chelation of Fe. LC-MS allowed the identification of several desferrioxamines from strain Arv20#4.1, in special desferrioxamine E, but no hit was obtained in case of strain A41C3 which might indicate that it is a novel compound. Bacteria and their culture supernatants showed ability to attract C. elegans. HPLC fractions of those supernatant-extracts of *Erwinia* strain A41C3, enriched with secondary metabolites such as siderophores, were able to kill pinewood nematode. These results suggest that metabolites secreted under iron limitation have potential to biocontrol *B. xylophilus* and for management of Pine Wilt Disease.

OP41. Interaction of *Staphylococcus aureus* with host cells: evaluation of the prevalence of the intracellular lifestyle

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Staphylococcus aureus is a major human pathogen responsible for a broad spectrum of life-threatening nosocomial and community-acquired infections. The diversity and severity of staphylococcal diseases, together with the advent of multi-drug resistant strains of *S. aureus*, explains the lack of therapeutic options making this bacterium a major public health concern worldwide. The versatility of this pathogen is illustrated by the numerous mechanisms to counteract immune response and the ability to produce numerous virulence factors that contribute to the clinical manifestations of *S. aureus*. Importantly, several studies indicate that *S. aureus* can invade, replicate and persist within a variety of human professional and non-professional phagocytic cells, providing a protective niche against antibiotics and a reservoir for chronic and recurrent infections. Nevertheless, the relevance of the intracellular lifestyle to *S. aureus* pathogenicity remains elusive and the bacterial and host factors required for this process are largely unknown. To determine how widespread is the ability of clinical *S. aureus* isolates to invade, replicate and persist inside host cells, we tested 215 clinically and genotypically well-characterized *S. aureus* isolates, collected from patients with bone/joint infections and infective endocarditis. Specifically, we characterized the interaction of the clinical isolates with three types of target cells frequently used as models for *S. aureus* infection: epithelial cells (HeLa), macrophages (differentiated THP-1) and endothelial cells (EA.hy926). Automated fluorescence microscopy-based infection assays and image analysis were performed to quantify the percentage of infected cells, the levels of intracellular *S. aureus*, the fraction of vacuolar vs. cytosolic bacteria and host cell viability, for each isolate at various times post-infection. This analysis revealed that over 95% of the *S. aureus* isolates were able to invade the three host cell models, and that more than 50% of were able to replicate within infected cells (>10% of cells with high bacterial levels). Furthermore, 88% of the isolates were able to persist within host cells at 48h post-infection. Overall, our results demonstrate that the intracellular stage is a common feature of *S. aureus* clinical isolates. Ongoing work is focused on further investigating this poorly understood lifestyle to identify novel strategies to counteract staphylococcal infections.

Flash and Poster Presentations

I1. Environmental Microbiology and Biotechnology

FP1. Co-production of phenazines, mcl-PHAs and EPS by the bacterium *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603 using glycerol

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Members of the *Pseudomonas* genus are known for their ability to produce multiple secondary metabolites, including bio active metabolites, such as antibiotics. *Pseudomonas chlororaphis* is a non-pathogenic bacterium widely used as plant growth-promoting rhizobacteria that has been reported to be able to produce three products of interest, namely, antibiotics (phenazines) and biopolymers (mcl-PHAs and EPS). Phenazines are heterocyclic nitrogenous compounds that can act as antibiotics, anti tumor, anti parasitic or even antiviral agents. Despite their high-value, the fermentative process for phenazine production is not completely understood. Mcl-PHAs (medium chain length polyhydroxyalkanoates) are a class of biobased and biodegradable polymers that depending on their composition and specific properties shows potential to be applied from thermoplastics to elastomers. EPS (exopolysaccharides) are a group of natural polymers mainly composed by sugars that due to their variety of physical and structural properties have many applications in industries such as food, pharmaceutical and cosmetics.
The main objective of this study was to develop and optimize the bioprocess for the co-production of phenazines, mcl-PHAs and EPS by *P. chlororaphis* subsp. *aurantiaca* DSM 19603, using glycerol as the sole carbon source. Considering phenazines synthesis is growth associated, the optimization strategy was to enhance cell growth by increasing both the nitrogen and the carbon sources. The ammonium and glycerol concentrations that guarantied the maximum specific cell growth rate were determined and used in bioreactor cultivation experiments. Such conditions resulted in significantly improved phenazines production and volumetric productivity by 5 fold. Moreover, the fed-batch process was developed. During the stationary phase, *Pseudomonas chlororaphis* subsp. *aurantiaca* DSM 19603 was able to accumulate 51% of mcl-PHA corresponding to 8.43 g/L and a volumetric productivity of 0.21 g/L.h.This study demonstrated that the bacterium *P. chlororaphis* subsp. *aurantiaca* DSM 19603 can be used for the synthesis of phenazines, mcl-PHAs and, EPS, all high-value products, in a cost-effective bioprocess based on the use of glycerol. Although some efforts still need to be put into phenazines purification and characterization, the developed bioprocess already revealed to be promising.

FP2. Dissolved oxygen limitation: friend or foe of neutral lipids production by *Alcanivorax borkumensis* SK2?

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Alcanivorax borkumensis is a marine hydrocarbonoclastic bacteria (HCB) capable of converting hydrocarbons (HC) into neutral lipids and therefore can be used for treatment and valorization of saline hydrocarbon-contaminated wastewaters. When submitted to stress conditions, HCB can increase neutral lipids accumulation. In this study, the effect of dissolved oxygen (DO) concentration on the production of bacterial lipids by *A. borkumensis* SK2 was investigated in a sequencing batch airlift reactor (SBAR) fed with oilfield produced water (PW). Periods of feast (carbon addition (2 g PW L^{-1} COD)) and famine (nitrogen addition (15 or 30 mg L^{-1})) were performed. Dissolved oxygen concentrations of 7 - 8 mg L^{-1} and 2 – 3 mg L^{-1} were tested. For all the conditions applied, intracellular lipids production was higher than extracellular lipids. The maximum intracellular lipids concentration attained (0.23 g L^{-1}) was achieved when lower COD/N ratios (79) and dissolved oxygen of 7 - 8 mg L^{-1} were applied (3 times higher than extracellular lipids concentration). Increasing the feast stage duration from 3 to 5 days led to an increase of the intracellular lipid concentration, from 0.07 g L^{-1} to 0.23 g L^{-1}. The application of 2 - 3 mg L^{-1} DO decreased the intracellular lipid production from 0.23 g L^{-1} to 0.10 g L^{-1}. Triacylglycerol (TAGs) and fatty acids (FA) were only detected at DO concentration of 7 - 8 mg L^{-1}. Throughout the reactor operation, a total petroleum hydrocarbon (TPH) removal efficiency up to 98% was achieved. This work shows that, although lipids production was decreased, the application of low DO concentrations did not compromise the biological treatment of PW in terms of hydrocarbons removal, which can be advantageous by reducing SBAR operation costs with aeration.

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I1. Environmental Microbiology and Biotechnology

FP3. Characterization of multi-drug resistant *Escherichia coli* in the UV-treated outflow of an Urban Wastewater Treatment Plant

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Wastewater Treatment Plants are sources of antibiotic resistance into aquatic environments, contributing to the spread of clinically-relevant antibiotic-resistant bacteria. The disinfection (e.g. UV-C irradiation) of the final effluent is a promising strategy to circumvent this problem. However, some clinically-relevant resistant bacteria are known to survive disinfection. In this work we characterized *Escherichia coli* strains isolated from a UV-C-treated wastewater effluent, aiming to infer possible human health risks associated with these effluents.
Multi-drug resistant (MDR) E. coli isolates (n=25) were genotyped (fingerprinting, Multi Locus Sequencing Typing and Clermont phylo-groups). Antibiotic resistance and virulence genes (ARGs and VG) were PCR-screened and plasmid transfer was assessed by mating assays. Cytotoxicity and invasion into mammalian cells were determined. The genome of 6 isolates was sequenced, and their survival in freshwater microcosms was evaluated.
Genotyping distributed the strains into 3 groups, corresponding to phylogroups B2-sgI (n=7 isolates), A (n=16) and C (n=2). Based on MLST analyses phylogroup B2 strains were classified as ST131, C strains as ST410, and A as ST155 (n=4), ST58 (n=1), ST453 (n=2), ST617 (n=2), ST744 (n=1), ST1284 (n=3), or as a novel ST (n=3). Nine of the 18 PCR-screened ARGs were detected: *sul1* (n=15 isolates), *sul2* (n=15), *tet(A*) (n=14), *blaOXA*-1-like (n=8), *tet(B)* (n=8), *aacA4-cr* (n=5), *aacA4* (n=2), *sul3* (n=2) and *qnrS1* (n=1). No VG were detected by PCR. Conjugal transfer of cefotaxime resistance was confirmed in 8/25 strains, in some cases yielding MDR phenotypes. Nine strains were significantly more cytotoxic than the positive control, and 10/21 strains were capable of internalization into Vero cells. Whole genome analysis evidenced the presence of additional ARGs (e.g. *catB3*, *aadA2*, *strA*, *strB*) and of VG encoding toxins, siderophores, and adhesion and invasion factors. After 28 days of incubation in freshwater microcosms, 4 strains were still detected by cultivation and/or qPCR.
This study demonstrates that MDR *E. coli* can survive UV-wastewater disinfection, maintain resistance and gene transfer capacity and, eventually, persist in freshwater environments. These evidences support the hypothesis that even disinfected wastewater, if not adequately controlled, may represent a risk for human health.

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I1. Environmental Microbiology and Biotechnology

FP 4. *Acetobacterium* sp. strain JM, a biotechnological platform organism for syngas conversion

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In the frame of a circular bio-economy, it is essential to find new ways to replace traditional fossil fuels with alternatives that besides fuel/chemicals production also foresee the conversion and treatment of waste materials. For instance, low-biodegradable wastes, such as lignocellulosic materials and municipal waste, are difficult to degrade. An alternative for these latter streams is performing gasification of the waste material, followed by a biological conversion of the generated syngas (CO, H_2 and CO_2). In turn, this gas mixture can work as a substrate for the anaerobic production of biocommodities. In this work, an anaerobic sludge was used to build an enrichment with syngas and acetate as main substrates, producing mainly methane, acetate and propionate. In this enrichment, *Acetobacterium* species were highly prevalent (87% of the bacterial clones sequenced), though *Methanospirillum* sp. and propionate-producing bacteria were also found. This data showed that *Acetobacterium* sp. should be the microorganism responsible for CO conversion. A novel acetogenic bacterium strain, strain JM, 99% identical (16S rRNA gene) with *Acetobacterium wieringae* (type strain) was isolated from this syngas-enriched culture. This strain was capable to convert up to 100% CO in the headspace, as sole carbon and energy source (no yeast extract added), producing acetate and ethanol. Interestingly, the type strain was not previously shown to grow on CO, and the related *A. woodii* was reported to grow on CO only in the presence of H_2/CO_2 or formate as a co-substrate, but not on CO alone. Furthermore, strain JM converts efficiently high concentrations of syngas, being able to convert 0.8 mmol d^{-1} of CO, while the type strain and *A. woodii* could only convert 0.2 mmol d^{-1} and 0.6 mmol d^{-1}, respectively. Genomic analysis showed that this organism has the complete Wood-Ljungdahl pathway, typical of acetogens for the conversion of one carbon compounds and that encodes an enzyme complex that shows high similarity to the bifurcational Fdh of *Clostridium autoethanogenum*, being potentially less sensitive to CO than the ones present in *A. woodii*.

I1. Environmental Microbiology and Biotechnology

P 5. Evaluation of synergy between *Saccharomyces cerevisiae* and two non-*Saccharomyces* yeast fermenters for sustainable biofuel production

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Environmental challenges associated with over-dependence of fossil fuels and its gradual depletion calls for development of renewable energy sources like bioethanol from plant wastes. Conversion of sugar constituents of plant wastes by the conventional fermenter (*Saccharomyces cerevisiae*) has not been efficient. *S. cerevisiae* is not known to convert pentose sugars to ethanol. Sugarcane bagasse is a readily available substrate for bioethanol production but it contains an important quantity of pentose sugar.
We evaluated the effect of synergy among *S. cerevisiae* Y10 (Accession number MG321589) and two non-*saccharomyces* yeasts on fermentation of sugarcane bagasse hydrolysate to ethanol.
Two non-*saccharomyces* yeasts *Pichia kudriavzevii* Y2 (MG321583) and *Candida tropicalis* Y5 (MG321586) were isolated from bagasse collected from a waste-disposal site of a sugar industry based on their ability to grow on xylose (pentose). Sugarcane bagasse hydrolysate was converted to bioethanol using single and coculture of *S. cerevisiae* Y10 and non-*saccharomyces* yeasts.
Ethanol yields of 12.03g/L, 12.46g/L and 12.15g/L from *C. tropicalis* Y5 singly, *P. kudriavzevii* Y2/*C. tropicalis* Y5; and *S. cerevisiae* Y10/*P. kudriavzevii* Y2/*C. tropicalis* Y5 combinations, respectively were not statistically different. Our results suggested that some naturally existing non-*saccharomyces* yeast like *C. tropicalis* may have promising traits needed in bioethanol industry. Scale-up experiments with single culture of *C. tropicalis* would be performed.

I1. Environmental Microbiology and Biotechnology

P 6. Conservation & Public Health clues - characterization of *Aeromonas* spp. collected from *Iberochondrostoma lusitanicum* reveals geographical and seasonal patterns of species composition and antimicrobial resistance signatures

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There is a knowledge gap concerning the role that infectious diseases play in the conservation of Portuguese endangered cyprinids, such as *Iberochondrostoma lusitanicum*. Establishing biosurveillance programs for common fish pathogens, such as *Aeromonas* spp., will provide crucial information for species and habitat management, being essential for public health investigations including the detection of environmental antimicrobial resistance (AMR) reservoirs.
To characterize species composition and AMR patterns in bacteria from freshwater ecosystems at the Lisbon district, a collection of *Aeromonas* spp. isolates from *I. lusitanicum* samples collected at distinct locations (Jamor, Laje, Lizandro and Samarra) and seasons (wet and dry) was used. Species identification was performed by multiplex-PCR targeting *A. hydrophila*, *A. veronii*, *A. media* and *A. caviae*. AMR profiling was evaluated by disk diffusion, regarding antibiotics selected based on their relevance for Human and Veterinary Medicine.
Aeromonas species distribution in *I. lusitanicum* shifted between locations and seasons ($p<0.001$). A high prevalence of *A. hydrophila*, considered the most pathogenic species was observed in samples collected in Jamor during the dry season; while in Samarra samples a low prevalence was reported. Regarding AMR, significant differences between multiresistant isolates prevalence in samples from different locations ($p=0.003$) and seasons ($p=0.004$) were observed. Higher prevalence of multiresistant isolates was found in samples from Jamor during the wet season. Significant differences were also observed for combinations of individual antimicrobials, susceptibility category, location and season.
Results show the potential of using *Aeromonas* spp. from *I. lusitanicum* as a conservation management tool and as bacterial indicators for AMR in aquatic ecosystems. Increments in Aeromonas species with higher pathogenic potential were observed in samples collected in critical life periods for fish survival, representing an additional risk for this species' conservation. Additionally, the spatial differentiation in AMR signatures in aquatic streams from the Lisbon district can be consequence of local demographic characteristics, rendering biosurveillance programs at the environmental level fundamental for wildlife conservation and public health protection.

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I1. Environmental Microbiology and Biotechnology

P 7. On the presence of microbial communities in caves: the case of LEYE CAVES

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Caves gives an insight into the life forms and geological formations below the Earth's surface. Karstic environments are one of the most diverse geomorphological occurrences. Speleothem, secondary mineral deposits, are one of the important characteristics of a karst caves. In this work microbial communities present in Leye caves in the Vezere valley in Dordogne, France that will be acceded by High-throughput sequencing (HTS).
In situ Fibre Optic Reflectance Spectroscopy (FORS) analyses allows us to examine the existence of microorganisms. In vitro culture confirmed the presence of bacteria, fungi and yeast and high throughput sequencing approaches allowed us to explore, compare and characterise the microbial communities in different parts of the cave. Bacterial community are mainly composed by *Proteobacteria*, *Actinobacteria* and *Firmicutes*, but Phylum like *Nitrospirae*, *Tenericutes* or *Spirochaetes* are also presents in the cave.
The study gives important contribution to assess the microbial communities present in limestone caves.

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I1. Environmental Microbiology and Biotechnology

P8. Monitoring antibiotic resistance genes by qPCR or metagenomics analyses: high sensitivity versus broad coverage?

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Antibiotic resistance is a major global human-health threat, with important connections to the environmental and veterinary domains. Detection and quantification of antibiotic resistance genes (ARGs) are therefore key players in any control measure. Two commonly used culture-independent approaches are quantitative PCR (qPCR) and metagenomics. While the first is a targeted method that screens a set of predefined ARGs, the second is non-targeted overview of ARGs/resistomes using sequence mapping to ARG database. It has been admitted that qPCR may be more sensitive compared to metagenomics, yet limited to the targeted ARGs coverage. Here, we compared the sensitivity of ARGs detection and quantification using qPCR and metagenomics in the same samples from two wastewater treatment plants (WWTP) at different treatment stages, surface water samples from rivers neighbouring the WWTP discharge points and a hospital effluent discharging into one of the WWTP, in addition to series of step-wise gradual increased volumes of pig and chicken faeces.
DNA was collected in duplicate and split into two groups; each was used for qPCR analyses and Illumina-sequencing metagenomics. The metagenomes were mapped against ResFinder and an in- house second ARG database, and the qPCR analysis targeted the 16S rRNA, *IntI1*, *uidA*, *incF* and 15 ARGs. Carbapenems, 3rd generation cephalosporins, aminoglycosides, sulfonamides, quinolones and glycopeptides ARGs were selected.
The resistome profiles identified 1099 ARGs with metagenomics, and the entire selected 15 ARGs for qPCR analyses. Animal faeces resistomes were markedly different in abundance and composition from the water samples. Genes with expected low abundance, e.g., carbapenem resistance gene, were detected with $bla_{\text{OXA-58}}$ being more abundant than $bla_{\text{OXA-48}}$ and bla_{KPC}, respectively, in raw influent. However, they were below the limit of quantification or in low abundance in animal faeces using either method. The ARGs bla_{CTX}, bla_{OXA} and bla_{SHV} were more dominant in wastewater, yet either absent or low in abundances in the animal faeces using either method. Finally, both methods were able to detect comparable dissimilarities in the resistome compositional profiles from wastewater and faeces. The metagenomics analyses were also able to gradually detect step-wise ARG differences in abundance and composition (10 folds) in complex matrices as animal faeces.

I1. Environmental Microbiology and Biotechnology

P9. Dynamics of carbapenem resistance in urban wastewater treatment plants and receiving water bodies: drivers and opportunities for action

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Urban wastewater treatment plants (UWTPs) are described as reservoirs of antibiotic resistance. It has become increasingly clear that antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) released by well-functioning UWTPs may have a negative impact on the environment, representing a threat for humans and/or ecosystems. Assessing risks where the environment and humans meet is therefore critical. This study has focused on antibiotic resistance loads and the epidemiological dynamics of resistance in natural water bodies receiving treated urban wastewaters.
Wastewater samples were collected from 4 full-scale UWTPs (PT1-PT4) located in Northern Portugal, at different stages of wastewater treatment, and river water samples were collected upstream and downstream of each treatment plant. All UWTPs have primary and secondary treatments, with PT1- PT3 reporting a tertiary treatment and PT2 an additional step of ozonation. PT4 reported the reception of hospital effluents. Samples collected in early Summer (SC1) and in early Autumn (SC2) were processed for total DNA extraction and isolation of cultivable bacteria resistant to meropenem or cefotaxime. Carbapenem resistance genes (*bla*$_{KPC}$, *bla*$_{OXA-48}$, and *bla*$_{OXA-58}$), class 1 integron- integrase (*intI1*) and 16S rRNA were quantified based on quantitative PCR.
The abundance of ARGs and *intI1* (per mL of sample) in the final effluent of all UWTPs could be ranked as *intI1*>*bla*$_{OXA-58}$>*bla*$_{OXA-48}$>*bla*$_{KPC}$, with a higher abundance of 16S rRNA gene, *intI1*, *bla*$_{OXA-48}$ and *bla*$_{OXA-58}$ observed in PT1. In PT2, *bla*$_{KPC}$ was not detected after secondary treatment and ozonation promoted a reduction of 1.44 to 3.85 log-units in genes abundance. Comparing river water upstream and downstream the UWTPs, the abundance of *bla*$_{KPC}$, *bla*$_{OXA-48}$ and *bla*$_{OXA-58}$ genes was higher downstream PT4, PT2 and PT3 in SC2, respectively. The genes *bla*$_{KPC-2}$, *bla*$_{CTX-M-15}$ and *intI1* were detected in isolates (*Klebsiella quasivariicola*) from a secondary effluent of PT3 and downstream the treatment plant (SC1). In addition, *bla*$_{KPC-3}$ and *intI1* were detected in 3 isolates (2 *Klebsiella pneumoniae*, and 1 *Klebsiella variicola*) after secondary and tertiary treatment from PT2, and downstream PT4 (SC1). The results demonstrate that although UWTPs are effective for removal of clinically relevant ARGs, some can persist in the downstream environment and associated with opportunistic pathogens.

I1. Environmental Microbiology and Biotechnology

P10. Microbiological characterization of biosolids for agronomic applications

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Wastewater is composed of materials that flow from household plumbing systems, including washing, bathing water, and toilet wastes. One of the most obvious reasons why wastewater must be treated before discharge is that pathogenic microbes can be transmitted in feces. Thus, once released into lakes, rivers or seas, contamination occurs, and posterior consumption of these waters could result in a serious public health problem.
Wastewater treatment plants (WWTP) use a series of processes, which end in the final obtention of a liquid fraction (effluent) that may be discharged into body water, and a solid fraction (sludge). This sludge, or biosolids, may be deposited in landfills or can be used in agriculture, contributing to fertilization of soils through nutrients (nitrogen and phosphorous) present in their constitution.
In order to use biosolids as fertilizers, without compromising public health, this work aimed to study the microbiological quality of biosolids, through the quantification of *Escherichia coli* and evaluation of presence or absence of *Salmonella* spp, as defined by law (Decreto–Lei nº 276/2009). In addition, some processes to improve sanitation of these biosolids, such as pasteurization and addition of adjuvants, were evaluated using quantification of *E. coli*.
Nineteen samples were collected from various WWTP. Quantification of *E. coli* was done according to ISO-16649-2, and the presence of *Salmonella* spp was determined by ISO 6579. Five samples complied with legal limits for the 2 microorganisms. *Salmonella* spp. was identified in 4 and quantification of *E. coli* was higher than allowed limit in 13 samples.
Using pasteurization at 70, 100 and 130 ºC and reducing the humidity to 50 and 30%, the better effect of removing *E. coli* was achieved at 130 ºC for both humidities studied. Addition of adjuvants [coal fly ash, dregs, eggshell, CaO and $Ca(OH)_2$] at room temperature during 24h, demonstrated that only CaO and $Ca(OH)_2$ led to elimination of *E. coli* below the legal limit for agronomic valorization.
Current processes used by WWTP are not efficient for the elimination of microorganisms. Therefore, other methods should be implemented in order to obtain biosolids that allow their subsequent use in agriculture without any public health problems/threats.

I1. Environmental Microbiology and Biotechnology

P11. A new important intermediate to the synthesis of fluorescent labels for biomolecules

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Some very important areas such as molecular biology, medicine and medicinal chemistry are dependent on very sensitive analytical techniques to detect and track biomolecules (amino acids, peptides, proteins, antibodies, oligonucleotides, nucleic acids, carbohydrates and other biological molecules). The fluorescent labelling presents several advantages, when compared to the other techniques, due to the high sensitivity of the fluorescence technique and also due of its non-destructive nature, that allows the use of small sample quantities and their fluorescent labels. The great development of fluorescent labelling techniques and the synthesis of new fluorophores combined with the enormous technological advances in the field of fluorescence microscopy allowed to deepen the structural knowledge of biomolecules.[1]
Coumarin derivatives represent one of the most important chemical classes of organic fluorescent materials. The application of coumarin derivatives as organic dyes has been hindered due to their colour spectra falling in the UV range and the relatively low intensity of their absorption bands. One solution to this problem arises from increasing the delocalization of the conjugated-electron system. A recurring strategy that became essential for the development of new organic dyes and that can be applied for coumarins, involves Donor–bridge–Acceptor molecules.[2]
Applying our knowledge on the conjugation extension of coumarins at position 3, we observed that the presence of electron-donating substituents in position 7 and electron-withdrawing moieties in position 3, contribute to coumarin derivatives with improved photophysical and spectroscopic properties, with high quantum yields.[2-4]
In this work, using 7-(diethylamino)-4-methyl-3-vinylcoumarin as intermediate, we developed a simple, low cost and effective synthetic strategy to produce new promising fluorescent labels for biomolecules.

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I1. Environmental Microbiology and Biotechnology

P12. Production of propionate from carbon monoxide by a synthetic co-culture

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Syngas, mainly composed of carbon monoxide (CO) is a polluting gas produced by several industrial sectors as the end product of gasification process. Fermentation of syngas/CO by carboxidrotrophic microbes allows the production of compounds with high economical and industrial interest. The interest in expanding its production frame towards more complex products is increasing. Propionate is a value-added compound with numerous industrial applications, e.g. as an antifungal agent in foods and feeds, and as a building block to produce plastics and herbicides, among others. Propionate is currently produced by chemical reactions, though, its production from syngas/CO represents a new approach on microbial syngas conversion. Some propionogenic bacteria have the ability of producing propionate from alcohols and organic acids such as ethanol and acetate, and these compounds are the main products of CO fermentation by acetogens. Consequently, CO can be used as substrate for propionate production, using a co-cultivation approach. In this work different co-cultures of acetogens were established together with propionogenic bacteria. A novel isolated syngas- fermenting organism, *Acetobacterium* sp. strain JM, and the type strain, *Acetobacterium wieringae* DSM 1911, were tested together with the propiogenic bacteria *Pelobacter propionicus* and *Anaerobacterium neopropionicum.* The co-culture composed by *Acetobacterium* sp. strain JM and *Anaerobacterium neopropionicum* was able to produce up to 24.3 mM propionate from CO fermentation. A proteomic analysis was performed to get insights into the physiology of CO conversion to propionate, and into the biochemical mechanisms and microbial interactions within the consortium. Differential protein expression was found throughout different phases of growth. At an early stage, the co-culture shows an acetogenic behaviour, where *Acetobacterium* sp. strain JM is more active, converting CO into acetate. The concentration of 30 mM of acetate triggers *Acetobacterium* sp. strain JM to produce ethanol from CO and acetate. At this point, protein expression in *Anaerobacterium neopropionicum* is increased, resulting in ethanol conversion to propionate. This synthetic co-culture couples the Wood-Ljungdahl and acrylate pathways to produce propionate from carbon monoxide, which engages an interesting way of broadening the production scope of CO fermentation to more complex and valuable products.

I1. Environmental Microbiology and Biotechnology

P13. New enzymes of *Gulosibacter molinativorax* ON4T are involved in the thiocarbamate herbicide degradation pathway

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Background: Enzymes are biological catalysts involved in all biochemical reactions in living organisms. They can be extracted from cells and applied in a wide range of catalytic biotechnological processes, playing an important role in the production or modification of different products [1]. Moreover, they can also be used in enzymatic bioremediation approaches, avoiding the introduction of allochthonous microorganisms in the polluted site, and consequently the potential disturbance of the native microbial communities. This technology relies upon the identification of the enzymes involved in the pollutant degradation pathway. *Gulosibacter molinativorax* ON4T is known to degrade molinate, a thiocarbamate herbicide [2]. Although the putative degradation products have been identified [1], only the enzyme responsible for the initial molinate breakdown (molinate hydrolase, MolA) was identified [1]. Objectives: This study aimed at identifying the enzymes involved in the transformation of the MolA products, namely azepane-1-carboxylate (ACA), by strain ON4T. Methods: A combined genomic and transcriptomic approach was used to identify the potential genes coding for the enzymes involved in the degrading pathway. Confirmation of activity has been carried out through recombinant protein expression in *Escherichia coli* BL21(DE3) using a pET system.
Conclusions: The analyses of the transcriptomic data from strain ON4T grown under two different conditions, with and without molinate, indicated that the genes encoding for Cytochromes P450 (*bioI* and *pipA*), hydantoinases A/B (*hyuA/B*), caprolactone hydrolase (*chnC*) and 6-oxohexanoate dehydrogenase (*chnE*) were over expressed in the presence of molinate. The work in progress will allow to determine their respective role in ACA degradation into caprolactam, 6-aminohexanoic acid and adipate, which can then enter the β-oxidation pathway, and further enable the kinetic characterization of these novel biocatalysts.

Acknowledgements
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I1. Environmental Microbiology and Biotechnology

P14. Analysis of virus' resistance to wastewater disinfection treatments

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Microbiological parameters on the current legislation for wastewater fail to account for the presence of enteric viruses. Conventional disinfection treatments applied in wastewater treatment plants are often inefficient to achieve virus removal, being the particles discharged into the environment at high concentrations (10^8 copies/L). These viruses are mainly transmitted via the fecal-oral route, or by ingestion of contaminated water and food, causing a variety of waterborne diseases.
Light emitting diodes (LEDs) recently emerged as an alternative to traditional UV mercury lamps due to their longer lifetimes, lower costs, lower energy, lack of harmful materials to discard, compact size and wavelength diversity. The combination of LEDs with membrane processes may prove to be an effective treatment of wastewater, since the virus will be retained by the membrane, and then be inactivated by the LEDs due to direct or indirect photolysis, if photocatalytic membranes are used.
Recently, silicon carbide ultrafiltration ceramic membranes modified by addition of homogeneous thin films of titanium dioxide, silicon dioxide and silicon carbide, proved to have photocatalytic activity, higher hydrophilicity, lower fouling potential and a desirable molecular weight cut-off.
This work aimed to evaluate different treatment processes efficiency - membrane filtration using unmodified and modified photocatalytic ultrafiltration membranes, LEDs emitting-light at wavelength 255, 265 and 365 nm, and the combination of the treatments. Effectiveness was evaluated in spiked wastewater matrices, regarding nucleic-acid and/or capsid damage, using PCR protocols (long-range PCR and quantitative PCR with enzymatic pre-treatment), and virus inactivation by infectivity assays with Hek 293 cell line cultures.
The experimental results obtained suggest that the three LED wavelengths combination is the more efficient regarding the tested viral genomes concentration (10^7 copies/mL of *Adenovirus* serotype 5 and *Mengovirus* strain vMC0), with a decrease of about 4Log10, after 2h exposure. However, infectivity assays for *Adenovirus* showed that the remaining virus particles are still infectious, being highly resistance to the applied treatments. Higher inactivation can be expected with a higher number of LEDs and lower distances between the samples and the radiation source.
The effectiveness of the combined treatment is currently being evaluated using a real wastewater matrix without virus spiking.

I1. Environmental Microbiology and Biotechnology

P15. Environmental monitoring of enteric viruses in wastewater from two Portuguese wastewater treatment plants

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Wastewater has essentially an anthropogenic origin and is mainly a combination of human faeces, urine, greywater and microorganisms, though it can also include industrial and hospital influents. Pathogenic and highly infectious viruses such as *Norovirus*, *Adenovirus*, *Enterovirus* and *Hepatitis A* virus are found in wastewater, reaching concentrations as high as 10^6 – 10^8 copies/L. Their persistence at high levels, despite the disinfection procedures conventionally applied in wastewater treatment plants (WWTP), can be due to their small size and structure.

To date, no regulations have been implemented to monitor viral concentrations in wastewater before it is discharged into a water body. Bacteria, such as *E. coli*, *enterococci*, and bacterial endospores, are normally used as indicators of faecal pollution for water quality monitoring, in spite of these bacteria being inadequate indicators of viral contamination due to completely different structure, composition, morphology, size, and survival characteristics.

Therefore, enteric viruses' monitoring for water quality assessment is crucial. However, classic methods for virus detection are based in cell culture, which is costly and time-consuming. Moreover, there is also a lack of effective cell lines to isolate some of the epidemiologically most important enteric viruses. Thus, nucleic acid-based methods such as multiplex quantitative real time PCR (qPCR) have been applied for several viral genomes detection at the same time in one single run, contributing to lowering costs and speeding up the analytical processes.

This study aimed at identifying/quantifying five pathogenic viral genomes (*Adenovirus*, *Polyomavirus*, *Norovirus* GI and GII and *Hepatitis A* Virus) by the optimization of two multiplex qPCR, along two WWTPs from Lisbon region (WWTP1 and WWTP2), during two seasons (autumn and spring), in order to track their fate and spread into the environment.

Our data suggests variances in the abundance of the target viruses regarding the seasonality, mainly in WWTP1. Also a decrease in some viral genomes concentration was observed after the conventionally applied disinfection procedures. Even so, the concentration of viral genomes released into the environment is extremely high becoming a potential source of human contamination and community- wide outbreaks, being a disregarded but crucial problem for public health.

I1. Environmental Microbiology and Biotechnology

P16. Phosphorus release from activated sludge by inversion of the biological P elimination in preparation for trapping in a Ca-fluidized bed

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Phosphate (P) is an essential building block for life and an indispensable plant nutrient. To avoid eutrophication of water bodies, several sewage treatment plants have implemented an enhanced biological P elimination. In the activated sludge P is taken up and stored as polyphosphate in the biomass of polyphosphate accumulating organisms. The 2017 amendment of the German Sewage Sludge Ordinance demands that sewage treatment plants recover P from either wastewater, sludge, or sewage sludge ash. In a circular economy approach, mining of rock phosphate could be replaced by sewage-borne P. Within the scope of the project Re-BioP-Cycle, the aim of this study was to evaluate the possibility of inverting the biological P elimination to achieve a release of intracellularly stored P for subsequent precipitation as P-fertilizer. Therefore, P-release studies were carried out as batch experiments using activated sludge samples incubated for 3-24 h at anoxic conditions. Ortho-P, acetate, and Fe content were determined photometrically by the molybdenum blue method, ion chromatography and atomic absorption spectrometry, respectively. Under anoxic conditions P was released if the sludge was supplemented with short chain fatty acids. The biological P-release with acetate was 135 times higher than without acetate. Up to 23% of the total P was released within 1-2 h. During the process acetate was taken up and the redox potential decreased. The acetate(uptake)/P (release) molar ratio was 1.77. It is known that polyphosphate accumulating organisms take up short chain fatty acids and store them as polyhydroxyalkanoates. The energy for this uptake is generated by decreasing the intracellular polyphosphate pool and thus releasing P into solution. However, inversion of the biological P elimination seems to be limited by the existence of a P-pool associated with iron (hydr)oxides. This is indicated by the observed co-release of P and Fe upon sludge extraction with ammonium oxalate. The results of this work provide the basis for further research on a sludge-based biological P-recovery route and will contribute to the development of a new sustainable adsorption technique for the production of a Ca precipitated P-fertilizer using (dolo-)limestone in a fluidized bed reactor.

P17. A novel *Enterobacter* sp. strain isolated from a petrochemical wastewater treatment plant effluent with potential use for urea removal in wastewaters

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The increasing global concern with the environment has led to the development of new approaches to environmental protection. In bioaugmentation, contaminant clean-up can be improved, in situ or ex situ, by the addition of microorganisms known to degrade or to expedite the degradation of those contaminants. These microorganisms can often be found in highly contaminated places, where contaminants act as nutrient sources, or as natural selection driving forces.
A bacterial strain (*Enterobacter* sp. BBC|003) was isolated from an effluent of a petrochemical wastewater treatment plant (WWTP) in Portugal, and was subjected to metabolic characterization (use of sugars, sugar alcohols, and esters, as sole carbon source), hydrolase screening (carboxylesterases, DNases, glycosidases, peptidases, and ureases), and growth optimization (temperature, pH, and salinity). The strain was also studied for its ability to grow on urea as sole nitrogen source, and its response to the presence of high concentrations of urea.
Strain BBC|003 grows better with sugars than sugar alcohols and shows weak growth with esters. The optimal growth is in the range 20-28 °C, pH 5-7, and 0-1% w/v NaCl, though growth occurred in the presence of 3% w/v NaCl. The strain produces carboxylesterases, being able to degrade non-ionic surfactants (several Tweens) and triglycerides (tributyrin), as well as DNases, β glucosidase, β galactosidase, xylanase, pectinases, amylase, and urease.
Strain BBC|003 is a fast urease producer, capable of urea removal in synthetic wastewater, and able to grow with urea as sole nitrogen source. Also, the strain can grow on rich-medium supplemented with up to 5.00% w/v urea and withstands concentrations up to 8.33% w/v urea with slow mortality rate.
Given these interesting features for innovative wastewater bioaugmentation, *Enterobacter* sp. BBC|003 can be a new asset in the biological treatment of wastewaters, especially those with high urea content.

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I1. Environmental Microbiology and Biotechnology

P18. First survey of microfungi from palm leaf spots in Portugal reveal a remarkable mycobiota diversity

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Palm trees are one of the most recognizable plants in the ornamentation of parks and gardens in Portuguese cities. Anything that diminishes their attractiveness, such as disfiguring leaf diseases, will negatively affect their aesthetic value. The recent impact of the red palm weevil, *Rhynchophorus ferrugineus*, on *Phoenix canariensis*, has highlighted the potential devastating effects of pests and diseases introduced onto a host growing outside of its native range.
Considering the almost complete lack of knowledge on the fungal assemblage on palms in Portugal, a preliminary assessment of their diversity was carried out. To this purpose and since fungi are one of the main causes of leaf diseases, the leaves from 50 diseased palm trees were sampled from 7 locations in the Lisbon district. A collection of more than 70 leaf spots was analyzed for associated fungi.
Following a strict isolation flow chart for a comprehensive survey of fungal diversity, a collection of more than 400 isolates was established. All isolates were characterized according to their macro- and micro- morphology and, when possible, assigned to genus level, and their genetic diversity was assessed by PCR fingerprinting (primers [GTG]5 and csM13). Representative or particularly interesting isolates were selected for molecular identification through sequencing of accepted molecular barcodes.
Overall morphological and genetic diversity revealed more than 30 genera of filamentous fungi, including 175 coelomycetous isolates and 235 hyphomycetes. A high level of genetic diversity was observed in most genera, consistent with the analyzed morphological traits. The most frequent coelomycetes belonged to genus *Phoma*, often observed sporulating in leaf dieback lesions. The most frequent hyphomycetes genera were *Alternaria*, *Cladosporium*, *Epicoccum* and *Stemphylium*, often co-occurring within a single lesion.
Sequence analysis of the selected isolates revealed a new genus in *Teratosphaeriaceae*, 2 new species in *Sporocadaceae*, and 2 new species in *Diaporthe*. Considering that only 2% of the isolates have been fully characterized, more novel species are expected to be found. Although this study was based on a relatively small sampling, it clearly confirms the already reported high diversity of palm mycobiota.

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I1. Environmental Microbiology and Biotechnology

P19. Study of kefiran production from cheese whey

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Cheese whey (CW) is the main by-product from cheese manufacture and its worldwide production is estimated as 180 to 190 million tons per year [1]. Due to its high organic matter content, high COD and BOD, it is considered a major pollutant. The interest of finding more options for its industrial utilisation, reducing its disposal problem and contributing to the circular economy model, is high. According to the biorefinery concept, CW can be used as a raw material for a wide range of commercial products, improving industrial profits. An interesting approach is the conversion of its lactose into value-added products through microbial fermentation [2].

CW fermentation can be carried out by kefir grains, a complex microbial community composed of different lactic and acetic acid bacteria, and yeasts, living in symbiosis, embedded within a polysaccharide matrix known as kefiran [3], an exopolysaccharide (EPS) with approximately equal amounts of D-glucose and D-galactose. Kefiran shows anti-bacterial, anti-fungal and anti-tumour activities with a wide range of medical applications, such as helping to modulate gut immune-systems and also as a food additive. The production of kefiran is mainly associated to the bacterium *Lactobacillus kefiranofaciens* present in kefir grains [4].

The main objective of this work was the use of cheese whey to produce kefiran with kefir grains. Different samples were used, namely cow CW and sheep CW. In order to optimise the process, different experimental conditions were tested, such as agitation and initial lactose concentration. After the fermentation step, kefiran precipitation, purification and quantification were performed. The produced kefiran was also analysed by analytical methods such as FTIR to better characterise the obtained product.

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I1. Environmental Microbiology and Biotechnology

P20. Development and validation of a new multiplex-PCR to detect prevalent species of house dust mites

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House Dust Mites (HDM) refer to small arthropods that live in close proximity to humans. Allergens from HDM, specifically from *Dermatophagoides* spp., are major etiological factors underlying allergic conditions that affect people worldwide.
Since HDM allergy is intricately linked to the exposure to mites themselves, the development of novel and optimized methods to search HDM in human environments, becomes crucial.
To counteract the fact that available techniques to search for HDM are time consuming or highly expensive, we propose the use of molecular based techniques, specifically Multiplex-PCR, with the full- length internal transcribed spacer from the rDNA gene cluster, as target for species-specific primer design.
Therefore, the aim of this work was to develop and validate a Multiplex-PCR assay to detect the two most common HDM species, *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp) in house dust samples.
Two novel primer sets for rDNA gene cluster were design and optimized first for Singleplex and after for Multiplex-PCR. The Multiplex reaction mixture contained 6.25 µl of NZYTaq 2x Green Master Mix, 0.125 µl of each forward primer (0.5 µM) and 0.250 µl of the common reverse primer (1 µM), and variable quantities of DNA template and nuclease-free water, in a total reaction volume of 12.5 µl. The reaction ran for 37 cycles at 94 ºC for 30 s, 56 ºC for 30 s and 72 ºC for 90 s. PCR amplicons and genomic DNA (gDNA) from house dust were used to evaluate the reaction limit of detection (LOD) and sensitivity. Sequencing was performed to confirm the method's specificity.
Two PCR products were obtained as expected with 800 bps for Df and 599 bps for Dp. The LOD were as low as 10^1 and 10^3 DNA copies for Dp and Df, respectively. The application of the new multiplex PCR assay to house dust samples also resulted in the identification of both species with amplicons of the expected sizes. The sensitivity using total gDNA extracted from house dust was 150 pg.
To conclude, the new Multiplex PCR here described was proven successful in identifying two common HDM species in house dust samples, with high sensitivity and specificity.

I1. Environmental Microbiology and Biotechnology

P21. Biodiversity and antimicrobial activity of *Actinobacteria* from Estremadura spur deep sea sediments

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The oceans cover 70% of the Earth´s surface and harbor most of the planet´s biodiversity. Among this biodiversity, the marine bacteria are an important and unexplored resource for drug discovery. A total of 14 sediment samples were collected off the Estremadura Spur, Continental Portugal coast, at 200 to 400 m depth using a ROV. The samples were processed for the targeted isolation of actinobacteria, and the recovered strains were identified and evaluated for their ability to produce natural products with antimicrobial properties against methicillin-resistant *Staphylococcus aureus* (MRSA, COL), methicillin-susceptible *Staphylococcus aureus* (MSSA, NCTC 8325) and *Escherichia coli* (K12). Eighty six actinobacteria were isolated and identified at the species level using protocols implemented in our lab.[1,2] Based on 16S rRNA gene sequencing, we observed that the genera *Streptomyces*, *Micromonospora* and *Saccharopolyspora* were predominant. Crude extracts were obtained from the 86 strains. Eleven of these crude-extracts showed antibacterial activity against MRSA, with MIC values ranging from 250 to 7.8 µg/µl, twelve were active against MSSA, with MIC values ranging from 250 to 1.9 µg/µl, and eleven revealed activity against K12, with MIC values ranging from 250 to 1.9 µg/µl. Three of these extracts showed antimicrobial activity against both Gram positive and Gram negative bacteria. We are currently determining the structure of the compounds responsible for these activities. These studies demonstrate that the Portuguese coast is a rich source of marine actinobacteria with potential applications for biotechnology.

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I1. Environmental Microbiology and Biotechnology

P22. Defining the root endosphere and rhizosphere microbiomes from the World Olive Germplasm Collection

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Up to date, the bacterial and fungal microbial communities from the olive (*Olea europaea* L.) root systems have not been simultaneously studied. In this work, we show that microbial communities from the olive root endosphere are less diverse than those from the rhizosphere. But more relevant was to unveil that olive belowground communities are mainly shaped by the genotype of the cultivar when growing under the same environmental, pedological and agronomic conditions. Furthermore, *Actinophytocola*, *Streptomyces* and *Pseudonocardia* are the most abundant bacterial genera in the olive root endosphere, *Actinophytocola* being the most prevalent genus by far. In contrast, Gp6, Gp4, *Rhizobium* and *Sphingomonas* are the main genera in the olive rhizosphere. *Canalisporium*, *Aspergillus*, *Minimelanolocus* and *Macrophomina* are the main fungal genera present in the olive root system. Interestingly enough, a high proportion of so far unclassified fungal sequences at class level were detected in the rhizosphere. From the belowground microbial profiles here reported, it can be concluded that the genus *Actinophytocola* may play an important role in olive adaptation to environmental stresses. Moreover, the huge unknown fungal diversity suggests that there are still some fungi with important ecological and biotechnological implications that have yet to be discovered.

I1. Environmental Microbiology and Biotechnology

P23. Arbuscular mycorrhizal fungi in bamboo under cerrado vegetation

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Many studies on bamboo are focused on assessing their production potential and utilization, but there are few studies on the correlation of associated mycorrhizal fungal species found in their rhizosphere. The objective of this work is to verify the established mycorrhizal community and its ecological relations with the bamboo species, *Actinocladum verticillatum* and *Bambusa vulgaris vittata*, under Cerrado vegetation. Root and soil samples of *Actinocladum verticillatum* and *Bambusa vulgaris* vittata were collected. For sampling, 12 points were chosen from the Gurupi - TO and Porangatu - GO microregions. The parameters mycorrhizal colonization rate, spore density and identification of associated genera were evaluated. There are no differences in mycorrhizal colonization rate values among the studied bamboo species, however *Bambusa vulgaris vittata* presented higher spore density values than *Actinocladum verticillatum*. The genera *Acaulospora*, *Claroideglomus*, *Diversispora*, *Scutellospora*, *Sclerocystis*, *Glomus* and *Gigaspora* were identified in the investigated rhizosphere, except for the genus *Sclerocystis*, all genera were identified in both bamboo species. The genera *Acaulospora*, *Diversispora,* and *Glomus* have the highest affinity for *Bambusa vulgaris vittata* and *Actinocladum verticillatum*.

I1. Environmental Microbiology and Biotechnology

P24. Screening of non-*Saccharomyces* wild yeasts for biotechnological applications

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In nature, a wide diversity of yeast species can be found even in wastes from food industries. The exploration of this yeast biodiversity has captured great interest from food, pharmaceutical and even fuel companies due to the interesting properties of such microorganisms [1]. These microorganisms can transform sugars present in raw materials into different valuable compounds as several chemical building-blocks and biofuels, in a process more sustainable than those based on fossil fuels and refineries [2]. Within yeasts, *Saccharomyces cerevisiae* is considered the model organism, being the most widely used industrially for the production of added-value products [3]. Properties that range from its simple cultivation, short replication period, sporulation efficiency, easy genetic manipulation and rare pathogenicity have turned it in an ideal organism for various biotechnological processes [3]. Nevertheless, other non *Saccharomyces* yeasts are being increasingly used for the heterologous production of valuable products [4]. In this work, a group of isolates from the TransBio collection (Project FP7 KBBE–Nº289603) was selected based on their ability to grow in organic acids. Microorganisms were identified by molecular typing (DNA sequencing of the ITS regions) and characterized regarding morpho- and physiological features. The morphological traits and sporulation patterns were evaluated for cell cycle determination. The selected yeasts revealed interesting physiological features regarding growth profiles using carboxylic acids as sole carbon and energy source. The full characterization of these wild yeast strains is underway.

Acknowledgments

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I1. Environmental Microbiology and Biotechnology

P25. Unveiling the ecological dynamics of antimicrobial resistance in wild ungulates

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Antimicrobial resistance (AMR) has been identified as a global problem for public health, animal and environment. The increasing incidence of AMR in humans and livestock has been linked to the emergence of AMR in wildlife and the global dissemination of resistant bacteria by wildlife has severe implications on ecosystem and human health. Wild ungulate populations have dramatically increased over the last decades in Europe, and Portugal is no exception. The present proposal will use the most widespread wild ungulates in Portugal as models to understand emergence, spread and persistence of AMR in the wildlife-livestock interface. These are perfect model species due to their ubiquitously, considerably large home ranges, unlikeliness of being treated with antibiotics and overlap their habitat with livestock and humans, serving as a link between humanize and natural areas. Also, they are emerging as source of foodborne diseases in humans due to the consumption of game meat. Our previous work demonstrated the presence of AMR bacteria and important foodborne diseases in wild ungulates. This raised several questions, concerning the ability of ungulates to act as reservoirs of AMR and on the mechanisms of AMR persistence. In this project, we will i) characterize the prevalence of AMR bacteria in wild ungulates, ii) understand the impact of human activities in the development of AMR in wild ungulates and iii) develop prediction models of AMR dynamics in the wild. The study of these patterns only makes sense in the light of landscape ecology. This project is pioneer by intersecting infectious disease ecology, landscape ecology, and microbiology, to infer emergence, transmission and the spatial drivers of AMR across space and species. Such approach will significantly contribute to disclose the dynamics of AMR in the wildlife-livestock interface. Such understanding is critical for identifying populations at risk, mapping high-risk areas and, consequently, targeting surveillance and designing proactive management programmes.

I1. Environmental Microbiology and Biotechnology

P26. Biodeterioration of easel paintings: a case study of Munch´s paintings

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Many artworks, including easel paintings, present several organic materials in their composition, namely protein compounds, used as additives in the different paint layers as well as support materials like wood, paper, fabric or parchment. These substrates, together with the presence of favourable environmental conditions, promote a set of microorganisms' development that among other factors can be responsible for different biodegradation processes causing aesthetic and structural alterations in these artworks. Thus, biodeterioration phenomenon is a relevant issue for preservation/conservation of the Cultural Heritage artworks [1-3].
This work focuses on the study of the biodeterioration process of Edvard Munch's easel paintings belonging to the Munch Museum in Oslo. Several microsamples were collected during the THE SCREAM Project campaign, using non-invasive/micro-invasive methods, in sterile conditions in zones with visible structural and chromatic alterations. The isolation, characterisation and identification of cultivable microbial population was performed. Several bacterial species found in these artworks were characterised revealing proteolytic capacity, an important biodeteriogenic characteristic for these paintings.
Two bacterial strains isolated with greater proteolytic potential were inoculated in paint prepared with casein as binder, the same used by E. Munch in these paintings, and monotonized for 6 months. Results show some degradation signs in paint models caused by these microorganisms activity.
Knowledge of the biodeteriogenic agents present in these E. Munch paintings under study have highest importance in order to develop appropriated mitigation strategies focused on effective interventions, and maybe be applied to other artworks with the same pathology.

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I1. Environmental Microbiology and Biotechnology

P27. BeachSafe: Occurrence of potential pathogenic vibrios in recreational water and human health risk outcome

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Naturally occurring in aquatic ecosystems around the world *Vibrio* species, can negatively impact human health and ecosystem services. Indeed, reported outbreaks of Vibrio-associated human illness linked to recreational bathing, and the ongoing climate changes have been increasing in the Northern Hemisphere. *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* are recognized human pathogens known to cause life threatening diseases, including diarrheal illnesses, wound infections and septicemia. The aim of this study was to investigate the prevalence of potential human pathogenic vibrios in bathing water, understand its dynamics in relation with environmental constrains, and therefore predict potential risk to human health. Monthly distribution of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* were investigated in 10 Atlantic beaches (NW Portugal) over a year, by means of MPN-polymerase chain reaction (MPN-PCR). Vibrios were detected in all the studied coastal beaches with abundances ranging 2.23-8.64 Log MPN/L. *V. cholerae* was detected in all months, although cholera toxin gene *ctxA* was found only once, in low abundance. Total *V. parahaemolyticus* was present throughout the year with higher expression of toxigenic genes *tdh* and *trh* during the bathing season. *V. vulnificus* showed abundances up to 8.64 Log MPN/L in the summer months, despite the scarce detection during the rest of the year. Temperature, salinity, and particulate matter appear to have a key role in the dynamics of these *Vibrio* species. The results highlighted the public health risk associated with the presence of potential pathogenic bacteria in bathing water that can be considered safe according to the European Union legal criteria. Monitoring and understanding the dynamics of these agents in a climate change scenario is essential to develop alert tools and consequently ensure a BeachSafe to users.

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I1. Environmental Microbiology and Biotechnology

P28. BeachSafe: characterization of *Vibrio parahaemolyticus* isolates in Portuguese bathing water

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Vibriosis outbreaks associated with recreational bathing are on the rise in the Northern Hemisphere linked to the ongoing climate changes. Since members of the genus *Vibrio* are gram negative bacteria autochthonous in marine and estuarine environments, its monitoring become essential for public health assurance. In the developed world, *V. parahaemolyticus* is recognized as a major agent causative of illness of non-cholera related *Vibrio* infections, including gastroenteritis and septicemia. Water samples were collected from 10 Atlantic beaches (NW Portugal) over a 15 months' period. Thiosulfate-citrate-bile salts-sucrose agar (TCBS) and Chromoagar *Vibrio* medium were used for presumptive identification and *V. parahaemolyticus* isolation. PCR targeting the *toxR* gene was used to confirm the identification of the *V. parahaemolyticus* isolates, and subsequent PCR targeting genes associated with coding toxin production were assayed (*tlh*, *trh* and *tdh*). Of the 80 confirmed *V. parahaemolyticus* isolates, the *tlh* gene encoding the thermolabile hemolysin was successful found in 16% of the environmental isolates, whereas 11 and 3% exhibited the thermostable direct hemolysin and thermostable-related direct hemolysin, respectively. The assessment of antibiotic susceptibility to 13 antibiotics was carried using the Kirby-Bauer disc diffusion method. The resistance patterns exhibited a wide variability with the majority of the isolates showing resistance at least 3 antibiotics. B- lactam, aminoglycosides and macrocyclic antibiotics revealed the highest number of resistant isolates. Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) and Multiple-Locus Variable-Number Tandem-Repeat Analysis (MLVA), used to identify differences in phylogeny among the pathogenic isolates, displayed higher diversity. The results of this study highlighted the potential public health risk associated with the presence of pathogenic antibiotic resistant *V. parahaemolyticus* in recreational waters. Furthermore, the study revealed the need for monitoring and alert tools development for these natural occurring agents in order to guarantee a BeachSafe to users, especially taking into account the climate change world reality.

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I1. Environmental Microbiology and Biotechnology

P29. Unravelling mechanisms involved in critical metal resistance by the strain *Rhodanobacter thiooxydans* $B_2A_1Ga_4$

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Critical high-tech elements such as indium (In) and gallium (Ga) have very high economic importance and demonstrate a high risk associated with their supply. Therefore, there is a challenge to increase recovery rates from secondary materials, to reduce the primary resource use and environmental impacts. This work pursuits to understand the biological mechanisms behind the bacterial-metal interaction, particularly In and Ga. This knowledge might be useful to the development of technical procedures to potentiate the bioremediation and biorecovery of these metals using bacteria as eco-friendly techniques.
A strain *Rhodanobacter thiooxydans* $B_2A_1Ga_4$, highly resistant to critical metals (particularly In), was used to study the potential mechanisms involved in metal resistance and metal stress. Mutants of strain $B_2A_1Ga_4$ were obtained using random transposon mutagenesis technique. The two mutants showing more susceptibility to In compared to the native strain were studied to disclose the bacterial strategies to cope with the critical metals. The mutant B2 had a decrease of the final bacterial growth (optical density, OD600nm) of 1.9 and 3.5 fold in the presence of 0.2 mM and 0.4 mM In, respectively, compared to the native strain. Aluminum led to a slight decrease in mutant growth. Moreover, this mutant lost its usual red pigmentation when grown in solid medium supplemented with In. The other selected indium- sensitive mutant, E32, revealed a gene encoding for the ferrous iron transport protein A (*feoA*) interrupted and demonstrated higher bacterial reactive oxygen species (ROS) levels with exposure to different metal concentrations and lower levels of reduced glutathione (GSH). The cells exposed to the critical metals resulted in a decrease of their cellular metabolic activity with reduction of 2.1 and 3.9 fold with 0.4 mM Ga and 0.2 mM In, respectively. This means that iron metabolism should have a role in cellular protection from the toxicity of some heavy metals. Altogether, those results indicate that the loss of metal resistance compromises the mechanisms to protect the cell and increase the intracellular metal stress with higher levels of oxidative stress and lower cellular viability comparing with the native strain.

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I1. Environmental Microbiology and Biotechnology

P30. Detection of *Endornavirus*: development of an ELISA assay

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Endornaviruses (family *Endornaviridae*, the genus *Endornavirus*) with a Double stranded RNAs (dsRNAs) approximately 2 to 15 kb in length are often found in plants, fungi and protozoa and have common properties that differ from those of conventional viruses: they have no obvious effect on the host phenotype and are efficiently transmitted to the next generation via the reproductive system. Endornaviruses spread symbiotically in host organisms and transmit vertically, they are unique viruses with symbiotic properties. Nucleotide sequencing and phylogenetic analyzes reveal that these dsRNAs are not transcribed from host genomic DNAs and encode a single long open reading sequence (ORF) with a viral RNA-dependent RNA polymerase domain and contain a site-specific nickname in the 5 'region of the coding chain and no coat protein. In this work monoclonal antibodies (Mabs) were developed against a conservative peptide sequence present in all viral RNA polymerase involved in *Endornavirus* replication. The Mabs obtained were used to develop an indirect ELISA assay capable to distinguish between plants infected with *Endornavirus* from healthy plants.

I1. Environmental Microbiology and Biotechnology

P31. Dynamics of bivalves' gut-associated microbiome induced by a marine harmful algal bloom event

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Bivalve molluscs are frequently exposed to harmful algal blooms being known vectors of marine phycotoxins. Toxicity varies greatly between bivalve species according to behavioral and physiological factors, including biotransformation capacity. In fact, microbiomes may have an important role on the metabolic capacity of natural toxins, in many cases potentially changing their efficacy and/or side effect profiles. However, despite the economic and ecological importance of this molluscs, little is known about the roles of microbe-bivalve interactions regarding phycotoxins resilience and elimination. In this study the bacterial diversity and community structure of the intestinal microbiome of three different bivalve species is characterized throughout a marine toxic algae outbreak. Sampling was done during a 2-month period at the Aveiro Lagoon and the algal bloom was mainly composed by diarrheic (DSP) and paralytic (PSP) shellfish poisoning-producing dinoflagellate species. The gut microbial consortium was profiled using high-throughput 16S rRNA amplicon sequencing (V3 region) with Illumina sequencing technology.
Preliminary results show clear differences between the bacterial communities associated with bivalve`s gut and those of seawater, besides the observed differences in gut microbial consortium among the 3 molluscs species indicating the gut-specific microbiota within the bivalves. Species-specific changes related to microbial diversity and structure were denoted according to toxic phytoplankton abundances.

I1. Environmental Microbiology and Biotechnology

P32. Assessment of drying conditions of a yeast-based solution for application on textile industrial wastewater treatment plants

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The textile sector is a worldwide industry that produces high amounts of harmful effluents that are discharged to the environment. These dyed effluents are resistant to biodegradation and potentially damaging to the aquatic and other ecosystems [1]. Classic chemical treatment methods are very costly and generate large quantities of sludge that need to be treated [2]. Biological methods are generally considered more environmentally friendly and of major relevance [3]. Biological alternatives to aid the decolourisation of dyes in textile wastewaters need to be implemented.
A yeast-based solution for decolourisation of textile industrial wastewater is under evaluation. A yeast strain, isolated from a textile wastewater treatment plant, was selected for its dye decolourisation capacity and was dried by freeze-drying. Skimmed milk and maltodextrin were used as cell protectors and the dried product was stored at cold and room temperature over time. Viability of the yeast cells and its decolourisation ability was assessed. Results showed that dried yeast cells maintained their viability and its decolourisation capacity.

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I1. Environmental Microbiology and Biotechnology

P33. Antimicrobial potential of *Lamiaceae* essential oils against heritage biodeteriogenic strains

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Biodeterioration caused by microorganisms is a major problem to be considered in the preservation of patrimonial assets, since these microbial agents can severely damage the goods that are intended to be preserved. Due to their toxic effects and health and environmental contamination risks, many of the most effective biocides, based on toxic chemical compounds and used to mitigate biological contamination, have been banned from market. It is therefore imperative to develop appropriate corrective actions for contaminated historic materials based on environmentally sound solutions. In this context, essential oils (OEs) obtained from aromatic plants, due to their antimicrobial properties and low toxicity, represent an alternative in the control of biodeterioration in the context of cultural heritage, without negative environmental and human impacts.

The aim of this study was to evaluate the antimicrobial activity of EOs of six flavouring herbs against biodeteriogenic microorganisms previously isolated from heritage artworks. Wild growth *Calamintha nepeta*, *Lavandula luisieri* and *Lavandula viridis* EOs were obtained from aerial parts of flowering plants by hydrodistillation in Clevenger-type apparatus and the other EOs of *L. luisieri*, *Rosmarinus officinalis*, *Thymus mastichina* and *Salvia officinalis* were provided by *Ervitas Catitas*, a local producer of biological aromatic plants. Chemical composition of EOs was evaluated by GC-FID. Antimicrobial activity was assessed by solid diffusion disk assays against mould strains of *Aspergillus niger*, *Cladosporium* spp., *Fusarium oxysporum* and *Penicillium* spp., yeast strains of *Rhodotorula* sp. and *Exophiala* sp. and bacterial strains from the genera *Bacillus* and *Arthrobacter*.

Results showed high antimicrobial activity of EOs. The most strains of *A. niger*, *Cladosporium* spp. and *Penicillium* spp. showed higher sensitivity to wild growth *L. luisieri* EO and *Rhodotorula* sp. and *Arthrobacter* sp. strains are sensible to *L. luisieri* and *S. officinalis* EOs, without growth.

The results suggest the potential use of OE in the safeguarding of cultural heritage, especially in murals, presenting as a greener and more advantageous alternative when compared to traditional biocides. This work is underway to study the mode of application of OEs as fungus control.

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I1. Environmental Microbiology and Biotechnology

P34. Diversity of isolates tolerant to multi-metals from mina Los Cóndores, Argentina

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Traditionally raw materials exploitation is focused in high-grade ore deposits, extracted and processed by conventional techniques. The metal recovery efficiency of these techniques was variable along time and framed by a minimum efficient scale approach. Consequently, a significant amount of metals was discarded to tailings dams, many of those in concentrations that exceed today's mines cut- off grades. It is also known that microorganisms interact with metals and minerals and bioleaching with autochthonous microbiome can be a sustainable approach.

In Argentina, the mine of Los Cóndores (Zona Departamento Chacabuco) was selected for evaluation for the presence of polymetallic resistant bacteria to be explored as bioleachers in this environment. The Los Cóndores mine was selected based on the antiquity of the exploitation mining processes, the volume of the tailing s (282.00 tons) and the richness in tungsten (0.8-1% WO_3).

The microorganisms were isolated in R2A medium, grouped by RAPD-PCR and each representative strain was sequenced (StabVida). The Minimum Inhibitory Concentration test was performed in R2A supplemented with arsenite (As^{3-}; 2, 3 and 5 mM), tungstate (WO_4^{2-}; 3, 5, 10 and 20 mM), gallium(III) (Ga^{3+}; 1, 2 and 3 mM), indium(III) (In^{3+}; 0.5, 0.75 and 1 mM) and tellurite (TeO_2^{3-}; 0.5, 1 and 3 mM).

A total of 121 colonies were isolated corresponding to 90 RAPD clones. The Identification by 16S rRNA gene sequencing of 60 strains showed that the majority belong to the genus *Bacillus* and the most abundant species was *Bacillus niacin*. Sampling area Arg05 showed the highest diversity with 14 species. *Brevibacterium frigoritolerans*, *B. aryabhattai* and *Dyella ginsengisoli* dominated the culture diversity in site Arg06. Sampling site Arg07 was characterized by the presence of the genus *Sinomonas* belonging to 2 different species *S. humi* and *S. atrocyanea*.

The distribution pattern between resistances was similar between samples Arg.05 and Arg.06, with most strains resistant to lower concentrations of all metals. Strains from sample Arg.04 were able to resist to all metals up to high concentrations. Strains from Arg.07 were only able to resist to gallium and tungsten. This is an on-going work to select resistant strains to be used to bioleach the Los Cóndores tailings for W.

I1. Environmental Microbiology and Biotechnology

P35. Expression of TupBCA from *Sulfitobacter dubius* for a better W accumulation in *Escherichia coli* cells

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Tungsten (W) is a valuable element with considerable industrial and economic importance that belongs to the European Union list of critical metals with a high supply risk. Therefore, the development of effective and new methods for W recovery is essential to ensure a sustainable supply. In recent years, many studies have been performed in order to construct bioaccumulators for several metal ions, since they do not require high quantities of chemical reagents and they minimize the volume of toxic sludge and other waste products.
In the present study, in order to obtain cells able to accumulate high quantities of W the *Sulfitobacter dubius* W transport system TupABC was explored in order to demonstrate both its functionality in *Escherichia coli* cells and to construct a bioaccumulator EcotupW. The complete gene cluster *tupBCA* or partial gene cluster *tupBC* were cloned in an expression vector and transformed into *E. coli*. Metal accumulation was evaluated when each construct strain was grown with three separate metal oxyanions (tungstate, molybdate or chromate). The specificity of the bioaccumulator was determined by competition assays using cells grown with mixed solutions of metal oxyanions (W/Mo and W/Cr).
The results showed the relevance of the TupA protein in the TupABC transporter system to enhance W-uptake, being essential to W-specificity uptake but also allowing Mo and Cr accumulations, although in lower amounts than W (1.7 and 2.9-fold lower, respectively). To identify the importance of the valine residue in the accumulation efficiency of the VTTS motif, site-directed mutagenesis of TupA was performed. A mutant with a threonine residue, instead of the respective valine, confirmed that W is mostly internalized in its native form (nearly double the amount).
In conclusion, the findings indicated that the cells carrying the native *S. dubius* TupABC system were great W-bioaccumulators and could be a promising and alternative tool for W recovery from their natural environments.

I1. Environmental Microbiology and Biotechnology

P36. Bioleaching of Gallium by bacteria isolated from two different Portuguese mines

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Gallium is used in microelectronic components containing either gallium arsenide (GaAs) or gallium nitride (GaN). GaAs is used in the manufacture of optoelectronic devices (laser diodes, LEDs, photo detectors, solar cells), and in the production of highly specialized integrated circuits and semiconductors, used for example in mobile phones. GaN is used in the manufacture of LEDs and laser diodes, power electronics, and radio-frequency electronics. Gallium metal is obtained mainly as a byproduct of bauxite ore processing for aluminum and sphalerite ore for zinc. Since there is an increasing demand for this critical metal, the development of effective recovery processes of gallium is of great importance. The aims of this study were: i) to screen bacterial isolates from two different Portuguese mines, Panasqueira and Urgeiriça for their ability to leach gallium from GaAs and GaN; ii) to optimize bioleaching conditions for strains that showed the highest efficiency for gallium leaching. Seventeen isolates were tested for potential bioleaching of gallium at 25 °C for 21 days, in 20 ml of modified R2A broth (mR2Ab) medium, pH6, and containing 10 mg of either GaAs or GaN. The amount of gallium was measured by ICP-MS. Six strains were able to leach gallium from both GaAs and GaN. A higher efficiency was obtained with GaAs, with 18% to 44% of gallium leached. For GaN, the percentage of gallium leached ranged between 12% and 28%. Strain *Arthrobacter* sp. A2-55 (Urgeiriça) and strain *Rhodanobacter* sp. $B_2A_1Ga_4$ (Panasqueira) showed the highest efficiency to leach gallium and were selected to optimize the bioleaching conditions. Preliminary results show that strain A2-55 leaches gallium more efficiently in mR2Ab (28%) when compared to LB medium (15%). Strain $B_2A_1Ga_4$ had a similar gallium leaching efficiency in both media (26-30%). However, both strains were more efficient in media with lower pH, (pH6), when compared to pH 7.5. Culture supernatants collected from the late stationary phase were the most efficient to leach gallium from GaAS, with 27% for strain A2-55 and 36% for strain $B_2A_1Ga_4$. However, none of the culture supernatants were efficient to leach gallium from GaN, reaching only 2-7% of leaching.

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I1. Environmental Microbiology and Biotechnology

P37. Modelling of hardwood spent sulfite liquor fermentation for 2nd gen bioethanol

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Biorefineries based on feedstocks of lignocellulosic biomass processing are considered one of the most promising alternatives for second-generation bioethanol production and thus, a sustainable alternative to fossil fuels [1]. Pulp and paper Industry produces a high amount of residues and some can be converted into valuable products [2]. This work advances the research done on bioethanol production from fermentation of Hardwood spent sulfite liquor (HSSL) with the yeast *Scheffersomyces stipitis*, aiming at evaluating and model the inhibitory effect of the acetic acid on the ethanol yield and productivity. For this purpose, experimental data of batch fermentations with *S. stipitis* was determined in synthetic medium with xylose, glucose and acetic acid.

12 kinetic equations based on the Monod equation [3,4] were used to develop a mathematical tool able to describe the biomass, substrates and product concentration profiles as function of time and calculate yields, productivities and other parameters that allow for a complete examination of the process under study. With the modelled profiles it was possible to conclude that the presence of acetic acid affects negatively both the length of the lag phase and the maximum ethanol concentration obtained, hence the ethanol productivity and yield on substrate.

Moreover, the increase in the initial acetic acid concentration causes the decrease in the substrate consumption rate, prolonging even further the time to reach the maximum ethanol concentration. This concentration value, however, is only significantly affected when the initial acid concentration is higher than 6 g/L.

The synthetic medium data was compared against a 60% HSSL sample showing that there are not only other inhibitory compounds present in the HSSL, besides the acetic acid, but also other sources of organic carbon, besides xylose, glucose and acetic acid.

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I1. Environmental Microbiology and Biotechnology

P38. Non-invasive monitoring of stress response of urban trees inoculated with EcM

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Urban trees play a key role in urban settings providing ecosystem services not only by improving the environment quality but also by contributing to population welfare and biodiversity. Ectomycorrhizal fungi (EcM) emerge as a helpful biotechnology tool for urban tree management conferring advantages to the host plant by improving its vigor and resilience under stressing conditions such as limited soil water availability.
The major aims of this study were (i) the analysis of the response of *Tilia tomentosa* inoculated with EcM under a water-stress scenario and (ii) the evaluation of bioindicators of water-stress.
A 9 months in-vivo experiment was established and consisted in the induction of water-stress on seedlings in two substrates (acid and alkaline pH) inoculated with 4 EcM. To induce water-stress, the watering system that irrigated the plants daily (before-stress point) was stopped for 2 weeks (stress- point). The plants were analyzed 2 weeks after resuming watering to assess its recovery from drought (recovery-point). SPAD levels and leaf proline content were measured. At the end of the assay specific leaf area (SLA) and leaf water content (LWC) were determined.
Inoculation with EcM significantly increased the SPAD values and proline content at the stress point of plants when compared to non-inoculated (control) plants in both acid and alkaline substrates, revealing the protection/mitigation effect of EcM by conferring the ability to the plants to maintain its normal activity during a drought period. The inoculated plants normalized their SPAD and proline levels during the recovery period. These two parameters can be used as bioindicators to evaluate the water-stress response and water-status of trees. The plants inoculated with EcM revealed a higher SLA and LWC indicating a promotion in the development of the aerial part of the plant and an improvement of tree vigor. These results represent an important contribution for the development and application of EcM- inocula to improve tree resilience and for the establishment of strategies for tree monitoring in urban context.

I1. Environmental Microbiology and Biotechnology

P39. Microbial communities involved in cork decay

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Cork is the main product of cork oak exploitation, with a great development in the Iberian Peninsula, especially in the regions of Atlantic influence. In Portugal, the Cork Oak is a dominant specie, forming multiple semi-natural systems, called “montados” [1]. Cork is a natural product that, due to its physical, chemical and mechanical properties, aroused interest for several purposes, but cork may contain some defects that influence the performance of cork products [2]. These defects when present in a cork stopper invalidate their use as a sealant. The ‘cork taint’ represents one of the most unpleasant offflavours spoiling bottled wines giving the wine a mold aroma. The compounds associated with taint include microbial metabolites which can be produced by the mycobiota found in association with the bark and cork throughout the often-lengthy processing, storage and cork transportation [3].
This work aims to develop a process for monitoring microbial communities in cork processing and contribute to understand the role of these agents in cork decay.
Cork samples with different pathologies (like yellow spots, blue spots, nail, TCA spots) were analyzed by Scanning Electron Microscopy (SEM-EDS) to confirm the presence of biofilms and the cultivable filamentous fungi were isolated from these samples, namely to the 2,4,6-trichloroanisol (TCA) yellow- spotted cork samples.
The structural characterization and chemical characterization of the samples showed the altered and unchanged cork structure, the presence of biofilms on the altered cork surface and the presence of chlorine in the contamination zones. The implementation of high throughput sequencing approaches allowed us to explore, compare and characterise the microbial communities, overcoming the limitations of culture dependent techniques, which only identify the cultivable population.

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I1. Environmental Microbiology and Biotechnology

P40. Potential of *Chlorella vulgaris* for urban wastewater bioremediation

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Eutrophication is a common phenomenon impacting on estuarine and coastal ecosystems, often related to enriched nutrient effluents discharges. The improvement of wastewater treatment methods to increase its quality, meeting the legislated parameters, is essential to address this problem, particularly of sensitive areas such as estuaries. The application of microalgae on wastewater treatment has proved to be an efficient method to reduce the organic load of the effluent, promoting an environmental and economical sustainability, through the nutrient recycling and decarbonization.

The aim of this study was to evaluate *Chlorella vulgaris* growth and bioremediation potential from a secondary effluent from an urban wastewater treatment plant located at Figueira da Foz (Portugal), to decrease the organic load released to Mondego river estuary.

Two cultivation methods, under optimal conditions, were tested to assess the best performance on *C. vulgaris* growth and their potential on effluent bioremediation: suspended microalgal cells and immobilized on alginate beads cultivation.

The immobilized cells on alginate beads contributed for NH_3, NH_4^+ and NH_3-N removal (58.6%, 65.1% and 70.7%, respectively), with a maximum biomass of 9×10^5 cells mL^{-1}. Comparing with the suspended cells cultivation the removal rate of NH_3, NH_4^+ and NH_3-N was 45.2%, 57.2% and 62.9%, with a maximum biomass of 1×10^6 cells mL^{-1}.

The removal rate of the suspended microalgal cells cultivation is lower in comparison with the immobilized cells cultivation. Furthermore, biomass harvesting in a suspended cultivation system is expensive and energy demanding, whereas the immobilized cells are easy to recover by sieving method.

I1. Environmental Microbiology and Biotechnology

P41. Lignocellulose degradation in wood and urban-waste composts analysed by functional metagenomics

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Few metagenomic analyses have been conducted to explore the compost highly efficient microbial system of lignocellulose biomass bio-recycling. These environments are sources of enzymes with high industrial value. The present work aims to uncover the differences on lignocellulose degradation in two metagenomes, derived from a wood-based compost pile (C18) (70 °C) and an urban waste composting tower (TL3) (46.3 °C) and characterize the enzymes involved in plant biomass degradation. Sequencing and assembly of bins from the two composts resulted in 19 bins for C18 and 47 bins for TL3. Taxonomy inferred from the bins indicated *Proteobacteria* as the dominant phylum in C18, while *Firmicutes* was the most abundant phylum in TL3. *Chloroflexi* was similarly identified in C18 and TL3. Degradation of lignocellulose was investigated by the CAZy annotation, blast searches and manually curation. Cellulases of the families GH5, 6, 9 were enriched on the C18 and TL3 was enriched in the GH10 family (xylanases). The major abundance of cellulases in C18 is indicative of a more active secondary cell wall degradation. On TL3 a more active primary cell degradation is suggested by the higher abundance of xylanases. The bacterial lignin degrading enzymes, laccases and peroxidades, were mainly present in TL3. We also searched the metagenomes for polyethylene terephthalate (PET) degradation. Indeed, and as expected, we only observed cutinases in the urban waste compost TL3. These enzymes were identical to cutinases of *Saccharomonospora viridis* and *Thermobifida fusca*. Detailed analysis of the identified enzymes is under way and may provide valuable information on enzymes for the degradation of lignocellulose with potential application in the biofuel industry. This work was supported by HORIZON 2020, under the project Metafluidics, ref. 685474.

I1. Environmental Microbiology and Biotechnology

P42. Ultrafiltration after ozonation of urban wastewater: tackling bacterial regrowth

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Water scarcity issues could be mitigated if treated wastewater was reused in different applications (e.g. agricultural irrigation and aquifer recharge). In some cases, after biological treatment (conventional activated sludge), the final effluent of urban wastewater treatment plants (UWWTPs) do not meet quality criteria for water reuse, mainly in terms of microbiological parameters, being required an additional tertiary treatment for wastewater disinfection.
Advanced oxidation technologies (AOTs), such as ozonation (O_3), have recently emerged as effective tertiary treatments for the removal of both chemical and biological contaminants in UWWTPs. However, previous studies demonstrated that some bacteria are capable of cell-injury repair and can regrow after the O_3 process [1]. A suitable approach that could remove these bacteria from treated wastewater would be a physical separation step, namely a membrane technology.
This study aimed at evaluating the ability of a pilot scale reactor to remove the secondary treated wastewater bacteria with ultrafiltration (UF) membranes after O_3. The UF step performed after O_3 contributed to remove coliforms and enterococci to values below the detection limit even after 7 days storage.
According to the Portuguese standards of water for irrigation (DL 236/98) [2], this stored treated water is suitable crop irrigation promoting a sustainable management of water resources and addressing water scarcity issues.

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I1. Environmental Microbiology and Biotechnology

P43. Rhizosphere microbiology of Ria de Aveiro salt-marsh plants: implications on phytoremediation and saline agriculture

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The rhizosphere of salt marsh plants is enriched in microbes that establish mutually beneficial relations with the hosts. The capacity of rhizosphere bacteria to degrade pollutants, produce phytohormones and stress mitigation enzymes, enhance the access to access to macro and micro nutrients and inhibit pathogens are generally referred as plant-growth promoting traits. The association with plant-growth promoting rhizobacteria (PGPR) represents a dual benefit to halophytes as it increases tolerance to environmental stress and enhances their potential for phytoremediation applications.
Microbe-assisted phytoremediation of hydrocarbons relies on the interaction of plants with hydrocarbon-degrading microbes. Metagenome analyses of the rhizosphere microbiome of Ria de Aveiro halophytes revealed that plant colonization is a major driver of the composition of bacterial communities in salt-marshes chronically exposed to hydrocarbons. Also, an enrichment in homologue genes related with hydrocarbon degradation, in relation to bulk sediment, was observed. In microcosm cultivation experiments, the inoculation of plants with a hydrocarbon-degrading isolate was accompanied by an improvement in plant photosynthetic parameters.
Salinization is one of the main causes of soil deterioration and represents a threat to agriculture in the near-future. The rhizosphere of halophytes has been regarded as a representative model for the understanding of the contribution of plant-bacteria interactions to the adaptative responses of plants to elevated salinity and has also been explored as a seed-bank for halotolerant PGPR. A collection of isolates from the rhizosphere of *Salicornia ramosissima* from Ria de Aveiro has been characterized in terms of plant-growth promoting and biocontrol traits. A halotolerant strain of *Bacillus aryabhattai* SP1016-20, expressing biocontrol effect against the phytopathogenic fungus *Alternaria*, was tested as inoculant on *S. ramosissima* seeds causing a significant attenuation of the negative effect of salinity on the germination efficiency.
The results of the research on the relation between halophytes and rhizosphere bacteria in Ria de Aveiro sustain the perspectives of rhizoengineering of salt-marsh plants as a strategy for widening the application and increasing the value of these plants either as phytoremediation agents or as alternative crops for saline agriculture.

I1. Environmental Microbiology and Biotechnology

P44. Microorganisms from sun-exposed stone wall microorganisms as a new source for the production of compatible solutes

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The survival of microorganisms in adverse natural environments implies their ability to adapt. The accumulation of compatible solutes is one of the most used adaptation mechanisms, either by the uptake of these compounds from the medium or by de novo synthesis. In this respect, consideration of new, unexplored or uncommon niches may be a source of new or different enzymes. We studied the microbial and functional diversity of a stone wall (SESW) directly exposed to the sunlight and the ocean water. In this microbial community, we searched for microorganisms that accumulate glycerate-related osmolytes or trehalose, among many others. According to the 16S rRNA gene metabarcoding, the microbial composition of SESW was dominated by the *Xanthomonadaceae* family (37%), which has not yet been linked to the production of osmolytes. However, the less represented families in this sample, namely *Halomonadaceae* (5%), *Rhodobacteraceae* (5%) and *Rubrobacteraceae* (7%), have been described to use compatible solutes as an osmoadaptation mechanism. Analysis of the functional metagenome of the SESW sample indicated the presence of enzymes involved in the synthesis of the compatible solutes glucosylglycerate and trehalose. Interestingly, the two hits for the glucosylglycerate synthesis were both found in actinobacterial-type bacteria, belonging to the genus *Rubrobacter*, already known for trehalose and mannosylglycerate accumulation, and the genus *Euzebya*, which has not yet been described for osmolytes synthesis. Moreover, annotation results show evidence of ABC transporters, suggesting the uptake of compatible solutes from the environment into the cell. Metagenome functional analysis allowed the reconstruction of the different osmolyte–dependent adaptation mechanisms in the SESW microbiome. It has been clear that there is a promising use for osmolytes in the biotechnology field and it will undoubtedly be important to identify enzymes from unusual habitats.

I1. Environmental Microbiology and Biotechnology

P45. Prevalence of antibiotic resistance in Ria de Aveiro

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Aquatic environments are relevant for the spread of antibiotic resistance, acting as reservoirs of antibiotic-resistant bacteria and resistance genes. In particular, estuaries are ecosystems that support high biodiversity and are often receptacles for contamination derived from anthropogenic sources. Several human activities take place in estuaries such as fishing and recreational activities. However, the prevalence of antibiotic resistance in estuaries has been poorly characterized.

We analyzed the prevalence of antibiotic resistance in Ria de Aveiro, an estuary of high economic and ecological importance. Resistance was assessed in several bacterial groups, including human and animals pathogens. Seasonal and spatial variations in antibiotic resistance prevalence were analyzed and correlated with environmental parameters.

Water samples were taken from 26 sites in three campaigns (Autumn, Spring and Summer). Water was was filtered, and the membranes placed in agar media specific for quantification of *Aeromonas* (GSP and GSP+cefotaxime), *Vibrio* (TCBS and TCBS+tetracycline), *Enterobacteriaceae* (m-FC and m- FC+cefotaxime, m-FC+ciprofloxacin or m-FC+imipenem) and *Enterococcus* (Slanetz-Bartley and Slanetz-Bartley+vancomycin). After incubation, colony forming units were counted in triplicate. Temperature, conductivity, salinity, dissolved oxygen, pH values were recorded for each sample.

High values of antibiotic resistance were recorded throughout the estuary, mainly for *Aeromonas* resistant to cefotaxime (from 1.6% to 100%). The highest prevalence values of resistant *Enterobacteriaceae* were recorded in urban areas, with maximum values of 55.8% for cefotaxime, 29.4% for ciprofloxacin and 6.1% for imipenem. As for *Vibrio* resistant to tetracycline, higher prevalence values were recorded in autumn (55.2%), followed by those recorded in Spring (11.8%) and Summer (6.3%). A seasonal variation was also registered for *Enterococcus* resistant to vancomycin, for which average prevalence values were 66.4% in Summer, 33.7 % in Spring and 4.2% in Autumn.

The lowest prevalence of tetracycline-resistant *Vibrio* (0.5%) was recorded at locations with the lowest salinity.

This study provides for the first time an image of the distribution of antibiotic resistance along Ria de Aveiro. High levels of resistance to antibiotics of critical importance to human and/or animal health were determined. We must highlight the occurrence of *Enterobacteriaceae* resistant to carbapenems, a group of antibiotics used as last resort for the treatment of severe infections.

I1. Environmental Microbiology and Biotechnology

P46. Analysis of the functional metagenome of two Portuguese hot springs identify the potential for compatible solutes synthesis

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Hot spring waters provide important ecosystems for organisms since they harbor unique bacteria and archaea due to water particular chemical properties and high temperature. Survival of microorganisms in these natural environments implies their ability to adapt to such adverse conditions. Accumulation of compatible solutes is one of the possible adaption mechanisms. Thermophiles are expected in hot springs, and they likely to accumulate glucosylglycerate (GG), mannosylglycerate (MG) or mannosylglucosylglycerate (MGG) osmolytes in response to high salt concentrations or temperature. Therefore, finding enzymes in a wide range of environmental conditions, such as high temperatures, has a great interest in different biotechnological applications. Here we studied the microbial and functional diversity of two Portuguese hot spring waters at Chaves and S. Pedro do Sul, focusing on the potential for the synthesis of compatible solutes. According to the 16S rRNA gene metabarcoding, the microbial composition of the water samples from Chaves and S. Pedro do Sul was similar, but while members of the *Nitrospirae* (62%) predominated in the water from Chaves, representatives of *Nitrospirae* (37 %) and *Aquificacea* (43%) dominated at S. Pedro do Sul water sample. Analysis of the functional metagenomes of the two hot spring samples indicated the presence of enzymes involved in the synthesis of glucosylglycerate and mannosylglucosylglycerate in Chaves but not in S. Pedro do Sul. The enzymes were similar to the enzymes of members of *Thermoanaerobaculum*, and *Chloroflexi*, *Nitrospirae* and *Deltaproteobacteria* phyla. The production of glycerate-based osmolytes has not yet been described for these organisms. The absence of the identification of genes for the synthesis of glycerate-related osmolytes in S. Pedro do Sul is unexpected, even though the two samples had similar temperatures, 65 ºC for Chaves and 63 ºC for S. Pedro do Sul and had similar phyletic composition. The presence of different species in the two samples may explain the differences observed. The physicochemical properties of the two hot springs are also different and may select for different microorganisms. Additional studies are required to understand the differences observed. This work was supported by HORIZON 2020, under the project Metafluidics, ref. 685474.

I1. Environmental Microbiology and Biotechnology

P47. Production of fatty acids by Oleaginous yeasts: overview of fermentative production and lipidic profile

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The possibility of waste utilization, both from industry and other human activities, has been increasing importance over the last decades. In this context, the VOLATILE project aims to embrace a circular economy by converting the heterogeneous urban biowaste streams into a volatile fatty acid (VFA) platform, which can be used as carbon source for different fermentation processes. In this work we tested the ability of selected yeast isolates to grow on different volatile fatty acids, as well as on a pre-treated dark fermentation effluent, and accumulate lipids in the form of single cell oil (SCO). For that we used four yeast strains and evaluated their lipid accumulation and growth in media supplemented with different volatile fatty acids, namely acetic, propionic and butyric acids. Globally, strains accumulated more lipids when more than one VFAs was employed as carbon source. When the effluent obtained from the anaerobic digestion of organic wastes was the fermentation media, all the used yeast strains were able to grow and produce valuable fatty acids, although lipid outputs of strains V139 and V213 were low. On the contrary, the strain of *Apiotrichum brassicae* consumed the seven volatile fatty acids available in the effluent and produced approximately 43% lipids by dry cell weight, reaching a lipid yield of 0.51 (g/g C). The yeast isolate of *Pichia kudriavzevii* metabolised 99% of the VFAs and had the highest lipid content (46.7% w/w). Regarding fatty acid composition, 57% were unsaturated, with emphasis in oleic acid production. Omega-3 and omega-6 fatty acids represented about 8% of the total fatty acids produced. Altogether, results showed that these two yeast isolates are potential candidates for SCO production through the use of an inexpensive carbon source, with economic and environmental benefits.

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I1. Environmental Microbiology and Biotechnology

P48. Surface wiping test to study biocide and cinnamaldehyde combination to improve surface disinfection efficiency

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Surface disinfection is crucial to improve the prevention and control of microbial contaminations. Nonetheless, the misuse of the disinfectants in routine practices has led to an increased concern on the selective pressure that the microorganisms are exposed to and consequently on their impact on bacterial resistance and cross-resistance. The aim of this work was to develop a formulation to be used for surface disinfection that is based on the combination of a natural product (cinnamaldehyde) and a quaternary ammonium compound (cetyltrimethylammonium bromide - CTAB) widely used in disinfecting formulations. The wiping methodology was developed according to the Wiperator test (E2967 − 15) and the European Standard EN 16615:2015. The development of a formulation tested for wiping was based on the screening of several phytochemicals by the checkerboard method where the best fractional inhibitory concentration index (FICI) and removal of sessile *Escherichia coli* and *Staphylococcus aureus* was obtained. Cinnamaldehyde-CTAB combination had a FICI of 0.9 for *S. aureus* and was synergic in the removal of sessile *E. coli*. In addition, after some steps of concentration optimization, the wiping of a contaminated surface, with 7.10 ± 0.06 log (total(CFU)) of *S. aureus* and 6.20 ± 0.21 log (total(CFU)) of *E. coli*, reductions of 4.27 ± 0.22 log (total (CFU)) and 4.35 ± 0.22 log (total(CFU)) were achieved when the wipe was impregnated with the formulation. Furthermore, the formulation prevented bacterial transfer to clean surfaces. The overall results highlight the potential of a combinatorial approach using old disinfectants with selected phytochemicals for fine-tunning disinfection formulations. This approach can also reduce the concentration of these old disinfectants and therefore reduce the potential environmental and public health burdens from their use.

P49. Exopolysaccharides production by aerobic granular sludge upon exposure to dual anthropogenic stresses

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Aerobic granular sludge (AGS) is a promising technology for the treatment of urban and industrial wastewater and its implementation at full-scale is growing worldwide. Extracellular polymeric substances (EPS) produced by the AGS microorganisms is crucial not only for granules formation and stability but also for cells protection against harsh conditions in the living environment which often occur in industrial wastewaters. Some industrial sectors, such as agro-food, petrochemical, textile, chemical manufacturing among others, use inorganic and organic salts in the process chain, producing streams difficult to manage due to their complexity.
In this study, the combined effect of salinity and different pharmaceuticals on the EPS production by AGS was evaluated in short-term (24 h) batch assays. Synthetic wastewater containing different salt concentrations and a pharmaceutical (diclofenac or carbamazepine at 8 mg L^{-1}) was inoculated with AGS. EPS production was assessed, and its biochemical characterization was performed. The microbial community was followed through 16S rRNA gene massive parallel sequencing. The pharmaceuticals removal was assessed revealing that the increase in salinity did not benefit the pharmaceuticals removal. Differences were found in the EPS production and composition upon exposure to different salt concentrations and pharmaceutical compounds. The impact of the stressful situations on the microbial community is under evaluation. Characterizing EPS compositions and microbial communities can help to elucidate the EPS´s function in the AGS process.

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I1. Environmental Microbiology and Biotechnology

P50. Genomic insights into the evolution of pathogenicity in a new walnut-associated *Xanthomonas* species

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Xanthomonas arboricola pv. juglandis (Xaj) is the causal agent of important walnut (*Juglans regia L.*) diseases, causing severe yield losses worldwide. The rise of novel plant-pathogenic strains threatens crops and trees, urging a deeper understanding of the evolutionary driving forces shaping adaptation to pathogenicity. Particularly for *Xanthomonas* genus, recombination and horizontal gene transfer (HGT) continuously drive the evolution of pathogenic strains. Recently, we observed the frequent occurrence of pathogenic and non-pathogenic xanthomonad lineages co-colonizing the same walnut host, suggesting that a sympatric lifestyle may contribute to genetic trade-offs related to pathogenicity in *Xanthomonas*. Five walnut-associated *Xanthomonas*, isolated from asymptomatic buds and symptomatic leaves of a single walnut host tree (strains CPBF367, CPBF424, CPBF426, CPBF427, and CPBF1521), were fully sequenced. Comparative genomics and average nucleotide identity (ANI) allowed identifying CPBF427 and CPBF1521 as Xaj, while CPBF367, CPBF424 and CPBF426 were not allocated to any known species of the *Xanthomonas* genus, likely belonging to a new *Xanthomonas* species. Pathogenicity assays in walnut revealed a pathogenic phenotype for CPBF424, while CPBF367 showed a non-pathogenic phenotype. Ongoing genomics studies uncovered differences regarding the repertoire of T3SS and T3E genes. While CPBF427 and CPBF1521 show a complete T3SS and a set of T3Es characteristic of typical pathogenic Xaj strains, the non-pathogenic CPBF367 and CPBF426 are deficient for T3SS and for most of T3Es. In contrast, the pathogenic strain CPBF424 possesses a complete T3SS and a T3E pattern (HpaA, HrpW, XopA, XopF1, XopZ2, XopM), that regardless being reduced in comparison to typical Xaj strains, did not impair disease in walnut. Blast analysis of T3E and T3SS genes present in CPBF424 revealed >76% identity values against the genomes of typical Xaj strains. Furthermore, synteny between the CPBF 424' T3SS cluster and its flanking regions with CPBF427 and other pathogenic Xaj suggested a HGT-mediated acquisition from Xaj strains. Interestingly T3SS operon seems to be highly degenerated for the non-pathogenic strains (CPBF367 and CPBF426) of this new *Xanthomonas* species, raising the hypothesis of an adaptation to an epiphytic lifestyle.

I1. Environmental Microbiology and Biotechnology

P51. Eco-friendly and integrated platform for the degradation of textile dyes using laccase and ionic-liquid-based surfactants

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The intensive use of water containing dyes by the textile industry, and consequently the contamination of soils and water, represents serious environmental concerns. Amongst the several processes applied in the treatment of textile effluents, biological-based processes, if designed to be cost-effective and eco-friendly, are promising alternatives to decolorize textile effluents. In this work we investigate and propose the novel use of ionic liquids (ILs) with surfactant characteristics to improve the degradation of the largely used and highly hydrophobic textile dye indigo carmine by laccase. An initial screening on the activity of laccase in aqueous solutions of twelve surfactant-based ILs from three different families, namely tetraalkylammonium- and imidazolium-based cationic surfactants and cholinium-based anionic surfactants, at different concentrations, was carried out. A high activity of laccase was observed with decyltrimethylammonium bromide, [N10111]Br, and 1-decyl-3- methylimidazolium chloride, [C10mim]Cl, at 75 mM (above the critical micellar concentration of each IL). These ILs were then investigated in aqueous solutions to simultaneously encapsulate laccase and IC for the in situ enzymatic biodegradation of the dye. The use of ILs remarkably increases the degradation rate of the dye and decolorization efficiency; a degradation efficiency of IC of 82% is attained in 0.5 h using aqueous solutions of [N10111]Br, whereas without IL only 6% of IC is degraded. Furthermore, at the end of 24 h, 93% of the dye decolorization was achieved in the presence of 75 mM of [N10111]Br. The overall gathered results show that it is possible to significantly improve the degradation of hydrophobic dyes by enzymes using appropriate surfactant-based ILs, while foreseeing the use of the treated water by the same textile industries in new dyeing steps and thus contributing to a substantial decrease of the economic input and environmental footprint of these industries.

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I1. Environmental Microbiology and Biotechnology

P52. Deep biosphere: aerobic microbial populations as source of biotechnological solutions

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Microorganisms constitute a major part of biosphere on Earth and a considerable portion of them is present in the deep biosphere: a set of habitats physically located below the surface of continents and/or the bottom of the oceans. Microorganisms living in these environments can be a leverage to better understand strategies of survival/growth in extreme conditions; however, only a small percentage can be recovered and cultured under laboratory conditions. Therefore, to get the closest idea of the aerobic microbial diversity, it is necessary to develop strategies to recover as many isolates as possible.
Alfaguara spring is a deep aquifer hyperalkaline spring, associated with serpentinization reactions, located at Peridotites of Ronda, South of Spain. To access the diversity of the aerobic heterotrophic bacteria present in this habitat, an extensive culture-dependent isolation process was conducted. Isolates were grouped by RAPD profiling, representative isolate(s) of each group were summarily characterized, and its phylogenetic placement was determined by 16S rRNA gene sequencing. The majority of the isolates was phylogenetically related to phylum *Firmicutes*, namely with genus *Bacillus*. Others were related to phylum *Actinobacteria*, specifically with species belonging to genera *Microbacterium*, *Micrococcus* and *Kocuria*.
The biotechnological potential of the isolates was accessed by screening their ability to degrade chitin, carboxymethyl cellulose, xylan and starch. All isolates were successful in the degradation of at least one of the substrates and some of them showed such ability at pH values superior to 9. The best degradation activity results were obtained for xylan, either by activity signal or number of positive isolates. None of the isolates degraded chitin. These results showed that aerobic heterotrophic communities can be considered a good source of interesting enzymes since several sets of microorganisms showed degradation activity for the three substrates used and this activity was in the alkaline spectrum. Strategies such as construction of microbial consortia may be used in the future to promote a higher rate of degradation activity for specific usage.

I1. Environmental Microbiology and Biotechnology

P53. Green roofs as a biotechnological solution to increase water retention in urban areas

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Urban world population has grown rapidly over the last decades. 74% of the European population live in urban areas, and that is expected to increase to over 80% by 2050. This rapid urbanization brings several environmental problems, aggravated due to climate change. Conservation and enhancement of green infrastructures in urban areas is imperative for sustainable urban development.
Green Roofs (GR), a multilayer technological construction that uses vegetation on top of buildings or structural slabs, are becoming a strong choice to promote urban greenery, using an area that accounts for ca. 50% of the impermeable urban surface area. Besides energy benefits to the building structure, water retention/runoff delay to the stormwater drainage systems is another ecosystem service provided by GR.
In the present study, a GR pilot system using aromatic plants and a commercial substrate has been studied regarding its capacity of water retention. Based on a previous water runoff model, rainwater retention by the system has been calculated to be ca. 30%. In previous studies, aromatic plants demonstrated that could be successfully used on GR in the Mediterranean region. Strategies to increase plant growth and minimize the adverse effects of the harsh environment on plant growth on the top of a building are important. Pot experiments using *Satureja montana* comprised inoculation of a selected mixture of plant growth promoting bacteria. Differences in the growth of plants in control and inoculated pots were followed to assess the potential of bacterial endophytes as bioinoculants in green roofs vegetation is under analysis.

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I1. Environmental Microbiology and Biotechnology

P54. The microbial diversity from deep-sea sediments of the Southern Gulf of Mexico

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Deep ocean sediments are the largest ecosystems on Earth covering approximately 65% of Earth's surface. In the Gulf of Mexico (GoM) the deep-water area can reach depths up to 4,000 m and comprises 20% of the basin. Although, there are some studies describing biological diversity in the GoM, very few of them involve microbial communities from the sediments, and even less from the deepest areas. To establish a baseline of the diversity of microorganisms inhabiting the Southern GoM, and to shed some light into its unknown microbial communities and their function, sediment samples were collected over different years by several oceanographic campaigns since 2015, as part of a sampling effort doing by the CIGoM consortium. Samples were taken at different depths, ranging from 550 to more than 3500 m. DNA was extracted from different sections of the sediment column, and the 16S ribosomal RNA gene was amplified and sequenced on our MiSeq (Illumina) platform and data analyzed with a set of bioinformatic programs including QIIME.

Our results show an unusual archaeal abundance not reported to date, reaching up to 40% of the total prokaryotes community, in particular, those from the deepest locations. This abundance seems to be correlated with depth since it is increased from shallow to deeper zone, but it is stable at the abyssal plain (1500 to 3500 m). Besides, it was observed a strong shift on the microbial assemblages along the sediment core. The archaeal diversity was higher and distinct than those from the upper part of the core and, the bacterial abundances changed. *Lokiarchaea* replaced *Thaumarchaea* and represented 20% of the total. And in the bacterial group, *Planctomycetes* and *Chloroflexi* increased their abundances while *Acidobacteria* almost disappeared from the bottom of the core (30 cm).

I1. Environmental Microbiology and Biotechnology

P55. *Porcellio dilatatus* guts as a potential hot-spot of plant-cellulosic-biomass degrading enzymes

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The plant biomass is the most abundant renewable biomass source and is essentially composed by lignin, hemicellulose and cellulose. Over the years, lignin has been considered an important source of bioenergy, however its recalcitrant structure is the central barrier for the development of environmental friendly alternatives of energy. Terrestrial isopods commonly known as woodlice are ubiquitous in soil and effective herbivorous scavengers. Their guts can be considered as natural enrichment environments for plant cellulosic biomass (PCB) degrading bacteria. In order to explore the biotechnological potential of *Porcellio dilatatus* guts microbiome as a source of PCB- degrading enzymes, we characterized the structural diversity of *Porcellio dilatatus* gut microbiome and predicted their functional diversity.
Porcellio dilatatus specimens were collected from four geographic sites of Portugal. Gut isopods total DNA was extracted and sequenced in an Illumina MiSeq V2 platform. The high-quality sequences were aligned, clustered into OTUs with a cut-off of 97% similarity. All high-quality sequences were phylogenetically identified through ARB-Silva taxonomic database. The putative metabolic functions of *Porcellio dilatatus* guts microbiome were predicted using the PICRUSt2 software and results analysed in KEGG database.
The good coverage and the tendency to the saturation point of the rarefaction curves demonstrated that bacterial diversity present in *Porcellio* gut samples were determined successfully. From 25 bacterial classified phyla, the most abundant were *Proteobacteria*, *Actinobacteria, Cyanobacteria*, *Tenericutes*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Patescibacteria* and *Chloroflexi*. The overall functional structure of the *Porcellio dilatatus* gut communities showed that the most abundant KEEG pathways were related to carbohydrate metabolism, amino acid metabolism, energy metabolism and metabolism of cofactors and vitamins. In KEGG category-4 analysis it was possible to predict the presence of several genes encoding enzymes of interest like glucosidases, xylanases, laccases, lignin peroxidases, among others.
In conclusion, these results showed the presence of well adapted bacterial communities, which are responsible for the host nutrient metabolism, and by so a potential source of PCB-degrading enzymes not to neglect.

P56. Performance of microalgae-bacteria granular sludge for nutrient removal of freshwater aquaculture wastewater

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Aerobic granular sludge (AGS) has been receiving increasing attention as one of the promising biotechnologies in wastewater treatment. Compared to conventional processes, AGS offers as main advantages its excellent nutrient removal capability, good settling properties, and the simplicity of operation [1]. At the same time, microalgae research in wastewaters has been blooming in the past few decades, largely owing to their capacity concerning the removal of nitrogen, phosphorus, and other elements from these effluents [2]. The combination of microalgae and bacteria within the same structure could improve wastewater treatment as the resulting metabolic diversity could make the process more efficient [3]. In a consortium, microalgae may provide oxygen for the aerobic bacteria which could biodegrade pollutants from wastewater and release carbon dioxide to be used by the microalgae in photosynthesis [4]. This study aims to investigate the effectiveness of microalgae- bacteria granular sludge in removing nutrients from aquaculture wastewater. A photobioreactor was operated in sequencing batch reactor (SBR) mode, which comprised the following successive phases: anaerobic feeding, aeration, settling and decanting. AGS from a full scale wastewater treatment plant was used as the seed for bacterial granules. Several microalgae were isolated from sludge collected at a freshwater aquaculture facility. A suspended microalgae consortia was added to the AGS granules during the aeration phase. Reactor performance concerning nitrogen and phosphorus removal was assessed throughout its operation. At startup, synthetic domestic wastewater was used as the feed, and its composition was gradually lowered to mimic the aquaculture facility's wastewater. Cycle times were adjusted to optimize nutrients removal. The aggregation of microalgae to bacterial granules was evaluated in the reactor throughout reactor operation by optical microscopy and BG-11 agar plating. Most of the ammonium present in the feeding wastewater was removed and converted into nitrate, without nitrite accumulation. Phosphate removal was instable throughout operation. This study is ongoing to better understand how the interaction of both microorganisms affects the removal processes.

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I1. Environmental Microbiology and Biotechnology

P57. Do *Klebsiella pneumoniae* environmental strains maintain clinically relevant genomic and phenotypic traits?

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Extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* are well-known pathogens, increasingly reported in the environment. This fact represents a human health concern since third-generation cephalosporins are frontline antibiotics used to treat infections caused by this species. A major question is whether environmental ESBL-producing *K. pneumoniae* can infect humans. To address this question, this study compared clinical and environmental *K. pneumoniae* strains regarding genetic and phenotypic traits and assessed their potential infectious capacity. Therefore, 59 isolates (25 environmental and 34 clinical) of cefotaxime-resistant *K. pneumoniae* were characterized based on antibiotic resistance phenotype, plasmids content, and horizontal gene transfer capacity. A subset of these isolates was tested for infection capacity in *Galleria mellonella* (23 environmental and 24 clinical) and for whole genome sequencing (7 environmental and 11 clinical). Most environmental (80%, 20/25) and clinical isolates (94%, 32/34) were multidrug resistant. Environmental isolates presented mostly 2 plasmids (48%, 12/25) while clinical isolates presented 1 or 2 plasmids (41%, 14/34 each), however bacterial conjugation was more frequent among clinical (76%, 26/34) than environmental isolates (40%, 10/25). *G. mellonella* health index was lower after infection with clinical (most of the infected isolates scored 1) than with environmental isolates (most of the infected isolates scored 6). A screening of the whole genome sequences, made in parallel with data available in public databases (in total 73 environmental and 78 clinical), targeting 6 groups of genes related to antibiotic and metal resistance, virulence, efflux systems, oxidative stress and quorum sensing, evidenced the existence of 1383 gene variants. A total of 438 genes out of the 1383 were common to all isolates, while 460 and 485 genes were found exclusively in environmental and in clinical isolates, respectively. A screening of the whole genome-deduced amino acid sequences demonstrated a common putative proteome, related with all functional categories, in environmental and clinical isolates (n=2715), although the number of exclusive amino acid sequences was higher for clinical isolates (n=577 in clinical vs. n=205 in environmental). These results suggest the adaptation of *K. pneumoniae* to environmental or clinical niches, although highlight that putative clinically relevant traits may persist in bacteria thriving in the environment.

I1. Environmental Microbiology and Biotechnology

P58. Aerobic granular sludge has EPS-producing bacteria able to tolerate salt

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The aerobic granular sludge (AGS) process is a promising biotechnology which relies on the formation of compact biomass granules. Granulation occurs due to the overproduction of extracellular polymeric substances (EPS) by some microbes in response to stress conditions. EPS protect bacteria from the effect of toxic or inhibiting compounds present in the wastewater, such as salts. One of the current challenges is to use the AGS process to treat high salinity wastewater, commonly produced by agro-food and chemical industries. The main objective of this study was to screen for EPS-producing bacteria bacteria in an AGS reactor treating synthetic saline wastewater contaminated with a toxic compound. Several bacterial isolates were obtained from the reactor biomass. Genomic DNA was extracted and isolates (30) were grouped according to species similarity, based on RAPD profiles. Isolates displaying unique profiles (15) were subsequently identified by 16S rRNA gene sequencing analysis. Bacteria highly related to *Pseudomonas*, *Aeromonas*, *Stenotrophomonas*, *Flavobacterium* and *Pseudoxanthomonas* were obtained. Isolates SG4 (*Stenotrophomonas*) and FG10 (*Flavobacterium*) belong to bacterial genera associated to EPS production in granules. These were selected for growth and biofilm formation assays with increasing NaCl concentrations (0 to 35 g L^{-1}). Both isolates were able to grow in the presence of 35 g NaCl L^{-1}, despite at a lower growth rate. Although salt increase affected biofilm production, SG4 was the best biofilm producer. EPS production by SG4 in the presence of 10 and 20 g L^{-1} of NaCl was compared. EPS was extracted and the content in proteins, humic acids and carbohydrates was quantified. SG4 was able to produce more EPS in the presence of 10 g L^{-1} (123 mg g^{-1} VSS) compared to 20 g L^{-1} of NaCl (77.6 mg g^{-1} VSS).
EPS-producing bacteria with ability to tolerate high salinity were retrieved from an AGS process treating synthetic wastewater. Further research is required to gain more knowledge on these bacteria and their importance for the robustness of a process treating saline wastewater.

I1. Environmental Microbiology and Biotechnology

P59. Bacterial diversity shifts in AGS reactor treating food industry wastewater

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Aerobic granular sludge (AGS) is a promising technology for treating industrial wastewater, possessing higher biomass retention and tolerance to toxic substrates than conventional activated sludge systems. AGS presents a diverse microbial community responsible for the simultaneous removal of carbon and nutrients. These communities are protected by extracellular polymeric substances (EPS) that allow for the compact structure of the granules. As a result, bacteria present in the aerobic granules are more resistant to variable wastewater composition, as commonly produced in food industry. The main objective of this work is to study the microbial community dynamics of an AGS reactor treating wastewater from a fish canning plant. The reactor was monitored during 220 days, divided into eight operational phases. COD, NH_4^+ and PO_4^{3-} removal were assessed and biomass samples were collected throughout time for microbiome profiling.
The reactor presented good COD, PO_4^{3-} and NH_4^+ removal during phases I, II and III, but decreased performance during phase IV, when a higher organic load was applied. The removal processes recovered after phase IV until the end of operation. *Proteobacteria* were dominant in the inoculum (relative abundance of 64.8%) and dominated almost all reactor phases. *Bacteroidetes* were second dominant in the inoculum (17.5%) as well in most reactor phases, being present with higher relative abundance (55.5%) than *Proteobacteria* (38.4%) during phase IV. Within *Proteobacteria*, *Gammaproteobacteria* were initially more abundant but *Betaproteobacteria* predominated after phase
IV. For *Bacteroidetes*, the community dynamics has also changed from phase IV onwards, with *Flavobacteriia* losing its high relative abundance to *Saprospiria* and *Cytophagia*. Several bacterial genera were detected throughout reactor operation, such as *Phenylobacterium* and *Flavobacterium*, while other were detected with higher abundance before (*Methylocaldum* and *Plasticicumulans*) or after phase IV (*Thauera* and *Paracoccus*). The relationship between bacterial community shifts and process performance was assessed. This study increases our knowledge on AGS technology application in real wastewater treatment.

I1. Environmental Microbiology and Biotechnology

P60. Evaluation and modeling of bacterial mobility and dissemination in terrestrial environments and role in the facilitated leaching of metal elements

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The rapid mobilization of inorganic pollutants by the colloidal fraction of soils, and in particular biotic colloids (bacteria, algae, fungi, viruses, etc.), is now identified as an important secondary transport process that can lead, under specific conditions, to an accelerated and potentially dominant transfer of pollutants to aquifers. However, the involved mechanisms remain poorly understood. In order to better understand the role of bacterial cells in metal leaching in porous media, we conducted a coupled study under static and dynamic conditions. First, we evaluated the biosorption of two important heavy metals, zinc (Zn) and cadmium (Cd) on active or inactive Gram negative bacteria (*Escherichia coli* and *Cupriavidus metallidurans* CH34) by characterizing the subcellular distribution of metals within the two bacteria at increasing metal loads. The quantification of Zn and Cd in the extracellular, membrane and cytoplasmic compartments of cells has shown that metals are unevenly distributed between the three cell compartments and also between the two types of bacteria. The internalization of metals appears to be the dominant accumulation process of metals (high cytoplasmic content). These results suggest that adsorption on the cell surface is only a first step in the management of metals by bacteria and that bacteria can internalize significant amounts of heavy metals, and can thus become important vectors. The thermodynamic reactivity constants determined in this way were used to model the leaching curves of metals in columns of a natural sand. These experiments on the transport of bacterial cells, metals or mixtures of bacteria and/or metals have shown that bacteria promote and accelerate the transport of Cd and Zn in porous media. It has thus been demonstrated that this transport process dominates the aqueous transport of metals and has been correctly modelled using a coupled transport and geochemical modelling approach. Overall, these results show that, under specific conditions, bacterial cells can increase the mobility of metal elements in soils, which can be harmful to the quality of the environment, but also interesting in terms of the prospects for the recovery of precious metals or the remediation of environments polluted with heavy metals.

I1. Environmental Microbiology and Biotechnology

P61. Structure of microbial communities on anaerobic digestion of olive mill wastewater complemented with piggery effluent

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The swine and olive oil industries are looking for practical and sustainable solutions to overcome existing environmental problems caused by their highly polluting discharges. Our previous studies have shown that piggery effluent (PE) is a highly energy-efficient substrate to be used directly by anaerobic digestion (AD) process [1]. Taking advantage of PE composition [pH 7, high total and ammonia nitrogen concentrations (5 and 3 g/L, respectively), and high total content of volatile fatty acids (6 g/L)], this was used to enable anaerobic digestion of OMW, a very recalcitrant acidic effluent, containing a high concentration of total phenols [2]. The present work assessed the microbiome from samples of PE, inoculum and tested conditions that provided the best biogas/methane production, by microbial profiling analysis.
Genomic DNA was extracted according Zhou et al. [3] and adapted by Eusébio et al. [4]. Samples were sent to StabVida (Caparica, Portugal) for Next Generation Sequencing (NGS). The microbiome was identified by PCR amplification of the V3 and V4 regions of bacterial and archaeal 16S rRNA gene using universal primers and sequenced on the Illumina NextSeq 300 platform.
For Bacteria domain, *Proteobacteria* (54.6%) and *Chloroflexi* (18.4%) were the dominant phyla present in the inoculum and maintained their predominance in all inoculated samples during AD. The microbial populations *Pseudomonadales* and *Anaerolineales*, belonging to those phyla, remained predominant in all samples that were inoculated. *Firmicutes* (65.1%) was the dominant phylum found in PE, maintaining its predominance in all essays complemented with PE. Archaeal populations were detected in inoculum and PE, mainly assigned to *Methanosaeta* (99.7%) and *Methanobrevibacter* genera (72.1%), respectively. At the end of anaerobic digestion of OMW complemented with PE, the genus *Methanosarcina* was dominant in all samples. The predominance of *Methanosarcina* and *Methanosaeta* is consistent with the higher biogas production obtained in the characterized samples.

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I1. Environmental Microbiology and Biotechnology

P62. Energetic valorisation of *Chrypthecodinium cohnii* lipid production wastes by anaerobic digestion

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The heterotrophic marine microalga *Crypthecodinium cohnii* produces high amounts of lipids (up to 70% w/w) with a high proportion docosahexaenoic acid (DHA), a high value w-3 fatty acid with many applications in pharmaceutical and food industry [1]. During the lipid production process from the microalgae, several wastes and effluents are generated, which may be energetically valorised by anaerobic digestion for biogas production.
C. cohnii biomass was produced in a 7L fed-batch bioreactor. After the microalgae cultivation, the culture medium was centrifuged and the biomass was taken for further intracellular lipids extraction. In this work, the deoiled biomass, and the remained supernatant obtained from *C. cohnii* cultivation, were used as anaerobic digestion substrates for biogas production.
Anaerobic digestion assays were carried out in batch mode, by using anaerobic glass reactors (about 70 mL total volume), in triplicate and under mesophilic temperature conditions (37±1 ºC). A substrate to inoculum ratio of 70% (v/v) was applied.
Total and volatile solids, chemical oxygen demand, total nitrogen concentrations were determined according to Standard Methods [2]. Biogas production was monitored daily and gas composition was analysed by gas chromatographic techniques according to ASTM Standard Method [3]. All gas volumes were adjusted to STP conditions (1 bar, 0 °C). Flow cytometry was used to monitor the microbial consortium cell viability during the anaerobic digestion, in order to understand the impact of *C. cohnii* lipid production residues on biogas production. As far as the authors know, such approach has never been reported before.

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P63. Microbiota profiling of sympatric intertidal marine sponges of the classes *Demospongiae* and *Calcarea* from the Portuguese coast

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Sponges (Phylum *Porifera*) are ancient invertebrate metazoans, with a fossil record dating back to 600 million years that host complex and diverse microbial communities such as archaea, cyanobacteria, heterotrophic bacteria, algae, fungi, and dinoflagellates. The sponge-associated microorganisms can contribute to nearly 40% of the total sponge biomass and may benefit the hosts with several functional roles. The effort to understand the sponge-associated microbial communities have been intensified by the potential pharmacological application of bioactive compounds isolated from sponges and/or associated microbes. Sponge-associated microbial diversity has been studied from wide oceans across the globe, mainly in subtidal regions, but the microbial communities from intertidal sponges remain mostly unexplored. In this study, sampling campaigns of distinct intertidal marine sponge species (n=18) belonging to the classes *Demospongiae* and *Calcarea*, and surrounding ambient water, were conducted at rocky beaches along the coast of Atlantic Ocean, Portugal. We performed microbiota profiling with 16S ribosomal RNA gene (V4 hypervariable region) utilizing the MiSeq sequencer. Raw reads of 16S rRNA gene amplicons (~460 bp) from a total of 78 samples representing the sponge species and seawater samples including the triplicates were processed using the QIIME2 (Quantitative Insights Into Microbial Ecology) pipeline. A significant difference (p- value=0.001) in microbial communities was observed between the sponges and surrounding water suggesting the possible microbial specificity among the different sponge species. Our study also highlighted the hidden microbial consortium associated with the co-occurring intertidal marine sponges belonging to different classes.

I1. Environmental Microbiology and Biotechnology

P64. In vitro effect of cell-cell signalling molecules on interkingdom biofilms between bacteria and filamentous fungi isolated from a DWDS

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Biofilms constitute one of the major microbial problems in drinking water distribution systems (DWDS) that most contribute to the deterioration of water quality. Knowledge of drinking water biofilms has been mainly obtained from studies on bacterial biofilms even though, under natural conditions, they are usually viewed as complex communities where different organisms are present, including filamentous fungi. The ecology of a biofilm is a complex function of different factors, including the presence of microbial metabolites and molecules (cell-cell signalling communication) excreted by the microbial inhabitants of the biofilm. Quorum sensing (QS) controls different population density- dependent processes, including biofilm formation. Eukaryotes have, however, the ability to interfere with bacterial communication by producing molecular signals that interact with bacterial QS. The aim of this study was to assess the effect of patulin, a fungal secondary metabolite, and N-(3-Oxododecanoyl)- L-homoserine lactone (3-Oxo-C12-HSL), a QS signalling molecule, on interkingdom biofilm formation between bacteria and fungi isolated from a DWDS.
The filamentous fungi *Penicillium expansum* and the bacteria *Acinetobacter calcoaceticus* and *Methylobacterium oryzae* were used as model species. Biofilm formation was performed using 96-wells microtiter plates under agitation (150 rpm) for 24, 48 and 72 hours. Two concentrations (2.5 and 25
M) of patulin and 3-Oxo-C12-HSL were tested on single and interkingdom biofilms. Biofilms were analysed in terms of biomass (crystal violet staining), metabolic activity (resazurin reduction assay) and bacterial colony forming units (CFU's).
The results showed that patulin reduced *M. oryzae* biofilm growth at the tested concentrations, while for *A. calcoaceticus* a slight increase in mass was observed. 3-Oxo-C12-HSL increased *M. oryzae* bacterial biofilm mass after 48 hours. In inter-kingdom biofilms, 25 M of patulin allowed the fungi to develop higher biofilm mass while the presence of 3-Oxo-C12-HSL had low effect. Metabolic activity per biomass revealed no significant changes regarding *P. expansum* exposure to the molecules at the tested concentrations. Bacterial CFU's allowed a correlation with biofilm mass.

I1. Environmental Microbiology and Biotechnology

P65. Degradation and detoxification of reactive blue 19 by *Oudemansiella canarii* laccase- mediator system

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Large amounts of synthetic dyes are released into the environment causing a serious environmental impact on the ecosystem. The removal of dyes from effluents prior to discharge into the environment is therefore a matter of great importance. Reactive blue 19 (Remazol brilliant blue R, RBBR) is an anthraquinonic dye widely used in the textile industry. As a toxic and recalcitrant organo- pollutant, its degradation is highly desirable. Among the different oxidative enzymes secreted by ligninolytic fungi, laccases (EC 1.10.3.2) have been widely exploited in dye degradation. Laccases can only oxidize phenols and aromatic or aliphatic amines that have lower redox potential than the laccase (<0.4-0.8 V) and are small enough to enter the active center of the enzyme. However, with the aid of low molecular weight substrate molecules as mediators, oxidation by laccases can be expanded to larger molecules unable to fit into the enzymatic pocket or even to non-phenolic compounds that are not actual substrates of laccases. The aim of the present study was to compare the ability of *O. canarii* laccase to degrade the RBBR in the absence or presence of violuric acid as a mediator *O. canarii* laccase alone was efficient in decolourizing the RBBR and after 24 h of incubation, and the relationship between initial decolourizing rate and dye concentration followed the Michaelis-Menten kinetics, with a KM value of 114.90 ± 15.63 μM and a Vmax value of 1.318 ± 0.100 μmol/min. In the presence of violuric acid, same descolorization was obtained after 2 h of incubation. Fourier transform infrared spectroscopy (FTIR) and mass spectrometry allowed to conclude that the *O. canarii* laccase-violuric acid acts not only on the dye chromophore group, but also that it cleaves different covalent bonds, causing an effective fragmentation of the molecule. The action of the laccase-violuric acid caused a significant reduction in toxicity, as indicated by the Microtox test. In conclusion, *O. canarii* laccase- violuric acid could be useful in future biological strategies aiming at degrading anthraquinonic dyes.

I1. Environmental Microbiology and Biotechnology

P66. Immobilization of laccase from *Oudemansiella canarii*: kinetic parameters, stability and application in the degradation of malachite green

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Diverse enzymes including laccases, have been immobilized using the crosslinked enzyme aggregate methodology (CLEA), which consists of controlled precipitation of the enzymes followed by crosslinking by using bifunctional reagents such as glutaraldehyde. CLEA is considered a simple, fast and low cost procedure for enzyme immobilization. The objective of this work was to immobilize the *Oudemansiella canarii* laccase using this methodology and to compare the kinetic constants and stability of free and immobilized enzymes, as well as their capability in degrading malachite green. Briefly, ammonium sulfate was added to the enzyme extract for a final concentration of 55%. Subsequently, 25% glutaraldehyde was added as crosslinking agent. The mixture was kept at 4 °C for 24 h and centrifuged for 15 min at 5000 rpm. The CLEAs were washed for removing excess ammonium sulfate and glutaraldehyde and stored at 4 °C in 50 mM sodium acetate buffer, pH 5.0. Laccase immobilization was efficient with 95% immobilization yield and 68% activity retention. The kinetic constants, KM and Vmax, with ABTS as substrate, were 0.21 ± 0.01 mM and 2.04 ± 0.04 µmol/ml.min for the free enzyme and 0.14 ± 0.01 mM and 1.25 ± 0.03 µmol/ml.min for the immobilized enzyme, respectively. The immobilized enzyme was more thermostable and presented a better storage stability than its free form. The free laccase efficiently decolourized the malachite green dye, producing substantial alterations in its chemical structure, as observed by Fourier transform infrared spectroscopy and mass spectrometry. On the other hand, the malachite green dye was not well decolourized by the immobilized laccase. However, the addition of the mediator violuric acid to the reaction medium resulted in a great improvement of decolourization. In the presence of violuric acid both free and immobilized lacases were equally efficient. The immobilized laccase plus violuric acid were repeatedly used six times with retention of more than 60% of its activity. In conclusion, laccase from *O. canarii* was efficiently immobilized using CLEA and can be used in the decolourization of malachite green dye in association with the mediator violuric acid.

I1. Environmental Microbiology and Biotechnology

P67. Hexadecane toxicity towards pure cultures of methanogens

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Petroleum industry generates large volumes of hydrocarbon-containing wastewater, that may be treated and valorized by anaerobic conversion to methane. This process is performed by complex microbial communities and is only thermodynamically feasible at low hydrogen partial pressure, which is generally accomplished by the activity of hydrogenotrophic methanogens. However, alkanes, polyaromatic hydrocarbons and BTEX were shown to inhibit methanogenesis in mixed microbial cultures. This may be due to a direct inhibition of the methanogens, or may result from indirect inhibition, by disrupting the microbial relationships in the complex communities. To get more insights on this topic, the toxicity of aliphatic hydrocarbons towards pure cultures of hydrogenotrophic methanogens was assessed in this work. Aliphatic hydrocarbons represent the largest fraction of crude oil or petroleum-derived products, and hexadecane (HC) was chosen as model compound. Methane production from H_2/CO_2 (80:20%, 1.7x105 Pa) by *Methanobacterium formicicum* and *Methanospirillum hungatei* was measured in the presence of increasing HC concentrations (1, 5, 15 and 30 mM), and was compared with the controls without HC. For both methanogens, the methane production rate was significantly lower (p30 mM could be estimated for *M. formicicum* and *M. hungatei*, respectively. Therefore, *M. hungatei* is more tolerant to the presence of HC than *M. formicicum*, possibly due to the differences in cell wall structure and membrane lipid composition of the two species. Moreover, the relatively high IC50 values obtained are most likely related with the low HC solubility. Considering the typical range of hydrocarbon concentrations in wastewater from the petroleum industry, toxic effects from aliphatic hydrocarbons towards hydrogenotrophic methanogens will not be expected to occur during the anaerobic treatment of these type of wastewater.

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P68. Microaeration in anaerobic digestion systems: effect of low oxygen concentrations on methanogenic communities

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In anaerobic digestion (AD) systems, the coordinated activity of different microbial groups leads to the conversion of complex organic matter into methane. The presence of oxygen may cause negative effects on these processes, by inhibiting the growth and activity of obligate anaerobes, namely methanogens. Nevertheless, the exposure to small amounts of oxygen (microaeration) was shown to improve the AD processes, mainly by enhancing the activity of facultative bacteria. These bacteria promote the hydrolysis and fermentation of the organic macromolecules into various intermediates, using oxygen as final electron acceptor, thus increasing the availability of substrates for syntrophic bacteria and methanogens. The effect of low oxygen concentrations towards these two microbial groups has been seldom studied and was investigated in this work. For that, anaerobic sludge was incubated in batch bottles with acetate, H_2/CO_2 or ethanol until reaching the exponential growth phase. Then, second substrate addition was performed and oxygen was added at increasing concentrations (0, 0.5, 1.0, 2.5 and 5%). Compared with the controls (0% O_2), O_2 exposure significantly decreased the substrate consumption and initial methane production rate (MPR) from H_2/CO_2 or acetate, at all the concentrations tested. At 0.5% O_2, MRP from these two substrates was inhibited by 31±5% and 39 ±10%, respectively. Nevertheless, the assays amended with acetate were incubated over 30 hours, and activity was recovered in the assays that received the lower % O_2. In the assays with ethanol, significant effects on ethanol uptake, acetate production and MPR were only observed at 2.5% and 5% O_2. At 2.5%, MRP inhibition was 36±7%. The lower impact of O_2 in these assays may be related to stimulation of facultative bacteria by the presence of ethanol, that enhanced O_2 removal from the media, allowing the methanogenic community to maintain its activity.

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I1. Environmental Microbiology and Biotechnology

P69. *Labrys portucalensis* F11 efficiently degrades Di-(2-ethylhexyl) Phthalate

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Phthalates, such as Di-(2-ethylhexyl) Phthalate (DEHP), are compounds extensively used as plasticizer. Due to the extensive usage, DEHP is found in many wastewaters, surface waters and soil. DEHP is persistent in the environment and the toxicity of the byproducts resulting from the degradation of DEHP sometimes exacerbates the parent compound toxicity. They are now becoming contaminants of emerging concern, considered as potential environmental endocrine disruptors, included in priority list of European Union water directive. The bacterial strain *Labrys portucalensis* F11 has shown to be able to degrade DEHP supplied as sole carbon source. Total degradation was achieved for concentrations up to 10 ppm. For 50 ppm, 60% of the compound was degraded in 30 days, with concomitant bacterial growth. The bacterial strain was also able to completely degrade Mono-(2-ethylhexyl) Phthalate (MEHP) and Phthalic acid (PA), which are considered as possible intermediates of DEHP degradation. Whole sample toxicity after degradation of the compound was reduced assessed through the inhibition of germination and growth of tomato and lettuce. Elucidation of the metabolic pathway of degradation is ongoing.

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I1. Environmental Microbiology and Biotechnology

P70. Fish-gut *Bacillus* spp. are potent inhibitors of aquaculture fish pathogens

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Aquaculture industry is the world's fastest growing food protein producer. Its sustainable profitability is however limited by frequent bacterial diseases outbreaks occurring during fish cultivation and maintenance. Bacterial infections are also associated with a misuse of antibiotics, posing serious threats to public health. One promising disease-preventive strategy is the use of probiotics. *Bacillus* species are the most attractive probiotics for aquaculture due to their endosporeforming nature and their production of natural antimicrobial compounds (NACs), active against important pathogens.
Harnessing the fish-gut microbial potential, we aimed to isolate and characterize *Bacillus* spp., from the gut of aquaculture fish, capable of producing NACs antagonistic of fish-pathogens growth, biofilm formation and communication (quorum-sensing). For that purpose, *Sparus aurata*, *Diplodus sargus*, and *Dicentrarchus labrax* were fed with the same commercial diet and their heat-treated intestinal contents were used to obtain aerobic sporeforming intestinal bacteria. All isolates were screened for antimicrobial, anti-biofilm and anti-quorum-sensing activities, using established protocols. Significance of inhibition was evaluated by repeated measures ANOVA or 1-way ANOVA.
A total of 176 isolates representing different colony morphologies and samples were obtained. Spore production was confirmed by phase-contrast microscopy, with 98% of the isolates producing endospores of different sizes and shapes. Screening for NACs production revealed that 52% displayed antimicrobial activity against at least one of the fourteen pathogens tested. By characterizing the localization (intra- or extra-cellular) of the inhibitory molecules, the cell-free supernatants of three isolates (identified as *B. subtilis* by 16S rRNA sequencing), significantly ($p<0.05$) inhibited the growth and biofilm formation of different *Aeromonas*, *Vibrio*, *Photobacterium*, *Tenacibaculum*, *Edwardsiella*, and *Shigella* species. Interestingly, cell-free supernatants of those three strains did not inhibit *A. salmonicida* growth, but significantly decreased its biofilm formation. Moreover, all three isolates produced compounds that interfered with acyl-homoserine-lactone signals, used in Gram-negative bacteria quorum-sensing.
These in vitro tests allowed the selection of three *B. subtilis* strains producing extracellular NACs with broad and potent antimicrobial, anti-biofilm and anti-quorum-sensing activities. These strains and their NACs are being further studied to be used as future probiotics or source of bioactive molecules as tools to prevent aquaculture fish diseases.

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I1. Environmental Microbiology and Biotechnology

P71. Culturable and non- culturable bacterial endophytes associated with *Crocus sativus*: diversity, community structure and functional characterization

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Crocus sativus is a triploid sterile plant, which produces apocarotenoids like crocin, picrocrocin and safranal. These compounds impart organoleptic properties to saffron (dried stigmas) making it world's costliest spice. Due to lack of breeding approach and poor disease management has led to declining trend in saffron production worldwide. One of the important factor is corm rot disease caused by various fungi particularly *Fusarium oxysporum*. Therefore it is imperative to characterize the bacterial endophytic microbiome of saffron plant and investigate the effect of bacterial endophytes in corm-rot inhibition and plant growth promotion. A total of 306 endophytic bacterial isolates were recovered from corm and shoot of *C. sativus* collected from four different geographical locations. Molecular phylogeny assigned them into 47 distinct OTUs which spread over 28 genera. The saffron microbiome was dominated by *Bacillus*, *Burkholderia* and *Pantoea* respectively. Metagenomic analysis through 16S rRNA V3 region profiling of the endophytic bacterial community indicated the microbiome was dominated by *Acinetobacter* and *Rahnella*. Among the culturable bacteria, several isolates displayed potential plant growth promoting properties and antifungal activities against the fungal pathogens of saffron. None of these endophytes caused any symptoms of disease when plants were treated with endophytes. Four endophytes, *Burkholderia gladioli*, *Bacillus halotolerans*, *Streptomyces achromogenes* and *Bacillus siamensis* increased the host root and shoot growth significantly. These endophytes had also a positive influence on the secondary metabolites content of the host. Thus, the short-listed endophytes are potential candidates for development of endophyte-based technologies for sustainable cultivation and enhanced productivity of Saffron.

I1. Environmental Microbiology and Biotechnology

P72. Fungal diversity in the salterns of the islands of Maio, Boavista and Sal (Cabo Verde)

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Cabo Verde has a large number of environments which remain virtually unexplored, including several hypersaline such as the salterns of the Islands of Maio, Boavista and Sal. Salterns represent extreme habitats because of their very high salinity, increased temperature and high exposure to UV and hold unique organisms adapted to these conditions.
Filamentous fungi (FF) are ubiquitous, have a vast and diverse occurrence in the environment and several of them are able to survive in harsh conditions. Estimates propose that there are 1.5 -3.8 million fungal species in the world with only 120,000 (3-8% of the total) being validly described, leaving much to be discovered, isolated, and characterised [1,2]. Furthermore, non-explored environments have a high potential as sources for new fungal diversity.
Most microbial studies on extreme biotopes tend not to focus on fungi. Our study aims to address this significant knowledge gap. In order to compare the abundance, richness and species diversity of the FF present within the salterns of Cabo Verde, we collected surface water, sediments and salt samples. These were plated in several different media to assess the FF diversity. The isolated fungal strains were morphologically characterised (photography, stereomicroscopy and microscopy).
The information collected allowed us to separate the strains into several taxa. Preliminary results, with our current set of isolation media, point to a predominance of *Penicillium* strains and suggest that the sample type and salinity, favours some species in detriment of others and are responsible for differences in the fungal communities. As expected, samples from lower salinity locations have a higher alpha diversity and abundance, and those correspond to sediment samples where higher subtract amount is available.

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I1. Environmental Microbiology and Biotechnology

P73. Genomic-based exploration of novel microbial isolates from hypersaline locations in Cabo Verde

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As part of the CHASE project (Cabo Verde Hypersaline Environments: Analysis, Survey and Exploration) we have collected sediment, salt, and brine samples across multiple hypersaline pools in the abandoned historical salterns of the islands of Sal, Maio, and Boavista. The goal of our project was to perform a wider-based assessment of their physical-chemical characteristics and variability within each site, their microbial diversity, followed by the first survey of their potential biotechnological relevance (focused on e.g. production of hydrolytic enzymes, biominerals, and anti-microbial compounds).
Our large-scale cultivation-based efforts have led to the isolation of a wide range of novel microbial strains and our preliminary screening revealed some noteworthy isolates for further studies. Within our collection of novel microbial strains, we have selected a total of 20 for whole-genome sequencing followed by genomic-based exploration of their capabilities (using RAST). Preliminary results have identified genes associated with production of bioplastics (PHA and PHB) in many of our strains, as well as genes implicated in heavy metal detoxification, and production of biominerals and potential antimicrobial compounds.
This is the first study of this kind on microbes isolated from Cabo Verde and has included sites that were never been previously studied from a microbiological perspective. Results of our genomic-based analysis highlight the enormous untapped potential of these locations and their microbial communities and provide important evidence on the need to protect and restore these sites before they are significantly altered or obliterated.

I1. Environmental Microbiology and Biotechnology

P74. Cabo Verde hypersaline environments: analysis, survey and exploration (the CHASE project)

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The CHASE Project (Cabo Verde Hypersaline Environments: Analysis, Survey and Exploration) focuses on the study of several hypersaline environments in Cabo Verde. The islands of Cabo Verde are a recognised biodiversity hotspot, which include a diverse range of ecosystems, including several extreme ones. Despite this, thus far no efforts have been made to assess and preserve existing microbial biodiversity or to explore their potential applications.

The decline in the economic importance of salt production in Cabo Verde and increasing pressure from tourism resulted in the existence of several abandoned traditional salterns, in varying degree of disrepair leading to several threatened hypersaline locations across the country. Our project aims to address this significant knowledge gap and provide additional visibility to this issue and scientific evidence of the biodiversity and biotechnological value of preserving these sites.

Here we present the preliminary results from our sampling campaign in the islands of Sal, Maio, and Boavista, and include data on the physical-chemical characteristics and variability found in these sites, their microbial biodiversity and results from our bioprospection survey. Preliminary results from our cultivation-based efforts included several isolates belonging to the Gammaproteobacteria (e.g *Salinivibrio*, *Salicola*, *Halomonas*, and *Marinobacter*), Firmicutes (genus *Bacillus*), and some archaeal isolates within the *Halobacteriaceae*. First results on screening for biotechnological potential have highlighted some amylase-producing strains, as well as a few biomineral producers. These are essential first steps of our ongoing efforts in assisting in mapping and protecting threatened extreme environments in Cabo Verde.

I1. Environmental Microbiology and Biotechnology

P75. Revealing the hidden diversity, opportunities and challenges of marine fungi in Portugal

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Blue biotechnology is becoming one of the main drivers for the development of sea economy. Biotechnological applications related to marine organisms, namely microorganisms, are increasingly leading to the generation of economic value and of innovative solutions. Fungi are ubiquitous members of ecosystems that are economically, biotechnologically and medically important. The total diversity of the fungi has been estimated to be 1.5–1.6 million species, while marine fungi have an estimated diversity around 12.500 species with less than 1500 species described so far over 500 genera. Marine fungi are frequently present in intertidal zones, salt marshes and mangroves but can also be found in extreme environments such as deep-sea sediments, ice and hypersaline waters. They act as pathogens and symbionts of other marine organisms, such as algae, corals and sponges and are ecologically relevant due to their performance in biochemical processes such as nutrient regeneration while acting as decomposers of organic matter.
Fungi from Portuguese marine environments are poorly studied, and therefore, their diversity is poorly known. Through culture dependent methods this study aimed to exploit the diversity of Portuguese marine fungi with characterization of the novel species for posterior studies on production of bioactive compounds focusing in a sustainable use of marine resources. During an extensive survey of the fungal diversity in marine and estuarine environments in Portugal, we obtained a collection of 615 isolates including 10 novel species and 1 new genus: *Neoascochyta fuci*, *Neocamarosporium aestuarinum*, *Neocamarosporium endophyticum*, *Neocamarosporium halimiones*, *Neodevriesia aestuarina*, *Poriferomyces lusitanum*. *Parasarocladium alavariense*, *Penicillium lusitanum*, *Trichoderma aestuarinum* and *Verrucoconiothyrium ambiguum*. Thirty-nine genera were found in our fungal collection of saline water, algae, sponges and driftwood samples. Overall, the most represented species belong to the genera *Cladosporium* and *Penicillium*. This study provides valuable insights into the marine fungal biodiversity creating opportunities for the study of their potential as sources of natural products or uses in biotechnology research.

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P76. Phylogenetic diversity of pathogenic *Colletotrichum spp*. retrieved from NE Portuguese olive orchards

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Anthracnose caused by species of *Colletotrichum* – a filamentous ascomycete genus – is a serious disease of more than 30 plant genera, being considered the 7th main plant disease worldwide. It is also one of the two major cause of olive-crop damage. Olive anthracnose causes fruit rot leading to its drop or mummification, resulting in important economic losses due to decreased yield and olive oil quality.
To define good strategies for disease prevention and management, it is thus paramount to know which *Colletotrichum* spp. are present in olive trees, and to correctly identify such pathogenic species. Yet, the taxonomy of *Colletotrichum* is intricate, even though the use of molecular data. For instance, it is now recognized that employing solely the nuclear ITS region in phylogenetic analyses is unlikely to resolve species delimitation within this genus. Accordingly, the current taxonomy is systematized by “species complexes” based on multilocus phylogenies.
This work up to date data regarding the phylogenetic diversity of cultivable *Colletotrichum* species already reported for Portuguese olive crops, with a special focus on NE Portugal (Trás-os-Montes), one of the most important olive growing regions of Portugal.

I1. Environmental Microbiology and Biotechnology

P77. Use of fungi in biocementation of sand

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Concrete is the most widely used construction material in the world being cement one of its main components. Cement production accounts for 5-8% of anthropogenic carbon dioxide (CO2) emissions into the atmosphere. Most of the world's infrastructures are produced from reinforced concrete and cracking is one of the major drawbacks for its durability. The cracks in concrete reduce their resistance capacity and allow the entry of harmful agents both for their microstructure and for the reinforcements located inside the structure. Sustainable solutions aimed at reducing costs and environmental impacts for this problem have been researched. The bioscience of precipitation mechanisms with microbiologically induced calcium carbonate (MICCP) is an alternative to traditionally used methods and a way to mitigate the environmental impact of using more cement and polymers. The biocement presents a more environmentally friendly alternative because it does not generates CO2 in its manufacturing process and when is produced, through metabolic conversion of calcium salts, the CO2 is converted in the calcium carbonate (CaCO3) mineralisation which can promote the improvement of the mechanical properties and durability of cementitious materials. Most of the biocementation studies present bacteria as microorganisms responsible for the CaCO3 induction process. Fungi are potentially better for the biocementation process because they have more biomass and are filaments, which may aid in the mechanical behaviour of the formed bioconcrete. Thus, the present work proposes the use of two urease-positive fungi (Penicillium chrysogenum MUM 9743 and Neurospora crassa MUM 9208) in the sand biocementation in column to produce sandstone. The microstructure and chemical constituents of biosandstone formed due to MICP were observed under Scanning electron microscopy (SEM). SEM showed fungal mycelia as bio-based fiber in bio- sandstones and cluster of probable calcite crystals on and around mycelia. These results envision a promising future use of fungal isolates in the maintenance of concrete structures.

I1. Environmental Microbiology and Biotechnology

P78. Wild mammals in Portugal: their associated enteric flora as a source of pathogenic bacteria and antibacterial resistance

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A high number of human infectious diseases arise from wildlife. These so called zoonoses are diseases shared between animals (including livestock, wildlife, and pets) and humans. In 2017 about 350 000 zoonoses were reported in EU by the EFSA and the ECDC. Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. infections were among the most reported causes of these zoonotic diseases. Additionally, since 2014, WHO considers antibiotic resistance (AMR) as an emerging global problem and a threat to the public health. However, wild animals are rarely exposed to antibiotics and therefore low levels of AMR are expected.

The main goal of this work is to characterize the AMR associated with wild mammals and its potential as reservoirs of pathogenic bacteria.

Faecal samples of mammal species with distinct phenology (wild boar, red deer, otter, and red fox) are being collected from areas under distinct anthropogenic pressures, in Portugal (Montesinho Natural Park, Lousã Mountain, Baixo Vouga Lagunar, Freita, and Tapada Nacional de Mafra). So far, a total of 220 samples were processed. Of these, 175 *E. coli* isolates were subjected to AST and according to clinical breakpoints resistance was detected for ampicillin (17%), streptomycin (9%), cefoxitin (9%), tetracycline (9%), co-trimoxazole (5%), ciprofloxacin (2%), nalidixic acid (2%), amoxicillin/clavulanic acid (2%), chloramphenicol (1%), tobramycin (1%), ceftazidime (1%), gentamicin (1%) and amikacin (1%). A multiresistant phenotype was detected in 10 isolates.

As regards potential pathogens, we have isolated 11 *Salmonella* spp. strains and 38 STEC, which will be further characterized (serotypes, virulome, and resistome).

Our preliminary results show that wild mammals are reservoirs and potential sources of pathogens and AMR and, considering the “One Health” concept, it is crucial to establish local monitoring programs worldwide that will benefit human, animal and environmental health.

I1. Environmental Microbiology and Biotechnology

P79. Response to vanadate toxicity in *Ochrobactrum tritici* strains

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Vanadium is a transition metal that has been added recently to the EU list of Raw Critical Metals. The growing needs of vanadium primarily in the steel industry justify its increasing economic value. However, because mining of vanadium sources (i. e. ores, concentrates and vanadiferous slags) is expanding, so is vanadium environmental contamination. Bioleaching comes forth as environmental sustainable strategy to deal with supply demand and environmental contamination. It requires organisms that are able to mobilize the metal and at the same time are resistant to the leachate generated. Here, we investigated the molecular mechanisms underlying vanadium resistance in *Ochrobactrum tritici* strains.
The highly resistant strain *O. tritici* 5bvl1 was able to grow at concentrations > 30 mM vanadate, while the *O. tritici* type strain only tolerated < 3 mM vanadate concentrations. Screening of *O. tritici* single mutants (at *chrA*, *chrC*, *chrF* and *recA* genes) growth during vanadate exposure revealed that vanadate resistance was associated with chromate resistance mechanisms (in particular ChrA, an efflux pump and ChrC, a superoxide dismutase). We also showed that sensitivity to vanadate was correlated with increased accumulation of vanadium intracellularly, while in resistant cells this was not found. Other up- regulated proteins found during vanadate exposure were ABC transporters for methionine and iron, suggesting that cellular response to vanadate toxicity may also induce changes in unspecific transport and chelation of vanadate.

I1. Environmental Microbiology and Biotechnology

P80. Monitoring morphological changes of suspended sludge during aerobic granulation process

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Aerobic granular sludge (AGS) technology depends on the growth of a self immobilizing microbial community to form granules, typically achieved through environmental and operational selective pressures. Long star-up periods and granules stability in long-term operation are described as the main drawbacks of this technology. During granulation, it is expected that most of the sludge achieve a granular form however often this is not the case. Microbial flocs and filamentous bacteria may endure in the system, potentially affecting granulation and/or granules stability.

Quantitative image analysis (QIA) has helped researchers to understand microbial population dynamics in activated sludge, e.g. identifying bad settling properties phenomena. In this work, we used QIA to monitor morphological changes of suspended sludge during an aerobic granulation process with two sodium acetate concentrations; i.e. R1: 250 $mgCOD.L^{-1}$; R2: 500 $mgCOD.L^{-1}$. R1 and R2 reached good settling properties, achieving sludge volume index (SVI5) of 36 and 55 $mL.g^{-1}$, respectively. However, granules were only visually observable in R1. QIA showed differences in aggregates with equivalent diameter of 100 µm ≤ Deq ≤ 650 µm, between both reactors in early stages of granulation. After 18 days, these aggregates were similar in key parameters such diameter, length and width, day from which a higher increase was observed in R1 than in R2. After 22 days, these aggregates represented 94% and 62% of the total projected area in R1 and R2, respectively. Interestingly, differences in aggregates appeared earlier in the reactors operation by morphological descriptors. In fact from day 11 onwards, aggregates in R1 exhibited higher compactness and robustness than in R2. Furthermore, the prevalence of filamentous bacteria in R2 might have been the reason why microbial aggregates could not achieve granular form. In R1, the total length of filaments (TL) remained below 3.3 $mm.\mu L^{-1}$ from day 25, whereas in R2, TL continued to increase up to 16.3 $mm.\mu L^{-1}$.

Overall, results showed that QIA could be used to monitor morphological changes of activated sludge during an aerobic granulation process. This could be particularly useful to decrease the long-periods required for granulation, and also to monitor AGS system stability in long term operations.

I1. Environmental Microbiology and Biotechnology

P81. Soil enrichment with plant-beneficial (micro)organisms in intensive horticultural production systems

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The predominant horticultural production systems for industry in Ribatejo (Portugal) are based on crops with high phytotechnic intervention, often in monoculture and with a high degree of intensification. At the soil level, these practices result in biodiversity imbalances and lead to the emergence of serious phytosanitary problems for which there is a growing lack of control methods. To counteract this trend, project MaisSolo aims to demonstrate the advantages of changing current monoculture systems to practices that include, for example, the installation of cover crops during the fall-winter period, preceding the main crop of the agricultural year. This approach is expected to contribute to increasing soil biodiversity and microbiological activity, favoring beneficial rhizospheric microorganisms, reducing the incidence of plant pathogens and weeds, and improving soil properties in general.
This work describes the evaluation of soil biological indicators in an experimental field in Golegã, where different cover crops were installed prior to potato cultivation: 1) biodiverse mixture of grasses and legumes, including rhizobia-inoculated clovers; 2) *Lolium multiflorum* (annual ryegrass), a mycotrophic grass that favors soil enrichment in endemic mycorrhizal fungi; and 3) *Raphanus sativus* (forage turnip), a biofumigant species which incorporation into soil contributes to eliminate phytopathogens. A control plot was maintained without any cover crop. Samples of rhizospheric soil from end-of-cycle potato were collected and the following indicators were evaluated: soil enzyme activities (dehydrogenase, alkaline phosphatase, β-glucosidase), total bacteria and fungi, symbiotic nitrogen-fixing bacteria (rhizobia), free- living nitrogen-fixing bacteria, phosphate-solubilizing bacteria and phytostimulant microorganisms. The mycorrhization of potato plants was also evaluated. The obtained results indicate that the introduction of cover crops, in particular the biodiverse mixture and annual ryegrass, increased soil microbiological activity, the abundance of beneficial rhizospheric microorganisms, and the mycorrhization frequency in potato roots. Additionally, nematode communities were evaluated as an indicator of the practices effect on the soil status. As a result, the beneficial nematode populations showed a marked increase, especially in the plots with the biodiverse mixture. Overall, the trend of the plant parasitic nematodes is to decrease its populations using the biofumigant *R. sativus*.

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I1. Environmental Microbiology and Biotechnology

P82. Selection of antibiotic resistance by metals in a riverine bacterial community

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Antibiotic resistance spread in the environment is a multifactorial phenomenon to which several environmental contaminants may contribute. This study aimed to investigate the role of metals in the selection of antibiotic resistance in complex bacterial communities. River water samples were exposed in microcosms to 50 μg L^{-1} and 100 μg L^{-1} of copper (Cu50, Cu100) and zinc (Zn50 and Zn100). After 20 days, colony-forming units were counted in PCA and PCA supplemented with antibiotics (cefotaxime, tetracycline, kanamycin). Cefotaxime- and kanamycin-resistant bacteria were selected from microcosms exposed to metals, and identified. DGGE analyses were performed to assess the metals effect on bacterial communities' structure. The abundance of bla_{CTX-M} (encoding cefotaxime resistance) and *intI1* (encoding class 1 integrases) was determined by qPCR.

After 20 days of exposure, the proportion of cefotaxime-resistant bacteria increased significantly in communities exposed to copper (2.3% of resistant bacteria in control microcosms to 9.5% in Cu50 and 16.8% in Cu100) and zinc (24.6% in control to 91.3% in Zn50 and 72.4% in Zn100). Exposure to Cu100 resulted in a significant increase of the proportion of tetracycline-resistant bacteria (0.03% in control microcosms to 0.23% in Cu100). Zinc exposure resulted in a significant increase of kanamycin-resistant bacteria prevalence (6.1% in control microcosms to 24.1% in Zn50 and 43% in Zn100). Cefotaxime- and kanamycin-resistant bacteria belonged to genera intrinsically resistant to these compounds, i.e. *Pseudomonas* and *Sphingomonas*. bla_{CTX-M} was below the detection limit (10^2 copies/mL) while *intI1* copies/mL reduced after exposure. DGGE analysis confirmed a strong effect on community phylogenetic composition, with non-exposed communities sharing low similarity with copper-exposed (55%) and zinc-exposed (60%) communities. Richness and diversity were significantly reduced in exposed communities.

Our results demonstrate a metal-imposed selection of antibiotic resistance in complex bacterial communities from a polluted river. Selection was observed for both metals (Cu and Zn) and for the two concentrations tested, which have been recorded in rivers worldwide. These effects varied according to the metal and the antibiotic tested. Selection seems to be associated with a community enrichment with bacteria intrinsically resistant to antibiotics, possibly encoding nonspecific resistance mechanisms that result in cross-resistance to metals and antibiotics.

P83. Wastewater treatment and lipid and carotenoid production by the oleaginous yeast *Rhodosporidium toruloides* grown in brewery effluent enriched with sugarcane molasses

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In the food and beverage sectors, the brewery industry is an important section of the economy of many countries. Actually, beer is the fifth most consumed beverage. The brewing process consumes large amounts of water and generates between 3 to 10L of wastewater per 1L of beer produced. This represents large volumes of brewery wastewater that must be treated. Considering this, the need for developing efficient methods for brewery wastewater treatment is imminent.
The brewery biological treatment can be carried out using oleaginous microorganisms that convert the biodegradable organic pollutants present in the wastewaters, into biofuels such as biodiesel, and high value-added products, such as carotenoids. Such strategy can reduce the overall costs of the process. In this study, secondary brewery wastewater (SBWW) supplemented with sugarcane molasses (SCM) was used to grow the oleaginous yeast *Rhodosporidium toruloides* NCYC 921 for lipid and carotenoid production.
Fed-batch cultivations were carried out in a 7 L (5 L working volume) bioreactor.
After 126.5 h, the culture attained a maximum biomass concentration of 42.48 g/L, and the maximum biomass productivity was 0.55 g/Lh at 48.25h. The maximum lipid content was 29.89% w/w DCW at 94h of the cultivation.
Flow cytometry was used to monitor the yeast cell viability in terms of membrane integrity. In the beginning of the essay the percentage of cells with permeabilised membrane was 25.8%, as cells were not yet adapted to the growth medium. At t=48.25h, the percentage of cells with permeabilized membrane increased as well as the concentration of sugars in the fermentation, suggesting that the high concentration of sugars was stressing the cells, and the feeding was stopped. For the rest of the fermentation, the proportion of cells with permeabilized membrane remained almost constant.
Relatively to the brewery wastewater treatment, after the batch phase, 45.84% of total Kjehdhal nitrogen removal, 81.72% of COD removal and 100% of sugar consumption were observed.
The approach here reported is simple, inexpensive and easy to implement. As far as the authors know, the use of brewery wastewater supplemented with sugarcane molasses has never been used to produce lipids and carotenoids by yeasts.

I1. Environmental Microbiology and Biotechnology

P84. Soil enrichment with arbuscular mycorrhiza fungal propagules in intensive horticultural production systems

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The predominant horticultural production systems for industry in Ribatejo (Portugal) are based on crops with high phytotechnic intervention, often in monoculture and with a high degree of intensification. At the soil level, these practices result in biodiversity imbalances and lead to the emergence of serious phytosanitary problems for which there is a growing lack of control methods. To counteract this trend, project MaisSolo aims to demonstrate the advantages of changing current monoculture systems to practices that include, for example, the installation of cover crops during the fall-winter period, preceding the main crop of the agricultural year. This approach is expected to contribute to increase soil biodiversity and microbiological activity, favoring beneficial rhizospheric microorganisms, reducing the incidence of plant pathogens and weeds, and improving soil properties in general.

This work describes the evaluation of the degree of mycorrhization as a soil biological indicator in an experimental field in Golegã (Portugal), where different cover crops were installed prior to corn (*Zea mays* (L.) cultivation: 1) biodiverse mixture of grasses and legumes, including rhizobia-inoculated clovers; 2) *Lolium multiflorum* (Lam.) (annual ryegrass), a mycotrophic grass that favors soil enrichment in endemic endomycorrhizal fungi; and 3) *Raphanus sativus* (L.)(forage turnip), a biofumigant species which its incorporation into soil contributes to the elimination of phytopathogens. A control plot was maintained without any cover crop. Samples of roots from corn were collected and the degree of endomycorrhization was evaluated. The obtained results indicate that the introduction of cover crops, in particular the biodiverse mixture and annual ryegrass, increased mycorrhization frequency in corn roots. The arbuscular mycorrhiza fungal propagules can be developed in the soil by mycotrophic plants and kept intact at the seeding of the corn crop by adopting appropriate tillage techniques. Altogether, this work points out the importance of crop rotation with green manure and other agricultural important species for plant protection and nutrition.

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I1. Environmental Microbiology and Biotechnology

P85. The fate of carbapenem and quinolone resistant bacteria and genes in two Portuguese full-scale wastewater treatment plants

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Carbapenems are an important class of β-lactam antibiotics, being used in the hospital settings as last-line agents for the treatment of persistent multi-resistant bacterial infections. However, their efficiency is threatened by the global emergence and spread of carbapenem resistant bacteria, which are usually also resistant to other groups of antibiotics, namely fluoroquinolones, due to the coordinated expression of different mechanisms of resistance. Although carbapenem resistance is increasingly being reported in clinical settings, it is currently recognized the importance of the environmental framework in the occurrence and fate of these resistant bacteria and genes. Wastewater treatment plants (WWTPs) are considered antibiotic resistance hotspots and are the greatest study representatives of the environmental contribution to the current antimicrobial resistance situation. In fact, it is well described that the conventional wastewater treatments are inefficient in the removal of antibiotic resistance genes, but very little is known about the fate of carbapenem resistance genes throughout the wastewater treatment train. Therefore, the main goal of this study is to characterize the bacterial community composition and to determine the prevalence, fate and removal of the corresponding resistance genes to carbapenems and fluoroquinolones in two Portuguese full-scale WWTPs. Four sampling points were defined and high throughput sequencing targeting the 16S rRNA V4 gene region was performed. Furthermore, identification and quantification of five carbapenem (*bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{IMP}$ and *bla*$_{VIM}$) and three quinolone resistance genes (*qnrA*, *qnrB* and *qnrS*) was achieved by three in-house TaqMan multiplex qPCR protocols. The results show that the bacterial community composition varied between and within the two WWTPs. Additionally, seven out of the eight resistance genes were detected in both influents at concentrations between 10^3 and 10^7 gene copies/ml and four were detected in both effluents at concentrations between 10^2 and 10^5 gene copies/ml. The high concentrations of carbapenem resistance genes in the studied WWTPs reflect the increasing resistance in the community towards this group of last resource antibiotics and that the conventionally applied wastewater treatments cannot prevent them from reaching the environment. All together, these results reinforce the importance of the environmental sector in the spread and increase of the antimicrobial resistance.

I1. Environmental Microbiology and Biotechnology

P86. Useful slime: biotechnological and biomedical applications of the extracellular polymeric substances (EPS) from cyanobacteria

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Many cyanobacteria produce extracellular polymeric substances (EPS) mostly composed of heteropolysaccharides, that can remain attached to the cell surface (capsular polysaccharides - CPS) or be released to the culture medium (released polysaccharides - RPS). The RPS are easier to isolate and therefore have particular advantages for industrial exploitation. These biopolymers have distinctive characteristics compared to those produced by other bacteria: (i) high number of different monosaccharides (wide range of structural conformations), (ii) uronic acids and sulphate groups (strong anionic nature), and (iii) deoxyhexoses and peptides (hydrophobicity and thus amphiphilic behavior), resulting in promising biopolymers for biotechnological/biomedical applications [1].
In our lab, we are studying the cyanobacterial biosynthetic pathways [2], optimizing the production and isolation of the biopolymers [3], characterizing the biopolymers produced by wildtype and mutant strains [4], and evaluating possible uses for these biopolymers and/or their tailored versions. Their unusual features endorse their use as vehicles for controlled drug delivery, namely of functional proteins [5] and vitamins [6], the development of coatings with anti-adhesive properties [7], as antitumor agents [8] and as emulsifying/thickener agents in food or cosmetic industries [9].

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I1. Environmental Microbiology and Biotechnology

P87. Photodynamic inactivation of *Xanthomonas citri* subsp. *citri*, the causative agent of citrus canker

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Citrus canker is caused by the Gram (-) bacterium *Xanthomonas citri* subsp. *citri* and is one of the most common diseases of citrus tree crops. The control of the infection involves mostly preventive measures, like the selection of less susceptible cultivars and the physical separation of orchards but also the chemical treatment with cupric bactericidal sprays.
Photodynamic inactivation (PDI) of phytopathogenic fungi and bacteria has been gaining interest because of the low environmental toxicity of this approach and the minimal risk of selection of strains resistant to chemical biocides.
In order to assess the efficiency of PDI of *X. citri* subsp. *citri*, tests of photosensitization of cell suspensions and biofilms with Toluidine Blue O (TBO)were conducted in vitro and ex-vivo.
A reduction of 5.8 log in the concentration of viable planktonic cells was achieved after 60 min irradiation with white light (400-700 nm; 150 mW cm^{-2}) in presence of 80 μM TBO. In similar conditions, biofilms were not susceptible to photosensitization with concentrations of TBO as high as 100 mM. However, a lower concentration of TBO (10 μM) in combination with a chemical coadjutant (10 mM KI) caused the complete eradication (> 6 log reduction) of viable cells of *X. citri* subsp. *citri*, in biofilms, both in vitro and ex vivo.
The results indicate that although the causative agent of citrus canker is quite resistant to PDI, KI enhances the efficiency of PDI and, in combination with TBO, represents a promising basis for a PDI- based protocol for the control of *Xanthomonas citri* subsp. *citri* infections.

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I1. Environmental Microbiology and Biotechnology

P88. Photodynamic control of the grapevine fungal pathogen *Lasiodiplodia theobromae* with natural and synthetic photosensitizers

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The need to reduce the environmental impact of chemical pesticides motivates the search for new low-toxicity strategies for the control of plant pathogens. Photodynamic inactivation (PDI) represents one of such alternatives. However, only few studies have addressed the control of phytopathogenic fungi and, to the best of our knowledge, none of them targeting *Lasiodiplodia theobromae*.
The objective of this work was to provide evidence that PDI is worth exploring for the phytosanitary treatment of grapevine plants.
Photosensitization with the synthetic photosensitizers toluidine blue O, methylene blue and the cationic porphyrin Tetra-Py+-Me and with the natural photosensitizer riboflavin, was tested either under natural daylight cycles or continuous PAR light irradiation, in 7-day assays.
Although limitations related with recovery of hyphal growth, once irradiation ceases, have been observed, the results demonstrate that PDI with the cationic porphyrin Tetra-Py+-Me or with the phenothiazines toluidine blue O and methylene blue significantly attenuates the growth of L. theobromae, and a reduction of the risk of trunk infection can be expected. The results are encouraging and justify further PDI studies with this relevant plant pathogen.

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P89. Comparative study of neighboring tree-associated belowground microbial communities subjected to different soil management

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It is well-known that different plant species, and even plant varieties, promote different assemblages of the microbial communities associated to them. Here, we investigate how microbial communities (bacteria and fungi) undergo changes within the influence of woody plants (two olive cultivars, one tolerant and another susceptible to the soilborne fungal pathogen *Verticillium dahliae*, plus wild Holm-oak) grown in the same soil but with different management (agricultural versus native). By the use of rRNA and ITS Illumina amplicon sequencing we determined that the native Holm-oak trees rhizosphere microbial communities were different from its bulk soil. Moreover, the agricultural management used in the olive orchard led to belowground microbiota differences with respect to the natural conditions both in bulk soils and rhizospheres. However, agricultural management removed the differences in the microbial communities between the two olive cultivars, and these differences were minor respect to the olive bulk soil. According to our results, and at least under the agronomical conditions here examined, the composition and structure of the rhizospheric microbial communities do not seem to play a major role in olive tolerance to *V. dahliae*.

I1. Environmental Microbiology and Biotechnology

P90. Water quality of the Fervença river hydrographic basin: microbiological impact of anthropogenic activities

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Microbiological monitoring of natural waters is of utmost importance for their evaluation in terms of public health.
In this study, the microbiological water quality of the Fervença river hydrographic basin, Bragança, Portugal, was evaluated. This river results from the confluence of streams and water lines of natural runoff on the north side of the Serra da Nogueira (rural environment), crosses the city of Bragança (urban environment with a downstream wastewater treatment plant, WWTP) and flows into the river Sabor (rural environment).
Sampling included 4 samples upstream of the city of Bragança, 4 samples in the city, one immediately after the WWTP, and 2 samples downstream of Bragança, being the last one at 10 km of the urban center. The samples were collected in the same day, transported in thermal suitcases, refrigerated and analyzed in the microbiology laboratory of the IPB, in the parameters: *Escherichia coli* and Intestinal *Enterococci*.
These parameters were evaluated considering the Portuguese Republic Decree-Laws on the quality of bathing water, nº 236/98 of 1 August and nº 113/2012 of 23 May, which are based on the microbiological analysis of *Escherichia coli* (recommended maximum limit of 1000 CFU/100 mL) and faecal *Streptococci* contents (recommended maximum limit of 400 CFU/100 mL).
Results showed that the levels of *Escherichia coli* in the urban environment waters exceed 4.0×10^3 CFU/100 mL, worsening after the WWTP, where the value of 1.3×10^6 CFU/100 mL was reached. In the case of fecal *Streptococci*, levels above 1.3×10^5 CFU/100 mL were obtained in all samples, except in sample of the Castanheira water reservoir.
The high microbiological load present in the waters sampled in the Fervença river hydrographic basin questions its use for bathing or for irrigation purposes, suggesting that the quality of the water is "Bad" which may constitute a risk to Public Health.

I1. Environmental Microbiology and Biotechnology

P91. Ochraoxigenic mycobiota in chilli *Capsicum annuum* L. and Chilean Merkén

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In Chile, *Capsicum annuum L.* berry fruits are used for the manufacture of a chilli powder known as Merkén. In January 2017, the Chilean Ministry of Health reported cases of Merkén samples available in the national market contaminated with Ochratoxin A (OTA). The main goal of this study was to search for the OTA potential of mycobiota on the whole processing stages of chilli used in traditional Merkén production, and for the OTA production potential of fungal strains isolated from chilli production process.
Capsicum annuum samples were provided by 8 farmers from 4 localities of the Chilean Region of La Araucanía. Chilli berry fruits were collected at three different sampling time points of production: (I) just at the day of ripe fruits harvest (II) after 30 days of harvest (drying process) (III) during the smoking process (IV) ingredients added to Méken (e.g., coriander seeds) and (V) final Merkén samples obtained from the 8 farmers and Merkén obtained from local markets. Cultivable microbiota was isolated on MEA, DRBC and DG18 media. Sequencing of β-tubulin or ITS region was used for fungal identification at species level. The ability of OTA production by isolated strains were assessed through HPLC.
A total of 225 fungal strains belonging to 9 fungal genera were identified. From these, *Aspergillus* (52) and *Penicillium* (118) were the predominant genera. While *Alternaria* (16) and *Fusarium* (15) are isolated mainly in the sampling point I. The variation in the relative abundance in the sample points can be directly influenced by abiotic factors, such as water activity, temperature and oxygen concentration. In addition, *Penicillium brevicompactum* was the only species isolated in all collection points. Regarding ochratoxigenic potential of strains, *P. verrucosum*, *A. flavus* and *A. niger* were identified. *Penicillium* and *Aspergillus* strains were evaluated (YES, 25 oC) for OTA production and its production was not detected under the analysed conditions.
Overall, potentially mycotoxigenic mycobiota and the presence of mycotoxins in Merkén consumed in Chile have already been reported indicating the susceptibility of this substrate. OTA evaluation in Merkén raw material is now under course.

I1. Environmental Microbiology and Biotechnology

P92. Monitoring the marine heterotrophic microalga *C. cohnii* stress response grown on low-cost substrates

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Microbial lipids are considered a sustainable alternative to first generation biodiesel obtained from terrestrial oleaginous crops. There are many studies reporting autotrophic microalgae as potential lipid producers, having the microalgal oil many applications. Nevertheless, heterotrophic microalgal cultures show many advantages over autotrophic microalgal cultures, namely higher growth rate and lipid productivities, less contamination risk and are easier to scale-up. The marine heterotrophic microalga *C. cohnii* can produce high amounts of lipids (up to 70 % w/w). In addition, these lipids are rich in docosahexanoic acid (DHA), an omega-3 compound that has many benefits on the human health, being used in pharmaceutical and food industries. Glucose, an expensive sugar, is the most used carbon source in media formulations to grow this microalga. Therefore, the use of low- cost carbon sources may reduce *C. cohnii* lipid production costs. However, such substrates may contain inhibitory compounds that negatively affect the microalgae growth and lipid synthesis. Therefore it is essential to monitor the cell stress response during the process development, in order to understand the cell adaptation mechanisms to such environments, which is rarely done. In this work, *C. cohnii* was grown on a medium culture containing different low-cost carbon sources (sugarcane molasses, vinegar industry effluent and glycerol from biodiesel industry). Biomass, lipids/DHA and carbon consumption were monitored during growth. The microalga cell viability was at-line monitored during the microalgal cultivation using flow cytometry coupled to propidium iodide (PI)/carbon fluorescein diacetate (CFDA) double staining procedure. Such information, obtained near real time during the process development, allows changing the growth conditions in order to achieve the highest lipid yields.

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I1. Environmental Microbiology and Biotechnology

P93. Chemical leaching versus bioleaching for P recovery from sewage sludge ash

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Phosphorus leaching from sewage sludge ash (SSA) is becoming an increasingly important topic for secondary fertilizer production. Release from the sparingly soluble P-phases present in the ash is mostly pursued with mineral acids. Bioleaching may be a promising alternative if the applied microorganisms are tolerant to the co-release of trace metals and are able to withstand the initially high pH of SSA suspensions. In this study, serveral strains of *Aspergillus* sp. and *Acidithiobacillus thiooxidans* DSM 11478 were used for bioleaching in stirring or shaking flask experiments with the aim of investigating their ability to solubilize P from SSA as compared to conventional acid leaching. Experiments were conducted with three different SSA. For *Acidithiobacillus thiooxidans*, a 2-stage bioleaching approach with a variable L/S-ratio (100:1-5:1) was chosen. The leaching media were pre- incubated to increase the bacterial density. Samples were brought to pH 80 % required pH values of ≤2.0. Results of bioleaching tests showed that strains of *Aspergillus* sp. were able to release approximately 30 % of the P content at a 1 % solids concentration within 8 days, reaching final pH values between 3 and 4. In the 2-stage bioleaching with *Acidithiobacillus thiooxidans* higher release rates could be achieved. P-recovery was more than 80 % at a maximum solids content of 16 % in up to 42 days. Comparable release rates could be achieved within 60 minutes using a solids content of 1 %. The ash content, alkalinity as well as the mineral composition, and resulting buffer capacity were recognized as limiting factors both for chemical leaching and the bioleaching of P from SSA.

I1. Environmental Microbiology and Biotechnology

P94. Toxicity assessment of ibuprofen on activated sludge by respirometric technique

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Micropollutants, including pharmaceuticals, have gained wide attention and concern due to the widespread usage and growing presence in aqueous systems. Activated sludge systems are widely used for wastewater treatment, being one of the processes that present greater efficiency for the removal of pharmaceuticals. The presence of these compounds affects the performance of the biological processes in wastewater treatment plants and is very important to quantify these effects. Among the available techniques for activated sludge control, for the particular case of toxic compounds' effects in the microbial community, respirometry can be considered a relevant technique allowing the evaluation of microorganisms activity. The respirometry test is obtained through the measurement of the oxygen uptake rate (OUR) that is determined by the slope of de dissolved oxygen (DO) concentration versus time during the biodegradation of the substrate. Combining OUR and volatile suspended solids yield the specific oxygen uptake rate (SOUR).
The toxicity was determined by the relative variation of SOUR when a pulse of control substrate is added before and after a pulse of toxic compound. This study aims to assess the toxic effect of ibuprofen on activated sludge using a respirometric test. These respirometric experiments were carried out with ibuprofen concentrations of 2.5, 10, and 20 mg/L. The results indicate a reduction in the respiratory activity of 48%, 54%, and 70%, respectively for the initial ibuprofen concentrations of 2.5, 10, and 20 mg/L. It can be concluded that for the concentrations of 2.5 and 10 mg/L there was similar toxicity, although the toxicity for 10 mg/L was slightly higher than 2.5 mg/L. Furthermore, at 20 mg/L the toxicity effect was considerably higher than the toxicity caused with 10 mg/L.
The results of the respirometry tests suggest that ibuprofen presents a non-negligible level of toxicity even at the lowest concentration studied, and at highest concentration, an accentuated reduction of biomass activity was observed. Globally, results showed that ibuprofen may have an important negative impact on the activated sludge microbial community.

I1. Environmental Microbiology and Biotechnology

P95. *Leptospira* spp. in soils and freshwater collections: a public health problem?

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Leptospirosis is an infectious zoonotic disease of worldwide importance. Rodents are the most common reservoirs in *Leptospira* spp. dissemination. When shedded in urine, spirochetes can survive for several weeks or months under favorable conditions (wet soil, water with neutral or slightly alkaline pH). Thus, contaminated water and soils become important vehicles of transmission to humans, which can be infected by penetration of these infectious agents into intact mucous membranes (nose, mouth, eyes), or to healthy or injured skin.
Environmental samples (N=250), of which 161 were obtained from different freshwater collections (surface of lakes, ponds, rivers, streams and public fountains), and 89 soil samples (near freshwater collections, dustbins or wooded areas) were collected in nine cities from Lisbon and Setubal districts. It was also evaluated certain chemical and physical parameters (e.g., pH, nitrites, water temperature, among others). After DNA extraction from all samples, two nested-PCR protocols with different primers were used. It was applied a first PCR protocol with universal primers based on *rrs* (16S) gene, for *Leptospira* spp. detection. Each sample with Leptospiral DNA amplification, was then evaluated in a second PCR protocol, with specific primers (targeting *lipL32* gene) for detection of pathogenic species, and sequenced.
The nested-PCR protocol using universal primers allowed the detection of *Leptospira*l DNA in 62% of water samples (100+/161), and 84% of the soil samples (75+/89). After further evaluation with “LipL32” protocol, it was detected pathogenic *Leptospira* DNA in 38+/100 water samples (38%) and 7+/75 soil samples (9%). Preliminary sequencing results showed the presence of *Leptospira borgpetersenii* serovar Hardjo-bovis and *Leptospira interrogans* serovar Copenhageni in water samples from different sites of Lisbon and Setubal districts. It was not found so far any link between soil and freshwater samples, when it comes to sequencing results.
These results show an unequivocal presence of pathogenic leptospires in more than half of the water samples as well as in most soil samples. This evidence constitutes an additional risk in the context of public health, particularly for populations most exposed to such environments, for both professional and leisure activities.

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P96. Rodents and leptospires: from the old relationship to current challenges

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Leptospirosis is a significant but neglected disease of humans and animals that is increasing worldwide. As it is well known, rodents and *Leptospira*s go “hand to hand” and human infection results from direct or indirect exposure to rodents urine. Thus, it is important to know the distribution of *Leptospira* spp. in their main reservoirs (rodents), namely the bacterial rate, geographic distribution and species diversity.
To achieve this objetive, a rodents’s capture effort was recently initiated, after obtaining all legal and ethical authorizations. A total (N=18) of rodents (rat and mice) was trapped in two districts of Portugal mainland (Setubal and Lisbon), in nine cities where environmental samples (freshwater and soils) were previously studied. Thus, the focus of rodents trapping was the proximity of lakes, rivers, bushes and trashcans. Preceding euthanasia, it was recorded rodents’ weight and morphometric parameters. During necropsy, were collected samples (blood, urine), and some organs (kidneys, liver, spleen and lungs) to determine bacterial rate.
After DNA extraction, a nested-PCR protocol with specific primers (targeting *lipL32* gene) was performed to amplify pathogenic *Leptospira* DNA and the products sequenced. Further, the renal tissue was cultured in EMJH medium.
Assessment with “LipL32” protocol, revealed *Leptospira*l DNA in 14 rodents (78%), and it was observed that the kidneys (n=13+/14; 93%), liver and lungs (n=12+/14; 86%) were the more colonized organs. No *Leptospira* DNA was detected in any blood samples. Among rodents colonized with *Leptospira* spp., all (n=14) were males, nine were mice (64%) and five came from sewers (36%). Preliminary sequencing results showed the presence of two species: *Leptospira borgpetersenii* and *Leptospira interrogans* in urine and kidneys samples of rodents from different sites of both districts prospected. Renal tissue cultures show so far no leptospires growth.
Amplification of pathogenic *Leptospira*l DNA in almost all rodents captured is not surprising when compared with previous results in environmental samples (freshwater and soils) where about half of them were contaminated with pathogenic *Leptospira* spp., confirming the role of rodents in *Leptospira* transmission to populations increasingly exposed to such environments. These results (although preliminary) are also a warning to public health authorities.

P97. A major threat; co-production of CTX-M-15 and MCR-1 among multidrug-resistant Enterobacteriaceae in pigs, Portugal

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The *mcr-1* gene has been identified as a source of acquired resistance to polymyxins in Enterobacteriacae, mainly in animals. The *bla*$_{\text{CTX-M-15}}$ ESBL gene has been widely detected among human isolates as a source of acquired resistance to cephalosporins, although the *bla*$_{\text{CTX-M-1}}$ gene is the most commonly identified ESBL gene among animal isolates. Our study aimed to prospectively evaluate the prevalence and the occurrence of the mcr-1 gene and ESBL genes among Enterobacteriaceae recovered from two pig farms in Portugal.
Methods. One-hundred and two fecal samples recovered from two different Portuguese pig farms were screened for polymyxin-resistant and ESBL-producing Enterobacteriaceae. Screening for *mcr*-like and ESBL-type genes was performed by PCR amplification and sequencing. Clonality was evaluated by Pulsed Field Gel Electrophoresis (PFGE) analysis and Multilocus Sequence Typing (MLST).

Results. Ninety-three ESBL-producing isolates (62 *E. coli*, 29 *K. pneumoniae*, one *E. aerogenes* and one *E. cloacae*) and eighteen colistin-resistant isolates (13 *E. coli*, 4 *K. pneumoniae* and one *E. cloacae*) were recovered. Among the 93 ESBL-producing strains, eighty (85%) produced CTX-M-15 (27 *E. coli*, 25 *K. pneumoniae* and one *E. cloacae*). The other isolates either produced CTX-M-1 or CTX-M -9. ESBL-producing *K. pneumoniae* isolates were all recovered from a single farm, and belonged to Sequence Type (ST) 1427. The 62 different ESBL-producing *E. coli* isolates belonged to thirteen different ST.
A total of 17 out of the 18 colistin-resistant isolates produced MCR-1, corresponding to 13 *E. coli* belonging to 7 clones and 3 *K. pneumoniae* belonging to 2 clones. Those MCR-1 producers were all recovered from a single farm, using colistin two years ago.

Conclusion. This study showed a surprising high prevalence of CTX-M-15 producers in two different pigs farms in Portugal. This result contrasts with reports from other countries where CTX-M-15 is mainly identified in human isolates, and CTX-M-1 among animal isolates.

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P99. Re-purposing of nitrogen in South African wastewaters using algal biotechnology for value addition

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Many countries, including South Africa, are currently facing potable water scarcities emphasising the need to protect existing water sources. The quality of ground and surface water, available for potable use, is also deteriorating due to the rise in nutrient deposits from overloaded wastewater treatment systems and run-off from agricultural activities. This results in surface water eutrophication which negatively impacts the environment and human health. Amending the already existing wastewater treatment infrastructure to improve the remediation of wastewater is costly and the acquisition of new wastewater treatment technologies is an economical burden on industries and communities. Therefore, there is a need to look for cost effective amendments to improve wastewater treatment and the resultant water quality. The use of microalgae as a water remediation tool and the concurrent production of potentially useful algal biomass may be a feasible solution.

We have developed a screening tool to assess the nitrogen remediation efficiencies of algal species in terms of specific nitrogen forms (ammonium, nitrate, nitrite, urea or complex amino acids) and tolerance to high concentrations. This has allowed us to screen 15 native South African microalgal species for potential remediation of nitrogen-rich wastewater. From these species, six species with similar or better nitrogen utilization rates as the well-studies genus, *Scenedesmus*, across the range of nitrogen forms tested have been identified. Studying the kinetic nitrogen utilization rates and tolerance of these species on the various nitrogen forms assists the selection of algal species based on their suitability to remediate specific wastewater streams, by efficiently utilizing the nitrogen forms present. A polyculture approach will be considered to facilitate the development of a robust algal biotechnology for efficient nitrogen removal from wastewater streams. This polyculture will be tested on a simulated and real wastewater stream to assess the efficiency of the technology and determine the quality of the treated water to inform the downstream use of water. The value of this research is highlighted by the fact that the algal biomass produced during wastewater remediation may be harvested and used for the production of potential high-value algal products, enhancing resource efficiency and resulting in job creation and the offset of infrastructure costs.

I1. Environmental Microbiology and Biotechnology

P101. Lignocellulolytic Activity of Soil Fungi Isolated From Different Sites of Conservation Agriculture

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The lignocellullolytic activity of soil fungi isolated from different sites of conservation agriculture at Ibere, Ikwuano local government area of Abia state, south east Nigeria. Samples of soil numbering 100 were collected from four different farming sites and analyzed using the pour plate technique and tannic acid/carboxyl methyl cellulose test for lignocellulolytic enzyme activity. Twenty eight (28) Fungal species were isolated namely *Penicillium* spp, *Aspergillius* spp, *Rhizopus* spp, *Mucor* spp, *Paraconiothyrium* spp, *Trichoderma* spp, *Fusarium* spp, *Curvularia* spp. Out of the 28 isolates, 12 isolates were positive for lignocellulolytic activity, 7 were lignolytic, 4 was cellulolytic and 1 was lignocellulolytic. After enzymatic screening, the fungal isolates were tested for their ability to degrade lignocelluloses residues (yam peel and corn stover) in solid state fermentation as well as various conditions suitable for the enzyme production. The temperature, pH and incubation were all significant in the production of enzymes. The isolates showed enzyme production best at temperature of 25ºC, pH of 9 after 96 hours of incubation and best degradation at pH of 6, temperature of 30ºC and time of 40 minutes. The molecular identification of the isolates were carried out using PCR and 16S rRNA. Sequencing and the isolates were identified as *Aspergillusniger*, *Penicilliiumrolfsii*, *Rhizopusoryzae* and *Paraconiothyrium brasilliense*. Therefore, the lignocelluloltic fungi isolated, could serve as useful source of commercial production of lignocelluloltic enzyme and also be applied to the soil to degrade organic residue, thereby improving the soil fertility and agricultural waste management in the environment.

I1. Environmental Microbiology and Biotechnology

P102. Diversity of endophytic fungi associated with macroalgae from Ria de Aveiro, Portugal

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In marine environments, fungi are abundant in the inner tissues of macroalgae, constituting the so-called algicolous communities. Fungi are known to be important members of endophytic communities, exhibiting a wide range of properties that can be beneficial for their hosts. Yet, the diversity of algicolous communities remains poorly understood. Evidence suggests that these fungal- algal associations are responsible for the production of numerous bioactive secondary metabolites with biotechnological interest, such as antimicrobial, cytotoxic, antifungal or anti-insect activities, useful for the pharmaceutical and agricultural industries. While studies of marine fungal diversity in Portugal are still rare, this study represents the first inventory of the cultivable endophytic mycobiota associated with macroalgae collected from an estuarine environment, Ria de Aveiro. Twenty-six algae samples were analysed, and the fungi were identified through a combination of morphological and molecular analyses: PCR and subsequent sequencing of the Internal Transcribed Spacer (ITS) of the representative species. The results showed a higher richness and diversity (Shannon and Simpson Indices) in brown and red algae than in the green algal group. In addition, the Ascomycota group dominated (99.4 %), followed by Mortierellomycota (0.4 %) and Basidiomycota (0.2 %). The most frequent genera were *Cladosporium* (30.25 %), *Alternaria* (6.38 %), *Penicillium* (6.17 %) and surprisingly, *Leptobacillium* (8.64 %). Also, in Ascomycota, new species were found, six *Acremonium* like-species, one *Cladosporium*, one *Hypoxylon*. A putative new genus was also identified. Statistical analyses did not reveal any significant influence of the algae hosts as well as of the sampling sites in the fungal communities. However, slight differences observed in fungal communities between sampling sites can be associated with the different physicochemical characteristics, mainly related to the salinity variation along the Ria de Aveiro. This study represents an important advance on the knowledge of endophytic mycobiota in algae. In addition, it emphasizes the need for more research to report putative new fungal taxa with potential beneficial abilities for algae hosts or biotechnological value.

I1. Environmental Microbiology and Biotechnology

P103. Treatment of surface water spiked with *Aspergillus* species using light-emitting diodes and photocatalytic membrane reactors

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Filamentous fungi are known to occur ubiquitously in nature such as soil, air and water. They have been associated with taste and odour problems, contamination of food and beverages, mycotoxin production, and health related effects. Their occurrence in drinking water sources has been reported in the past years, so new treatments of water utilities are needed.(??) Currently, low pressure and medium pressure mercury lamps are the most common UV sources used for water disinfection. If proven effective, light emitting diodes can replace mercury lamps since they are mercury free, are compact and robust, have longer lifetimes, do not need stabilization time, lead to a low energy consumption and can be constructed with a diversity of wavelengths. This study addressed the effectiveness of light emitting diodes to achieve inactivation of three different *Aspergillus* species (*Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus*) spiked in surface water. The LEDs that emit light at 265 nm were found to be more effective than the 255 nm, achieving 3-log, 1-log and 5-log inactivations of *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* using less than 20 mJ/cm^2 (13,97 mJ/cm^2; 7,28 mJ/cm^2; 19,74 mJ/cm^2).
Coupling UV photolysis, stable photoactive TiO_2 layers and water filtration in a single photocatalytic membrane reactor can be beneficial to achieve high quality drinking water since the membrane retains the microorganisms whereas the photocatalytic treatment decreases fouling components, treats the concentrated retentate and inactivates the retained microorganisms. Two photocatalytic membrane reactors were therefore used to evaluate the combination of UV disinfection with membrane filtration for drinking water treatment (Oliveira et al. 2019b). Ceramic supports were used unmodified and modified with titanium dioxide and silicon dioxide (Huertas et al, 2017) to evaluate the effect of membrane retention, direct photolysis and photocatalysis. Results showed high percentages of adsorption and retention of the spores for all treatments and that UV photolysis effectively achieved retentate treatment.
The mode of action of all the treatments applied on the fungal spores were addressed taking into account the effects on fungal spores' morphology, cell wall permeability, enzymatic activity, DNA integrity and proteomics.

I1. Environmental Microbiology and Biotechnology

P104. Potential of Native Rhizobia in Enhancing Nitrogen Fixation of Legume Pastures in the Montado Ecosystem

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Montado is a Mediterranean agro-forestry-pastoral ecosystem dominated by cork and holm oaks and combines the exploitation of cork with livestock, pastures and other uses. In Portugal, in soils where the Montado is the dominant production system, there are acid soils with low fertility. In these soils, natural pastures are usually degraded and the success in the installation of sown pastures is often reduced. Symbiotic nitrogen-fixing bacteria can play an important role in the sustainability/recovery of low-fertility soils, through the establishment of biodiverse legume pastures using native rhizobia as biofertilizers. The project - Improving pasture production in acid soils in the Montado: Chemical and biological approach - aims to study strategies to increase the productivity of legume pastures in the most important soils associated to the Montado.
A field assay was installed in a Montado area (acidic soil) and different treatments were applied to the soil (addition of dolomitic limestone and/or cellulosic sludges). The evaluation of natural rhizobial population size and genetic diversity (by REP-PCR) were performed during 3 years. Symbiotic nitrogen-fixing capacity of rhizobial population, using two species of annual clovers as host plants, was also assessed. Each year, bacteria were isolated from root nodules of these *Trifolium* species. Selected strains were evaluated for symbiotic effectiveness and identified by 16S rDNA and *recA* gene sequencing. Experiments in greenhouse were conducted in pots containing acidic soil and seeded with annual clovers inoculated with the selected bacteria. The results point to an increase of the rhizobial population over time and to the presence of highly (genetic) diversified and effective strains among the natural population. Also, symbiotic effectiveness was high among natural population. A group of strains showing the best performance in nitrogen fixation capacity were selected and identified as *Rhizobium leguminosarum*. Results obtained in the greenhouse experiment showed the beneficial effect, of the selected bacteria inoculation, in the shoot dry weight and in the nodule numbers of clovers plants. In conclusion the selected bacterial consortium could be used in a future development of a biofertilizer for annual clovers adapted to acid soils.

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P105. Extracellular metabolites from marine *Aquimarina* (Bacteroidetes) species possess promising anticancer activity

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Aquimarina (Flavobacteriaceae, Bacteroidetes) is a recently-described bacterial genus whose potential for pharmaceutical and industrial applications only now begins to be unveiled. In genomic surveys, *Aquimarina* isolates have been shown to possess a large variety of secondary metabolite biosynthetic gene clusters, including polyketide, terpene, non-ribosomal peptide and fatty acid synthase genes. All the abovementioned compound classes are associated with previously reported anticancer activities. Additionally, a novel polyketide named cuniculene has been recently described from one of our in-house *Aquimarina* isolates. Given that polyketides are one of the most prevalent classes of natural products in the field of cancer therapy, the genus *Aquimarina* emerges as a potential source of novel anticancer compounds. To access this underlying potential, six distinct *Aquimarina* strains isolated from marine sponges and soft corals were investigated. To this end, 120 mL culture medium supernatants from two incubation timepoints (2 and 10 days, respectively, corresponding to early and late stationary phase) were collected, sterile-filtered, and solid-phase extracted. The activities of the concentrated (240x) methanol-water extracts were assessed against three distinct human cell lines, MCF-7 (breast cancer cells) and A549 (lung cancer cells) and HBE, a healthy epithelial cell line. Cell viability upon 72 hours of exposure to different v/v concentrations of the extracellular *Aquimarina* extracts was assessed using PrestoBlue® assays. All *Aquimarina* extracts reduced viability of the cancer cells to some extent, whereby extracts from 10 days of incubation were usually more effective than culture extracts harvested after 2 days. At 1% v/v extract concentration, cell viabilities were reduced by (until) 55% compared to solvent-only controls, being the human lung cancer cell line A549 the most susceptible followed by breast cancer cell line MCF-7. The viability of healthy HBE cells was mostly unaffected. The activities of extracts obtained from the soft coral associate *Aquimarina* sp. EL33 were most reproducible, especially against cell line A549. We conclude that the marine bacterial genus *Aquimarina* produces (potentially novel) secondary metabolites with promising anticancer activities. Future studies are now required to consolidate these findings and to chemically identify the underlying active metabolite(s) as well as to discern their modes of action.

P106. *Aquimarina* species (Flavobacteria, Bacteroidetes) possess antimicrobial activity towards human pathogens and opportunistic marine bacteria

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The emergence of antimicrobial resistant pathogens is accelerating worldwide. Bacterial species such as multi-resistant *Staphylococcus aureus* (MRSA) are associated with persistent hospital- acquired infections and mortality, accounting for >50,000 deaths each year in Europe and the USA. Likewise, infections caused by *Candida* spp. (fungi) are increasing to an extent that 75% of all women will develop vaginal candidiasis at least once in their lifetime. Additionally, intensified agri- and aquaculture to meet the worldwide increasing food demand imposes new challenges regarding infection control in livestock. Hence, the urge to find new effective drugs is greater than ever. Marine organisms are a rich source of chemically novel, bioactive metabolites and a key target in current drug research. In this study, the antimicrobial activity of a panel of nine distinct strains of the marine bacterial genus *Aquimarina* was evaluated, following newest genomic insights into their complex yet unexplored secondary metabolism. The inhibitory activity of *Aquimarina* was tested against five human pathogenic bacteria (*S. aureus* ATCC 6538, MRSA JE2, *Pseudomonas aeruginosa* PAO1, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* SL1344), two fungal pathogens (*Candida albicans*, *Candida glabrata*) and twelve marine bacteria, including diverse *Vibrio* spp. (n = 9) as a proxy for aquaculture pathogens, two marine *Alphaproteobacteria* and one *Actinobacterium*. Cross-streak diffusion plate assays revealed inhibitory activity of most *Aquimarina* strains against eight out of nine *Vibrio* spp., both *Alphaproteobacteria* species and *Candida glabrata*. *Aquimarina muelleri* stood out as the most bioactive strain with nearly 100% growth inhibition of most marine bacteria and *C. glabrata*. In a second (ongoing) experiment, extracellular extracts from 120 mL culture medium supernatants from all *Aquimarina* strains were prepared by solid-phase extraction (HLB-cartridges) and antimicrobial activities of these 240x concentrated methanol-water extracts were assessed in minimum inhibitory concentration (MIC) assays. Preliminary results showed that the extract (at 10% v/v concentration) of *Aquimarina* sp. Aq135 inhibited the growth of *S. aureus* ATCC 6538 and *S. aureus* MRSA JE2 by 65% and 51%, respectively. In conclusion, *Aquimarina* spp. are a promising source of (novel) antibacterial and antifungal metabolites. Further studies are now required to isolate and identify the underlying bioactive compounds.

FP108. Quality control of stable RNA mediated by Hfq and RNase R

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Surveillance mechanisms that oversee the quality of stable RNA molecules are critical for the cell since the accumulation of RNA fragments derived from such molecules is often deleterious. In this work we describe novel quality control pathways in *Escherichia coli* mediated by the RNA chaperone Hfq and the 3'-5' exoribonuclease R. These RNA-binding proteins were found to associate in a previously unrecognized protein complex being critical for the elimination of aberrant rRNAs, processing of rRNA precursors and degradation of tRNAs. Inactivation of both Hfq and RNase R leads to the strong accumulation of 16S- and 23S-derived rRNA fragments, readily detected on agarose gels. Hfq and RNase R were also found to cooperate in the maturation of the 16S and 23S rRNA precursors which correlates with the severe ribosome assembly defects and the sharp reduction in 70S ribosome levels observed in the Δ*hfq*Δ*rnr* mutant. In addition, Hfq and RNase R were found to control tRNA levels in the cell. This is the first demonstration that the well-conserved Hfq and RNase R proteins interact in vivo, unravelling previously unknown mechanisms of stable RNA quality control with important consequences for translation and cell survival. Given the high conservation of Hfq and RNase R throughout evolution this pathway may represent a broader mechanism of RNA quality control.

FP109. Deciphering *Mycobacterium bovis* phylogeny using genome-wide SNP data

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Mycobacterium bovis is an important pathogen, responsible for causing animal tuberculosis (TB) in livestock and wild vertebrates, as well as humans. In Portugal, *M. bovis* is maintained in a multi-host scenario with wildlife species, particularly red deer and wild boar, implicated in the spread and persistence of TB in cattle populations. However, the ecological processes driving transmission among wildlife reservoirs and sympatric livestock populations are not fully understood.

To improve understanding of *M. bovis* signatures, cross-species transmission and spatial spread in a TB hotspot area in Portugal, whole genome sequencing and single nucleotide polymorphism (SNP) variant calling was performed for 44 isolates representative of *M. bovis* population diversity, previously assessed by combining standard genotyping techniques and Bayesian clustering methods. SNP-based phylogenetic clustering support the branching of this population into five main genetic clades, with a discernible geographic structure. A deeper analysis allowed the establishment of a catalogue of SNPs specific to each clade, supporting their use as phylogenetic markers. Core genome alignment of SNPs also allowed the reconstruction of a transmission network, with suggestion of recent inter- and intra-species transmission events. Further work using molecular clock phylogenetic analyses to estimate time of most recent common ancestor (MRCA) and to quantify the probability of *M. bovis* transmission among host species was performed. Preliminary results, considering the best-fit clock model, estimate that the common ancestor of *M. bovis* isolates existed nearly 400 years ago. This whole genome sequencing approach of clinical *M. bovis* isolates has provided insights into infectious disease epidemiology, allowing the recovery of novel evidences regarding *M. bovis* demographic history in Portugal, adding information regarding geographic and host specificities.

FP110. Engineering of AceTR membrane transporters to improve organic acid production in yeast

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Organic acids are industrially relevant chemicals obtainable from renewable feedstocks via microbial cell factories. Microbially produced organic acids have a wide variety of applications, including bioplastic synthesis. Thus, they possess the potential to replace petroleum-derived commodity chemicals that are obtained through unsustainable production processes. Yeasts commonly represent the organisms of choice for microbial production of organic acids, namely due to their tolerance of low pH environments. Such production conditions allow for direct formation of the desired protonated form of the acid and thus cut downstream processing costs. Efficient product export over the plasma membrane in low pH conditions is particularly demanding, therefore expression of membrane transporters with adequate substrate specificity and transport mechanism is often the determining factor at acquiring competitive product titres. Our current objective is to deepen the knowledge on organic acid transporters from the AceTR family (1,2,3). We performed functional characterization by studying transporter kinetics, energetics and specificity as well as site-directed mutagenesis to acquire insight into the structural features of transporters. Finally, we aim to improve organic acid production in *S. cerevisiae* cell factories via expression of engineered AceTR transporters with altered activity and substrate specificity.

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FP111. A dimeric redox module present in sulfate reducers reveals a likely widespread mechanism of energy conservation

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Sulfate reducing prokaryotes are widespread in anoxic environments, as marine sediments or the human gut. In natural habitats, hydrogen is a main electron source for respiration with sulfate as the terminal electron acceptor. In Bacteria, respiratory chain membrane complexes often rely on redox loops with substrate and quinone binding sites on opposite sides of the membrane, in order to build a proton motive force for ATP synthesis.

The QrcABCD membrane complex is a striking example of a multi-redox protein that links periplasmic hydrogen and formate oxidation to the menaquinone pool. This complex is highly conserved in *Deltaproteobacteria* sulfate reducers with abundant multiheme cytochromes and hydrogenases or formate dehydrogenases that lack a membrane subunit for direct quinone reduction.

Here, we report the reconstitution of *the Desulfovibrio vulgaris* Hildenborough QrcABCD membrane complex in liposomes and show that the complex is electrogenic, as protons and electrons required for menaquinone reduction are extracted from opposite sides of the membrane1. Although the complex does not act as a H^+-pump, a structural homology model for the dimeric redox module QrcCD, allowed the identification of a conserved proton channel leading from the N-side up to the quinone pocket.

Our work reveals how energy is conserved during the dissimilatory reduction of sulfate, and suggests mechanisms behind the functions of related bacterial respiratory complexes in other bioenergetics contexts.

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P112. Involvement of *Geobacter sulfurreducens* ImcH and CbcL in extracellular electron transfer for bioelectricity production

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Fossil fuel combustion is heavily associated with greenhouse gas emissions, which in turn are one of the main factors of global warming and climate change. Moreover, human population constant demand for energy is depleting fossil fuels. Hereupon, the search for new sustainable and renewable energy sources are now a global urgency. Microbial fuel cells (MFCs) are seen as promising clean energy sources. Studies with *Geobacter* spp. have shown that MFCs have the potential to efficiently convert organic compounds into electricity. *Geobacter* cells can attach to electrodes and completely oxidize organic substrates for long periods of time, while transfer electrons to generate electricity (1,2). Therefore, bacterial extracellular electron transfer (EET) requires electron to flow from the cytoplasm, cross the inner membrane, periplasm and finally the outer membrane, going towards the extracellular acceptor. In *Geobacter sufurreducens*, two inner membrane multiheme cytochromes, ImcH and CbcL, were, pointed to be determinant, for ETT to high and low potential acceptors, respectively (3). However, the mechanism behind their function remains elusive. The main goal of this work is to clone, overexpress and purify *Geobacter* ImcH and CbcL for their biochemical and kinetic characterization, and further understand their involvement in energy conservation mechanism. Both proteins structural homology models predict two domains: a soluble multiheme domain and a multiple transmembrane helices domain, essential for interaction with the inner membrane quinone pool. Here, we report studies on the expression of these proteins in different bacterial systems, and their characterization.

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P113. Characterization of novel plasma membrane carboxylate transporters from non- conventional yeasts

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Carboxylic acids (CAs) are a group of organic compounds that play a central role in cellular metabolism of many organisms, using it as unique sources of carbon and energy [1]. In order to replace conventional petroleum-based methods for the obtainment of CAs, alternatives are required for more sustainable way of producing these compounds. The exploitation of yeast biodiversity has received great interest from food, pharmaceuticals and even fuels companies, due to the interesting properties of some microorganisms in producing these compounds in a “greener” trait [2]. Non-*Saccharomyces* yeasts, also called unconventional yeasts, have recently gained prominence in the biotech industry, and are increasingly being used for the heterologous production of valuable products [3]. New strategies for increasing the production of bio-based organic acids are based on the expression of carboxylate transporters in *Saccharomyces cerevisiae* strains. In this work, we focus on the identification of new carboxylate transporters present in several yeasts. The strategy involved the search of homologs to known carboxylate transporters characterized in several microorganisms from yeast, fungi and bacteria. The *S. cerevisiae* IMX1000 Δ25 strain, without carboxylate uptake capacity, was used as a host for the heterologous expression of putative genes encoding CAs transporters [4]. Transport activity was determined by growth phenotypes in different medias containing sole carbon and energy sources, namely mono, di and tricarboxylic acids. The full characterization of the newly identified putative CAs transporters is currently undergoing.

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II2.Molecular Microbiology and Microbial Physiology

P114. Haem metabolism in human pathogens

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Campylobacter jejuni is a Gram-negative pathogen, and the most common cause of gastroenteritis worldwide being responsible of 1/4 cases of diarrheal disease that can be fatal in high risk groups (1). The increasing amount of infections due to *C. jejuni* demands a better understanding of the molecular mechanisms allowing its survival within the host.

Haem is an iron-protoporphyrin molecule that is essential to all living organisms. The survival of pathogens within the host also depends on haem accessibility as this cofactor endows the function of several essential proteins. In microbes, haem can be internally synthesized, by the Haem Synthesis Pathway, or acquired directly from host haemoglobin with dedicated Haem Acquisition Systems (2).

The first haem biosynthesis pathway discovered, denominated “classical pathway” starts with the universal tetrapyrrole precursor δ-aminolevulinic acid and requires at least eight enzymes to form haem. However, we have previously shown that the “classical pathway” is not the only possible pathway for haem formation. Indeed, primitive sulfate-reducing bacteria and pathogens, such as *Staphylococcus aureus*, produce haem through two other distinct pathways that involve different enzymes and intermediates (3,4).

In *C. jejuni*, little is known about its capacity to synthesize haem and which of these three different pathways are active. Moreover, and like other pathogens, *C. jejuni* genome encodes systems to capture haem from the host (5). This work focuses on the poorly understood haem biosynthesis pathway along with understanding mechanisms that maintain haem homeostasis and its importance for *C. jejuni* pathogenicity. In this way, potential drug targets for controlling *C. jejuni* infection would be unveiled.

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P115. MurJ is the lipid II translocase of the gram-positive pathogen *Staphylococcus aureus*

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Peptidoglycan (PGN), the major component of bacterial cell walls, is assembled as long glycan chains of repeating N-acetyl-glucosamine (GlcNAc) and N-acetyl muramic acid (MurNAc) crosslinked via short peptides. Synthesis of PGN is initiated in the cytoplasm with the conversion of uridine diphosphate-GlcNAc (UDP-GlcNAc) to uridine diphosphate-MurNAc (UDP-MurNAc)- pentapeptide, which is then converted to lipid I by attachment to the inner side of the cytoplasmatic membrane via the lipid carrier bactoprenol. This precursor is subsequently converted to lipid II by the MurG-mediated addition of UDP-GlcNAc. In *Staphylococcus aureus* lipid II is further modified with the addition of five glycine residues to the stem peptide before being flipped across the membrane to allow incorporation into the existing cell wall.
Translocation of lipid II-Gly5 was proposed to be mediated by one of two essential membrane proteins, FtsW or MurJ. FtsW from *E. coli* was shown to flip lipid II *in vitro*, but in vivo experiments failed to identify flipping activity. On the other hand, inactivation of MurJ leads to accumulation of lipid II in vivo and decreased incorporation of new PGN in the cell wall, in agreement with a flippase role.
Here, by analyzing the lipid fraction of FtsW and MurJ conditional mutant strains, and the incorporation of newly synthesized PGN precursors in the cell wall, we show that, while depletion of both FtsW and MurJ leads to cell enlargement, only the absence of the latter results in an accumulation of PGN precursors on the inner side of the cell membrane. This leads us to propose that MurJ is the lipid II flippase in the gram-positive pathogen *S. aureus*.

P116. Improved Method For The Extraction Of Humic Acid-Free DNA From Lignocellulosic Residues For Metagenomic Studies

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The isolation of a high molecular weight DNA is essential for successful metagenomic studies aiming to screen and exploit the variety of microorganisms inhabiting an environment [1]. In lignocellulosic rich samples is common to find contaminants like humic substances that are formed by the decomposition of plant, animal and microbial biomass [2]. In this sense, four DNA extraction methods were evaluated with respect to the quality and purity of DNA extracted from samples collected in a composting unit which handle lignocellulosic residues. In the methods A, B and C, the same chemical and enzymatic cell lysis and purification protocol was used, differing only in the type of humic substance removal agent (HSRA) added: (A) no agent [3]; (B) Cetyltrimethylammonium bromide (CTAB) 1% + β-mercaptoethanol 0,2%; (C) CTAB 1% + Polyvinylpyrrolidone (PVP) 1%. In method D, sodium phosphate was added to keep the DNA integrity, and CTAB 1% + $CaCl_2$ were applied as HSRA [4]. Furthermore, chloroform/isoamyl alcohol (24:1) and isopropanol were used in the purification step. Humic acid was extracted from the initial composting sample by acid precipitation [5] and quantified, as well as the humic acid content in DNA solution, by absorbance measurements at 340 nm. Metagenomic DNA of good quality was efficiently isolated from the composting sample using the method D (54.59 µg/g of compost). The average DNA yield obtained with the other methods was only 7.80 µg/g of compost. An assessment of the extracted DNA purity demonstrated that method D provided the purest DNA with an absorbance ratio $A_{260/280}$ of 1.86 and $A_{260/230}$ of 1.80. DNA solution obtained from the method B presented the highest humic acid content (1.482 µg/g), thus justifying the low absorbance ratio $A_{260/230}$ observed (0.86), and highlighting consequently the low efficiency of this method in the humic acid removal (46.29%). The method D ensured higher yield of good quality contamination-free DNA in comparison to other methods evaluated in this study. The addition of $CaCl_2$ allowed the binding of humic substances to the calcium ions which greatly contributed to eliminate humic impurities prior to cell lysis.

P117. CRIPR-Cas9 based strategy to engineer *Saccharomyces cerevisiae* towards the production of curcumin from ferulic acid

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Curcumin is a secondary metabolite produced in the rhizome of turmeric (*Curcuma longa*). Several biological activities and therapeutic effects have been associated to curcumin being the anticancer activity the most well documented. Since it is synthetized in low amounts, difficult to isolate and hard to chemically synthesize, its heterologous production could represent a rapid and easy method to obtain large amounts of this bioactive compound. Two pathways are involved in the curcumin biosynthesis, (a) first, the phenylpropanoid pathway where the amino acid phenylalanine is converted to ferulic acid and activated through condensation with a malonyl-CoA and (b) second, the curcuminoid pathway where two activated ferulic molecules condensate to form curcumin. This work aims to design and construct an artificial biosynthetic pathway to produce curcumin in *Saccharomyces cerevisiae*. Herein, we intend to produce curcumin from ferulic acid as a precursor being the enzymes involved the 4-coumarate-CoA ligase (4CL) and the type III polyketide synthases (PKSs), diketide-CoA synthase (DCS) and curcumin synthase (CURS). Curcuminoid synthase (CUS) from *Oryza sativa* is also a PKS able of catalysing the “one-pot” synthesis of curcuminoids. Curcumin has been previously produced in *S. cerevisiae*, as proof-of-principle, by our research group using episomal plasmids. However, these type of plasmids present some limitations namely the instability, as well as the variations in gene expression within the population. Therefore, an artificial biosynthetic pathway carrying the 4CL from *Arabidopsis thaliana* and CUS was constructed and was further integrated in the *S. cerevisiae* genome. In order to achieve high recombination efficiencies and to allow a multicopy integration, a CRISPR-Cas9 based strategy was used to successfully insert the heterologous pathway at Ty transposon deltas sites in the yeast genome.

P118. Identification of 2CS-CHXT operon signature of chlorhexidine tolerance among *Enterococcus faecium*

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Background: Chlorhexidine-gluconate (CHX) activity against *Enterococcus faecium-Efm* is scarcely documented, with most available data not addressing the clonal background of the strains (clades A1-infection derived strains, A2-mostly animals, B-human commensal). A P102H-mutation in a conserved DNA-binding-response-regulator (ChtR) has been associated with chlorhexidine tolerance among strains of *Efm* clade A1, although the operon remained unidentified (PMID:28242664). Here, we evaluated CHX activity, the distribution of ChtR-P102H, the predicted ChtR operon and its variability among *Efm* from diverse sources and clades.

Methods: *Efm* (n=106) from clades A1 (n=48; human/animal/food/environment), A2 (n=43; human/animal/food) and B (n=15; human/animal/environment) (1995-2016; 5-countries; multidrug-resistant:72%) were included. CHX susceptibility (range:2-32mg/L) was determined by broth- microdilution. *Efm* MIC distribution was analysed by ECOFFINDER-tool (http://www.eucast. org/mic_distributions_and_ecoffs/). Thirty-seven *Efm* were sequenced (Illumina-NextSeq platform/2X150bp paired-end). DOOR-2.0 operon database (http://csbl.bmb.uga.edu/DOOR/index.php) predicted ChtR operon. Amino-acid mutations in ChtR and other operon proteins were identified by comparison (BLASTp-NCBI) with the CHX-tolerant reference strain ChtR-P102H-Efm-E1162 (EFF34003.1; PMID:28242664).

Results: CHX-MIC ranged between ≤2-32mg/L, with the MICs fitted curve slightly deviated to the left comparing to raw data distribution, suggesting the presence of a non-wild-*Efm* population. Most *Efm* with MIC≥8mg/L (89%-n=25/28; 3 clades; 54% of clade A1) presented the ChtR-P102H, while most isolates with MIC≤4mg/L did not (89%-n=8/9; clades A2/B). The predicted 4086bp-operon associated with *chtR* included a previously identified sensor-histidine-kinase as well as a genes coding for proteins related to a glucose:proton symporter and an amino acid permease of the Amino acid-Polyamine- organoCation (APC) family, firstly described here. The complete operon was present in all 37 *Efm* sequenced. Most of 28 *Efm*-MIC≥8mg/L exhibited operon sequences identical to ChtR-P102H-Efm- E1162, contrasting with diverse amino-acid mutations identified in the sensor-histidine-kinase and/or in the two new transporters proteins identified in isolates with a CHX-MIC≤4mg/L and lacking ChtR- P102H.

Conclusions: The complete characterization of the ChtR-P102H-operon, highly conserved among *Efm* with high CHX-MICs, is here firstly described. The ChtR-P102H mutation associated with CHX tolerance is spread in *Efm* from different sources and clades, but mostly from clade A1. The role of each ChtR-operon protein in the CHX-tolerance as well as the occurrence of other CHX tolerance mechanisms in isolates with MIC≥8mg/L and lacking ChtR-P102H deserves further research.

P119. Potential role of a RND-efflux pump in resistance and virulence of *Arcobacter butzleri*

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The genus *Arcobacter* belongs to the family Campylobactereaceae and the class Epsilonproteobacteria and currently includes 29 recognized species. Among these, *A. butzleri* is associated with enteritis in humans, with symptoms such as diarrhea, abdominal cramps or nausea. Among Campylobacter-like organisms, it is considered the fourth most frequent species found in diarrheic stool samples, and, in addition, it integrates the list of microorganisms considered a serious hazard to human health by the International Commission on Microbiological Specifications for Foods. *A. butzleri* isolates have shown resistance to various classes of antibiotics and even resistance to multiple drugs, mediated through various mechanisms including efflux pumps. Efflux pumps are protein structures that represent important elements belonging to the microbial repertoire of bacteria. The efflux pumps of the resistance nodulation cell division (RND) family are only present in Gram negative bacteria and have a relevant role in multidrug resistance. Various genes coding for RND-efflux pumps were identified in the genome of *A. butzleri*. In order to evaluate the contribute of a RND efflux system, named *areDEF*, on *A. butzleri* resistance and virulence, we first constructed a mutant by deletion of the *areE* gene from the AreDEF operon. Afterwards, we verified the resistance profile of native and mutant strain to different antimicrobials agents. Lastly, the effect on virulence was evaluated by growth curves, strain fitness, motility, cellular hydrophobicity and survival under adverse conditions. Regarding the antimicrobial resistance profile, the efflux pump AreDEF was associated with an increased resistance to cephalosporins, tetracyclines, macrolides, gentamicin, streptomycin, levofloxacin, sodium cholate and benzalkonium chloride. The results also showed that the *areE* deletion had no effect in bacterial growth but decreased the fitness cost. Regarding virulence, the *areE* mutant showed a change in cellular hydrophobicity associated with a decrease in motility. When comparing the survival of the mutant with its parent strain, we observed that the mutant was more susceptible to oxidative and osmotic stresses. Moreover, the mutant strain was more susceptible to chlorhexidine and bile extract. In conclusion, the efflux pump AreDEF likely contributes to the resistance, survival to stress and bacterial virulence in *Arcobacter butzleri*.

P120. Phenotypic and Genotypic plasticity of the most frequent *Staphylococcus haemolyticus* clonal lineage

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Staphylococcus haemolyticus, an emerging cause of nosocomial infection, is now the second-most frequent coagulase-negative staphylococci in blood cultures. *S. haemolyticus* is remarkable for its genome plasticity; however, the mechanisms of evolution and adaptation of *S. haemolyticus* are still poorly understood.

To further explore the origin of genetic diversity in *S. haemolyticus* we analyzed a strain belonging to the most prevalent *S. haemolyticus* clonal type for genetic and phenotypic stability after serial passages *in vitro*. Cultures were characterized by PFGE and Southern hybridization with a probe for IS*1272* and also for oxacillin and cefoxitin susceptibility, hemolysis, mannitol fermentation and biofilm production. To assess variability within the cell population, five colonies at seven time points during stability assays were analyzed for phenotypic characteristics and WGS (MiSeq). Genomes were annotated (Prokka) and a pangenome was constructed (Roary). Alignment of contigs was performed (Mauve) and draft genomes were constructed. Phylogenetic analysis was performed based on SNPs (CSI phylogeny).

A high instability was observed both in *Sma*I PFGE and IS*1272* hybridization patterns during serial passage *in vitro*. Most of the changes in the *Sma*I-IS*1272* patterns paralleled those observed in *Sma*I- PFGE profiles and alterations in mannitol fermentation, hemolysis, biofilm formation were also identified. Phenotypic and genotypic variants were detected within individual colonies taken from the same population at the same time point. Also, alteration in the number of viable cells and population growth rates were observed from day to day. Analysis of single colony WGS data showed the occurrence of large-scale genomic deletions within the *oriC* environ. A significant number of genes encoding for amino acid and metal transporters, resistance, virulence, mannitol, metabolic processes and IS elements were deleted. Small deletions located outside the *oriC* were also identified. All the strains with large-scale deletions were grouped in the same genomic cluster. Our results suggest that *S. haemolyticus* populations are composed of subpopulations of genetic variants that might be affected in their growth, gene expression level, stress resistance, specific metabolic processes and virulence. The maintenance of subpopulations of cells in different physiological states might be a strategy for adaptation to the host/hospital environment.

P121. High Clonality of *Pseudomonas syringae* pv. *actinidiae* from North of Portugal inferred by MLSA and pathogenicity-related phenotypic traits

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Pseudomonas syringae pv. *actinidiae* (Psa), a quarantine phytopathogenic bacteria included in the EPPO1 A2 list, is the etiological agent of the bacterial canker in kiwifruit plants, causing major economic loses worldwide. The disease is characterized by angular necrotic spots in leaves, bacterial ooze and cankers in trunks and branches. Aiming to address the epidemiology of this infectious disease, twenty-one isolates of Psa obtained between 2013 to 2017 from several kiwi orchards located in the north of Portugal (Amarante; Amares; Felgueiras; Prado; Penafiel; Valença; Vila do Conde), were genotyped by MLSA. In addition, to unveil the occurrence of particularly virulent lineages, the mobility and Indoleacetic Acid (IAA) production, two traits related to pathogenicity, were evaluated among the studied Psa isolates. After confirming Psa identity by duplex-PCR1, four housekeeping genes (*gapA* [564bp], *gltA* [435bp], *gyrB* [425bp] and *rpoD* [434bp]) were selected for MLSA according Sarkar and Guttman2. Concatenated sequences (1858bp) were used to construct a neighbour-joining phylogenetic tree (Geneious, USA). Psa biovar 3 type (CFBP 7286, Italy - 2009) was used as reference strain. No polymorphisms were found between the 21 concatenated sequences used for MLSA, neither with strain CFBP 7286. These data showed a high clonal population structure for these Psa isolates, and suggested they belong to Psa biovar 3, recognised as the most virulent biovar and the only one so far reported to be present in Portugal. Furthermore, the mobility and Indoleacetic Acid (IAA) production phenotypic analysis did not show statistically significant differences ($p>0.05$) between the isolates. These results indicate a high clonality of Psa occurring in the sampled kiwi orchards, regardless of year of Psa isolation and geographical distance. Further studies are needed to fully understand the transmission routes and the ecological reservoirs of this phytopathogen. A better understanding of the type and epidemic behaviour of these bacteria will be essential to implement strict phytosanitary guidelines to prevent its dissemination.

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P122. A novel regulator of biofilm formation and virulence in the foodborne pathogen *Listeria monocytogenes*

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Polynucleotide phosphorylase (PNPase) is a 3'-5' exoribonuclease, widely conserved from bacteria to metazoans. This enzyme takes part in a multitude of RNA surveillance mechanisms that are critical for translation accuracy across species. Remarkably, it has been associated to disease-related processes, from bacteria to human. Indeed, PNPase mutants of many pathogenic bacterial species were found to be less virulent than their wild-type counterparts. However, the mechanisms behind this phenotype remain mostly elusive.

Listeria monocytogenes is an important intracellular pathogen that is able to breach the host's intestinal, blood-brain and placental barriers during infection. In this work, we studied the role of PNPase in *L. monocytogenes* virulence traits. We analysed *Listeria* PNPase expression and found that its levels are highly regulated by different growth conditions, namely stationary phase. Stationary phase bacteria are more closely related to sessile bacteria found in biofilms, rather than exponential phase bacteria. Biofilms are sessile multicellular microbial consortia that are attached to a surface and are embedded in a self-formed extracellular matrix in order to survive harsh environmental conditions, such as nutrient deprivation. Inactivation of PNPase in *L. monocytogenes* resulted in reduced levels of biofilm formation. Strikingly, the morphology of the biofilm is also affected, suggesting that the matrix composition is altered in the PNPase mutant. In both cases, complementation fully rescued the wild- type phenotype. Biofilm architecture is largely dependent on swimming motility, which prompt us to further study this trait. Our results clearly showed that PNPase mutants were less motile. The strong effect of PNPase in biofilm and motility suggested that PNPase could affect the virulence traits of *L. monocytogenes*. Accordingly, we performed invasion assays with PNPase mutants, using different mammalian cell lines and we observed that PNPase inactivation impaired the invasion of the different types of host cells studied.

Overall, results show that PNPase has an impact in biofilm formation and eukaryotic cellular invasion, which indicates that PNPase is advantageous to successfully infect the host.

P123. Exploring the *Cyberlindnera jadinii* transportome for the identification of novel carboxylate transporters

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Considering the global problems of resource scarcity and environmental damage, new technologies based on renewable biological sources are needed as current model of natural resource exploitation is unsustainable. Novel strategies to boost bio-based production of organic acids are based on the expression of carboxylate transporters in microbial cell factories.

In this work we have focused on the identification and characterization of novel carboxylate transporters in the *Cyberlindnera jadinii* yeast. The transportome of *C. jadinii* was analysed by two approaches. First, the *C. jadinii* homologs of the carboxylate transporters Jen1p (Major Facilitator Superfamily) and Ady2p (AceTr Family) were identified and expressed in *Saccharomyces cerevisiae*. The *S. cerevisiae* strain W303 1A *jen1*Δ *ady2*Δ, lacking carboxylate uptake capacity, was used for the heterologous expression. Genes were identified through sequence alignment and homology prediction. In a parallel bioinformatic approach, the proteome from *C. jadinii* NRRL 1542 was downloaded from NCBI database and explored using a pipeline developed together with the CBMA bioinformatic team. This tool was designed to retrieve data from a specific database: a) that contains a single representative genome/proteome on the species level; b) where multiple matches within a species directly reflect homologs within the same genome, and c) e-values from BLAST searches that are statistically more reliable. A set of genes were selected using this tool and expressed in the IMX1000 strain, which is deleted in 25 genes related to carboxylic acid transport [1]. GFP-fusions versions were used to determine protein expression and localization. Transport activity was determined through growth on different carbon sources and measurement of the uptake of several radiolabelled CAs. The full characterization of the Ady2 and Jen1 homologs as well as others candidate CAs transporters from *C. jadinii* is ongoing.

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P124. Structural and functional relationship of Repair of Iron Centre (RIC) proteins present in human pathogens

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During the infection process the host innate immune system produces Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which damage important pathogen's structures such as Fe-S containing proteins. To cope with those stresses, pathogens developed many strategies, including a set of protective proteins, in which the Repair of Iron Centre (RIC) proteins are included.
RIC are di-iron proteins widely spread among bacterial species, and also present in some human eukaryote pathogens (1,2). We previously showed that *E. coli* RIC is able to restore the activity of stress damaged on Fe-S containing proteins, which is due to its capacity to give iron for repairing the Fe-S centre (3,4).
RIC proteins contain a di-iron centre inserted in a four helix bundle fold. The two iron atoms are coordinated by highly conserved residues, four histidines and two glutamates. The glutamate residues form two μ-carboxylate bridges that are essential to the formation of a functionally active di-iron centre (5,6).
More recently, we reported that RIC interacts with another important protein of *E. coli*, namely the DNA binding protein from starved cells Dps, and that this interaction is important for the *in vivo* role of RIC (7).
Now, several site-directed mutants of RIC were constructed and analysed by X-ray crystallography and iron release assays. The results provided important insights into the molecular structural basis of RIC that enables its iron donor capacity.

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P125. Characterization of methicillin-resistant *Staphylococcus aureus* isolates colonising Portuguese pigs with different exposure to antibiotics

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In 2016, very high rates of methicillin-resistant *Staphylococcus aureus* (MRSA)-ST398 (99%) were found in two Portuguese pig farms that used colistin and amoxicillin as feed additives (Farms A and B). Since then, those farms actually banned the use of these antibiotics. The aim of the present study was to evaluate the impact of the ban of colistin and amoxicillin on pig MRSA carriage rates, clonal types and antimicrobial resistance, compared to the results obtained in 2016.
In 2018, 103 pigs (52 from Farm B using amoxicillin only as a feed additive and 51 from Farm C where no antibiotic was used) were nasally swabbed for MRSA colonization. Isolates were tested for antimicrobial susceptibility, and characterised by spa typing, SCC*mec* typing and MLST. Whole genome sequencing (WGS) was performed for representative isolates.
Overall, 96% of the pigs swabbed in 2018 carried MRSA, mostly ST398-SCC*mec* V-spa types t011/t108. MRSA from pigs not receiving antibiotics in the feed regimen showed susceptibility to a higher number of antibiotics, namely erythromycin, ciprofloxacin, gentamicin, and chloramphenicol. Notably, most of these isolates (n=52) presented an unusual erythromycin-susceptibility/clindamycin- resistance phenotype. WGS showed that these isolates lacked the *erm* and the *lnu* genes encoding resistance to macrolides and lincosamides, respectively, but carried the *vgaALC* gene encoding resistance to lincosamides, which is here firstly identified in *S. aureus* ST398.
In conclusion, after two years the ban of colistin and amoxicillin as feed additives had no significant impact on the MRSA nasal carriage rates. Nevertheless, the MRSA strains circulating in those farms showed resistance to a lower number of antibiotic classes.

P126. Epidemiology of invasive meningococcal disease due to serogroup B ST-213 clonal complex in Portugal

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Introduction
Neisseria meningitidis is a Gram-negative commensal of the human nasopharynx that occasionally cause serious invasive infections including fatal sepsis and meningitis.
In recent years, epidemiology of *N. meningitidis* changed with the emergence and expansion of new clones. Example of this is the increase in the prevalence of invasive Serogoup B ST-213 clonal complex (cc), which are predicted to be not covered by the 4CMenB vaccine (Bexsero). The opacity proteins (Opa) give *N. meningitidis* the ability to colonize the nasopharyngeal mucosa by adhering to specific receptors on host cells. These interactions appear to be important in asymptomatic colonization and in the incidence of meningococcal disease.
The goal of this work is to study the phylogenetic evolution of Portuguese strains serogroup B (MenB) cc213 and to know the distribution of *opa* genes within hyperinvasive cc.
Methods
MenB genomes and sequences of *opa* loci data are available at PubMLST database (until 22th August 2019). For visualization of genomic relationships, the ITOL free software available on Neisseria PubMLST database was use.
Results
During the 10-year period from 2009 to 2018, 441 cases of IMD due to MenB (70,5% of all cases) was reported in Portugal, including 43 cases assigned to cc213 mostly with the genotype B:P1.22,14:F5-5: cc213. From 2014 to 2018 it was observed an increasing trend in the number of cases.
Phylogenetic analysis of MenB cc213 isolates showed a high heterogeneity that differed across countries and over time. Phylogenies of *opa* genes showed a heterogeneous distribution in the isolates cc213. Interesting one isolated cc213 appear to be close to MenC cc11 virulent strains.
Conclusion
Our data indicates a scenario quite similar to the one observed in other European countries, where it has been reported an increasing number of IMD due to cc213.
Considering the inclusion of Bexsero in the immunization plan in several countries, it will be prudent to maintain a high level of vigilance to monitor MenB cc213 epidemiology in order to develop real-time public health prevention strategies.
Further studies should be performed to analyze the role of Opa in the cell adhesion and their implications on the expansion of new clones.

P127. Benzo[a]phenoxazine C9 as an antifungal agent: uncovering its targets and antiproliferative mechanisms

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Benzo[a]phenoxazines have been the subject of increased research due to their antiproliferative properties, with potential applications as antitumor and antimicrobial agents. Previous studies in our laboratory have shown that a newly synthesized phenoxazine derivative (BaP1) induces yeast cell death through a regulated process mediated by vacuolar membrane permeabilization, without the involvement of autophagy or mitochondrial pathways. The interesting potential of this compound prompted us to study another compound of the same family (C9) differing from BaP1 in a single substitution of its extended aromatic system, which increases its antifungal activity. In this study, we aimed to characterize the intracellular distribution and the molecular targets underlying C9 antiproliferative activity using the yeast *Saccharomyces cerevisiae* as a model. The results showed that the C9 compound accumulates in the vacuolar membrane and the perinuclear endoplasmic reticulum. Due to its accumulation at the perinuclear endoplasmic reticulum, we hypothesized that C9 could deplete ergosterol or even disturb its metabolism, impairing yeast cell growth. However, C9 did not lead to differences in ERG gene expression. In addition, we observed that addition of external ergosterol to the medium increased C9 toxicity of a specific C9 concentration. Moreover, this compound did not appear to induce endoplasmic reticulum stress. On the other hand, we observed that C9 induced cell death in *S. cerevisiae* accompanied by vacuolar membrane permeabilization and involvement of Pep4p, which seemed to play a pro-death role. C9 also induced extensive mitochondria fragmentation, implicating this organelle in the cell death process. Overall, results indicate that C9 induces regulated cell death mediated by vacuolar permeabilization and involving mitochondria. This work provides new insights in the use of phenoxazine derivatives as antifungal agents, by characterizing their activity in order to evaluate their potential application.

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P128. Bioengineering nisin to target biofilms of *Streptococcus uberis* that possess nisin resistance proteins (NSR)

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The emergence of new multi-drug-resistant organisms has created an urgent need to find new therapeutic options to eliminate pathogenic microorganisms. An alternative to this method would be to use antimicrobial compounds such as the bacteriocin nisin. However, some bacterial strains possess resistance to nisin. The nisin resistance protein (NSR) cleaves the peptide resulting in a significantly lower bactericidal efficacy. This resistance protein was identified in different pathogenic strains including *Streptococcus uberis*, a worldwide pathogen that causes mastitis in dairy cattle. Mastitis is considered one of the most frequent and expensive diseases in the dairy industry. The ability of *S. uberis* to form biofilms increases the resistance to antibiotics and protection against host defences. The objective of this study was to investigate the ability of a nisin peptide termed PV, a derivative that was bioengineered to be impervious to NSR, to eradicate and inhibit biofilms of *S. uberis* ATCC700407 and DPC5344 compared to the wild type (WT). The effect of nisin PV and WT were evaluated using crystal violet assays to determinate biofilm cell quantity, XXT assays to estimate viability and confocal microscopy using LIVE/DEAD® BacLight™ staining which revealed the viability as well as the architecture of the biofilm. A significantly greater reduction in biofilm formation as well as metabolic activity was observed for both strains in the presence of nisin PV compared to WT. When pre-established biofilms were assessed, both peptides partially reduced the amount of biofilm. However, the metabolic activity of cells was significantly lower following treatment with nisin PV compared to WT. Furthermore, the confocal microscopy analysis revealed a higher number of dead cells on top of the biofilm and a reduced thickness after treatment with PV vs. WT. These results suggest that nisin PV is a promising and potent alternative to effectively reduce biofilm formation of *S. uberis* strains that possess NSR. Additionally, we demonstrate that it is possible to increase the spectrum of action of nisin on clinically relevant pathogens that possess such resistance mechanisms and that produce biofilms. Consequently, such nisin derivatives may have future applications for the elimination of problematic biofilms and associated infections.

P129. Structural determinants of substrate specificity in family 15 Carbohydrate Esterases

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Glucuronoyl esterases (EC 3.1.1.-) are microbial enzymes that cleave the ester bonds between glucuronic acid residues that decorate xylans and lignin alcohols. By untangling the lignin- polysaccharide network, glucuronoyl esterases facilitate the access of cellulases and hemicellulases to their target substrates thus promoting biomass recycling in nature. Although several glucuronoyl esterases have recently been characterized the mechanism of substrate recognition by these highly specific carbohydrate esterases remains relatively unexplored. Here we describe the isolation of 20 bacterial and fungal glucuronoyl esterases and their recombinant expression in *Escherichia coli*. Eleven enzymes revealing the expected glucuronoyl esterase activity were efficiently produced and purified. The structure of the glucuronoyl esterase displaying the highest catalytic activity, TtGE15A from *Teredinibacter turnerae*, was solved. TtGE15A's structure revealed a classical α/β hydrolase fold observed in several esterases and typical of the family 15 Carbohydrate Esterase family. Structural alignment studies with other CE15 members revealed a peculiar active site occluded by two large insertions that will probably limit the access to large polymeric lignin-carbohydrate substrates. This hypothesis is supported by the high activity displayed by TtGE15A on small artificial substrates that mimic the structure of small xylo-oligosaccharides esterified with lignin alcohols. The different active site topologies in CE15 glucuronoyl esterases suggest that some enzymes, such as TtGE15A from *T. turnerae*, act on esterified xylo-oligosaccharides, while others may target high molecular mass lignin-carbohydrate complexes (LCCs).

P130. Revealing the crosstalk between autophagy and proteasome in the yeast aging model

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Aging is a complex multi-factorial process that results in the progressive accumulation of molecular alterations and disruption of cellular homeostasis. Several hallmarks of aging that represent age-common denominators in different model organisms have been proposed, including deregulated nutrient-sensing and loss of proteostasis. Disruption of proteostasis during aging is mainly caused by a decline on autophagy and the ubiquitin-proteasome system (UPS) activities. Caloric restriction (CR) is still the most effective non-genetic intervention known to promote longevity associated with the modulation of the proteolytic systems in different aging model organisms. In the present work, we used the yeast *Saccharomyces cerevisiae*, a simple and powerful model organism, to understand the ties between proteasome and aging under the beneficial effects promoted by CR intervention. Proteotoxic stress was elicited by the heterologous expression of human α-synuclein (aSyn), a protein associated with synucleinopathies, age-related diseases. The results obtained show that CR promotes a coordinated regulation of UPS and autophagy activities during aging. Indeed, CR boosts UPS activity, reversing its decline aggravated by aSyn in aged cells, and keeps autophagy at homeostatic levels. Autophagy inhibition upregulates UPS activity, pointing to a compensatory mechanism. However, UPS inhibition was not associated with enhancement of autophagy activity. Maintenance of autophagy at homeostatic levels appears to be relevant for UPS activity and for the mechanism underlying rescue of cells from aSyn-mediated toxicity by CR.

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II2.Molecular Microbiology and Microbial Physiology

P131. *Candida parapsilosis* virulence attributes are regulated by NDT80

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Candida parapsilosis is typically a commensal of human skin and its pathogenicity is limited for those who are immunocompromised. This fungus is the predominant species along non-albicans species responsible for invasive candidiasis in a particular patient groups at risk, including a neonatal age patients, transplant recipients, having prior antifungal therapy (mainly fluconazole), and patients receiving parenteral nutrition. In fact, its prevalence results from its notorious capacity to grow in parenteral nutrition, to adhere and form biofilm to central venous catheters and other medically implanted devices, to be horizontally transmitted from the hands of health care workers and to its persistence in the hospital environment for a long period of time. Such behavior indicates that adhesion, biofilm formation, and additionally, the ability to shift phenotypic morphology are keys factors for *C. parapsilosis* establishment in clinical setting and infection.
In *C. albicans*, Ndt80 is one of the transcription factors involved in virulence regulation. In this species, it controls biofilm formation, hyphal growth and the expression of genes related with cell wall organization. In *C. parapsilosis* this transcription factor is still poorly studied, thus we aimed to understand the role of *NDT80* (CPAR2-213640) in *C. parapsilosis* virulence attributes. Deleting *NDT80*, even one copy of the gene, substantially changes colony and cell morphologies from smooth and yeast-shaped to crepe and pseudohyphal elongated forms. Both *ndt80*Δ and *ndt80*ΔΔ mutants exhibit increased adherence to polystyrene microspheres and enhanced biofilm formation than the wild type strain. In addition, we identify ALS7, UME6, CPH2, CWH41, ACE2, MKC1 transcription factors to be under NDT80 negatively regulation, justifying the virulence factors exhibition under *NDT80* knockout. Additionally, we explore the impact of Ndt80 regulation in the interaction of fungal-host immune system by assessing macrophage mediated response. *ndt80*ΔΔ mutants, in their natural pseudohyphae phenotype, were more efficient in macrophages killing.
Our findings provide the first evidence for a direct role of Ndt80 as a repressor in the regulation of *C. parapsilosis* virulence attributes, culminating in an increased capacity to neutralize immune response.

P132. Elusive roles of an RNA chaperone in the membrane damage stress response

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The RNA chaperone Hfq is an important bacterial post-transcriptional regulator. In Gram- negative bacteria, Hfq acts mostly as an RNA matchmaker promoting the interaction between non- coding sRNAs and their mRNA targets. However, the role of Hfq in Gram-positive bacteria is controversial, as it seems to play a minor role in riboregulation.
In this work we analysed the role of Hfq in the foodborne pathogen *Listeria monocytogenes*, a low GC- content Gram-positive bacterium. Previous work established that Hfq-mediated regulation of RNA is induced under stress conditions in the model organism *Escherichia coli*. Here, we analysed a variety of biochemical stress agents and found that Hfq is critical for the integrity of the cell membrane in *Listeria*. The deletion mutant of Hfq was more sensitive than the wild-type strain to membrane disruption agents, both in solid and liquid media. Furthermore, this effect was observed to be dose dependent. Remarkably, the protein levels of Hfq were found to be growth phase-regulated and to increase in bacteria exposed to membrane damage agents. Remodelling of the membrane is important for cell adaptation to the surrounding environment. Interestingly, inactivation of Hfq resulted in the attenuation of virulence in the invasion of mammalian cells, suggesting a role for Hfq in infection, possibly through regulation of the membrane integrity.
Overall, we show that Hfq is a stress-induced protein particularly important in the membrane damage response and pathogenicity. This shades light on the elusive roles of the conserved RNA-binding protein Hfq in Gram-positive bacteria.

P133. The Unrevealed Phylogeny And Sources Of CMY-2 Beta-Lactamases

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Background
The precise origin of CMY-2-like, CFE and LAT acquired AmpC (qAmpC) betalactamases within the *Citrobacter* genus has been obscured by a suboptimal identification of *Citrobacter* species. We have recently proposed accurate molecular markers (*recN*, *qnrB*) that supported the description of novel *Citrobacter* species. In the present study, we aim to elucidate the origin of CMY-2-like, CFE and LAT β- lactamases within *Citrobacter* spp.

Materials/methods
145 *Citrobacter* spp. genomes and genes encoding 180 CMY-2-like (83 qAmpC, 52 crAmpC, and 45 new putative crAmpC identified in *Citrobacter* genomes), 2 LAT, and 1 CFE-1 were collected from public databases (NCBI/PATRIC/http://www.bldb.eu/). Phylogenetic analyses were performed for accurate species identification (*recN*) and affiliation of all AmpC genes and β-lactamases studied. Genetic surroundings of blaCMY-2-like were compared by BLAST/Clustal Omega.

Results
Phylogenetic tree of blaCMY-2-like showed 7 main clusters and 1 branch (sharing ≤94.5% identity, bootstrap ≥96%), each cluster comprising crAmpC from a given *Citrobacter* species (Figure 1), revealing additionally that not all *Citrobacter* species described to date (n=13) carry blaCMY-2. The topology of phylogenetic tree based on amino acid sequences was similar (data not shown). Interestingly, the majority of CMY-2-like variants (57%; n=105/183, cluster I), including most (93%; n=77/83) of qAmpC (such as the worldwide spread CMY-2), were highly similar to crAmpC from the newly described *C. portucalensis* (sharing 98.3% identity, bootstrap ≥96%). The second and third clusters with the highest number of CMY-2 alleles included crAmpC from *Citrobacter freundii* (26%; n=47/183, cluster II) or *Citrobacter braakii* (9%; n=17/183, cluster IV), respectively (Figure 1). qAmpCtypes previously designated as LAT-1/-3 and CFE-1 were identified in clusters I and III, respectively, suggesting that they should be regarded as CMY-2-like variants (Figure 1). The same genetic context (AmpR-blaCMY-2-blc-sugE) surrounding chromosomal blaCMY-2-like was observed, irrespectively of CMY-2-like cluster.

Conclusions
This study provides a state of the art of CMY-2-like phylogeny and furthermore establishes for the first time that each CMY-2-like cluster is associated with a particular species within the *Citrobacter* genus. We also demonstrate that *C. portucalensis* seems to be the source of most acquired CMY-2-like enzymes.

P134. The importance of the cytosolic termini of Jen1 lactate transporter for function, regulation and trafficking

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Biotechnological applications usually require a detailed characterization of microbial eukaryotic nutrient transporters physiology, including its function, structure and regulation. Indeed, the identification of trans-acting effectors and cis-acting domains controlling transporters subcellular regulation is obviously critical to improve or optimise microbial cell factories. In this work, we have investigated the role of the cytosolic N- and C-termini of the Jen1 lactate transporter of *Saccharomyces cerevisiae* in respect to subcellular trafficking, transport activity and turnover, through rational design and functional analysis of specific Jen1 truncations and chimeric transporters based on Jen1 and two other transporters of *S. cerevisiae*, Gap1 and Fur4, or the purine transporter UapA from *Aspergillus nidulans*. Our results show that both cytosolic termini are critical for Jen1 function. The N-terminus proved critical for subcellular localization and essential for transport activity and endocytic turnover. The direct involvement of the N-terminus in Rod1-dependent, glucose-elicited, endocytic turnover was strongly supported by its ability to confer glucose-elicited vacuolar turnover to the UapA, an *A. nidulans* transporter that is normally stably expressed in *S. cerevisiae*. The C-terminus of Jen1 was also shown to be essential for transport activity and critical for signal-elicited endocytosis. Results presented herein and in a recent report, point to the idea that the cytosolic termini of Jen1 interact and together determine Jen1 endocytic turnover.

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P135. Oxacillin tolerance among clinical isolates of *Staphylococcus aureus*

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Staphylococcus aureus is an important human opportunistic pathogen causing both hospital- and community-acquired infection in humans and animals. The acquisition of an acquired genetic determinant – usually *mecA* – encoding for a low affinity penicillin binding protein that enables continuous cell wall synthesis even in the presence of beta-lactams in the medium, is the most common strategy used by *S. aureus* to survive the action of beta-lactam antibiotics. Other strategies to survive antibiotics' killing action have been already described in *S. aureus*. In one of those strategies called "tolerance" *S. aureus* is able to survive transient exposure to high concentrations of oxacillin above its minimum inhibitory concentration (MIC) without changing it. Hospitals routinely test strains for resistance to antibiotics, but not for tolerance due to the lack of appropriate and fast methods to detect tolerant strains; this may lead to misclassification of tolerant strains as resistant, or vice versa, resulting in ineffective treatments.
In the work described here, strains identified as methicillin-susceptible *S. aureus* (MSSA) in microbiological laboratories of Portuguese hospitals were tested to detect their ability to develop tolerance to oxacillin. The recently described "Replica Plating Tolerance Isolation System (REPTIS)" was used. This method, based on agar replica plating, allows detection of tolerant bacteria, through the supply of nutrients in new antibiotic free replica plates, which were completely consumed after the antibiotic incubation period. The results obtained so far seem to indicate that at least 13 of the 30 strains tested were able to develop tolerance, as they were able to tolerate high concentrations of oxacillin without changing the MIC and showed prolonged lag phases before exponential phase, another feature that characterizes tolerant strains. More tests are under way in order to confirm their tolerant phenotype and whole-genome sequencing is planned in order to detect involved mutations. It is essential to better understand the mechanisms responsible for the survival of bacteria in the presence of bactericidal antibiotics in order to develop new and effective antibiotic therapies to eradicate them.

P136. Worldwide dissemination of linezolid resistant *Staphylococcus epidermidis* clones

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Introduction

Linezolid is an important antibiotic for the treatment of serious infections caused by two of the most relevant nosocomial pathogens - methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE). Although resistance to linezolid remains rare among *S. aureus* and *Enterococci*, the emergence of linezolid resistant *Staphylococcus epidermidis* (LRSE) is a source of concern. This is important not only due to the increasing clinical relevance of *S. epidermidis*, but also because of the possibility of transfer of this resistance to MRSA and VRE. In spite of its clinical significance, LRSE clonal lineages and resistance mechanisms that are contributing to the spreading of linezolid resistance are poorly explored.

Material and Methods

Ninety-three LRSE isolates from infection and colonization, collected in 2004-2016, from six different countries (Germany, Greece, Italy, Spain, Poland and USA), were analyzed. Linezolid resistance was evaluated by disk diffusion and minimum inhibitory concentration (MIC). Whole genome sequencing was performed with NextSeq Illumina. Raw data were assembled with INNUca v3.1 pipeline and molecular characterization performed using available resources in CGE and/or ABRIcate. Phylogenetic analysis was performed by CSIPhylogeny v1.4.

Results

Seven sequence types (ST) were identified: ST2-III and ST22-III comprised more than 75% of the collection, with 49.5 and 28.6%, respectively; the prevalence of the remaining STs (ST5, ST23, ST185, ST186 and ST797) ranged between 1.1 and 6.6%. SCCmec III was present in 80% of the isolates, while SCCmec type IV was the second most common, present in 7.5%. ST2-III and ST22-III clones were distributed throughout five countries; interestingly clones isolated in different countries could differ by less than 12 SNPs.
Approximately, half of the isolates presented a minimum inhibitory concentration (MIC) for linezolid above 256 µg/ml, and all *S. epidermidis* were multidrug resistant. A single isolate harbored the *cfr* resistance gene, suggesting that mutation was the main mechanism responsible for linezolid resistance.

Discussion

Linezolid resistance in *S. epidermidis* was mainly associated with two clonal types related to clonal complex 2 (ST2-III and ST22-III), which were disseminated geographically among five different countries. Mutation was identified as the main mechanism generating linezolid resistance worldwide.

P137. *Staphylococcus epidermidis* – coping with the host immune system

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Staphylococcus epidermidis is a common colonizer of the human skin and mucous membranes. This bacterium is an important nosocomial pathogen that infects particularly patients with a compromised immune system. Its pathogenesis is characterized by a strong ability to form biofilms on implanted medical devices, such as catheters and prostheses (1). This survival mechanism confers increased resistance to the host immunity and to antibiotics, and hampers the pathogen eradication.
One of the main defenses of the host against pathogen infections is the production of reactive oxygen and nitrogen species (ROS and RNS) by the major phagocytes of innate immunity. ROS and RNS are highly deleterious as they target key components of the pathogen, such as lipids, DNA, amino acids and protein metal centers. Pathogen survival depends on mechanisms to overcome these attacks (2-4) and the strategies used by *S. epidermidis* to resist the host innate immunity remain to be elucidated. Therefore, in this work we investigated the behavior of *S. epidermidis* exposed to oxidative and nitrosative stress by studying growth rate, survival and biofilm formation. We show that this pathogen is resistant to high concentrations of stress imposed by hydrogen peroxide and nitric oxide (NO) donors. The current results will be presented and discussed.

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P138. Inter-species hybridization followed by massive gene loss of one progenitor promoted *Saccharomyces cerevisiae* adaptation to the niche of processed olives

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A few yeast species show signs of adaptation to artificial environments created by human activity. In some circumstances, yeasts have been unconsciously selected by humans for thousands of years for their relevant properties in the fermentation of different beverages and foodstuffs, a process known as microbe domestication. The yeast *Saccharomyces cerevisiae*, the workhorse to produce wine, beer and other important produces, is probably the most well-known case of microbe domestication. The availability of both domesticated and wild populations allows for detailed comparisons aiming at a better understand the genetic underpinnings of domesticated phenotypes [1,2].
In this study, a hybridization event between *S. cerevisiae* and its closest relative *S. paradoxus*, an exclusively wild yeast, is explored in the context of adaptation to a man-made artificial habitat associated with processed olives. Genomic analyses showed that the hybrid strains have lost most of the non-cerevisiae sub-genome, only keeping fragments of the *S. paradoxus* parental strain. This unbalanced genomic architecture, appears to be beneficial in the harsh environment of processed olives, with high levels of salt and phenolic compounds. We observed that these hybrid strains performed better than the *S. cerevisiae* parental strains. Additional features like the increase in copy number of some genes also seem to have played a major role in adaptation to the processed olives environment. In order to understand in more detail, the genomic changes that occurred after hybridization we created in the laboratory a 1:1 homoploid hybrid using ascospores of *S. cerevisiae* and *S. paradoxus* and plan to investigate its adaptation and genome evolution in olive brine.

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P139. Evolutionary history of alcoholic fermentation – a new pathway for mannitol metabolism in fructophilic yeasts

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The yeasts belonging to the *Wickerhamiella* and *Starmerella* genera (W/S clade), presently comprising more than 100 species, share the metabolic unusual trait known as fructophily, that is, the preference for fructose over all other sugars, including glucose. The common ancestor of the W/S clade seems to have lost genes essential for alcoholic fermentation (encoding pyruvate decarboxylase and alcohol dehydrogenase). Alcoholic fermentation was subsequently reinstated in this clade through horizontal acquisition of a bacterial alcohol dehydrogenase.

The preference for fructose of these yeasts seems to be linked to the fact that fructose can be directly converted into mannitol; in a reaction that regenerates NADP+. Production of mannitol in this clade seems to play a role so crucial for the metabolism of these yeasts that *St. bombicola* (and other W/S- clade species) established a novel pathway to convert glucose into fructose, thereby allowing the yeast cells to be able to produce mannitol even when glucose is the only available carbon source.

These results suggest that mannitol production in the W/S clade most likely had an essential contribution to alleviate the negative effects on the redox balance that resulted from the ancient loss of alcoholic fermentation in this clade.

P140. Influence of peptidoglycan amidation on the cell wall hydrolytic machinery

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Peptidoglycan (PG) is a dynamic macromolecule that undertakes several secondary modifications during its biosynthesis. The MurT-GatD enzymatic complex is necessary for the amidation of glutamate of the stem peptide of *Staphylococcus aureus* PG, a reaction that influences critical processes of the bacteria, such as growth rate, beta-lactam and lysozyme resistance (1). Yet, the mechanisms through which it influences *S. aureus* physiology stand unknown.

S. aureus strain COL and isogenic mutants defective for PG amidation were studied for patterns of cell wall hydrolysis and autolysin expression by performing: Triton-X 100 induced autolysis assays; hydrolysis of heat-inactivated cells with recombinant PG hydrolases; zymographic assays, using autolytic extracts of parental and mutant strains to digest cells with amidated and non-amidated PG; Western Blot with antibodies raised against Atl catalytic domains; and promoter fusion assays to assess *atl* and *sle1* transcription.

Our results demonstrate that the rate of autolysis for living cells is decreased upon impairment of PG amidation. However, non-amidated PG is more prone to digestion by the autolysin Atl, as shown by hydrolytic assays of suspensions of heat-inactivated cells. Zymograms containing mutant cells also showed that non-amidated PG is more efficiently digested by autolysins. Furthermore, fewer hydrolytic bands developed for the autolytic extracts of the amidation mutant strains, suggesting an altered expression of autolysins when PG amidation was impaired. Amidation mutants were confirmed to have reduced transcriptional and translational expression of Atl and Sle1, as well as reduced Atl processing. These results suggest that the autolytic system of *S. aureus* could be regulated in response to the lack of PG amidation, conferring protection to cells with non-amidated PG, which is more prone to digestion.

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P141. Mutagenesis and functional analysis of a bacterial multitask nucleotide-binding domain of type I ABC carbohydrate importers

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ATP-binding cassette (ABC) proteins are generally part of complexes that mediate transport of molecules across cellular or organellar membranes. ABC transporters consist of two transmembrane domains (TMDs) and two cytoplasmic nucleotide-binding domains (NBDs or ATP-binding domain). Bacterial ABC-type I importers comprise an additional dedicated extracytoplasmic substrate-binding protein (SBP). MsmX is an orphan NBD from *B. subtilis* that we have recently shown to interact with several distinct ABC type I sugar importers. Since sharing of an ATPase among carbohydrate ABC transporters in both Gram-positive and Gram-negative bacteria seems to be a common strategy for adaption and survival it may represent novel therapeutic approaches for drug targeting because ABC importers are exclusive to prokaryotes.
In this work, the primary sequence of MsmX was aligned with several NBDs, from both Gram-positive and Gram-negative bacteria. The group of proteins used in this bioinformatic analysis comprised both NBDs that were able to substitute functionally MsmX in *B. subtilis*, and NBDs unable to perform this function. The multiple sequence alignment highlighted amino acids conserved in all sequences of NBDs from different species (*B. subtilis*, *B. thuringiensis*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Clostridium difficile*) able to substitute functionally MsmX but not present in proteins (from *Escherichia coli*) unable to execute this function. The majority of these residues is located between position 60 – 179 of MsmX, which comprises the region for NBD-TMD interactions. The targeted amino acids were substituted by alanine by site-directed mutagenesis and the impact of the mutation in the functionality of each MsmX variant evaluated in vivo. By determination of the growth kinetic parameters in the presence of the substrate of the AraNPQ importer, the results revealed a different degree of capacity of each mutant NBD to act as energy generator of the transporter. In addition, accumulation of the MsmX variants was detected by Western-blot analysis. Our work presents, for the first time, a site-directed mutagenic study of a multitask NBD domain of a bacterial ABC-type I importer. We identified amino acid residues important for the MsmX function as energy generator of the AraNPQ sugar transporter. Furthermore, the construction of chimeric MalK*(E. coli)*-MsmX*(B. subtilis)* proteins provided insights into the map of MsmX functional domains.

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P142. Identification of Novel Multitask ATPases of ABC-type I importers through interchangeability studies among different bacteria

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ABC-type transporters comprise one of the largest and most diverse transporter superfamilies. They are characterised by a highly conserved ATP-binding cassette (ABC), which couples substrate transport across the membrane by hydrolysis of ATP. Contemporary in all living organisms, these transport systems share a common molecular structure composed by the transmembrane domain (TMD) and the nucleotide-binding domain (NBD, ABC or ATPase). Although NBDs were thought to exclusively energize specific transport systems, different ABC-type I importers sharing the same energy-generating component were discovered. Recent studies performed in our laboratory revealed that, unlike other NBDs, *Bacillus subtilis* ATPase MsmX interacts with different ABC sugar importers and thus it is considered to be multitask. Sharing of an ATPase among carbohydrate ABC transporters in bacteria seems to be a common strategy for adaption and survival, and may portray novel therapeutic approaches for drug targeting, since ABC importers are exclusive to prokaryotes.

To characterise multipurpose ATPases and assess their intra- and interspecies interchangeability, we engineered a genetic system in *B. subtilis* for controlled gene expression in trans. The functionality of multitask ATPase alleles from *B. subtilis*, *B. thuringiensis*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Clostridium* difficile was assessed by their ability to complement the role of MsmX as energy generator of distinct sugar importers in a *msmX*-null background. All NBDs were able to energize AraNPQ and GanSPQ sugar importers. Growth kinetic parameters were determined in the presence of both importers' substrates, revealing the ability and different degrees of efficiency of each NBD to substitute MsmX in the *msmX*-null mutant. Moreover, heterologous NBD accumulation in *B. subtilis* was confirmed by Western Blot analysis. These results establish *B. subtilis* as model for the study of bacterial multitask ATPases from Gram-positive pathogens involved in carbohydrate transport. Thus far, in our laboratory interchangeability of ATPases has been established within the Firmicutes phylum. Here, we also show that a putative NBD from cyanobacteria *Synechocystis* sp. is able to substitute *B. subtilis* MsmX suggesting interspecies interchangeability of multitask ATPases beyond the Firmicutes phylum.

P143. The role of the multicopper oxidase in copper homeostasis in gram positive pathogens

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Copper ions are present in all cells, as an important cofactor for several enzymes and also as part of the immune system response to surpass infection. In addition, copper is used in animal food as an additive and is a promising antimicrobial agent due to the continuing emergence of multiresistant strains. However, free copper ions are highly toxic due to their ability to generate reactive oxygen species, via Fenton-type reactions, which damage lipids, DNA and proteins. For this reason, it is crucial for any organism to control tightly the intracellular concentration of free copper ions, a role played by dedicated homeostasis systems, described extensively in gram negative bacteria (*cop* and *cus* operons). In *Staphylococcus aureus*, the homeostasis system is composed by ATPase copper transporters, copper chaperones, copper transcription regulators and more recently a multicopper oxidase and a surface-exposed lipoprotein were described as also playing a role in resistance.
The aims of this study are to biochemically characterize the multicopper oxidase and establish its involvement in the copper homeostasis in this gram positive pathogen, as well as in *Enterococcus faecium*. The multicopper oxidase genes were cloned for heterologous expression in *Escherichia coli*, purified to homogeneity, and biochemically characterized. In addition, the *in vitro* phenoloxidase and peroxidase activity was studied for the purified protein, as well as for cell extracts. The minimal inhibitory concentration (MIC) to copper was estimated for different *S. aureus* strains in aerobic, microaerobic and anaerobic growth conditions. Copper resistance was observed to be oxygen- dependent and strain-dependent. The biofilm formation in the presence of copper was also analysed and preliminary results show that for some strains the biofilm formation increased in the presence of copper ions, suggesting that copper may enhance *S. aureus* pathogenicity.

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II2.Molecular Microbiology and Microbial Physiology

P144. Pneumococcal adaptation: from colonization to disease

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Streptococcus pneumoniae is a Gram-positive colonizer of the human nasopharynx that is responsible of several diseases such as pneumonia, bacteremia and meningitis. The transition from asymptomatic colonization to disease is accompanied by drastic changes in host physiological conditions such as temperature, nutrient availability and pH, and in the mode of growth of the pathogen – from biofilm to planktonic. The pneumococcal within-host adaptation mechanisms during this transition are still poorly understood.

We aimed to investigate the differences in the phenotype of invasive (isolated from normally sterile body sites) and carriage (isolated from nasopharyngeal aspirates) pairs of strains obtained from patients with invasive pneumococcal disease. We characterized strains obtained from ten patients: in eight cases the carriage and invasive strains obtained from an individual had the same capsular type and genotype; in the other two cases two different strains were isolated in each individual. Strains were minimally passaged in the laboratory in order to retain their original characteristics. Whole genome sequencing analysis was carried out for all strains. Phenotypic characterization consisted on determining planktonic growth rates, capacity to form biofilm and ability to disperse from biofilm.

Zero to five single nucleotide polymorphisms (SNPs) were detected when strains with a matching serotype from the same individual were compared. Despite these minimal genomic alterations, significant differences were observed on the phenotypic assays. Planktonic growth rates and maximum optical densities in liquid medium varied across pairs of strains with no general tendency. Still, significant differences were found between strains of four pairs. In the biofilm model, invasive strains had higher or comparable cell counts than the corresponding carriage strain. In all cases but one, invasive strains had higher biofilm dispersal rates. Further characterization is ongoing.

The results suggest that *in vivo* adaptation occurs in *S. pneumoniae* during the transition from carriage to invasive disease resulting in strains with different phenotypes, which are unlikely to be explained by genomic differences alone.

P145. New Staphylococcal Transporter Involved in Survival to Cell Wall Damage

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Studies on peptidoglycan biosynthesis and in resistance to β-lactams in the human pathogen *S. aureus* led to the construction of a *murF* mutant in the background of the MRSA strain COL. The MurF protein is a Mur ligase responsible for the addition of the terminal D-alanyl-D-alanine residues to the stem peptide of peptidoglycan. This mutation is responsible for an abnormal incorporation of tripeptide in the peptidoglycan that results in decreased cell viability and β-lactam antibiotic resistance. A transcriptomic analysis of the *murF* mutant, by microarrays, allowed to identify a gene, annotated in the databases with unknown function, to be overexpressed, suggesting to be related with the capacity of the mutant to survive to the cell wall damage. To address this possibility, *in silico* analysis was performed and a conditional mutant was constructed in order to functionally characterize this gene which was named *scwd*, standing for Staphylococcal cell wall damage. *In silico* analysis revealed that *scwd* encodes a transmembrane protein with 10 spanning domains which can form a pore across the membrane. Moreover, the protein is predicted to have two EamA domains of opposite folding, which is a signature of the drug/metabolite exporter family.
A conditional mutant COLpcadscwd was constructed and the resistance to β-lactam antibiotics was assessed by oxacillin disk diffusion testing. The results showed that the impairment or absence of Scwd did not have an impact on growth neither on the β-lactams resistance level. However, the expression of the gene was essential for the normal growth of the mutant in the presence of sub-inhibitory concentrations of oxacillin in liquid medium. The fact that Scwd has a role in resistance to oxacillin in liquid medium, but not in solid medium, suggests a role for Scwd as an exporter of an unknown component to the external medium, that would be important for *S. aureus* survival to a cell wall stress.

P146. Functional characterization and structure prediction of Igt1, a new dual-affinity transporter from *Torulaspora delbrueckii*

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Torulaspora delbrueckii is a yeast phylogenetically related to *Saccharomyces cerevisiae* that is receiving increasing attention from the biotechnology industry, with particular relevance in the wine, beer and baking sectors. Most biotechnological applications of yeast rely on their ability to efficiently ferment a great variety of sugars. This property is closely related to their sugar transport capacity, which has been widely considered a rate limiting step of sugar metabolism. However, little is known about *T. delbrueckii* sugar transporters and their sugar transport capacity. Actually, only one glucose transporter, Lgt1, has been characterized so far. Here, we report the identification and characterization of a second glucose transporter from this yeast, Igt1. Kinetic analysis of sugar transport mediated by Igt1 in a hexose transport null mutant strain of *S. cerevisiae* showed that this transporter mediates uptake of glucose with intermediate and high affinity, depending on the sugar concentration. It is also able to mediate fructose and mannose uptake. Despite the different kinetic behaviour of the two transporters, Lgt1 and Igt1 display a high degree of sequence similarity. A whole-genome bioinformatics analysis of the reference strain *T. delbrueckii* CBS 1146, showed that LGT1 and IGT1 are probably part of a larger cluster of hexose transporters. This work contributes to a better characterization of glucose transport in *T. delbrueckii*, with relevant implications for its exploitation for the conduct of mixed or sole fermentations in the food industry.

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P147. Physiological and genomic approaches of *Shewanella algae* and *Shewanella xiamenensis* in decolorization of azo and antraquinones dyes

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Background: Textile industry frequently use azo dyes due to its high performance and low cost, despite being the most difficult group to degrade; described as a possibly mutagenic, carcinogenic and recalcitrant agent. Given this problem, the genus *Shewanella* emerges as a possible bioremediating agent due to its versatility to use a wide variety of substrates as final electron acceptor. Objective: The present study aimed to identify genes involved in azo and anthraquinones dyes degradation process.
Methods: The strains used were *Shewanella algae* 2NE11 and *Shewanella xiamenensis* LC6, to which growth kinetics and decoloration kinetics were performed. Dyes evaluated were Direct Blue 71, Methyl Orange, HEXL Proction Yellow and Remazol Bright Blue at 100 mg/l. The total DNA of the two strains was sequenced by SMRT RSII and the genomes were assembled with Unicycler and annotated with Prokka. Genes related to decolorization were identified and aligned with ESPript. The phylogeny of azoreductases was obtained from Neighbor joining, Maximum likelihood and Bayesian inference consensus with 10000 bootstraps.
Results: Both strains reach 90% decolorization rate after 12 hours of exposure to all dyes. Genome analysis allowed to identify the presence of an FMN-dependent NADH-azoreductase, FMN-dependent NADPH-reductase and heme-dependent Dyp peroxidase in both strains suggesting that it would be related to decolorization process of azo and anthraquinones dyes as previously described. Analysis of alignment and phylogeny of aminoacidic sequence reveals that an ACP phosphodiesterase gene in *S. algae* 2NE11 is close related to *azoR* (<50% similarity). Additionally, the active site and c-terminal sequence are conserved, suggesting that it could encode a new type of azoreductases.
Conclussion: *S. xiamensis* LC6 and *S. algae* 2NE11 decolorizate azo and anthraquinones dyes efficiently. An ACP phosphodiesterase gene in *S. algae* 2NE11 could belong to a new type of azoreductase not described previously.

P148. The role of DsrD in dissimilatory sulfate reduction

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Dissimilatory sulfate reduction (DSR) is an ancient biogeochemical process, which occurs in sulfate-rich anoxic environments, such as marine sediments. Sulfate-reducing organisms (SRO) are ubiquitous in these habitats and are able to perform DSR to generate energy, using sulfate as terminal electron acceptor and oxidizing hydrogen or a wide variety of organic compounds, linking the Sulfur and Carbon cycles [1,2].
In this pathway, sulfate is phosphorylated and reduced to sulfite. Then, DsrAB and its cosubstrate DsrC catalyse the four electron reduction of sulfite to a S^0 valence state, in the form of a trisulfide bound to DsrC by two strictly conserved cysteine residues [3]. This DsrC-trisulfide is suggested to be later reduced to sulfide and DsrC by the DsrMKJOP transmembrane complex through menaquinol oxidation, which allows coupling to proton translocation [3].
Despite years of research on this metabolic pathway, there are still fundamental questions left to answer, such as the role of DsrD. This is a small protein of ≈ 9 kDa that contains no cysteine residues, unlike DsrC, which makes it unlikely to be involved in electron transfer or sulfur chemistry. The importance of this protein in sulfite reduction is deduced by the conservation of the *dsrD* gene in all organisms that have a reductive DsrAB, and its presence in the same operon as the *dsrAB* genes, generally in a *dsrABD* arrangement [2].
Here we report studies on the physiological function of the DsrD protein, using a *dsrD* deletion mutant and *in vitro* studies with the isolated proteins. The results provide the first detailed characterization of the function of DsrD, showing by *in vivo* and *in vitro* assays that DsrD plays an important role, together with DsrAB/DsrC, on the respiratory metabolism of SRO.

P149. *Giardia lamblia* modulates LPS-induced pro-inflammatory response in macrophages through cleavage of NF-κB p65RelA by proteases

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Introduction: The protozoan *Giardia lamblia* is the most common cause of parasitic gastrointestinal infection worldwide. The parasite developed sophisticated, yet not completely disclosed, mechanisms to escape immune system and maintain a favorable habitat for gastrointestinal growth. To further understand the interaction of *G. lamblia* with host immune cells, we investigated the ability of parasites to modulate the canonical activation of macrophages (Raw 264.7 cells) triggered by the TLR4 agonist, lipopolysaccharide (LPS).
Material and Methods: Western blot analysis was performed to evaluate the effect of *G. lamblia* (trofozoites and extracts) on the activation of MAPKs and NF-κB signaling pathways, and to evaluate the levels of iNOS and COX-2. Gene transcription of TNF-α, IL1β, IL6, IL10, CCL3 and CCL4 was analysis by quantitative Real Time RT-PCR (qPCR). Proteinase activities in *Giardia* extracts were examined by zymographic assays.
Results: We observed that *G. lamblia* impairs LPS-evoked pro-inflammatory status in macrophages through inhibition of cyclooxygenase-2 and inducible nitric oxide synthase expression and subsequent NO production. This effect was in part due to the activity of three *G. lamblia* proteases, a 135kDa metalloprotease and two cysteine proteases with 75 and 63kDa, that cleave the p65RelA subunit of the nuclear factor-kappa B (NF-κB). Moreover, TNF-α and CCL4 transcription was increased in the presence of the parasite. Overall, our data indicates that, in order to successfully colonize small intestine, *G. lamblia* could modulates macrophages inflammatory response through impairment of the NF-κB, thus silencing a crucial signaling pathway of the host innate immune response.
Conclusion: The knowledge of the intracellular signaling profile modulated by *Giardia* parasites in host immune cells highlight putative molecular targets for further development of new therapeutic strategies against giardiasis.

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P150. The relevance of taxonomy in biotechnologically important microalgal class Eustigmatophyceae

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The biotechnological importance of microalgae is no longer new to the biotechnological academic and industrial sectors. Applications of microalgae and their derivatives in all fields, from nutrition to health, are implemented in society. New strains are being studied and tested as novel sources of biomolecules. The taxonomy and species names of the organisms are sometimes not yet fully clarified and validated, which creates instability in the species names use in non-taxonomic work. This may originate replication of mistakes in the use of species names and this may impact on the scientific output of the study. Taxonomic studies are therefore critical to provide a baseline for the correct use of species names.

Before molecular methods were routinely employed, the taxonomic diagnostic was performed relying almost solely on morphological characteristics. Cell structures difficult to perceive by microscopy, morphological plasticity, different outputs achieved with preparative techniques or microscopes are limitations of morphologically-based only taxonomic determinations. Recent molecular taxonomy studies revealed however, that molecular data must be complemented with morphological data in order to properly establish microalgal taxonomy.

The Eustigmatophyceae is a class of nearly ubiquitous yellow-green microalgae, with a peculiar story. The class was established to accommodate misplaced xanthophytes. Its deployment has progressed slowly but consistently, either by the transfer of misplaced taxa or by the isolation of new strains. The extensive eustigmatophyte collection held at the Coimbra Collection of Algae (ACOI) has recently been a key source of strains for taxonomic studies, with the description of a new family with new genera and the clarification of the taxonomy and phylogeny of genera *Characiopsis* and *Pseudostaurastrum*.

There is a growing interest of the biotechnology community to explore their potential. Long-term focus on oleaginous *Nannochloropsis* (and *Microchloropsis*) for biodiesel, food and feed has diverged. New eustigmatophytes are being surveyed and results show a wider application of the compounds found in the tested biomass namely lipids, carotenoids and antioxidants.

The disclosed biotechnological value of Eustigmatophyceae highlights the importance of taxonomical clarification of the class, in order to provide an accurate and consensual taxonomic base of understanding linking academic and applied research with these organisms.

P151. Screening of Lactic Acid Bacteria strains for biopharmaceutical-grade plasmid and recombinant protein production

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Production of recombinant proteins and DNA based vaccines in microbial systems have revolutionized the biopharmaceutical industry since the beginning of the century. Lactic acid bacteria (LAB) has gained increasing interest in this field for their GRAS status and for usually carrying food- grade vectors. Challenges on the efficiency of LAB as microbial cell factories were observed in studies with *Lactococcus lactis* spp. *lactis* LMG 19460 in terms of low concentration and fast degradation of heterologous plasmid production. The screening of several LAB strains was made to find a better candidate for plasmid replication with a high yield, high quality and low degradation rate as a result. Growth conditions were tested on *Lactococcus* and *Lactobacillus* strains, including temperature (30º-43ºC), medium composition (MRS and M17 media) and agitation (0 and 100 rpm). Growth rates in *Lactobacillus* are higher with MRS medium, and with M17 in *Lactococcus*, a streptococcus specific media used in the studies with LMG 1946. *Lactobacillus plantarum* CCUG 61730 is the most temperature resistant strain, showing the least growth rate alterations with temperature variations. As heat stress is commonly encountered by many LAB and many genome editing systems use heat strategies, this may be a relevant advantage. Antibiotic susceptibility screening from distinct classes of antibiotics was performed and demonstrated a high resistance (>2000 mg/mL) of all strains to Neomycin, Apramycin and Spectomycin except the two *Lactococcus lactis* spp. *cremoris* tested in which the MIC was significantly lower (200-500 mg/mL). Erythromycin and Chloramphenicol showed particularly low MIC values making them appropriate to use as resistance markers. Finally, transformation feasibility was analyzed by electroporation, in order to evaluate which strain delivered the highest yield and best quality pDNA. *Lactococcus* showed better transformation outcomes and at least one strain (*Lactococcus lactis* spp. *cremoris* MG 1363) was capable to produce higher pDNA concentrations when compared to our reference strain. Evidence from this work suggest that a more suitable candidate is available, and the screen of its physiological and molecular properties can lead to an efficient gene expression protocol with a high range of applications in the fields of bioengineering.

P152. Robust industrial strains as platform for de *novo* resveratrol production from carbon sources: establishing grounds for an integrated process

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The search for robust microorganisms is essential to design sustainable and industrially attractive processes. *Saccharomyces cerevisiae* has a wide range of applications in the food industry, being also a valuable platform as a robust cell factory to yield several chemicals of interest, from biofuels to high-value natural products. Resveratrol is a polyphenolic antioxidant compound, generally extracted by a complex and low-efficiency process from the roots of Japanese knotweed, which makes it dependent on the supply of plant resources and environmental factors. Alternatively, it can also be synthesized chemically, but this method is also very complex and polluting. Considering this, its production through microbial biosynthesis can be a valuable alternative to side these drawbacks, nonetheless is generally achieved at the cost of expensive substrates as p-coumaric acid. *de novo* resveratrol production from glucose was recently reported in laboratory haploid yeast strains[1]. Stemmed on this knowledge, here, a set of robust industrial diploid strains was engineered using the CRISPR/Cas9 system and screened for its aptitude for resveratrol production. Initially, strains were engineered with Tyrosine ammonia lyase, which converts tyrosine to p-coumaric acid, in order to evaluate p-coumaric production, a key precursor in the resveratrol pathway. From here, p-coumaric- producing strains were selected and successfully engineered for *de novo* resveratrol production using glucose as sole carbon source, by overexpressing four plant genes, named resveratrol central pathway (RCP). The influence of the pentose phosphate pathway (PPP) on the *de novo* resveratrol production was then assessed. An alternative version of the top-producing strain, where four genes of the PPP were previously overexpressed, was used as platform for the integration of the RCP genes. The combination of these genes revealed a significant improvement in resveratrol titre. Altogether, this work establishes grounds for the development of an integrated biomass-to-resveratrol process in an industrial context.

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P153. Staphylococcal clonal lineages associated with skin and soft tissue infections in humans and pets

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The aim of this study was to evaluate the genetic diversity of staphylococci associated with skin and soft tissue infections (SSTIs) in humans or pets and to identify the main clonal lineages present in the collec-tion studied. The collection comprises all isolates associated with SSTIs identified as *S. aureus* (n=55), *S. epidermidis* (n=14) or *S. schleiferi* (n=28) in two veterinary research laboratories in Lisbon, over 19 years (1999-2018); as well as isolates of *S. aureus* (n=34) associated with human SSTIs identified in a clinical diagnostic laboratory in Lisbon from February to June 2014.
All staphylococcal isolates were subjected to molecular typing by *Sma*I-PFGE. The *Sma*I macrorestriction profiles, resolved by pulsed-field gel electrophoresis (PFGE), were analysed with the aid of the Bionumer-ics software, using the UPGMA algorithm. *S. aureus* isolates representative of each identified pulsotype were selected for further characterization by multilocus sequence typing (MLST), performed according to the scheme available in PubMLST database. For representation of the possible relationships between clonal lineages the Phyloviz freeware was used with goeBURST algorithm.
The *S. epidermidis* and *S. schleiferi* associated with SSTIs in pets showed high genetic diversity, with 11 and 10 pulsotypes identified, respectively. Regarding *S. aureus*, the isolates of human origin presented a higher genetic diversity when compared with *S. aureus* of animal origin (16 vs 15 pulsotypes for 34 vs 55 isolates, repectively). The *S. aureus* clonal lineages identified by MLST corroborated *Sma*I-PFGE typing. Amongst the isolates of human origin, the lineages belonging to CC5 (ST5, ST105) were the most fre-quent (11/34 isolates) whereas CC22 (ST22) was the most frequent lineage among animal isolates (25/55 isolates). Some of the clonal lineages found (CC5, ST7, ST72, ST97, ST15, CC22) were detected among *S. aureus* of both human and animal origins.
This study indicates the high diversity of various staphylococcal species associated with SSTIs in pets or humans. Of utmost importance, our findings pinpoint the frequent presence of nosocomial associated *S. aureus* lineages among strains from human or animal SSTIs outside the hospital environment.

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P154. Exploring the efflux-mediated multidrug resistance in staphylococci

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In the last ten years, our group has been dedicated to the study of the efflux-driven re- sponse of staphylococci to the challenge of antibiotics and non-antibiotic, such as ethidium bromide (EtBr), a substrate of a wide range of bacterial efflux pumps (EPs). We have shown that exposure of two reference strains, *Staphylococcus aureus* ATCC25923 and *Staphylococcus epidermidis* ATCC12228 to EtBr resulted in two adapted strains, ATCC25923_EtBr and ATCC12228_EtBr, with a multidrug resistance (MDR) phenotype, which included resistance to fluoroquinolones and decreased susceptibility to biocides (eg., cetrimide and benzalkonium chloride). These MDR phenotypes were accompanied by an increased efflux activity which was linked to overexpression of the *norA* EP gene [1,2].

To further explore the pathway leading to the *norA* efflux-mediated response, we now studied the transcriptomic profile of both pairs of isogenic strains, in the presence of a sub-inhibitory concentration of EtBr. Total RNA was isolated and rRNA depleted. A cDNA library was prepared and sequenced in a HiSeq instrument (Illumina). The resulting single-end reads were checked for quality control and assembled against the respective reference genome with HISAT2 v2.1.0. Two approaches were used in parallel for differential expression analysis, one based on NOISeq package v2.26.1 and another on Gfold v1.1.4. Transcripts were considered differentially expressed between the adapted and parental strains if log fold change was higher than 1. Only transcripts identified by both approaches were considered differentially expressed and further analysed for functional classification. The preliminary analysis of data suggests alterations in the carbohydrate and aminoacid metabolism for both species, as well as variations in membrane transport and quorum-sensing genes for *S. aureus* or varia-tions in lipid and energy metabolism for *S. epidermidis*.

This approach may provide new light into the cellular pathways that allow the bacteria to cope with stress to EtBr, a model molecule for EP substrates. Moreover, it allows the identification of common and differential pathways that lead to EP activation in these two important staphylococci.

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P155. Comparative analysis of *Shewanella xiamenensis* LC6 transcriptome during degradation of two azo dyes

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Background: Textile industry generates the largest volumetric amount of residual effluents. One of the most concentrated pollutants in textile effluents is the synthetic dyes used during dyeing. Recently, efficiently and suitable method for remove dyes has performed through bacterial biodegradation. Due to its great biodecolorization activity, the genus *Shewanella* has been studied intensively.
Objective: Profile the *Shewanella xiamensis* LC6 transcriptome during decolorization of two azo dyes with different polarity.
Methods: A comparative study of the *S. xiamenensis* LC6 transcriptome was performed while growing in minimal medium before (Control) and after challenged with two separate dyes: Methyl Red (MR) and Methyl Orange (MO) under microaerophilic conditions. cDNA (previously rRNA depleted) was sequenced by NovaSeq (Illumina®), insert length was 250 bp.
Results: From at least 4200 mapped genes, 417 differentially expressed genes (DEGs) were obtained under MO/Control conditions and 183 DEGs under MR/Control (log2FC> 1, FDR <0.05). 112 genes were upregulated under both comparative conditions. Notably *azoR*, an azoreductase related to the specific cleavage of azo bond, was more intensely expressed under MR respect to MO exposure, suggesting its main intracellular activity. Additionally, genes coding for efflux pumps were overexpressed (*bepE*, *bepF*) suggesting greater intracellular stress during MR compared to MO conditions. On the other hand, during degradation of MO, the intensified use of Fe (as heme groups) would correspond to an intense activity of the decahaem cytochromes from Mtr pathway (genes such as *hutX*, *hutZ* and *HxuC*). Other pathways such as glucose metabolism (*pgcA*), tricarboxycyclic acid cycle (*acnD*) and urea cycle (*argF*) were altered. Finally, at least 5 hypothetical gene and 1 new transcript with unknown functions were overexpressed in both conditions.
Conclussion: *Shewanella xiamenensis* LC6 uses both pathways during the decolorization process: azoreductase-mediated and extracellular electron transfer process. Although the intensity of these responses will depend on the polarity of the dye.

P156. Diversity of *Diaporthe* species on *Vaccinium corymbosum* plants in Portugal

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The blueberry (*Vaccinium corymbosum*) is a non-native and increasingly cultivated crop in Portugal and represents an important part of the economy of the country, particularly in the Sever do Vouga region. The fast growing of this crop, due to its health benefits, has been associated with the emergence of several fungal diseases. Although twig blight and dieback symptoms typical of the genus *Diaporthe* have been observed in Portuguese blueberry growing areas, the identification has been merely based on the morphology of the symptoms. Given that no exhaustive studies have been carried out to identify the etiological agents of the symptoms observed, this study aimed to fulfill this gap by studying the diversity of *Diaporthe* species associated with blueberry plants in Portugal and evaluate their pathogenicity to this host. For this, a collection of 90 fungal isolates obtained from asymptomatic and symptomatic blueberry plants was characterized. Fungal isolates were initially subjected to Microsatelite-Primed PCR (MSP-PCR) fingerprinting to evaluate their overall genetic diversity. From this analysis, 22 isolates representative of each cluster were selected for further molecular characterization based on a multi-locus analysis of the rDNA internal transcribed spacer region (ITS) and the protein-coding genes: translation elongation factor 1-alpha (*tef1-α*), β-tubulin (*tub2*), calmodulin (*cal*) and histone 3 (*his3*). The phylogenetic analyses placed the isolates into six distinct clades representing two known *Diaporthe* species (*D. foeniculina* and *D. rudis*) and four new species (*Diaporthe phillipsii*, *D. crousii*, *D. rossmaniae* and *D. vacuae*) with *D. crousii* being the most abundant. The new species were fully characterized in terms of morphology, ability to grow at different temperatures and their mating strategy. This study revealed the occurrence of a diverse assemblage of *Diaporthe* species associated with twig blight and dieback of blueberry plants in Portugal. However, their impact as pathogens of blueberry is not yet understood.

P157. Metabolic adaptation of *Staphylococcus aureus* to antimicrobial nitrosative stress imposed by host innate immunity

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Staphylococcus aureus is a major cause of life-threatening infections due to the widespread occurrence of strains that are resistant to antibiotics. The infection of human phagocytes by *S. aureus* stimulates the production of nitric oxide (NO.), but *S. aureus* has unique abilities to resist the deleterious effect of this molecule (1-3). The human nasopharynx is the primary colonization niche of *S. aureus*, where this bacterium binds to the nasopharyngeal mucus mucins. In this environment, *S. aureus* encounters high concentrations of NO.. In contrast to other niches, the mucus mucins of the nasopharynx contain lower amounts of glucose, which is the preferable carbon source of *S. aureus*, but instead are rich in slow-metabolizing carbon sources such as galactose. Here, we have used deep- sequencing transcriptomic analysis (RNA-Seq) and 1H-NMR to uncover how *S. aureus* survives NO stress when grown on galactose and the impact on the virulence of this human pathogen. We show that this resistance is achieved through a distinct metabolism that relies on the increased production of amino acids, such as glutamate, threonine, and branched-chain amino acids (BCAAs) (4). Furthermore, we found that the *S. aureus* α-acetolactate synthase (ALS) enzyme, which converts pyruvate into α- acetolactate, contributes to *S. aureus* resistance to NO stress. ALS is also proposed to prevent intracellular acidification, to promote the production of BCAAs and to activate the TCA cycle. Moreover, ALS is shown to successfully contribute to the infection of murine macrophages. Interestingly, ALS contributes to the resistance of *S. aureus* to beta-lactam antibiotics such as methicillin and oxacillin.

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P158. Using metabolomics to assess the effect of the antimicrobial carbon monoxide-releasing molecule-3 in *E. coli* central metabolic enzymes

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The lack of alternative antibiotics is undermining our capacity to treat the infections caused by emergent antibiotic resistant bacteria. Carbon monoxide-releasing molecules (CORMs) are an alternative class of antibiotics attested for the capacity to kill several bacteria, including antibiotic resistant strains (1, 2). These molecules consist mostly of transition metal carbonyl complexes that have bound carbon monoxide (CO) and other stabilizing ligands. Importantly, they release CO in a controlled and targeted way in specific tissues but not in blood and they are innocuous to several eukaryotic cells (3). Altogether, these characteristics make CORMs promising antibiotics, however the lack of knowledge regarding their mode of action hampers their development. By using a metabolomics approach, that combines metabolite profiling by 1H–NMR analysis with liquid chromatography-tandem mass spectrometry, and enzymatic assays studies, we show that independently of the atmosphere to which *E. coli* is exposed, the prototype CORM-3 targets iron-sulphur enzymes of the tricarboxylic acid (TCA) cycle, e.g., aconitase, and fumarase, and also glutamate synthesis (4). Supplementation of the *E. coli* growth medium with the products of the activity of these enzymes revert the toxic effect of CORM-3. This inhibition is not observed when a control compound that lacks CO (iCORM3) is used instead of CORM-3, indicating that the inhibitory effect of CORM-3 is dependent on CO. In accordance, the use of the turn-on fluorescent probe COP-1 detected significant amounts of CO released intracellularly in *E. coli* exposed to CORM-3, while no CO was detected in iCORM-3 exposed cells. In order to recover from the CORM-3 stress, *E coli* cells activate glycolysis to establish an energy and redox balance (4). Altogether, this work reveals that the antimicrobial action of CORM-3 results from intracellular glutamate deficiency and inhibition of nitrogen and TCA cycles.

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P159. Using flow cytometry to monitor the stress response of yeast and microalgae populations in mixed cultures

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Recently, the use of yeast and microalgae mixed cultures have been widely used in biological effluent treatments and biofuels production, since such cultures show many advantages over pure cultures, specifically their symbiotic relationship that reciprocally favors both microorganisms (Dias et al., 2019). However, since effluents contain toxic compound that may inhibit cell (yeast and microalgae) growth, it's important to evaluate the cell stress response when growing in such conditions. Flow cytometry (FC) is a technique that provides single cell information in microbial populations near real time, by means of light-scattering and fluorescence measurements. By analyzing individual cells, FC detects a variety of physiological and metabolic states of different subpopulations, using light scattering or fluorescence emission. There are two types of light scatter: the forward and the side scatter (FSC and SSC, respectively). FSC is proportional to cell-surface area or size and SSC is proportional to cell granularity or internal complexity. Based on this information, it's possible to differentiate yeast and microalgae cells as they have different sizes and levels of internal complexity. Therefore, when working with mixed yeast and microalgae cultures, FC allows distinguishing the two populations of microorganisms, in a rapid and easy way. In this work, FC coupled with fluorescent stains was used to characterize the cell stress response of *R. toruloides* and *S. obliquus* cells in a mixed culture. The mixed culture was subjected to different conditions to induce different physiological states: metabolic active exponential growing cells, heat-treated dead cells and cells growing under nutrient starvation. Then cells from these mixed cultures were analyzed by FC after stained with two dyes: SytoxGreen, to evaluate the cell integrity membranes, and Carbofluorescein diacetate (CFDA) to assess the cell enzymatic activity. Using this approach, it's possible to differentiate, in a mixed culture, *R. toruloides* cells with permeabilised membrane from *S. obliquus* cells with permeabilised membrane when stained with SytoxGreen, as well as cells with enzymatic activity of the two microorganisms, when stained with CFDA. This work describes a simple and easy method to monitor individual stress response of *R. toruloides* and *S. obliquus* growing in a mixed culture, using FC.

II2.Molecular Microbiology and Microbial Physiology

P160. A synthetic biology approach using a cyanobacterial chassis for the production of specialty compounds, namely compatible solutes

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Cyanobacteria are promising “low-cost” cell factories since they have minimal nutritional requirements, high metabolic plasticity and use sunlight and CO_2 as energy and carbon sources. The unicellular *Synechocystis* sp. PCC 6803 is the best studied cyanobacterium and is already considered the “green” *Escherichia coli*1. In order to use this organism as an efficient and robust photoautotrophic chassis, a customized and well-characterized molecular toolbox is being developed. In this context, a set of heterologous and redesigned promoters was characterized in *Synechocystis* showing a wide range of activities, and the use of three self-replicative vectors from the SEVA repository2 was also validated for this cyanobacterium3. Currently, these tools are being used for the assembly and implementation of synthetic devices meant for the production of specialty compounds, namely compatible solutes. Compatible solutes are a group of low-molecular weight organic compounds, usually with no charge, that are synthesized as a mechanism to cope with environmental stresses such as temperature, salt or drought4. These compounds have commercial interest for cosmetic, biomedical and food industries due to their interesting protective and stabilizing properties5. As a strategy to redirect the metabolic fluxes of the chassis for the production of heterologous compatible solutes, knock-out *Synechocystis* mutants in the main native compatible solutes’ pathways (sucrose or/and glucosylglycerol) were generated. These mutants are being characterized in terms of growth/fitness (OD, chl a); total carbohydrates, capsular and released polysaccharides; and glycogen production. Moreover, the levels of transcripts of the genes involved in the production of compatible solutes are being evaluated, and the amount of compatible solutes produced is being quantified by NMR. In parallel, a *Synechocystis* genome-scale metabolic model (an updated version of the iSyn811)6 is being used to simulate/predict the behavior of *Synechocystis* wild-type and our chassis (knock-out mutants).

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P161. Adaptation of a one-plasmid genome editing system for increased production of biopharmaceutical grade recombinant molecules in *Lactococcus lactis*

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In recent years, the species of the *Lactococcus* genus have become subject to increasing focus as cell factories of heterologous proteins and DNA. Their extensive use in the food industry and therapeutics, has led to the increase in the molecular tools available for their manipulation, which, alongside their Gram-positive nature and generally regarded as safe status have turned them into prospective alternatives to the widely used *Eschirichia coli* as cell factories. These same characteristics have also led many to employ them as live bacterial vectors.
However, the *Lactococcus* spp. have notable drawbacks, as they typically have disappointing yields when producing heterologous protein or DNA. This is, most likely, due to the presence of endogenous nucleases and proteases and, as such, genome editing of *Lactococcus* spp. is required in order to fully utilize these bacteria. In this work, a one plasmid system, previously shown to be effective in generation of knockout mutants in *Streptomyces coelicor*, has been modified to allow it to function in *Lactococcus lactis*, with the gene of the lactococcal nuclease nth chosen as the target. This plasmid is composed of a Cas9 gene sequence, a sgRNA sequence and two regions termed homology arms (HAs) designed to substitute the targeted gene through homology-directed repair mechanisms. The necessary modifications were done using Gibson Assembly and included changing the vector backbone, whose low copy number origin of replication proved inefficient in *L. lactis*, and the promoter responsible for Cas9 expression, whose inducer was non-functional in *L. lactis*. The new vector backbone was the high copy number pTRKH3 plasmid whose origin of replication, pAMβ1, grants it remarkable stability. Additionally, it also possesses an erythromycin resistance marker proven to work in *L. lactis*. The promoter responsible for Cas9 expression was changed to the xylose inducible PxylT promoter due to its strong and tightly controlled expression. All necessary modifications have been achieved and attempts at the knockout of the *nth* gene are underway. The successful knockout of this gene would potentially provide us with a strain capable of increased production of recombinant molecules that would compensate for *L. lactis* strains most notable drawback.

P162. Extracytoplasmic function sigma factors: diversity, evolution, mechanisms and synthetic biology applications

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Bacteria frequently inhabit complex and fluctuating environments. Hence, continuously monitoring the environment and use that information to adjust their behaviour is crucial for survival. The three most abundant signal transduction systems in bacteria are one- and two-component systems, and extracytoplasmic function (ECF) sigma factors. These alternative sigma factors, alter the RNA polymerase promoter specificity and allow transcription of specific regulons in response to particular environmental stimuli. Here, I will summarize the latest results of our integrative approach to study the third pillar of bacterial signal transduction.

Comparative genomics studies concerning ECF sigma factors of Actinobacteria and Planctomycetes supported the expansion of the pre-existing ECF classification to include a larger number of sequences from previously unexplored phyla. Additionally, such analyses have revealed the existence of membrane-bound ECF sigma factors and those containing large N-terminal extensions.

This knowledge stimulated research on the mechanisms of activation of actinobacterial ECF sigma factors containing C-terminal extensions. Studies on ECF41 and ECF42 of *Streptomyces venezuelae* showed that the C-terminal extensions are necessary for the sigma factor mediated transcription initiation of small regulons.

The abundance, diversity and widespread use of ECF sigma factors by bacteria has inspired the exploration of their potential for synthetic biology applications. Several heterologous ECF sigma factors were implemented in *Bacillus subtilis* and their activity as genetic switches characterized in detail. Furthermore, the first ECF-based autonomous timer circuits were successfully developed.

Finally, the evolutionary history of the ECF protein family, the most abundant and diverse family of alternative sigma factors was investigated. A dynamic evolutionary history that includes adaptive duplications, sequestration of other genes, and horizontal gene transfer was revealed.

P163. High variability in the composition of the biofilm formed by *Staphylococcus saprophyticus* from different origins

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Background. *Staphylococcus saprophyticus* is frequently associated with urinary tract infections (UTI) in young women. One of the most important factors in the success of *S. saprophyticus* as uropathogen is its capacity to form biofilm but its composition and genetic basis remains unclear.
Methods. From a large collection of 460 isolates we selected 63, representing all sub-clusters and belonging to two *S. saprophyticus* lineages; the isolates were recovered from UTI (n=42) and food-related environment (n=21). The biofilm composition was assessed using biofilm detachment assay applying Proteinase K, DNase I, and sodium-periodate as disruptors. Wholegenome sequencing was performed by Illumina Miseq. Genomes were annotated (Prokka), pan-genome constructed (Roary), and association determined between genomic, demographic and phenotypic data using GWAS (Scoary).
Results. A total of 99% of *S. saprophyticus* (n = 62/63) were biofilm producers. Five types of biofilm matrix components were identified: protein-eDNA-polysaccharide (44%, n = 27/62); protein (35%, n = 22/62); protein-polysaccharide (13%, n = 8/62), protein-eDNA (6%, n = 4/62) and one strain with polysaccharide only. There was no significant association between specific matrix composition and *S. saprophyticus* lineages. However, biofilm composed of only protein and protein-polysaccharide was significantly associated with isolates from UTI (49%, n = 20/41, p = 0.0003; 17%, n = 7/21 p = 0.01 respectively) while biofilm containing proteineDNA- polysaccharides was significantly linked to food-related isolates (62%, n = 13/21, p = 0.0043). Genes putatively associated with biofilm formation such as *aas*, *atl*, *ebpS*, *uafA*, *sasF*, *sasD*, *sraP*, *splE*, *sdrH*, *sdrE* and *sdrC* were found in different frequencies but there was no correlation between their presence and biofilm production level or its composition.
Conclusions. There was a high variability in the composition of the biofilm formed by *S. saprophyticus*. All strains produced biofilms composed of protein, but the most common type contained protein, eDNA and polysaccharides. The biofilm components appear to differ between food-related and infection isolates, suggesting that modulation of biofilm composition could be a key step in *S. saprophyticus* virulence.

P164. Copper-mediated Growth and Morphological defects in *E. coli* MG1655 are caused by pleiotropic effects

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Copper (Cu) is an essential element, but toxic when excessed. Mechanisms for Cu toxicity include generation of reactive radicals (ROS), displacement of Fe from Fe-S cluster proteins or alternate metal ions from other proteins.

Here, we found that Cu (and not other metals ions) produces growth and morphology defects in *E. coli* MG1655 in a medium-dependent manner (1000 times more Cu is needed in LB than M9). In M9 plus low Cu (1-6 μM), growth was slightly affected but cells became smaller and round (fail to elongate but still dividing). At higher Cu (8-10 μM), growth was severely affected with larger cells. Cu higher than 10 μM was lethal with exacerbated morphology.

First, by using MG1655 mutants affecting ROS and Fe-S cluster proteins pathways (*sodA,B, C, cueO*, *iscA* and *sdaAB-tdcG*) we determined that Cu did not cause the aberrant phenotypes by inhibiting these pathways.

Next, we reasoned that penicillin-binding proteins (PBPs) could be involved, but due to the unexpected capacity of Cu to complex with Bocillin-FL, a direct relationship cannot be determined.

We confirmed that Cu produced outer membrane damage (activating the Cpx stress response) and the possible role of cell permeability for Cu-mediated defects (abnormalities were absent in *E. coli* 2443 (functional LPS) when compared to MG1655 (LPS-defective)).

Morphological effects of Cu cannot be explained by the most popular hypothesized mechanisms for Cu toxicity. Our results suggest a more complex situation in which Cu affects cell elongation and division, either directly or indirectly by mechanisms yet to be identified.

P165. Genome editing for improvement of food-grade lactic acid bacteria

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Lactic acid bacteria (LAB) are a heterogeneous group comprising several GRAS status, nonpathogenic and food-grade bacteria, such as *Lactococcus lactis*, that bring quality, safety and cost effective advantages as cell-factories, as an alternative to the widely used *Escherichia coli*. Thus, making a promising tool in new biomedical approaches as producers and deliverers of pharmaceutical- grade molecules.
This study aimed at the improvement of a *L. lactis* strain for generation of a high yielding plasmid DNA-producing mutant. The *L. lactis* LMG19640 strain is a good candidate for optimization for such applications due to its plasmid free status, however, it possesses a high rate of degradation of exogenous DNA. The silencing of an important endonuclease, encoded by the *nth* gene, is a suitable primary approach for obtaining an improved strain for production of pharmaceutical-grade plasmid DNA and heterologous proteins.
A λ-Red recombination system, designed by Datsenko & Wanner (2000) and usually applied in Gram-negative bacteria, was optimized for use in the *L. lactis* LMG19460 strain to remove the endonuclease *nth* gene. The recombinant proteins encoded in the pKD46 plasmid, were induced by L-arabinose to integrate a kanamycin resistance cassette, designed to contain homology arms to the *nth* gene and flanked by flippase (FLP) target sites, into the target site in the genome. Optimal conditions of growth and antibiotic concentrations were determined to allow efficient transformation and selection of the necessary plasmids in the Gram-positive bacteria.
The results reveal that the *nth* gene was successfully knocked-out by incorporation of the kanamycin gene. For a food-grade mutant, removal of the antibiotic resistance marker is necessary. Attempts of removing the cassette using a helper plasmid carrying FLP are being implemented. The effect of the *nth* knockout on the pDNA quantity and quality will be evaluated using several molecular biology techniques, after the mutant strain being transformed with the well characterized pTRKH3 plasmid.
A *L. lactis* strain with a knocked-out endonuclease would facilitate subsequent mutations and, thus, the generation of an improved food and pharmaceutical-grade cell-factory for application in novel therapies and biomedical strategies.

P166. Inhibition of Peptidoglycan Biosynthesis as a Strategy Against Drug-resistant *Mycobacterium tuberculosis*

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains as one of the top ten causes of death worldwide and is currently the most lethal infection. Ending the tuberculosis epidemic by 2030 is contemplated in the Sustainable Development Goals of the United Nations. However, the alarming rise of multidrug-resistant (MDR-) and extensively drug-resistant tuberculosis (XDR-TB) hinders this achievement. MDR-TB is caused by a strain resistant to, at least, isoniazid and rifampicin, while XDR-TB strains have additional resistance to any fluoroquinolone and at least one injectable agent.
Since isoniazid and rifampicin are the most successful first-line anti-TB drugs, resistance to these antibiotics is a major concern and requires the use of less efficient and tolerable second-line drugs. Thus, there is an urgent need to reexamine the therapeutic options against MDR-TB/XDR-TB. One strategy consists in evaluating the potential use of antibiotics that are not usually considered for TB treatment, like beta-lactams. This class of antibiotics comprises potent inhibitors of the penicillin-binding proteins that synthesize peptidoglycan (PG) and includes penicillins and carbapenems, which target D, D- and L,D-transpeptidases, respectively. Historically, beta-lactams have been excluded from standard TB management because *M. tuberculosis* is considered innately resistant to these antibiotics, mainly due to the presence of non-classical transpeptidases and a potent beta-lactamase, BlaC.
Here we present a screening performed on a collection of Portuguese clinical isolates of *M. tuberculosis* with diverse drug susceptibility patterns and one reference strain, H37Rv. The Minimal Inhibitory Concentration (MIC) to several beta-lactams, with or without a beta-lactamase inhibitor, clavulanate, was determined. Our preliminary results suggest that: (i) generally, addition of clavulanate reduces the MIC for beta-lactams by two to eight-fold; (ii) amoxicillin and ertapenem are the least efficient antibiotics, but when combined with clavulanate, amoxicillin is as potent as some carbapenems; (iii) within carbapenems, meropenem and biapenem have the lowest MIC values, combined or not with clavulanate. These findings are in accordance with previous studies, strengthening the notion that transpeptidases and beta-lactamase are therapeutic targets for *M. tuberculosis* eradication. Future assays with more clinical strains and mutants for PG biosynthesis genes will further elucidate on the clinical role of beta-lactams in TB.

P167. Targeting of the mycobacterial peptidoglycan: how mycobacteriophage Ms6 hydrolase compromises this cell barrier

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Mycobacteriophages are viruses that infect and lysis mycobacterial hosts at the completion of the lytic cycle in order to release progeny phage particles. The nature of the mycobacterial cell wall unique structure, where the highly cross-linked peptidoglycan is attached to an outer mycolic acids layer via the polysaccharide arabinogalactan, represents a striking challenge to mycobacteriophages which have evolved towards acquiring specialized and regulated lytic proteins that target the integrity of the different mycobacterial cell envelope layers.
Mycobacteriophage Ms6 accomplishes lysis by producing amongst other proteins, a peptidoglycan hydrolase (LysA) with N-acetylmuramoyl-L-alanine amidase activity that holds a central peptidoglycan- recognition protein conserved domain. Remarkably, the *lysA* gene generates two products designated Lysin384 and Lysin241 (produced, in frame, from an internal translation initiation codon), according to the size of the polypeptides produced. Ms6 mutants producing only one of the forms of LysA are viable, albeit defective in the normal timing, progression and completion of host cell lysis which suggests that both proteins act synergistically for complete and successful lysis of mycobacteria. These data indicate an intriguing and accurate regulation of these enzymes during the lytic cycle in order to achieve an optimal peptidoglycan hydrolysis. A bioinformatic analysis of several mycobacteriophage endolysins has predicted for Ms6 endolysin a modular structure organized into three domain types: an N-terminal domain with potential peptidase activity, a central Ami-2A catalytic domain and a C-terminal cell wall binding domain. Based on these predictions we further analyzed the catalytic activities of both Lysin384 and Lysin241 by HPLC. We observed different HPLC profiles for each enzyme consistent with muramidase and amidase activities in the case of Lysin384 and only with amidase activity in the case of Lysin241. Both enzymatic activities are required for efficient degradation of the extremely cross- linked and modified mycobacterial peptidoglycan and seem to be tightly regulated as they act sequentially.
This work uncovers a complete novel mechanism of peptidoglycan degradation and illustrates that bacteriophages have evolved a plethora of mechanisms adapted to efficiently destroy the different bacterial cell wall targets which may help designing strategies to defeat bacterial pathogens such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis.

P168. The role of bacteriocins in pneumococcal intra-species interactions

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Streptococcus pneumoniae is responsible for high morbidity and mortality worldwide. Disease is incidental and is preceded by asymptomatic nasopharyngeal colonisation in the form of biofilms. There are over 95 serotypes described but only around ten are prevalent in colonisation, suggesting the existence of intra-species competition for niche occupancy. How intra-species interactions shape the pneumococcal population structure and the molecular mechanisms determining these interactions remain poorly studied. Bacteriocins encoded by the bacteriocin-like peptide (*blp*) locus were previously implicated in these interactions. The aim of this study was to identify pneumococcal intra-species interactions and to investigate the contribution of bacteriocin production to these interactions. A collection of 20 epidemiologically relevant and three laboratory strains was studied. Each strain was transformed with fluorescent constructs containing either gfp (green fluorescent protein) or rfp (red fluorescent protein), generating two labelled strains. Single- and dual- strain biofilms of labelled strains in a 1:1 ratio (one GFP- and one RFP-labelled) were grown for 72h. Interactions between strains were assessed using flow cytometry to compare cell counts of each strain in single- and dual-strain biofilms. Three types of interactions were found: commensalism, amensalism and competition. The strongest phenotype was a negative interaction (amensalism) where one strain (PT1990) led to a 1000-fold decrease in the cell counts of another (PT7031). Deletion mutants for the bacteriocin immunity region (BIR) of the *blp* locus were constructed in the inhibitory strain PT1990 and tested in dual-strain experiments with the inhibited strain, PT7031. While the mutant still inhibited strain PT7031, it showed a 10-fold attenuation of the amensalism phenotype. In conclusion, we identified three types of pneumococcal intra-species interactions using a dual-strain biofilm model. The *blp*- bacteriocins seem to have a role in negative interactions although other factors are likely at play. Our study improves our understanding of the determinants of pneumococcal dynamics during colonisation and may guide alternative strategies to prevent pneumococcal infections.

P169. Molecular methods as a first assessment tool in the surveillance of cyanobacteria and cyanotoxins

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Cyanobacteria are an ancient group of photosynthetic microorganisms that cause severe damage to ecossystems and human health through the production of dense blooms and the release of harmful cyanotoxins with higher impact in freshwater systems (drinking, irrigation, recreational). In the study of these microorganisms microbiological methods englobe the collection of plankton net samples to the isolation and sub-culturing of cyanobacterial strains in order to identify the taxa and of the cyanotoxicity of the studied ecossystems. In molecular microbiology genetics has played an important role in the evaluation of the taxa composition and cyanotoxicity through the high availability of tools (primers) in its detection even directly from water samples. Therefore in this study water samples of small volume were submitted to molecular tecnhiques (genomic DNA extraction, PCR amplification and gel electrophoresis) to evaluate the presence of certain toxic cyanobacteria strains (one genus and three species) and of the cyanotoxicity of the sampled water samples through detection of toxic genes belonging to the cyanotoxins microcystins, cylindrospermopsins, anatoxin-a and saxitoxins. In total 24 water samples were collected from 12 distinct locations with a wordwide representation (Costa Rica, Cuba, Fiji, France, Indonesia, Mali, Portugal, South Africa, Spain, Thailand, USA, Vietnam) including new unsurveilled areas (e.g. Fiji and Mali). Percentage of detection, co-detection of cyanobacteria and cyanotoxins and relation to the type of system were evaluated. Results indicate that new identifications were obtained in the previous surveilled areas as well as in the unsurveilled areas. Also the relation of the presence of these microorganisms and their toxins with the type of system highlights concern to the risks that humans are exposed particularly regarding the socio-economic activities such as recreational and tourism that are undertaken in the analyzed ecosystems. This study reflects the need of applying molecular methods as a tool in the first assessment of toxic microorganisms obtained from environmental samples.

FP170. A host-carried Cr(VI) Whole-Cell Biosensor for detection of environmental contamination transfer to the food chain

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Cr(VI) is a highly toxic metal produced by anthropogenic activity which may impact the environment, affecting plants and animals. In plants, literature reports that chromium affects negatively plant growth but is generally poorly translocated after being absorbed by roots. Metal whole-cell biosensors (WCBs) have been reported as very useful tools to detect and quantify the presence of bioavailable fractions of certain metals in water and soil samples. In the current work, bacterial WCBs able to report Cr(VI) presence and plants growing on Cr(VI)-enriched soil/medium were used to assess the potential transfer of this metal to organisms of higher trophic levels, and the risk of transfer to the food chain (MEC/MCTI/CAPES/CNPq 400756/2012-9). Strains of *Ochrobactrum tritici* and *Nitrospirillum amazonense* were modified to become Cr(VI) WCBs by introducing plasmid pCHRGFP2. Upon exposure to Cr(VI), WCBs express GFP and emit a quantifiable fluorescence. Tests of functionality were performed with pure WCB cultures. *In vitro* toxicity tests were performed on plants (three rice varieties and one maize variety), using ranges of Cr(VI) concentrations and WCB doses. These tests showed that plants had different tolerances to the metal toxicity and inoculum. Finally, the functionality of the WCBs within tissues of inoculated plants in contact with Cr(VI)-contaminated soil and water (25 µM) was studied *in vitro* and in a controlled greenhouse environment. Inoculation of each WCB into plants exposed to Cr(VI) showed GFP expression within plant tissues. WCBs penetrated the root tissues and later colonized the shoots and leaves. In general, a higher fluorescence signal was detected in roots, together with a higher Cr content (observed by AAS) and denser WCB colonization. By analyzing colonized tissues, both WCBs allowed the detection of Cr(VI) contamination in soils and its transfer to plants commonly used in crops for human diet. The detection of fluorescence in the upper parts of the plants was indicative of contamination by hexavalent chromium, and therefore consumption of plants exposed to contaminated soils constitute a risk for the health of consumers.

FP171. Isolation And Characterization Of Thermostable Amylases Compatible With Detergent Applications

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Bio-based economy is a worldwide strategy to address the global challenges and bio-catalysis is a key process in this approach. Keeping this in mind, our lab devoted to exploit microbial diversity from hot springs and volcanic areas searching for enzymes producers of particular interest in bio-catalysis. A screening of 120 bacterial isolates belonging to the thermophilic collection of CBA for the production of heat-stable carbohydrate-degrading enzymes allowed the selection of a *Geobacillus stearothermophilus* isolate Azth62 with high capacity to hydrolyse starch. From the supernatant of this isolate we purified by FPLC two proteins with amidolytic activity, one with 60 kDa identified by MS/MS with significant homology score as alpha-amylase (EC 3.2.1.1) and the another with 65 kDa identified as oligo-1,6-glucosidase (EC 3.2.1.10). Both enzymes presented an optimum of activity at 70°C and pH 7, retaining more than 80% of catalytic activity after 24 hrs under these conditions. Kinetic analysis of the alpha-amylase estimate a Km of 3.13 g L^{-1} and a Vmax of 0,24 g $L^{-1}min^{-1}$ in starch whereas oligo-1,6-glucosidase presented a Km of 0.46 g L^{-1} and Vmax of 0.024 g L^{-1} min^{-1}. The combination of both enzymes allowed to a Km 3.86 g L^{-1} and Vmax of 0.17 g L^{-1} min^{-1} in starch and a Km of 1.43 g L^{-1} and Vmax of 0.066 in amylopectin. An enhancing activity of around 50% and 30% was obtained in the presence of Mn and Mg, respectively. Furthermore, an increment in the activity was observed in the presence of non-ionic, and zwitter-ion detergents like Triton X-100 and CHAPS and a great tolerance to the strong detergents like SDS. Amylases are currently used in the formulation of about 90% of liquid detergents. Tests in clot washing taches showed increase of efficiency of regular detergents in about 81% in ketchup, 64% in peanut butter, 42% in mustard and 22% in chocolate. The properties of the purified enzymes such as thermostability, pH profile, pH stability, and non Ca-independency, support the conclusion these enzymes has potential in laundry.

FP172. D-Lactic acid production from pretreated *Cistus ladanifer* by D-lactogenic *Escherichia coli*

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Renewable lignocellulosic industrial residues are ideal biorefinery feedstocks, due to environmental, logistic and economic reasons, as they are already concentrated in an industrial facility. As an example of unconventional feedstocks laying in this category, are the biomass feedstocks for the cosmetic industry, which are typically processed in high amounts.

In this work, *Cistus ladanifer* (Cl) residues were used as a complex lignocellulosic feedstock. Cl is an endemic shrub in Mediterranean type climates that is used for the production of high value essential oils. Lignocellulosic residues from this process are currently used for combustion. The Cl residues (after oil extraction) were pretreated with autohydrolysis for hemicellulose recovery, followed by an alkaline treatment to solubilize the lignin. The remaining treated biomass was used for studying the effect of solid loading (SL: 2-10%) and enzyme loading (EL: 6.34-23.66 FPU/g DB) for saccharification using a Doehlert experimental design; followed by fermentation with a metabolic engineered *Escherichia coli* to produce only D-lactic acid. Biomass pretreatment enable to increase cellulose content with a saccharification yield increased to 76%. The maximum lactic acid titer obtained was 370 mM with the highest SL; whereas the highest productivity and D-lactate yield from glucose were obtained with the maximum EL and 8% SL (2.17 g L^{-1} h^{-1} and ~100%). These results clearly indicate that this feedstock and process may be a future alternative for a bulk product route in the biorefinery framework.

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FP173. Valorization of aqueous and ethanolic extracts from *Pinus pinaster* bark: chemical and biological properties

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Natural compounds such as phenolics, flavonoids, carotenoids, etc have fostered interest in pharmaceuticals and food industry. Pine bark (PB) is a by-product of the wood industry representing 10-20% of pine trunk. This waste is normally discarded or used for energy production. Only a low percentage is used for high value and industrial applications. Polar solvents such as ethanol and its aqueous mixtures are frequently used for the recovery of phenolic compounds from plants. In this context, the aim of this study was to characterize the PB extracts obtained at different concentrations of ethanol (0-90%) under same conditions of extraction (115 min, 82°C, 6g PB/40mL, previously optimized). Chemically, the amount of total phenols and flavonoids were evaluated and quantified by colorimetric and liquid chromatography methods. The antioxidant activity (FRAP and DPPH) and in vitro cell viability against normal mouse fibroblast (L929), human embryonic kidney (HEK293T) and human lung cancer (A549) were evaluated. The results show that extracts obtained with hydroethanolic solvents have a higher content of phenolic and flavonoid compounds than aqueous extracts, as expected. However, extracts obtained using 50 and 70% ethanol have the highest amount of these compounds. 18 phenolic compounds were detected by UHPLC-DAD in the PB extracts. The compounds with the highest concentration are: taxifolin, ellagic acid, 3,4dihydroxybenzoic acid, catechin and narigenin (hydroethanolic extracts) and gallocatechin (aqueous and EtOH 30% extracts). The extracts presented high antioxidant activity (both for DPPH and FRAP; TROLOX standard as a reference), particularly the extracts obtained with the 50% ethanol. The cytotoxic effect of these extracts was evaluated, and in non-tumor cell lines (L929 and HEK293T) the extracts showed non-toxic effects observing a high percentage of cell viability. In addition, a cell growth effect was also observed at the lowest doses tested (75 and 125 µg/mL). This growth effect was not observed in the cancer cell line (A549) and the cell viability was lower than in the non-tumor cells. This effect is more pronounced for the hydroethanolic extracts (50 and 70%). In conclusion, pine bark extracts are rich in phenolic and flavonoid compounds and show high antioxidant and no cytotoxicity.

P174. Modeling the impact of microbial inhibitors on the upgrade of lignocellulosic biomass using *E. coli*

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E. coli is one of the most promising industrial biocatalysts for biofuels production. Understanding how individual inhibitors affects *E. coli* physiology it is an important step to understand how can we cope with these inhibitors, in order to minimize their effect during fermentation process. Since no isolated inhibitors is present in lignocellulosic hydrolysates, studies with multiple inhibitors are also essential to understand their potential synergetic effect on the microorganism physiology.

In this work, a selected host strain of *E. coli* (Tuner) was cultivated in mini-fermenters, at controlled pH of 7.0 in a chemically defined medium containing xylose as sugar source to mimic the concentrations found in hydrolysates. Based on our previous work, with isolated inhibitors and considering the impact on the *E. coli* physiology and the concentration in the hemicellulosic hydrolysates, acetic acid (0-10 g/L) and syringaldehyde (0-0.25 g/L) were used as model inhibitor compounds and studied using a statistical experimental design to explore the entire concentrations ranges.

Fermentation performance was followed for 72 – 96 h, and the collected data were modelled using the modified Gompertz equation to quantify growth parameters, and kinetic-base empirical models (Monod and Luedeking-Piret) were used to describe substrate, inhibitors and product profiles.

The main effects and interactions of the inhibitors concentrations were studied for the estimated model parameters, and the results clearly indicate that the interaction effects play an important role on the biomass production, and on sugar assimilation, especially for the initial phases of the growth curve.

A discussion on the use of this full set of models for the analysis of fermentation data in industry, as well as in physiological studies is presented and are the basis for a further discussion of the impact of metabolic inhibitors on the economic performance of the fermentative processes within the biorefinery framework.

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P175. Successful valorisation of carbon-rich and nitrogenous waste streams: Methods, challenges and solutions

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Achieving zero waste to landfill requires creative, innovative and realistic solutions for the valorisation of a wide range of waste streams. These wastes are generated by various industries as well as agricultural and human activities. Here we investigate the most appropriate process, or processes, for the valorisation of diverse waste streams. These streams include sugar-rich food wastes, nitrogen containing industrial and agricultural waste waters and cellulosic-rich sludges. The most effective valorisation route for each waste stream is informed by the composition and physico- chemical characteristics of the waste stream and the conversion efficiency of the components of interest. Additional to the process efficiency we also consider the presence of contaminants and persistence of these through the chosen valorisation route and the associated risks of downstream product use. Persistence of contaminants is especially unwanted in products that may have contact with human users. We present methods to assess the microbial load within potential feedstocks, critical points within the process and presence within product streams. Knowledge of the microbial load and identification of the key microbial species present will also help inform disinfection strategies. An understanding of the pathogenic load within the feedstock should be included in the decision-making process on the suitable route of valorisation.

P176. Exploitation of pathogenic molecules from the *Steinernema carpocapsae* with bioinsecticidal potential for sustainable agriculture development

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Entomopathogenic nematodes are insect parasitic nematodes used as biological control agents against a broad range of insect pests, because they actively seek and kill the host rapidly after the contact. Despite of that, they have some drawbacks, like the cost of production, limited shelf-life, storage problems and low resistance to environmental extremes. Currently, the pathogenicity of these organisms is ascribed to excretory/secretory products (ESP), released by the infective nematode.
Our group identified and characterized some virulence factors produced by *Steinernema carpocapsae*, underlying the nematode success as an insect pathogen.
Among those, we found several nematode derived disulphide-rich peptides, such as ShK-like domains and saposins, that have putative biological function that resembles other insecticidal biomolecules.
ShK peptide was successfully produced in *E. coli* expression host by fusion with Disulfide- bond isomerase (DsbC) which is a chaperone tag that is reported to enhance the solubility of these peptides while promoting correct disulphide-bond formation. The results from insecticidal assays using *Drosophila melanogaster* as a model, shows that this peptide can kill insects at a dose dependent manner with a LD50 of 74 ng per adult. At sub-lethal doses (<10 ng) the locomotor activity was statistically significantly lower on treated group (7.8 ± 3.5 meters) compared with untreated groups (16.4 ± 6.6, p= .0014).
The alternative use of biodegradable nanoparticles functionalized with natural insecticides, will overcome the limits of this biological control agent and will contribute to sustainable agriculture by reducing the usage of conventional pesticides that are broadly toxic and thus minimizing risks to both environment and human health.

III3. Bioeconomy and Sustainable Development

P177. Arbuscular Mycorrhizal Fungi: biotechnological potential of association with soybean plants submitted to water deficit period

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The objective of this work was to verify the association of Arbuscular Mycorrhizal Fungi (AMF) of the genus *Gigaspora* sp. with soybean plants submitted to a water deficit period. The plants (variety ANTA82) were grown in pots, which were distributed in a completely randomized design with 4 replications and 3 plants per pot with the following treatments: 1-ANTA82 without inoculating irrigated, 2-ANTA82 without inoculating deficit, 3 plants inoculated with *Gigaspora gigantea* under irrigated irrigation, 4-ANTA82 inoculated with *Gigaspora gigantea* deficit, 5-ANTA82 inoculated with *Gigaspora margarita* irrigated, 6-ANTA82 inoculated with *Gigaspora margarita* deficit. Inoculation occurred in the 3 g inoculum seed hole (containing 3.5 g-1 spores) and the plants were grown until the third trifolium was fully expanded. 180 mL of water was added to the irrigated pots, plants under stress were added 60 % of water at field capacity (CC). The first evaluation was at 40 days with plants under water deficit for 10 days, subsequently re-irrigated for 7 days and evaluated again. The results show that plants inoculated with fungi were superior to those not inoculated, in the biometric and physiological evaluations in which the plants were under water deficit and later re-irrigated, the fungus *Gigaspora margarita* stood out in relation to *G. gigantea*. It was concluded that inoculated soybean plants supported the water deficit and the fungi were able to associate symbiotically with the roots. The use of AMF, especially *G. margarita*, provided plant recovery after deficit more efficiently than *G. gigantea*.

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P178. Low-cost alternative culture media for fungal pigments production

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Natural pigments have several industrial applications, namely in the textile industry for dyeing cloths, or in the food industry, as colouring agents. Pigments can also be used in cosmetics, leather or in the pharmaceutical industry. More recently, other applications were found for pigments like in histological staining, in solar cells or as pH indicators.1 Conventionally, the natural pigments are extracted from flowers or insect tissues. However, microbial production of natural pigments has been considered a promising alternative. Filamentous fungi are known to produce many different pigments. Recently, some *Penicillium* species, such as *P. chrysogenum* and *P. purpurogenum*, were described as effective pigment producers.
In this work, the production of extracellular pigments by *Penicillium* sp. was evaluated under submerged fermentation conditions using a synthetic medium2 and alternative fermentation media containing cheese whey (CW) and corn steep liquor (CSL). Preliminary results indicated that pigment production was favoured when lactose was used as carbon source. Since CW, a by-product from cheese industry, contains high lactose content, it was used as an inexpensive alternative fermentation medium to induce the pigment production. On the other hand, CSL, a major by-product from cornstarch process, has been identified as a potential nitrogen source in biochemical industries. A mixture of three pigments (yellow, orange and red with λmax=400, 470 and 500 nm, respectively) was obtained. To determine the best conditions for pigment production the sum of the absorbances obtained for the three wavelengths was considered. After 12 days of fermentation, the synthetic medium and media with CW supplementated with 4 g/L yeast extract/peptone (4 g/L) or CSL (1 g/L and 8 g/L) presented the highest pigment production (Figure 1). Furthermore, the supplementation of CW with yeast extract and peptone or CSL allowed a pigment production similar to that obtained with the synthetic medium. These results show that extra supplementation of CW or the use of CSL as alternative source of nitrogen can be a promising strategy to improve pigment production in low-cost fermentation medium.

P179. The potential of ohmic heating for agar extraction from seaweed

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Agar is a polysaccharide with a high commercial value due to its wide range of applications in several industries. *Gracilaria* is used as the raw material by the agar production companies, being around 54% of the agar commercialized worldwide obtained from this seaweed. Traditional extraction is a process that requires high solvent and energy consumptions and generates large amounts of waste creating the need for greener and more efficient technologies. Ohmic heating consists in a process where an electric current is passed through materials heating them uniformly with a rapid rate. The process has high energy conversion efficiencies, resulting in lower operational costs and in an environmental-friendly system. The ohmic heating can be applied to obtain sub-critical water conditions for extraction of compounds from vegetal tissues, joining the energy and time efficiency with the selective power of sub-critical water. In this context, the aim of this work was to evaluate the effect of ohmic heating on seaweed agar extraction, as an alternative extraction technology. The extraction was performed using a frequency of 25 kHz at 82 °C, during 1 and 2 h, for different proportions of water: ethanol. The extraction yield and sugars composition were identical in all extracts when compared with a conventional extraction, under the same conditions of solvent, temperature, and time. However, the gel strength of agar was significantly higher for the extracts obtained with ohmic treatment in water. These results show the potential of ohmic technology as a low cost and an environmental-friendly alternative for agar extraction from seaweed.

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P180. BIONANOSCULP: development of chitosan-based coatings as an eco-friendly strategy for conservation of outdoor sculptures

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Urban outdoor sculptures, which are an immeasurable part of our cultural heritage, are easily vulnerable to chemical and physical alterations due to their exposure to weather elements and air pollution. These alterations are aggravated by the colonization of degrading microorganisms. Nowadays, the products used for biodeterioration control of such cultural objects lack versatility or are toxic to the environment and the human health. In order to find new alternatives based on non-toxic and sustainable materials, the BIONANOSCULP project proposes the development of an antimicrobial protective layer for the preventive conservation of outdoor sculptures. For that, chitosan has been tested as a base biopolymer for the synthesis of protective coatings due to its proven antimicrobial properties. Different concentrations and molecular weights of chitosan, as well as the addition of adjuvants and cross-linking agents were tested to obtain coatings with relevant properties for application on metal and stone sculptures. Antimicrobial activity testing, together with physical and chemical assessments are being performed to characterize and evaluate the suitability of the coatings as protective layers able to slow down the growth of relevant microorganisms. Permeability assays and compatibility of the coatings to stone and metal samples are also being considered in order to analyze their performance on different materials while not causing alterations to the original aesthetics of the sculptures.

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P181. Biological and chemical valorization potential of portuguese seaweeds: *Ulva rigida, Gracilaria* sp. and *Fucus vesiculosus*

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The seaweeds are an interesting source of nutrients and their rich composition allows them to be applied for different purposes. The aim of this study was to optimize the conventional solid-liquid extraction for three different seaweeds (*Ulva rigida*, *Gracilaria* sp. and *Fucus vesiculosus*) and characterize the chemical profile and antioxidant activity of the different extracts. An experimental design using a central composite was chosen for the optimization process. Four independent parameters were selected: type of solvent (different water:ethanol ratios), ratio (1:10-100 solid:liquid), time (0.5-9 h) and temperature (5-95 °C). Besides the nutritional characterization, the extraction yield, total phenolic content and antioxidant activity (DPPH and FRAP methods) were also evaluated. It was possible to verify that the use of water as solvent presented the highest extraction yield for the three seaweeds when performed with temperatures above 75 °C, times above 1 h of extraction and different ratios, and that these extracts were rich in polysaccharides. For *U. rigida*, the extraction conditions of 2 h, 85 °C, water as solvent and ratio 1g:60 ml was the one that showed higher yield (45.65 %) and also higher sugar content (49.76 mg/g, Glucose Equivalent/dry weight). However, for this seaweed, the extractions performed with ethanol concentrations above 50% and low temperature (under 45 °C) presented higher content of total phenolic and antioxidant activity. For *Gracilaria* sp., the extraction condition of 7 h, 75 °C, ethanol 50 % and ratio 1g:90 ml showed highest protein and total phenolic content, as well as antioxidant activity, but water as solvent with temperature above 75 °C was the condition with higher yield and sugar content. Similar results were obtained for *F. vesiculosus*, but with values of antioxidant activity 10-fold higher than obtained for *Gracilaria* sp and *Ulva rigida*. In conclusion, a sequential extraction procedure using a hidro-ethanolic solvent at mild temperatures to extract an antioxidant fraction and water at high temperatures to extract a polysaccharide-rich fraction can be considered. These fractions with different added value features can be used to develop new food products such as functional foods and nutraceuticals, improving the local bioeconomy.

P182. The ABC transporter Pdr18 is a determinant of yeast thermo- and osmo- tolerance: underlying mechanisms and biotechnological implications

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The development of sustainable bioprocesses for production of biofuels and bio-based chemicals is essential to reduce society dependence on the petrochemical industry. For this, the development of superior industrial yeast strains more tolerant to multiple chemical and physical relevant stresses is crucial. Yeast performance depends on ethanol tolerance, thermotolerance, osmotolerance and tolerance to several other fermentation inhibitors either produced during fermentation or present in the media.
The expression of plasma membrane transporters that confer resistance to multiple drugs/xenobiotic compounds (MDR/MXR) is among the mechanisms by which yeast overcomes multiple stresses. Although traditionally considered drug efflux pumps, the physiological role of these transporters is still a matter of debate [1,2]. Some of them mediate the reduction of intracellular accumulation of toxicants while playing crucial roles in yeast cell physiology, such as in lipid transport [1]. This is the case of the ABC transporter Pdr18, that confers tolerance to a wide range of chemical compounds of biotechnological interest, such as alcohols, organic acids, heavy metals and agricultural pesticides [2– 5]. Pdr18 is involved in ergosterol transport at the plasma membrane, contributing to the maintenance of its organization and reduced permeability under acetic acid stress [4].
The present work reports new roles for Pdr18 in yeast tolerance to osmotic and thermal stresses and their combined effects. Expression of PDR18 is advantageous to attain higher final titers of ethanol produced under challenging conditions (300g/L at 30ºC and 40ºC), in molasses fermentation and in media mimicking lignocellulosic hydrolysates. However, yeast cells devoid of Pdr18 exhibit a higher ethanol production rate and yield under unstressing or mild growth inhibitory conditions. The underlying mechanisms were investigated, focusing on the analysis of ergosterol biosynthetic pathway genes expression and the influence of plasma membrane ergosterol content in the kinetics of glucose transport.

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P183. Kraft pulp bioethanol production according to the circular economy model

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Due to the demands of the modern lifestyle, fossil fuels such as coal, oil and natural gas are overused, which leads not only to their depletion but also to serious environmental issue. The production of second generation biofuels obtained from nonedible feedstocks can mitigate these problems, also contributing to the reduction of discarded wastes. Lignocellulosic biomass is a potential source for the production of second generation bioethanol, mainly due to the presence of high levels of cellulose and hemicelluloses, its large availability, and relatively low cost.

Portuguese pulp and paper (P&P) industry is a growing sector of great importance for the country's economy. In this work, wastes from the P&P industry were used in order to produce bioethanol according to a circular economy model. *Eucalyptus* barks, resulting from the preparation of raw materials for pulp and paper production, were processed using the kraft pulping, a well-established and commercially proven technology, as biomass pre-treatment. This process resulted in a pulp similar to that obtained from the wood, which is generally intended for the production of paper and other pulp derivatives. The pulp, rich in celluloses and hemicelluloses, was subsequently hydrolyzed by an enzymatic consortium of cellulases to obtain fermentable sugars. Fermentations with the yeasts *S. cerevisiae*, *S. stipitis* and a co-culture of both yeasts were firstly carried out in batch mode in Erlenmeyer flasks and lately in the bioreactor. Biomass concentration and pH were monitored during the fermentation. The concentration of glucose, xylose and ethanol was analyzed by HPLC.

This work aims to serve as a proof of concept of the feasibility of using *Eucalyptus globulus* bark, a waste feedstock, as a substrate in the production of bioethanol. In *S. cerevisiae* fermentation assays a concentration of 18.1 g/L of ethanol was achieved, with a yield (gEtOH/ginicial sugars) of 0.342, corresponding to 75.0% of the maximum possible yield.

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P184. Assessment of the effects of a bionematicide 1,4-naphthoquinone emulsion on soil microbial community by PLFA: a method to determine ecotoxicity indicators

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Environmental concerns for organic farming management have fueled a worldwide demand for bionematicides. Here, we developed a work aiming to evaluate the potential impacts of these compounds on soil non-target organisms (FCT - PTDC/AGR-PRO/2510/2012). The main goal of the present study was to evaluate the impact of 1,4-naphthoquinone (NTQ), a nematicidal compound that can be found in natural products, such as walnut husk, on soil microbial community, and determine ecotoxicological indicators in order to follow and quantify the effect of this compound. The effects on diversity and metabolic state of the microbial community were evaluated by an ISO-derived PLFA method. Tests were conducted using a natural uncontaminated soil. A range of NTQ concentrations, as well as comparable solution controls containing solubilization enhancer Triton X-100 (TX100) was prepared to spike the soil. NTQ impacted the soil microbial community, causing significant changes at 12 mg/Kg and with strong diversity (structural) changes occurring at 96 mg/Kg. TX100 also caused a significant effect on the microbial community global profile, but only at 192 mg/Kg NTQ-equivalent. The microbial profile modifications induced by either NTQ or TX100 were distinct and possible to discriminate by PLFA analysis, as different indicators were affected. The effects of NTQ on microbial community were quantifiable for several indicators, and the calculation of half maximal effective concentrations (EC50) was possible for a diversity of parameters. The PLFA method therefore proved to be suitable for the assessment of NTQ ecotoxicity, and should be considered for any soil ecotoxicological analysis.

P185. Polar lipidome of microalgae *Emiliania huxleyi* and its biotechnological potential

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Emiliania huxleyi is one of the most abundant microalgae in the oceans, which so far does not have a high commercial value. Detailed knowledge of the chemical composition may contribute to its valorisation as a sustainable source of compounds with biotechnological potential. In the present study, polar lipidome of *E. huxleyi* was characterized in-depth for the first time using a mass spectrometry-based lipidomic approach. This allowed the identification of more than one hundred polar lipid species distributed over six classes of glycolipids, six classes of phospholipids and two classes of betaine lipids. Several molecular species identified are carriers of n-3 polyunsaturated fatty acids with high nutritional value, namely stearidonic acid (SDA, 18:4n-3) and docosahexaenoic acid (DHA, 22:6n-3) that accounted respectively for 7.3% and 7% of total fatty acids. Also, polar lipid species have been reported as bioactive compounds with anti-inflammatory, anti-microbial and anti-proliferative activity, among others. These results highlight that *E. huxleyi* is a good candidate to promote bioeconomy and sustainable development, namely as a source of high value polar lipids with different potential applications, such as functional food ingredients and bioactive food packaging.

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FP186. Structural And Functional Diversity Of Biodeteriorated Stone Biofilms Samples Collected From Four Chapels Of The Old Cathedral Of Coimbra

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In 2013, UNESCO classified the 'University of Coimbra – Alta and Sofia' (Portugal) as World Heritage site which encompass some limestone monuments like the Old Cathedral of Coimbra. The Old cathedral of Coimbra (Sé Velha) was built during the 12th and 13th Centuries and nowadays it is the only Portuguese Romanic cathedral from the Reconquista times which survived relatively intact. To promote the preservation and conservation of this historic stone monument, the objectives of the present work were to assess the structural diversity of degraded limestone walls at Sé Velha, by NGS, and to explore the correlation between microbial populations and specific biodegradation typologies and to predict the metabolic functions of associated bacterial communities. Ten biofilm samples were collected, total DNA was extracted and 16S rRNA genes was sequenced in Illumina MiSeq V2 platform, for domains Bacteria and Archaea. To establish the existence of correlations between bacterial or archaeal populations and biodegradation patterns, a PCA was performed. The functional potential of different microbial communities was predicted by the PICRUSt2 pipeline, results were assigned to KEGG Orthologs. For domain Archaea, only nine samples provided results, while for domain Bacteria all samples were successful. For both domains, coverage values were at minimum 99.8% which showed that the bacterial diversity were successfully determined. From 29 classified bacterial phyla, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria* comprised 71-94% of sequences of each sample. For Archaea, the majority of the populations were distributed by classes *Haloarchaea* and *Nitrososphaeria*. PCA allowed to assign the presence of some populations to specific biodegradation typologies and provide evidences of possible symbiotic relationships. The overall functional structure of the Bacterial and Archaeal communities presented in limestone suggested that the most abundant KEGG pathways were related to cellular processes and to genetic information processing. In conclusion, it was possible to establish cause-effect between the microbial populations and the occurrence of specific biodegradation patterns. Results provided evidence for the presence of well-adapted microbial populations to this semi-open extreme ecosystem. Data collected will be important to for the development of the best conservational methodologies for this specific site.

FP187. Microbial community structural and functional dynamics in basins of tungsten mine tailings: a vertical profile analysis

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In a circular economy concept, where more than 300 million tons of mining and quarrying wastes are produced annually, those can become a valuable resource, supplying metals that are extracted today by other processes. Innovative methods and processes for efficient extraction of these elements are needed. The Portuguese mine of Panasqueira is one of the largest operating tungsten mines in Europe and has produced several million tons of residues during its almost 120 years of operation. Some of the residues consist of a fine ground material produced by the ore processing plant. These materials may have interesting grades in tungsten and other metals depending on the efficiency of the technologies applied throughout the life span of the mine. The mine residues have been deposited in two tailing basins. The first basin was closed in1985 (Basin 1) and only the new one is currently receiving the mine tailings (Basin 2). This work aims to assess the microbiological and chemical spatial distribution within these two tailing basins from Panasqueira mine, using a MiSeq approach targeting the 16S rRNA gene, in order to relate microbial composition with chemical variability, thus providing information to enhance the efficiency of the exploitation of these secondary sources. The tailings sediments core microbiome is comprised by members of family *Anaerolineacea* and genera *Acinetobacter*, *Bacillus*, *Cellulomonas*, *Pseudomonas*, *Streptococcus* and *Rothia*, despite marked differences in soil physico-chemical properties. The higher contents of Al and K shaped the community of the older Basin 1, while As-S-Fe contents were correlated with the microbiome composition of Basin 2. The microbiome was rich in genes related with metabolism pathways and environmental information processing as signal transduction and membrane transport. An understanding of the tailings microbiome and its metabolic capabilities across different depths can provide a direction for the management of tailings disposal sites and maximize their potential as secondary resources.

FP188. The microbiome of xylem sap associated with almond leaf scorch disease caused by *Xylella fastidiosa* in South-East Spain

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Xylella fastidiosa (XF) is one of the most harmful emerging plant pathogenic bacteria and represents an important threat to agriculture, forestry and landscape worldwide. In June of 2017 XF was reported in almond trees in the province of Alicante, Spain. Metagenomics is a valuable methodology to study the impact of causal agents of plant diseases and their interaction with others naturally occurring microorganism, as part of innovative approaches to mitigate or control the disease. Moreover, endophytic bacteria seem to be a promising biocontrol solution. The objective of the study was to compare healthy and diseased almonds trees infected by XF subsp. multiplex to identify groups of microorganisms that could potentially modulate the almond leaf scorch disease. Almond plots were selected in five municipalities within the demarcated area of XF outbreak. A total of 93 trees were selected and characterized as positive (52) and negative (41) for the presence of XF using official EPPO standard qPCR protocols. The bacterial microbiota was determined from DNA extracted from xylem samples of wood chips based on the V5-V6 region of the bacterial 16S rRNA gene using Illumina's MiSeq sequencing. There was a clear concordance between qPCR results and identification of XF reads in the samples. Within the diseased plants, the relative abundance of XF varied from 0.34% to 92% of total bacterial reads. In total, 152 OTUs were assigned to 11 phyla, 53 families and 86 genera. A core microbiome of 77 OTUs common to healthy (qPCR negative) and diseased (qPCR positive) almond trees was determined were 5 genera accounted for most of the diversity. Disruption in the frequencies of these OTUs occurred on the diseased tree since those predominant genera were less abundant due to the emergence of *Xylella*. Furthermore, 32 and 38 OTUs were unique to healthy or diseased trees, respectively. Our results allow to better understand the interaction between XF and the xylem sap microbiome identifying potential bacteria that could act through direct inhibition or through niche displacement of XF envisioning innovative strategies to control the almond leaf scorch disease.

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FP189. The study of viruses in urban sewage by metagenomics: a tool for public health surveillance

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Raw sewage contains a large variety of pathogenic and commensal viruses, excreted from thousands of inhabitants, being municipal wastewater treatment plants (WTTPs) one of the most important routes for propagation of viruses from humans to the environment. Thus, influents from these WTTP can indeed reflect traits of the human population microbiome, or more specifically of their virome. Since viruses do not have conserved molecular markers, such as 16S rRNA, that are shared across all species hampering the study of viral metagenomes, the application of random-primer-based sequencing approaches in combination with next-generation sequencing (NGS) techniques has opened a new path for viral discovery/characterization. Viral metagenomics application to sewage provide excellent tool for monitoring and identifying potentially known and unknown viral pathogens that circulate among the human population, contributing to the public health surveillance.
Therefore, this study aimed to characterize the virome by Illumina MiSeq from two Lisbon' WWTP, by analyzing four sampling points: influent, effluent after the secondary treatment, discharged effluent, and reutilized effluent. Virus identification from NGS data was performed with Genome Detective Virus Toll as a first approach, and preliminary results reveled more than 20 different viral families in both WWTPs. Bacteriophage families *Siphoviridae*, *Myoviridae* and *Microviridae* show a higher diversity in wastewater samples. Viral plant *Virgaviridae* species, as well as reads related to viruses belonging to the *Circoviridae* and *Picobirnaviridae* families were obtained. Furthermore, important human viral pathogens taxonomically assigned to *Astroviridae*, *Caliciviridae* and *Hepeviridae* were also detected. Virome information from urban sewage may constitute an important database for known, novel and emerging viral strains circulating in the human population. High throughput techniques, such as NGS, is a valuable tool to pinpoint major pathogens present in the community and environment, and provide data for epidemiologic studies and public health surveillance. In addition, the knowledge on the pathogenic composition of the wastewater virome can raise the awareness for the necessity to modify WWTP processes, to efficiently halt virus dissemination.

I4. Microbiomes Structure and Function

P190. Female urinary microbiota: who´s coming, who´s going, and who´s staying

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Over the past years the composition of the female urinary microbiota (FUM) has been intensively inspected in disease-associated states reporting diverse bacterial community. Still, we have incomplete knowledge on healthy community profiles and their stability over time. Using extended culturomic approach at two time points, we aimed to evaluate FUM stability of healthy reproductive-age women.

Ten healthy reproductive-age women provided, at two time points within 2,5 years interval, midstream urine samples (n=20) that were subjected to extended culturomics (100 ul urine; blood agar and supplemented chromogenic agar; 48h of incubation; different atmospheric conditions) with isolate identification to the species level by MALDI-TOF/MS and/or suitable genotypic biomarkers (16S rRNA, *pheS*, *rpoB*, *recN*).

The isolates characterized (over 2000 isolates, range of 17-321 isolates/sample) were assigned to 5 phyla, 45 genera and 111 species. A stability in detected phyla between 1st and 2nd sampling was observed comprising Firmicutes (51% of species in both samples), Actinobacteria (38%, 33%), Proteobacteria (6%, 11%), Bacteroidetes (3%, 3%) and Fusobacteria (1%, 2%). A high bacterial load was observed (10^4-10^8 CFU/ml), which in 70% samples varied up to 10^2 CFU/ml between paired samples. The *Staphylococcus* and *Lactobacillus* were the most prevalent genera in both sampling points present in 18 out of 20 (18/20) and 17/20 samples, respectively. Stability at species level was only observed for 35 bacterial species comprising previously identified as predominant e.g., *Staphylococcus epidermidis* (8 out of 10 individuals, 8/10), *Micrococcus luteus* (6/9) and *Streptococcus anginosus* (6/7) mostly in low relative abundance (RA) and occasionally scoring up to 15% RA. Also, opportunistic pathogens associated with urogenital tract health were detected e.g., *Gardnerella vaginalis* (3/5) or *Streptococcus agalactiae* (1/4), maintaining high RA at both sampling points (up to 91% RA and 98% RA, respectively). Additionally, one individual presented highly abundant Enterobacteriaceae member in both samples (1st sample with 99,98% RA *Citrobacter koseri*; 2nd with 90,76% RA *Escherichia coli*) together with *Lactobacillus jensenii*.

Our data corroborate previously reported diversity within FUM community. Moreover, a set of bacterial species detected as highly prevalent are stably maintained, that might constitute patterns for the identification of a healthy FUM.

I4. Microbiomes Structure and Function

P191. Effects of long-term exposure of oxytetracycline in zebrafish and water microbiome and in a post-exposure scenario

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Oxytetracycline is one of the most used antibiotics in aquaculture to prevent fish diseases. Due to poor antibiotic absorption by fish, approximately 90% of the compound is excreted in its unaltered form. Consequently, antibiotics may enter the aquatic environment affecting non-target organisms at several levels, including the microbiome, with unknown effects to the organism itself. Our work studied the long-term impact of OTC exposure in zebrafish and water bacterial communities. Zebrafish adults were exposed for two months to three concentrations (0; 10 and 10000 µg/L) of OTC via water exposure. After this period, organisms were transferred to clean water for one month to assess reversibility of effects. Samples were collected at four time points: 5 days and 2 months of exposure and at 5 days and 1 month of post-exposure period. DNA was extracted from gut and water samples and changes in bacterial communities' structure were observed through DGGE analysis. Our results revealed that both zebrafish gut and water bacterial communities were affected by OTC exposure with effects being detectable after 5 days of exposure. In the water samples, effects on the bacterial communities were evident both during the exposure and during the post-exposure period for both concentrations used. On the other hand, changes in fish gut bacterial communities' structure due to OTC exposure were more evident at the highest concentration. In addition, after 1 month of post- exposure, fish gut bacterial communities' structure shared a high similarity (70%) with the control group, suggesting a microbiome recovery. Overall, our results suggest that in zebrafish gut effects in bacterial communities' structure seemed reversible, while in water samples changes in the communities may last for longer periods. Thus, our work revealed that bacterial communities may react differently after the exposure ceased with possible negative impacts on ecosystem functioning. Also, since in our work changes were only observed at structure level, future works should be done for a deeper analysis of which bacterial phylotypes were affected. Therefore, realistic scenarios like post-exposure periods should be considered for a better understand of the real ecological impact of antibiotic exposure.

I4. Microbiomes Structure and Function

P192. Study of vaginal microbiome in portuguese sex workers

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Sex workers are a highly active sexually group, who are at risk of having a higher prevalence of sexually transmitted infections (STI) such as HIV, human papillomavirus (HPV) infections and bacterial vaginosis (BV). In most cases, the presence of pathogens is related to the imbalance of the vaginal flora. Therefore, previous studies have associated the absence or decrease of lactobacillary flora with the presence of STIs. In fact, Lactobacillus species are responsible for maintaining a healthy vaginal ecosystem, in healthy fertile women, protecting them from acquisition of vaginal and cervical infections.
This study aims to characterize the microbiome of sex workers from the Lisbon area, in Portugal, and relate it with the *Lactobacillus* spp. group.
Sixty-two vaginal exudates were obtained from sex workers from Lisbon region, that attended at IHMT. The samples were classified according to Nugent score and used for DNA extraction. Bacterial 16S rRNA gene V1-V2 hypervariable regions were amplified and sequenced with Illumina MiSeq next generation sequencing platform using the v3 kit. Sequenced data was analyzed using QIIME2. Finally, 16S metagenomics results were used to study the dominance pattern of lactobacillary flora.
Experimental results showed that most prevalent microorganisms (with relative abundance higher than 10%) are *Lactobacillus iners* (n=33), *Gardnerella vaginalis* (n=23) and *Lactobacillus crispatus* (n=18). *Escherichia coli*, *Enterococcus faecalis*, *Atopobium vaginalis*, *Enterobacter cloacae*, among others were detected in some samples. Most of identified microorganisms were compatible with clinical status characteristic of BV or AV (Aerobic vaginitis), according with the determined Nugent score. *L. iners* co- exists with *G. vaginalis* in most of the studied cases (n=17). Overall, it was found that the population had a low prevalence of healthy-associated *Lactobaclillus* spp., such as *L. gasseri* and *L. jensenii* in addition to *L. crispatus*. However, some women (n=12) showed dominance of *L. crispatus* in their vaginal flora, indicating some heterogeneity in the microbiome of this specific population. Thus, there is a low prevalence of healthy-associated *Lactobacillus* spp. supporting the clinical status related to the development of infections or STI. Furthermore, *L. iners* appears as prevalent microorganism in this population suggesting its relationship to vaginal dysbiosis.

I4. Microbiomes Structure and Function

P193. Biodegraded walls bacterial isolates as a tool to develop target specific preservation and conservation methodologies: the old cathedral of Coimbra case study

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The historic monuments are constantly exposed to abiotic and biotic factors (such as weather conditions, pollution and the colonizing microorganisms) that contribute to their deterioration. By interacting with minerals, microorganisms induce the dissolution of minerals by producing organic acids and promote the precipitation of secondary mineral deposits, which provoke aesthetical alterations and physical damages in monuments. In 2013, UNESCO classified the 'University of Coimbra – Alta and Sofia' (Portugal) as World Heritage site which encompass some limestone monuments like the iconic Old Cathedral of Coimbra.

The Old cathedral of Coimbra (Sé Velha), built during the 12th and 13th Centuries, is the only Portuguese Romanic cathedral from the Reconquista times which survived relatively intact, and it is considered a semi-open space, with an open central area surrounded by five lateral chapels. In order to develop new and more efficient preservation and conservation methodologies, this historic stone monument was subjected to the characterization of the microbial structural diversity, by culture- dependent methodologies. With that purpose, wall areas that showed important signs of biodeterioration were sampled. Isolates were identified and their capacity to produce stone dissolving acids was ascertained.

A total of sixteen samples were collected from wall areas (distributed by four different chapels) that showed clear visual signs of biodeterioration. Enrichment cultures were inoculated, by spread plate method, in different culture agar media (R_2A; TSA; HM), at different pH values and different salinity percentages. The pure cultures were grouped by RAPD analysis and representative strains were identified by 16S rRNA gene phylogeny. A total of 879 isolates were processed and distributed mainly by genera *Acinetobacter*, *Actinoplanes*, *Aliihoeflea*, *Arthrobacter*, *Bacillus*, *Brevundimonas*, *Devosia*, *Halobacillus*, *Halomonas*, *Lentibacillus*, *Mesorhizobium*, *Micrococcus*, *Paenibacillus*, *Paenisporosarcina*, *Sphingomonas*, *Virgibacillus*. Some isolates may represent novel species and/or genera.

After a careful analysis, circa120 isolates were selected to test their capacity to produce dissolving acids. These isolates were inoculated in different specific media ($CaCO_3$ glucose agar, $CaCO_3$ MEA, $CaCO_3$ B4 and CACO). The preliminary results are promising and will be an important starting point for bioreceptivity studies and will provide relevant data for contra-measures development.

I4. Microbiomes Structure and Function

P194. Molecular characterization of the preputial microbiome in bulls (*Bos taurus*)

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Improvement of current diagnostic methods for bovine venereal diseases rely on novel research on bulls' preputial and penile microbial communities, namely concerning molecular diagnostic methods for detection of bacterial pathogens. Studies focused on bovine preputial microbiome are scarce and mainly rely on culture-based approaches, not capable of identifying fastidious and unculturable microorganisms as part of the preputial microbial community.
The objective of this study was to explore the composition of bulls' preputial microbiome, using a high-throughput sequencing analysis.
Preputial samples were collected from 6 bulls housed in five different herds by a washing/scraping technique infusing 10-20 ml of phosphate-buffered saline into the preputial cavity. Total DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany). Each sample was subjected to 16S rRNA V3-V4 hypervariable regions amplification and products were sequenced using the Miseq ® platform (Illumina ®) at the GenoInSeq (Cantanhede, Portugal).
Sixty three different operational taxonomic units (OTU) were identified, with a mean OTU number/sample of 29 ± 3. Six of the identified OTU were found in all animals and were considered core, which include the genera *Porphyromonas*, *Clostridum*, *Pasteurella*, *Fusobacterium* and unclassified genus from Leptotrichiaceae and Erysipelotrichaceae families. A considerable inter-sample variation regarding the relative abundance and the overall OTUs identified, which may reflect the influence of distinct environmental (management, diet, climate) and biological (breed, age, individual variation) factors on preputial microbiome. Phyla with higher relative abundance were Proteobacteria (31.6 ± 11.5%), Fusobacteria (21.4 ± 3.9%), Bacteroidetes (18.6 ± 7.4%) and Firmicutes (18.5 ± 2.9%), composing more than 90% of the preputial bacterial community. Genera most prevalent in the prepuce were *Pasteurella* (18.0 ± 9.8%), *Porphyromonas* (12.6 ± 5.4%), *Fusobacterium* (10.1 ± 3.3%), *Prevotella* (4.7 ± 1.6%), *Clostridium* (4.5 ± 0.9%) and *Ureaplasma* (3.8 ± 2.1%). Genera that integrate the bulls' preputial microbiome (e.g. *Porphyromonas genus*), previously unnoticed with culture-based techniques, were also identified in this study.
In conclusion, this study provided novel knowledge on the complex and individually diverse microbial community that constitutes the bovine preputial microbiome.

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I4. Microbiomes Structure and Function

P195. Reacquisition of central metabolic pathways in fructophilic yeasts through multiple horizontal gene transfers from bacteria

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Horizontal gene transfer (HGT) is recognized as one of the most important mechanisms of genome evolution in bacteria. In eukaryotes, and more specifically in fungi, HGT events from different sources have been occasionally described, however in much lower number when compared to bacteria. In yeasts such events are apparently even less common, nevertheless we recently showed that in a fructophilic yeast lineage comprising species from the Wickerhamiella/Starmerella (W/S) genera, an outstanding number of bacterial genes (~500 genes) were transferred into their most recent common ancestor (MRCA) (1). We also found that the MRCA of the W/S clade lost several central and ubiquitous metabolic pathways such as alcoholic fermentation (1) and thiamine biosynthesis (2). The ancestral loss of these essential pathways was later counteracted by the reacquisition of bacterial orthologues that contributed to their reassembly. The loss of the two alcoholic fermentation enzymes, alcohol dehydrogenase (Adh1) and pyruvate decarboxylase (Pdc1), was resolved by the acquisition of bacterial ADH1 and PDC1 orthologues in independent events in one W/S-clade species. In the remaining species of this clade only ADH1 was acquired while a pre-existing decarboxylase, Aro10, changed its substrate specificity and is currently substituting Pdc1.
Thiamine biosynthesis, on the other hand, was reinstated by the acquisition of entire operons from bacteria encoding three to four thiamine-metabolism related genes. We found that adaptation of the acquired operons to an eukaryotic-like transcription involved the increase in the length of intergenic regions possibly to accommodate de novo promoters in some cases. However, in three instances, evolution of de novo promoters was circumvented by fusion of adjacent genes giving rise to different and new multifunctional proteins. We showed that additional acquisition of single genes was essential to widen the range of compounds that can be used by these yeasts to produce thiamine, resulting in a mosaic pathway composed of native yeast genes and bacterial genes of multiple origins. Our results show that HGT occurred recurrently in this yeast lineage and was crucial for the re-establishment of lost functions.

[1] C. Goncalves et al., eLife 7:e33034 (2018)
[2] C. Goncalves & P. Goncalves, PNAS, in press

I4. Microbiomes Structure and Function

P196. Endophytic bacteria on strawberry tree (*Arbutus unedo L.*): orchards vs. wild populations

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Strawberry tree (*Arbutus unedo L.*) is an evergreen Mediterranean tree from cosmopolitan Ericaceae family, well adapted to biotic and abiotic stress. It can grow on poor marginal lands and thrives in the Mediterranean forests due to its sprouting ability after forest fires. It's a source of bioactive compounds with antioxidant activity and its round edible berries are the main income for growers, who demand high quality plants for a rapidly increasing production area. However, clone selection is based almost exclusively on its genotype and phenotype, while other important plant components, such as the microbial endophytic community, are usually ignored. Endophytic bacteria have a great impact on host plants by promoting their growth and increasing their fitness and resistance to biotic and abiotic stresses. Several are even considered effective biocontrol agents and can be efficiently used as alternatives to chemical control. Therefore, endophytic bacteria are one of the most crucial elements in plant micro-ecosystems. Still, our knowledge about this interaction is very limited and needs to be considerably improved as such information can be used in biotechnological applications and agricultural practices. Thus, the objective of this work was to identify and compare cultivable endophytic bacterial diversity from plant's leaves grown in orchards with phyllosphere bacterial microbiome of wild trees. From each plant, 10 leaves were handpicked and combined as composite samples (3 plants per sample) that were used for microorganism isolation and total genomic DNA extraction. Almost 100 isolates were obtained, selected from previous determined RAPD groups and further identified by partial sequencing of 16S rRNA gene. Based on the results, isolates were identified as bacteria belonging to *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Sphingomonas* and *Staphylococcus* genus. In general, the structure composition between wild and orchard plants was very similar. However, the genus *Sphingomonas* was only found on orchard plants and some of the identified species were also exclusively of a single group (e.g., *Bacillus megaterium* in wild plants and *Bacillus safensis* in orchard plants). Its interaction and potential antagonism effect were studied, which may contribute to a better knowledge of endophytic bacteria role on disease defense mechanisms and strawberry tree growth.

II5. Bioprocess Integration and Intensification

FP197. Chromatographic purification of single stranded DNA scaffolds for biomanufacturing DNA-origami nanostructures

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DNA nanotechnology encompasses the self-assembly of nucleic acids into complex nanostructures by exploring Watson–Crick base pairing. These DNA nanostructures are expected to find application as components of nanomachines, nanopores, drug delivery systems and biosensors. Usually, asymmetric PCR (50 µL scale reactions) is used to generate 500-3500 base pair (bp), object-specific, single stranded DNA (ssDNA) scaffolds using the DNA of the M13 phage as template. Each scaffold is typically purified by agarose gel extraction after electrophoretic separation (1-5 pmol yields). This process is inherently laborious, limited, not scalable, presents low recovery yields and results in a low quality product as the recovered scaffolds are usually co-purified with agarose-gel residues. Here we present a chromatography-based method to purify ssDNA scaffolds from asymmetric PCR mixtures, which can be further used to assemble DNA nanostructures via DNA-origami techniques. Asymmetric PCR was performed using the M13mp18 phage genome as template, to generate 449 bp-long single and double stranded DNA (dsDNA). In order to isolate the target ssDNA from PCR impurities (dsDNA, unused primers and nucleotides), anion-exchange (Q-ligand) and multimodal chromatography (CaptoTM adhere ImpRes) were explored. In both cases, a stepwise gradient with increasing NaCl concentrations was used. The unused primers and oligonucleotides were washed-out in the flowthrough due to their low charge density. In anion exchange chromatography, the less-charged ssDNA was eluted before the dsDNA. In multimodal chromatography, however, the elution pattern was reversed and both species eluted at higher salt concentration, highlighting the importance of hydrophobicity when multimodal ligands are used. Gel electrophoresis revealed that ssDNA-containing fractions are homogeneous and impurity free. Finally, the recovered fractions were used to assemble 31-bp edge length tetrahedrons using site-specific short oligonucleotides (staples), thermal annealing and high magnesium concentrations. The assembled nanostructures were separated from the unused staples by centrifugal filters. Agarose gel electrophoresis showed high assembly yield and purity. In conclusion, chromatography was successfully used to purify ssDNA fragments with high yield and purity. As a scalable technique, the developments achieved with this type of purification hold great promise not only to produce high quality DNA nanostructures but also to enable the increase of product titer.

II5. Bioprocess Integration and Intensification

FP198. Culture systems: a comparison based on *Nannochloropsis oceanica* productivity and nutritional value

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Industrial microbial cultures can be carried out using different approaches: batch, continuous and semi-continuous operation modes. Each of them presents advantages and disadvantages mostly related to culture productivity, cost of maintenance and risk of contamination. In this study, three 2.5 m^3 outdoor tubular photobioreactors (PBRs) were used to access the three mentioned cultivation modes in order to select the most suitable for the production of *Nannochloropsis oceanica*. Each of the operating modes was implemented in the three different PBRs for *N. oceanica* cultivation at three different time frames, in order to minimize the influence of external factors. The experiments were performed in Allmicroalgae facilities at Pataias (Portugal) during Spring/Summer time intending to optimize the microalgae production occurring at that moment. In what concerns biomass productivity, the results clearly show an advantage of both continuous and semi-continuous. These two operating modes presented productivities 1.5-fold higher than the batch mode (0.153 and 0.165 gCDW/L/day, compared to 0.108 gCDW/L/day, respectively). However, the continuous and semi-continuous modes were the ones using a higher volume of water, 495 L/day and 447 L/day, respectively, compared with the 173 L/day used in batch. Additionally, the nutritional content of the obtained *N. oceanica* biomass did not show significant differences regarding proteins, fatty acid profile and elemental composition; thus not compromising its commercial value. Thus, according to the present study, for *N. oceanica* culture in tubular PBRs, an investment in water volume results in an increase of the biomass productivity. Further studies are needed to evaluate if the culture performance is maintained when the scale is increased from pilot (2.5 m3) to industrial (10 and 100 m3), however, this study represents an important step in the design and implementation of culture systems for industrial cultivation at Allmicroalgae.

II5. Bioprocess Integration and Intensification

FP199. Improvement of recombinant proteins purification by the use of ionic liquids as adjuvants in aqueous two-phase systems

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The advent of biopharmaceuticals in modern medicine brought enormous benefits to diverse human diseases and improved the well-being of many people worldwide. Among these biopharmaceuticals is interferon alfa 2b (IFNα2b), who plays a significant role in antiviral immunological responses and in the treatment of oncological diseases such as hairy cell leukemia and malignant melanoma. Aiming at finding cost-effective, efficient and sustainable technologies for the purification of IFNα2b from *Escherichia coli* BL21 cultures, novel polymer-polymer aqueous two-phase systems (ATPS) with ionic liquids (ILs) as adjuvants were investigated in this work. IFNα2b was effectively solubilized from inclusion bodies, the sample was dialyzed and the partition of IFNα2b was evaluated in the studied ATPS. Several ILs at 5 wt% with distinct chemical structures were investigated to improve the systems selectivity for IFNα2b. Higher partition coefficients of IFNα2b were obtained using ILs composed of aromatic cations and anions with higher hydrogen-bond basicity, in which hydrogen bonding and π-π interactions may account for the increased performance of these systems. Overall, it is here shown that the presence of ILs in low concentrations leads to an increase in the selectivity of polymer-polymer ATPS.

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II5. Bioprocess Integration and Intensification

FP200. A chromatography approach for clinical-grade purification of mesenchymal stem/stromal cell-derived extracellular vesicles

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Secreted into the outer space by most of the cells, extracellular vesicles (EVs) are found in almost all bodily fluids, participating in many cellular processes, particularly in cell-cell communication, through the exchange of proteins and nucleic acids. Previously described as cellular waste, former studies unveil that EVs' role goes beyond homeostasis maintenance, helping also in diseases propagation. Therefore, EVs have received a lot of attention from the scientific community due to their potential in diseases diagnosis, prognosis and therapy, working as drug delivery vehicles.

Despite the recognized potential, clinical usage demands reproducible scaled-up purification platforms, that confers reliability in EVs isolation from complex biological samples, with high efficiency and purity. So far, different downstream strategies have been implemented, being the differential ultracentrifugation, the most popular method used for EVs purification. Although efficient for EVs' isolation from cell culture supernatants, the high centrifugal forces can damage EVs integrity, while proteins are prone to aggregate, thus decreasing the purity of the final product. Adding to these disadvantages, the expensive equipment and the limited sample volumes processed, make this technique unfeasible for a scalable application.

In order to construct a scalable downstream process platform suitable for clinical trials, chromatography emerges as a method of choice, since it allows higher selectivity, reproducibility and cost-effectiveness. Nonetheless, the purification of these large biomolecules using chromatography remains a challenge due to low binding capacity and inability of working at higher flowrates using traditional packed-bed resins. In this work, we evaluated the performance of new chromatographic alternatives - gigaporous resins versus traditional agarose resins and the new-in-market CIMmultusTM EV monolith kit in the purification of MSC-derived EVs. Chromatography performance was assessed comparing the resolution and resin capacity by evaluating the recovery yields, and impurities (DNA and protein) removal. The quality of the final product obtained was evaluated.

This study shows that chromatography is a scalable, cost-effective and robust alternative purification method for EVs isolation. Its implementation in a downstream purification platform provides a highly purified product that can meet the requirements imposed by the agencies for phase I clinical trials.

II5. Bioprocess Integration and Intensification

P201. Integration of aqueous biphasic systems and ultrafiltration for purification of green fluorescent protein and solvent recycling

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The advances in biotechnology and molecular biology allowed the development of remarkable biomolecules, with industrial and medical applications. However, despite many breakthroughs in the upstream stages, the downstream processes still account for most of the production costs for biomaterials requiring high purification like the biopharmaceuticals. The association of different methods and the development of integrated platforms for downstream process can help to optimize the purification of biomolecules and to reduce costs. The goal of this study was to develop an integrated platform using Aqueous Biphasic Systems (ABS) and ultrafiltration to purify and polish proteins (particularly, the Green Fluorescent Protein, GFP) and recycle the solvents used in the process. Firstly, different biocompatible ABS composed of polymers, buffers and cholinium chloride ([Ch]Cl) were selected and the GFP extraction efficiency and purification potential was assessed. Two ABS presented high purification potential (GFP with > 97% purity): 1) a single-step ABS composed of polypropylene glycol 400 (PPG-400) and [Ch]Cl; and 2) a two-step back-extraction ABS with a first extraction using sodium polyacrylate 8000- (NaPA-8000), polyethylene glycol 600- (PEG-600) and [Ch] Cl-based ABS, followed by a PEG-600 and [Ch]Cl ABS extraction. Additionally, the PPG-400/[Ch]Cl ABS was associated with an ultrafiltration step to allow the recovery of GFP in an aqueous medium (GFP polishing) and to recycle 60% of the solvents used in the process. In conclusion, the integration of different downstream techniques can improve the purification of biomolecules and allow the development of more sustainable and potentially cheaper integrated processes.

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II5. Bioprocess Integration and Intensification

P202. Biocompounds recovery from Spirulina by conventional and ohmic heating methodologies: chemical and biological properties

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Extracting the totality of bio-compounds with industrial interest from Cyanobacterium is often prevented by the intrinsic rigidity of its cell wall. In this sense, the present study focuses on evaluating the influence thermal batch extraction (conventional extraction technologies) and ohmic heating (OH) assisted extraction (considered a greener alternative technology) in blue green microalgae *Arthrospira platensis* (Spirulina) cell disruption for bioactive fractions recovery.

The proximal composition of Spirulina was initially determined. The maximum protein content (i.e., C-Phycocyanin), total carbohydrates (TC) and total phenolic compounds (TPC) extracted in water at different times (30-120 min) and temperatures (30-51 ºC) was quantified after the conventional and OH- assisted extraction. The freeze-thawing process was used as control. The antioxidant activity (i.e., FRAP and DPPH assays) of the obtained extracts was assessed.

Results showed that with the freeze-thawing process, traditionally used for the recovery of bio- compounds from Spirulina, the concentration of C-phycocyanin was approx. 42 mg/g of Spirulina, 26 mgGlcE/g Spirulina of TC and 9 mgGAE/g Spirulina of TPC. Using OH-assisted extraction, the maximum of C-Phycocyanin content obtained was 45 mg/g of Spirulina (obtained at 37 ºC, 30 min), the maximum carbohydrates' content was 40 mgGlcE/g Spirulina and the maximum TPC was 10 mgGAE/g Spirulina. On the other hand, using conventional thermal treatment it can be observed that, under the same conditions, the bioactive compounds recovery decreased to 35 mg/g, 20 mgGlcE/g Spirulina for C-phycocyanin concentration and TC ($p<0.05$), respectively. The concentration in phenolic compounds is not so affected, but even so the ohmic heating potentiates the extraction of these secondary metabolites.

The antioxidant activity of the extracts there was not different between conventional treatments andOH.

Thus, the results indicated that OH is a good alternative to conventional methods aiming at the extraction of intracellular components with a decrease in processing time and energy costs associated with the extraction process, which together with an easy upscale make OH an interesting methodology for use in the industrial production of microalgae colorants and bioactive supplements.

II5. Bioprocess Integration and Intensification

P203. The effect of agitation in a heterogeneous integrated fermentative process with eucalyptus bark kraft pulp

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Currently, the industrial scale production of bioethanol from lignocellulosic biomass has been widely explored, since the raw materials used in the production of second generation biofuel are originated from a renewable energy source.
In this study, the raw material used for the tests was the kraft pulp of Eucalyptus globulus bark[1] with a kappa index of 32 (approximately 4.8 wt% lignin content), provided by RAIZ. The process used for testing was batch saccharification and simultaneous fermentation (SSF). SSF assays were performed at 38°C, 9 wt% consistency, 20 FPU per gram of carbohydrate (CH) of Cellic® CTec2 enzyme solution and the microorganism used for alcoholic fermentation was *Saccharomyces cerevisiae* (ATCC® 26602TM) yeast. The evaluated parameters were the agitation system configuration (orbital and mechanical), type of agitation and scaling up in order to investigate a variety of design configurations and to obtain higher results in bioethanol production. The tests were performed in agitated tank bioreactors (STBR) and in Erlenmeyer flasks, with working volumes from 50 to 1225 mL. Four agitators were used, the three-blade radial, the three-blade axial, the three-segment blade and the helical ribbon impellers.
Both the bioreactor configuration (STBR vs. Erlenmeyer) and the stirring system of the lignocellulosic biomass conversion process were identified as very important factors for high ethanol yield. The best SSF performance was obtained with dual helix ribbon impeller for larger volumes.

[1] Pedro C. Branco, Inês Mota, Paula C. O. R. Pinto, Eucalyptus globulus bark for fermentable sugars: preliminary results on the effect of pre-extraction and severity of pulping, 5th European Workshop on Lignocellulosics and Pulp (EWLP 2018), Aveiro, Portugal, oral communication.

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II5. Bioprocess Integration and Intensification

P204. Ethanolic fermentation of steam-exploded eucalyptus bark, under different conditions

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bioconversion of lignocellulosic biomass into bioethanol, pretreatment of the material prior to enzymatic hydrolysis is essential to obtain high yields of fermentable sugars and hence bioethanol. The main goal of pretreatment is to increase cellulose digestibility by improving enzymatic accessibility.
In this study, the effect of steam explosion pretreatment, under different conditions, alone or in conjunction with mild orgonosolv (70 wt% EtOH), as a second pretreatment, on *Eucalyptus globulus* bark valorization was evaluated. Different strategies were also studied for bioethanol production, including separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). The SHF was applied to previously obtained enzymatic hydrolysates, using *Saccharomyces cerevisiae* (ATCC® 26602TM) to convert the monosaccharides to bioethanol. In the SSF strategy, the solid pretreated bark was used as substrate, with both cellulase enzyme complex Cellic® CTec2 (to produce the fermentable sugars) and *S. cerevisiae* (ATCC® 26602TM) yeast. The effect of lignocellulosic biomass consistency (6 and 9 wt.%) and enzymatic load (20 and 40 FPU per gram of carbohydrate, CH) were also explored.
The results obtained showed that the SSF process is a viable alternative to SHF configuration considering the higher amounts of bioethanol obtained.

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II5. Bioprocess Integration and Intensification

P205. In the path of DHA producers: lab-scale heterotrophic growth of *Aurantiochytrium* sp. 0043

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Thraustochytrids, a heterotrophic fungus-like clade of Stramenopiles are well-reported by their outstanding ability to produce and accumulate PUFAs, particularly DHA. The biochemical composition of the obtained biomass is directly related to culture conditions. Aiming high biomass rates and DHA yields, medium and fermentation parameters were optimized for *Aurantiochytrium* sp. 0043. Medium optimization was literature-based, focused on carbon and nitrogen sources screening, its concentration, ratio and feeding strategies. Fermentation optimized parameters involved initial inoculum conditions (volume, age and growth phase), light intensity, oxygen transfer rates, and pH. Lab-scale optimization of *Aurantiochytrium* sp. strain 0043 allowed a 2-fold increase in biomass yield and improved lipid and DHA content from 22.78% and 1.25% to 31.14% and 29.66%, respectively. Although several conditions were tested in the 5L bench-top reactor, cultivation was not successful due to the shear-sensitive cells of this species and further trials on fermentation parameters and reactor design are required to enable the scale-up. Further studies are needed in order to verify the up-scaling viability of the designed media, however, the obtained results are very promising in what concerns to the optimized use of *Aurantiochytrium* sp. 0043 as a source of DHA.

II5. Bioprocess Integration and Intensification

P206. Reversible aqueous biphasic systems for biotechnology applications

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Liquid-liquid separation processes usually require the use of volatile organic solvents. In this field, aqueous biphasic systems (ABS) are promising alternatives, since these systems are majorly composed of water. ABS are ternary systems formed by water and two polymers, a polymer and a salt, or two salts, which above given concentrations undergo phase separation. The introduction of ionic liquids (ILs) into ABS offers several advantages when comparing with conventional polymer-based ABS, such as improved extraction performance and selectivity due to the possibility of designing their chemical structures according to the target application. ILs can be divided into aprotic ILs (AILs) and protic ILs (PILs). Although AILs have been the most studied ILs, in recent years, PILs are gaining momentum since they tend to be low-cost fluids. Accordingly, in this work, four ethanolammonium- based PILs combined with the chloride anion were synthetized and studied to form IL-based ABS with polypropylene glycol with a molecular weight of 400 g·mol-1, aiming the development of stimuli- responsive ABS (temperature- and/or pH-driven). The corresponding phase diagrams were determined at three temperatures (25,35 and 45 °C) and different pH values (4, 7 and 9). Based on the obtained results, it was observed that the studied ABS do not present temperature dependence, but depend on the pH. Therefore, considering their non-temperature dependence and strong influence of the pH, these systems may be relevant for the development of new integrated biotechnological platforms.

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II5. Bioprocess Integration and Intensification

P207. Continuous Atps Extraction Using Oscillatory Flow Reactor

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The continuous downstream processing can bring many advantages such as: automation and integration of unit operations, lower operator interaction with the equipment and consequently lower chance of errors; possibility of recycling buffers and auxiliary material; improvement of product quality, due to the greater uniformity of the process time; and increase of the process productivity. Biotechnology has allowed the development of completely new products from health to food industry. The quality of the process since cell culture until formulation is crucial in their application. Nowadays, almost all biological products are still produced entirely in batch mode, which brings some drawbacks that can be overcome, operating in continuous mode. The usage of aqueous two-phase systems (ATPS) have been proven as an efficient operation for the clarification and purification of biological products, but despite this fact ATPS have had a limited use at large scale. On the one hand, ATPS extraction uses aqueous solutions that provide an ideal environment for biological molecules, on the other, the high salt concentrations and the cost of some polymers are the main drawbacks that make this process less cost-competitive comparing with others; however the majority of ATPS extractions are still done in batchwise mode. Oscillatory flow reactor (OFR) is a type of tubular/channel reactor that has been used in processes as liquid-liquid reaction, polymerization, flocculation and crystallization. One of the most important features of this type of reactor is the uniform mixing that is provided by the combination of the periodically spaced restrictions and the oscillatory motion of the fluid. Therefore, the continuous mode and the particular characteristics of OFR could be the answer for a more cost-competitive ATPS extraction. In this work, the properties of different polymer-salt ATPS operated in a continuous oscillatory mode are being studied and compared with the batchwise type, highly described in the literature. The OFR will then be explored for the continuous ATPS extraction as a primary clarification step in the purification of food enzymes and antibodies, Yields and the purification factors obtained with the OFR will be compared with the batchwise operations, envisaging a more cost-effective process.

FP208. Improving minicircle DNA production in *E. coli* – in vivo analysis of recombination efficiency after re-design of *ParA* resolvase 5'-UTR

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Minicircles (MCs) are DNA delivery vectors that enable higher eukaryotic transgene expression in vivo than conventional plasmids. MCs are produced in *Escherichia coli* by intramolecular recombination of a parental plasmid (PP) into two circular DNA molecules: (i) a MC comprised by the eukaryotic cassette of interest and (ii) a miniplasmid containing the prokaryotic backbone. This process is mediated by a recombinase that recognizes two sequences located on the PP. The efficiency of recombination requires high-levels of recombinase expression. From the recombinase systems reported for MC production, the *parA* resolvase under control of the *PBAD*/*araC* system has showed high efficacy.
This work focuses on optimization of the *PBAD*/*araC-parA* system, based on evidence that in *E. col*i the expression of heterologous proteins is inversely correlated to the stability of the secondary structure of their mRNA 5' untranslated region (5'-UTR). To optimize *parA* expression, we used an *in silico* thermodynamic model to iteratively probe and re-design the original 5'-UTR of *parA*, generating four sequences with higher translation initiation rates (TIR). According to a predictive analysis of the initial 77-nt mRNA subsequences, the engineered 5'-UTRs (*PBAD/araC-par2A* to *PBAD/araC-par5A*) were expected to have a TIR between 63 to 366-fold higher than the original system. The *ParA* expression systems were then evaluated in vivo by inserting each cassette into a helper plasmid that was transformed into a PP-harbouring strain. Recombinase expression under limiting induction conditions was measured by qRT-PCR of the *parA* mRNA and the recombination efficiency with densitometry analysis of agarose gels.
Although all engineered sequences led to a faster recombination process, the highest values were obtained for the *PBAD/araC-par3A* cassette which enabled a 10-fold increase of recombination efficiency over the original cassette and was 2-fold more efficient than the other optimized systems. Insertion of a single copy of the original, *PBAD/araC-par2A* or *PBAD/araC-par3A* cassettes into the bacterial chromosome resulted in a better performance by the *PBAD/araC-par3A* containing strain which, with a recombination efficiency 2-fold higher than the one with *PBAD/araC-par2A*, was considered the most promising strain for MC production.

FP209. Alternative strategies to fight antibiotic-resistant pathogens resulting from the combination and modification of natural antibacterial functions

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It is widely recognized that in a near future bacterial infections may become very difficult to treat because of antibiotic resistance. This problem, associated to a very limited pipeline of truly new therapies from the pharmaceutical industry, has been driving research on alternative antimicrobials, preferentially on those with modes of action that minimize resistance development. Among such alternatives are phage-derived lytic enzymes (lysins) that degrade the bacterial cell wall (enzybiotics).
Bacteriophages produce endolysins to destroy the bacterial host cell wall (CW) at the end of infection for virion progeny release. In their natural context, endolysins need to escape the cytoplasm to reach the CW (lysis from within). A key phage-encoded factor involved in endolysin translocation across the cytoplasmic membrane (CM) and/or its activation in the CW is the holin. Holins form holes in the CM that cause fatal dissipation of the membrane proton-motive force (PMF) and provide a conduit for endolysin passage to CW compartment. Besides channel formation, we have shown that the holin PMF-dissipating action can be essential to fully sensitize cells to the endolysin lytic action. Consequently, when bacteria are under PMF-supporting conditions they may present a certain level of tolerance to exogenously added endolysins (lysis from without), which might result in limited efficacy of the enzybiotics.
As a strategy to improve the lytic action of endolysins added from without, we have reasoned that the holin key action could be replaced by that of antimicrobial peptides (AMPs), which typically act by injuring the bacterial cell envelope. AMPs have also been presented as antibiotic alternatives, but some hurdles in (pre)clinical trials have been hampering their translation into effective antimicrobials. By using model bacteria, which include staphylococcal and enterococcal species, we show that AMPs used either in combination with endolysins or genetically fused to the enzymes significantly enhance the bacteriolytic action of the enzybiotics. We expect that fusing selected AMPs to endolysins will generate a new class of potent antibacterial agents, that we named AMPLys, which combine in a single polypeptide CW-degrading and cell envelope-damaging activities. Importantly, it is anticipated that AMPLys will overcome major limitations of the parental molecules.

FP210. A powerful and cost-effective strain typing tool: the ideal solution for real-time control of *Klebsiella pneumoniae* infections

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Background: *Klebsiella pneumoniae* (Kp) infections are increasingly more challenging to treat and control, particularly in the hospital setting. Bacterial typing is fundamental to support surveillance and control of infections but available methods are complex and have inadequate time-to-response, including whole genome sequencing. We used molecular genotypic, comparative genomics, biochemical and phenotypic data to support the usefulness of a promising but underexplored spectroscopic method [Fourier Transform Infrared (FT-IR) spectroscopy] for simple, quick and cost-effective *K. pneumoniae* typing.
Materials/methods: 154 representative and previously characterized multidrug resistant Kp isolates involved in human infections in Europe and South America (2002-2015) were selected to validate the approach. Bacterial types were defined by gold-standard methods including PFGE, MLST, PCR/sequencing of specific genetic markers, and subsequently by whole genome sequencing (n=9; Illumina), assembly (SPAdes) and annotation (PROKKA). FT-IR spectral data were obtained and analyzed by multivariate data analysis, and complemented with genomics and biochemical (polysaccharide composition and structure) data on capsular (K)-types, which were on the basis of FT-IR discriminatory ability.
Results: Using a very simple and inexpensive protocol, we were able to obtain a reliable (~95% correct predictions) FT-IR-based discrimination of main multidrug resistant *K. pneumoniae* nosocomial lineages (n=21) on the basis of their corresponding K-types. These were perfectly distinguished by FT-IR analysis in two multivariate data analysis models that capture spectral diversity, in line with variation on K-type polysaccharide structure/composition. The correlation established between K-types and Kp lineages identified in different geographic regions over time supports the value of the method for precise strain typing. Real-time application of the method in the field allowed the correct identification of MDR Kp outbreak strains in less than 24h, which represents a 75% reduction in time-to-response compared to competing methods.
Conclusions: The FT-IR-based approach developed gathers an unprecedent combination of features (high resolution, speed, low-cost, simplicity) that provides enough information to support real-time epidemiology and infection control decisions. Thus, it represents the ideal solution to control Kp infections in the nosocomial setting.

III6. Health Microbiology and Biotechnology

FP211. Cod skin collagen extraction and characterization

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Collagen is a structural protein present in different animal tissues and has a wide range of applications in the health-related sectors. Regarding its industrial exploitation, collagen has mainly bovine and porcine origins. However, due to religious beliefs and infectious diseases, other collagen sources are being debated. In this regard, the use of collagen with marine origin is being considered highly attractive by the industry as an important alternative source. Fish residues may account for an average of 55% of the total fish weight; of this material, up to 30% may be skin and bone. Fish skin has more than 80% of its total protein content as collagen. Thus, a general methodology to isolate collagen from fish by-products was applied for cod skin collagen recovery: a sodium hydroxide (NaOH) pre- treatment to remove non-collagenous proteins, pigments or fats, followed by the extraction phase using an acid solution to obtain the Acid Soluble Collagen (ASC). Finally, collagen was precipitated adding NaCl and collected by centrifugation, dissolved in acetic acid and dialyzed. The ASC extracted from cod skin was then partly characterized. SDS-PAGE analysis showed the characteristics bands which represents the typical structure of type I collagen, including two different kinds of α chains, β chains (dimers) and γ chains (trimers).The UV absorption data of ASC from cod skin was a maximum absorption at 213 nm, confirming the typical absorptions near 210–240 nm of collagen. No absorbance measurements were obtained at 280 nm due to low concentrations of aromatic amino acids in ASC. FTIR analysis presented the main collagen characteristic absorption peaks contained amide A, amide B, amide I, amide II, and amide III. The amide III band (1238 cm^{-1}) revealed the presence of a helical structure and the ratio of absorbance between amide III and the 1400–1454 cm^{-1} wavelength was 1.08, which revealed that the triple helical structure of collagen was intact. The SEM images confirmed that the collagen fibrils remained intact after the extraction process. Therefore, cod skin collagen may be an alternative to terrestrial mammalian collagen and may enhance the added value of this fish species.

III6. Health Microbiology and Biotechnology

P212. ESBL-positive Enterobacteriaceae in dogs from praia, Cape Verde – a public health issue

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Antimicrobial resistance is a major global health concern, responsible for increased morbidity and mortality worldwide. In developing countries, such as Cape Verde, there are basic sanitation problems, facilitating the development and transmission of resistant bacteria between humans, animals and the environment. However, bio-surveillance programs on antimicrobial resistance dissemination are lacking in this country, particularly in the veterinary setting. Among resistant bacteria, Extended Spectrum Beta-Lactamases (ESBL)-positive Enterobacteriaceae are of special importance, being classified by World Health Organization in 2017 as critically relevant.

Forty-four fecal samples were collected in Cidade da Praia, Cape Verde, from dogs with (n=25) and without (n=19) gastrointestinal signs of disease, using AMIES swabs. Samples were kept refrigerated until transport to the Laboratory of Bacteriology of the Faculty of Veterinary Medicine, Lisbon, where they were processed for the detection of ESBL-positive isolates using CHROMID®ESBL agar (bioMérieux) according to the manufacturer's instructions. Statistical analysis was performed to evaluate the relation between ESBL-positive samples and dog characteristics.

From the tested samples, 13 (29.5%) isolates were ESBL-positive, with the clear majority being identified as *Escherichia coli* (n=10, 76.9%). No dependency was observed between the prevalence of ESBL-positive isolates and the age (p=0.599), sex (p=0.322) and manifestation of gastrointestinal signs of disease (p=0.067) of the sampled animals. However, dogs presenting ESBL-positive isolates had a 1.65 times higher risk (95% CI: 1.015-2.677) of displaying gastrointestinal signs of disease in comparison to dogs with ESBL-negative samples.

Considering that most dogs in Cape Verde are outdoor animals, i.e. range freely in the streets, the current work shows their potential as disseminators of important resistant isolates to Public Health, independently of their health status. Despite the current dog population control measures, the establishment of effective antimicrobial resistance surveillance programs in this species would be of major importance for the country's health system, requiring an investment in laboratory equipment, technology and training.

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P213. Can antimicrobial peptides induce the development of resistant mutants? – a pilot study with staphylococci from Diabetic Foot Infections

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Worldwide mortality rate associated with infections promoted by multi-drug-resistant (MDR) bacteria continuous to increase. Antimicrobial peptides, such as nisin, represent promising alternatives for treating chronic infections promoted by MDR strains such as Diabetic Foot Infections (DFI), a major complication of Diabetes mellitus. However, there are no studies available regarding its influence in resistant mutants' development. Mutant prevention concentration (MPC) corresponds to the antimicrobial concentration that impairs the formation of mutant strains, which combined with the Minimum Inhibitory Concentration establishes the mutant window selection (MSW), a range of concentrations that may promote MDR evolution. Our goal was to determine nisin MPC and MSW values regarding a collection of *Staphylococcus aureus* isolates (n=24) obtained from DFI patients previously characterized by our research team. Nisin concentrations ranging from 11.25 to 720 µg/mL were applied onto Mueller-Hinton agar plates, followed by inoculation with 1010 CFU/mL bacterial suspensions. After incubation (72h, 37ºC), plates were observed to detect the development of resistant isolates. All assays were performed in duplicate and repeated twice.
Results distribution was as follows: 2 isolates presented an MSW ranging from 11,25 to 360 µg/mL; 3 isolates an MSW from 11,25 to 540 µg/mL; and 1 isolate an MSW from 11,25 L to 720 µg/mL. For the remaining 18 isolates, the MSW interval could not be determined, since they were able to grow in the presence of the highest concentration of nisin tested (720 µg/mL).
Results show the importance of MPC and MSW determinations to guarantee an antimutant dosing strategy, allowing to establish innovative treatment protocols based on appropriate antimicrobial doses. To our knowledge, this is the first report of nisin's MPC and MSW determination regarding DFI staphylococci, being a relevant step in the R&D of new antimicrobial strategies. Nevertheless, results are in accordance with the previously determined MSW interval for vancomycin regarding *S. aureus*, which is relevant since the mode of action of these two antimicrobials are similar.

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P214. Cutaneous mycobiota of pet rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*)

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Pet rabbits and guinea pigs are increasingly popular. Among the zoonotic infections that affect these species, dermatophytosis is the most frequent, and these animals have already been referred as asymptomatic carriers of dermatophytes. Therefore, studies characterizing their cutaneous mycobiota are of major importance for safeguarding Public Health.
We conducted an assessment of rabbit and guinea pigs cutaneous mycobiota in two veterinary hospitals located in Lisbon, Portugal and Barcelona, Spain. The sampled animals included x guinea and y pigs (118 animals in total) presented for routine evaluation. Samples were collected by (1) pulling hairs around cutaneous lesions (if present) or along the animals' body, or (2) using the Mackenzie's technique. Samples were inoculated in (1) Sabouraud Chloramphenicol Agar and (2) Dermatophyte Test Medium, incubated at 27ºC for 21 days. Cultures were observed daily and fungal species identified through macro and microscopic characterization of the colonies.
No dermatophytes were identified; however, saprophytic fungi such as *Aspergillus*, *Penicillium* and *Scopulariopsis*, similar to those found on the skin and hair of other animals, were frequently isolated.
Fungal species identified were already reported as belonging to the cutaneous mycobiota of healthy domestic animals, such as dogs and cats, and were also reported as causing mycotic infections in animals and humans.

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P215. Detection of ESBL and Carbapenem-resistant *Klebsiella pneumoniae* from hospitalized patients with bacteremia in Portugal

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The emergence and dissemination of carbapenem resistance among Enterobacteriaceae, especially *Klebsiella pneumoniae*, constitute a serious threat to public health [1, 2]. *K. pneumoniae* is one of the most important infectious agents that commonly causes nosocomial infections and contributes to substantial morbidity and mortality. Carbapenem resistance was first reported a decade ago and has subsequently emerged worldwide [3, 4]. The aim of this work was to determine the carriage rate of carbapenem- resistant and ESBL-producer *K. pneumoniae* from hospitalized human patients in Portugal, and the type of enzymes implicated.

Twenty-two cefotaxime/ceftazidime-resistant *K. pneumoniae* isolates obtained from blood cultures of patients of a Portuguese hospital during June 2017-July 2018 were included in this study. The MALDI- TOF MS method was applicable in this study to confirm bacteria identification. Antimicrobial susceptibility was performed by disk-diffusion test (CLSI, 2018) and ESBL phenotype was analyzed by the double-disk test. The presence of bla_{CTX-M} (different groups), bla_{TEM}, bla_{KPC}, bla_{SHV}, bla_{NDM}, tet_A, tet_B and int1 were tested by PCR/sequencing.

All the isolates are resistant to ceftazidime, amoxicillin + clavulanic acid and cefotaxime (100%) and 21/22 were resistant to ciprofloxacin and sulfamethoxazole. ESBL-producers were detected in 50% of the isolates (11/22), all of them associated with CTX-M-15 encoding gene. Furthermore, we found class 1 integron (n=3) and its association with tetA gene (n=8). All ESBL-positive isolates contain SHV, specifically SHV-1(n=9), SHV-11 (n=1) and SHV-27 (n=1). We also detected KPC2/3 in one ESBL producer isolate and NDM in another one. Ten ESBL negative isolates were positive for the same gene (91%) and in most cases associated with SHV-1 (n=4) and SHV-106 (n=1).

The findings demonstrate that CTX-M-15-producing K. pneumoniae are frequent in nosocomial infections implicated in bacteremia in the tested hospital, and in most of the cases associated with carbapenem resistant related to KPC gene.

P216. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* traits may determine the presence of these species in the hospital microbiome

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Healthcare-associated infections - HAI covers a set of emerging infections in patients from the community with a history of exposure to pathogens in healthcare. Bacteria belonging to the ESKAPE group are usually associated with HAI and comprises the majority of the isolates whose resistance to antimicrobials presents serious therapeutic dilemmas for human health. *Pseudomonas aeruginosa* is involved in 11% of infections acquired in hospitals. This species is often found in humid environments, including hospital facilities as tap or sinks, as part and involved of a biofilm formation. On the other hand, *Klebsiella pneumoniae* is associated with cases of pneumonia and with broad resistance to antibiotics. The objective of this work is to determine the antibiotic resistance pattern, biofilm formation and polymer degradation ability of hospital environment bacteria to understand the impact of these strain characteristics in the definition of the hospital microbiome. Samples from non- critical equipment were collected, by swab, over three consecutive months from two public hospitals in Portugal. A total of 56 strains, belonging to the species *P. aeruginosa* and *K. pneumonia* were isolated, purified and their antimicrobial susceptibility determined according to the method disc diffusion (EUCAST, 2017). The strains were also screened for their ability for polymer degradation using a standard method modified (van der Zee, 2011) and for their ability to form biofilm (standard microtiter). The antibiotic resistance pattern of the isolates was different according to the hospital origin. All strains from hospital 1 were resistant to six antibiotics, and all strains from hospital 2 were multiresistant to seven antibiotics, but the resistance pattern was different. None of the strains was able to completely degrade the polymers tested (silicone and food cover film). The presence of silicone in the medium promoted the growth of *K. pneumonia* HST102BK and HST118CP *P. aeruginosa* when comparing to control. Biofilm formation was observed for *Pseudoxanthomonas indica* (HST12DK), *P. aeruginosa* (HST118CP and HST244P) and *P. nitroreducens* (HST 222aK) from 35 strains used in duplicate. In conclusion, the *P. aeruginosa* and *K. pneumonia* strains isolated from the two hospital environments seems to share characteristics that promote their presence in the hospital microbiomes.

III6. Health Microbiology and Biotechnology

P217. Evaluation of complex of Base Schiff plus copper in Visceral Leishmaniasis model *in vivo*

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Introduction: The leishmaniases are a group of diseases caused by protozoan parasites, the WHO estimated that over 616 million people living in endemic areas at risk of Visceral Leishmaniasis (VL), and 300.000 new cases occur annually. The objective of this work was evaluate the leishmanicidal activity of complex of Schiff Base plus copper (N-Salicilidenoanilina + Cu) *in vivo* model. Material and Methods: Young male Golden hamsters (110 g) were infected intraperitoneally (i.p.) with L. (L.) infantum chagasi promastigotes (1.107 /animal). Sixty days after the infection, the treatment with N-Salicilidenoanilina + Cu (400mg/kg/day) was administered intraperitoneally for eight consecutive days. Five hamsters were uninfected and untreated, five were infected and untreated, and five were infected and treated with amphotericin B (20mg/kg/day). The animals were euthanized with isoflurane after the treatment. A tissue sample of the liver and bone marrow was collected and used for DNA extraction, and the spleen was used for DNA and RNA extraction. The parasite load was analyzed from the three tissues with quantitative PCR (qPCR) performed with JW11 and JW12 primers using SYBER GREEN®. Also the Reverse transcription PCR (RT-PCR) was performed with primers and specific probes for IFN-ɣ and iNOS and for the cytokines IL-10 and IL-4.
Results: The parasite load decrease in treated animals with amphotericin B and complex of Schiff Base plus copper. There was no significant difference for parasite load reduction in the three organs analyzed. The treated group with the complex of Schiff Base plus copper showed a significant increase in iNOS expression when compared to the infected and amphotericin B treated groups. The IL-4 expression was significantly increased in complex of Schiff Base plus copper treated group compared to the amphotericin B group. There was no differences in IFN-ɣ or IL-10 expression when compared the groups.
Conclusion: Golden hamsters treated with the complex of Schiff Base plus copper increased the expression of gene encoding inducible nitric oxide synthase (iNOS), which is an important factor for VL resolution. However further *in vivo* assays are needed to evaluate the toxicity and the dosage of this compound.

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P218. A molecular biology platform for the production of cytokines and cytokine-functionalized materials

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Leukemia inhibitory factor (LIF) and Oncostatin-M (OSM) are two pleiotropic cytokines from the IL-6 cytokine family with implications in health and disease. Their biotechnological exploitation finds applications in the treatment of different diseases such as cardiac and neurological pathologies, and even cancer. In recent years, several patents and research papers were published regarding their heterologous expression, however problems with production yield, protein stability and activity can significantly hinder the applicability of these cytokines. In this work, we developed a molecular biology platform for the cloning of cytokines LIF or OSM with different recombinant protein-based polymers aiming at their production and subsequent processing into functional materials. As polymers, we used a variant of an elastin-like recombinamer (ELR) and another from silk elastin-like proteins (SELPs). A combination of ELR-intein proteins was used as production, purification and cleavage tag for the cytokines, which can be encapsulated into organic and inorganic particles. On the other hand, a SELP-6xGlycine module was devised to facilitate further processing into a variety of different materials including films, fibres and scaffolds. In this system, we cloned both murine and human forms of the cytokines, to allow their use in different studies and models. Heterologous protein expression studies, using *Escherichia coli* as cell factory, indicate that all the designed proteins are overexpressed although at different levels depending on the nature of the cytokine, though always suitable for lab-scale utilization.
We believe that the modular system here discussed will endow us with sufficient plasticity to design materials adjusted to the pathologies where LIF and OSM are implicated and may have a positive therapeutic impact.

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P219. Geospatial distribution of toxoplasmosis and its association with hepatitis and HIV infection in pregnant women in Luanda (Angola)

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Introduction: Toxoplasmosis is widely distributed worldwide infecting almost a third of the world's population. To date, no research has explored the overall *Toxoplasma gondii* infection seroprevalence among women in Angola, nor have the risk factors associated with the infection been examined in region context. Therefore we aim to study the seroprevalence of toxoplasmosis in pregnant women in Luanda and to establish the potentially risk factors.
Material methods: Specific anti-T. gondii IgG and IgM antibodies were quantified from 878 whole blood samples of pregnant women. Demographic and clinical data were collected by questionnaire after written consent. The address of the individuals was collected during the interview allowing the identification of the place of residence. This information was converted into geographic coordinates through the www.google.pt/maps/. The spatial distribution of pregnant women was assessed through a Kernel Density Function. The statistical and descriptive analysis of the data was developed using SPSS software version 20.0. Statistical significance was considered when P <0.05.
Results: Anti-T. gondii antibodies were quantified in 878 pregnant women and 346 (39.4%) samples were IgG positive, 2 (0.2%) positive for IgM and IgG and 530 (60.4 %) are negative for both immunoglobulins. The prevalence of *T. gondii* varies by municipalities, most of the pregnant women (435 of 878; 49.5%) live in Luanda municipality, followed by Viana (207; 23.6%), Belas (120; 13.7%), Cazenga (64; 7.3%) and Cacuaco (52; 5.9%). Regarding other infections, 226 (25.7%) were positive for hepatitis B and 652 (74.3) negative while 118 (13.4%) were HIV positive and 760 (86.6) were negative. Pregnant women positive for hepatitis B (p=0.013) and/or HIV positive (p=0.005) were associated with an increased risk to toxoplasmosis.
Conclusions: The study showed that there are a high number of women in Luanda that are not immunized for toxoplasmosis and are at risk of acquiring the primary infection. The consequence of primary infection is the potential vertical transmission to the fetus, resulting in congenital toxoplasmosis. Therefore, it is crucial establishing the diagnosis of maternal primary infection as well the appropriate diagnosis of congenital toxoplasmosis. Prenatal counseling should include education on prevention of toxoplasmosis, hepatitis and HIV infection.

P220. Companion animals with Skin and Soft Tissue Infection share multidrug-resistant *Escherichia coli* strains gut colonization with their human household members

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Objectives: This study aims to characterize the antimicrobial resistance (AMR) among Enterobacteriaceae colonizing the gut of companion animals (CAs) with urinary tract infections (UTIs) and skin/soft tissue infections (SSTIs) and their household members.
Material/methods: Between February 2016 and July 2019, nine households (HDs) with companion animals (cats-2; dogs-7) diagnosed with UTI and 19 healthy humans were enrolled. Additionally, eight HDs with CAs diagnosed with SSTIs (cats-1; dogs-7) and 20 healthy humans were obtained. Fecal samples were inoculated on MacConkey agar with 1.5µg/mL cefotaxime and 1.0µg/mL meropenem, and CHROMID® OXA-48 plates. Susceptibility tests were performed by disk diffusion. Beta-lactam genes were screened by PCR and sequencing. *Escherichia coli* was identified by PCR and clonality assessed by rep-PCR.
Results: In 22% (n=2/9) of the UTI HDs and in 63% (n=5/8) of the SSTIs HDs, third-generation-cephalosporins-resistant *E. coli* strains were isolated from the gut of human and CAs. Regarding UTI HD1 and 2 there was no sharing. Susceptibility testing of this companion animals and humans *E. coli* isolates revealed a multidrug resistance phenotype.
Concerning SSTIs HDs, 3 HDs showed gut colonization in humans and CAs that shared multidrug resistant *E. coli* strains with the same rep-PCR pattern (100% similarity). Bla genes were harbored in those strains: i) in HD3, $bla_{\text{CMY-2}}$ gene was detected in both dog and 2 owners; in HD4 the $bla_{\text{CTX-M-15}}$ gene was also detected in both dog and 2 owners; in HD5 the $bla_{\text{CTX-M-65}}$ and the $bla_{\text{TEM-1}}$ genes were detected in both dog and 1 owner. The rep-PCR pattern was different in *E. coli* isolates, as well as, the bla genes content in HD6 and HD7, demonstrating a probable lack of AMR sharing.
Conclusions: These results are crucial to demonstrate the importance of interventional measures to avoid antimicrobial transmission between sick pets and humans. To the best of our knowledge, this is the first description of $bla_{\text{CTX-M-27}}$ gene in *E. coli* from companion animals in Portugal.

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P221. Sharing of ESBS/pAmpC-producing *Escherichia coli* in healthy companion animals and their household human members

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Objectives: The aim of this study was to identify the possible sharing of ESBL/pAmpC and carbapenemases beta-lactamases-producing Enterobacteriaceae in healthy companion animals (CAs) and their household members.
Material/methods: During April 2016 to August 2019, fecal samples were obtained from fifty households constituted of healthy humans (n=72) living with healthy CAs (n=70; 24 cats and 46 dogs). Owners informed consent was obtained. Fecal samples were inoculated on MacConkey agar plates containing 1.5µg/mL cefotaxime, 1.0 µg/mL meropenem, CHROMagarTM Acinetobacter and CHROMID® OXA-48 plates. ESBL/pAmpC genes were detected by PCR and sequencing. *Escherichia coli* identification and phylogenetic groups were determined by multiplex PCR. Clonality was investigated in *E. coli* isolates using the repetitive element sequence-based PCR (rep-PCR).
Results: All cultures from the fecal samples studied were negative for multidrug-resistant Acinetobacter and carbapenemase-producing–Enterobacteriaceae. In 4% (n=2/50) of the households (HD), third- generation-cephalosporins-resistant *E. coli* strains were shared between human and CAs. Regarding ESBL/pAmpC genes, the CTX-M group-1 was the main ESBL found in E. coli shared in the households. In HD1 the *E. coli* isolate from the cat harbored the $bla_{\text{CTX-M-1}}$ group and $bla_{\text{CMY-2}}$ genes and the *E. coli* from the human additionally harbored a bla_{TEM} and bla_{ACC} genes. Both isolates belonged to phylogroup A. Regarding HD2, both *E. coli* isolates from the dog and the human harbored the $bla_{\text{CMY-2}}$ gene. Additionally, the dog *E. coli* also harbored the bla_{TEM} gene. Both isolates belonged to phylogroup B1. Concerning clonality, in both HDs the *E. coli* isolated from humans and CAs shared identical rep-PCR profiles (100% similarity).
Conclusions: This study demonstrates that *E. coli* isolates colonizing the companion animal and human gut living together can be shared by direct contact. Also, simultaneous carriage of ESBL/pAmpC between companion animals and their owners in these bacteria even in a small number of households is a worrying finding that poses concerns to Public Health.

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P222. ESBL/Carbapenemase-producing Enterobacteriaceae in healthy companion animals in close contact with humans

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Objective: The purpose of this study was to evaluate the presence of extended-spectrum β- lactamases (ESBL) and carbapenemase-producing Enterobacteriaceae bacteria faecal carriage in healthy companion animals in close contact with humans.
Material and Methods: Between January 2016 and August 2019, 71 healthy companion animals (47dogs and 24 cats) living in close contact with humans in 50 households, were enrolled in this study. The pet owners were informed of the procedures and conducted the fecal sample collection from their companion animals with sterile gloves, containers and plastic bags. Informed consent was obtained. Fecal samples were inoculated on MacConkey agar plates containing 1.5µg/mL cefotaxime, 1.0 µg/mL meropenem, and CHROMID® OXA-48 plates. Regarding, third-generation cephalosporin (3CG) resistant Enterobacteriaceae, three serine-β-lactamases molecular class A families of genes: *bla*$_{SHV}$ and *bla*$_{TEM}$ and the *bla*$_{CTX-M}$ genes, were screened by PCR with specific primers. The genes encoding the serine-β-lactamases molecular class C family of AmpC β-lactamases *bla*$_{CIT}$, *bla*$_{LAT}$, *bla*$_{ACT}$, *bla*$_{MIR}$, *bla*$_{FOX}$, *bla*$_{MOX}$ and *bla*$_{DHA}$ were screened by multiplex PCR.
Results: Third-generation cephalosporin-resistant Enterobacteriaceae were detected in 14.9% of the dogs (n=7/47) and in 12.5 % of the cats (n=3/24). In Dogs, among these 3CG-resistant Enterobacteriaceae, two were positive for the *bla*$_{CTX-M}$-group-1 gene, one for *bla*$_{CMY-2}$ gene, one for both *bla*$_{TEM}$ + *bla*$_{SHV}$ genes and one was simultaneously positive for the *bla*$_{CTX-M}$-group-1 + *bla*$_{CMY-2}$ + *bla*$_{TEM}$ genes. Among cats, 3CG-resistant Enterobacteriaceae only one harbored the *bla*$_{CTX-M}$-group-1 + *bla*$_{CMY-2}$ genes. In both groups of companion animals carbapenemase-resistant Enterobacteriaceae were not detected.
Conclusion: The isolation of multidrug-resistant Enterobacteriaceae in healthy companion animals is an emerging problem and dissemination of resistant bacteria through fecal contamination of the environment should not be neglected and is a concern towards animal and human health.

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P223. Public health impact of ESBLs/pAmpC- producing *Escherichia coli* causing urinary tract infections in non-related companion animals and humans

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Objectives: This study aimed to characterize third-generation cephalosporin (3GC)-resistant *Escherichia coli* causing urinary tract infections (UTI) in human from the community (H) and in companion animals (CA).
Material and Methods: 3GC-resistant *E. coli* (companion animals n=35; humans n=85) isolated from patients with UTI were tested against 14 antimicrobials. PCR-based assays were used to detect the major *E. coli* phylogenetic groups, Pathogenicity associated-islands (PAIs) (n=8), urovirulence genes (n=8), ESBLs/pAmpC) resistance genes. ESBL/pAmpC-producing *E. coli* isolates were typed by multi locus sequence type (MLST). The ST131 clonal group and subclades C2 (H30-Rx) and C1 (H30-R1) were identified by PCR. Fisher exact test was used for statistical analysis. Results were considered significance if $p < 0.05$.
Results: The frequency of resistance against fluoroquinolones (CA=74.3%, H=88.2%), trimethoprim/sulphamethoxazole (CA=71.4%, H=74.1%) and gentamicin (CA=40%, H=37.6%) were higher in 3CG-resistant *E. coli* from both groups. All isolates were susceptible to carbapenems. Considering phylogenetic group 3GC-resistant *E. coli* strains from companion animals and humans mainly belonged to group-D and B2 (48.6%, 67.1%, respectively). The most frequent PAIs and virulence genes among isolates from companion animals and humans were: PAI IV536 (CA=72%, H=91.8%, p=0.017) and PAI ICFT073 (CA=54.3%, H=78.8%, p=0.013); ecpA (CA=100%, H=100%)
and iucD (CA=48.6%, H=83.5%, p=0.0002). MLST typing of the ESBL/pAmpC-producing *E. coli* strains revealed a heterogeneous population of *E. coli* clonal groups in companion animals and in humans with UTI. Companion animals and humans *E. coli* strains shared two MDR high-risk clonal lineages: ST131, and ST648, an emergent virulent lineage. The $bla_{\text{CTX-M-15}}$ and the $bla_{\text{CMY-2}}$ genes were the most frequently detected in companion animals and human isolates. ST131 strains from companion animals and humans mostly belonged to subclade C2 (H30-Rx).
Conclusion: The cross-species sharing of important multidrug-resistant high-risk clones is a public health concern. Considering that companion animals with UTI are generally treated at home by the owners, measures should be implemented to avoid the spread of these bacteria to the environment.

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P224. *Gardnerella vaginalis* enhances *Atopobium vaginae* viability *in vitro*

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Background
Bacterial vaginosis (BV) is a clinical condition characterized by a dramatic shift in the vaginal microflora from the beneficial lactobacilli to a polymicrobial flora, consisting of strictly and facultatively anaerobic bacteria. It is noteworthy that a hallmark of BV is the presence of a highly structured polymicrobial biofilm on the vaginal epithelium, presumably initiated by a facultative anaerobe, *Gardnerella vaginalis*, which then become a scaffold for other species to adhere. While not much is known about multi- species interactions within BV biofilms, *Atopobium vaginae* is often associated with *G. vaginalis* biofilms and is rarely detected without *G. vaginalis*.

Methods
This study assessed interactions between *G. vaginalis* and *A. vaginae*, analyzing both mono- and dual-species cultures. Firstly, we evaluated the impact of *A. vaginae* on a pre-established *G. vaginalis* biofilm, by determining the total biofilm biomass using the crystal violet method. Furthermore, the bacterial distribution and biofilm structure were evaluated by Peptide Nucleic Acid Fluorescence in situ Hybridization (PNA-FISH) and confocal laser scanning microscopy analysis. Afterward, quantification of viable bacteria within pure or dual-species planktonic cultures was performed by using specific *G. vaginalis* or *A. vaginae* PNA probes, using the FISH method.

Results and Conclusion
We observed that, in our in vitro conditions, *A. vaginae* was not able to establish a single-species biofilm but easily incorporated a pre-formed *G. vaginalis* biofilm. Interestingly, *A. vaginae* lost viability after 48 hours of single-species planktonic growth but was able to maintain viability when co-cultured with *G. vaginalis*. This demonstrated that in vitro *A. vaginae* is dependent on *G. vaginalis* to survive, providing an explanation of the co-occurrence of these two species *in vivo*. Overall, this study underlined the importance of the ecological interactions between these BV-associated species, which might delineate the development of BV.

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P225. Elastin-based tags for the biotechnological production of antimicrobial peptides

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Due to the misuse and overuse of antibiotics, antimicrobial resistance continues to be as a global threat and new therapeutic approaches are crucial against bacteria. Antimicrobial peptides (AMPs) are promising candidates but their recovery from natural sources or their chemical synthesis usually requires cumbersome, inefficient and expensive process. These limitations can be overcome using recombinant DNA technology for the biotechnological production of AMPs using microbial cell factories. However, AMPs are often expressed in inclusion bodies and susceptible to proteolysis. Furthermore, purification is often cumbersome and time consuming. The use of elastin-like recombinamers (ELRs) as fusion partners represents a promising alternative to traditional chromatographic purification methods. Apart from increasing solubility, their most relevant characteristic is the reversible temperature transition behavior: in solution, ELRs acquire a disordered structure, but above a specific temperature threshold they self-assemble, segregating from solution, in a totally reversible process. This phenomenon can be used for the purification of recombinant proteins by simple hot and cold cycles that can be up-scaled. Owing to their unique physical and biological properties, we developed a method based on the use of ELRs as fusion partners, for the production and isolation of antimicrobial peptides. The AMP-ELR proteins demonstrated to be efficiently overexpressed in *Escherichia coli*, here used as microbial cell factory, and easily purified by simple temperature cycles. The minimum inhibitory concentration of isolated AMPs was determined against different bacteria in accordance with EUCAST/CLSI Antimicrobial Susceptibility Testing Standards, showing activity against Gram-negative and Gram-positive bacteria. This provides the basis for the development of a biotechnological platform for the production and purification of antimicrobial peptides.

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P226. *OmpL1* and *LipL32* genes as pathogenic *Leptospira* markers – relevance in leptospirosis diagnosis

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Leptospirosis is a worldwide zoonosis caused by spirochetes of *Leptospira* genus, which affect more than one million people/year. Spirochetes are spread in the environment, mostly by the urine of rodents, their primary reservoirs. The Azorean archipelago (Portugal) is an endemic region with a high annual incidence, representing a public health problem.
The aims of this work were: i) characterize the pathogenicity of *Leptospira* spp., cultures (isolates), previously obtained from rodents captured in São Miguel and Terceira islands (Azores), by detection of ompL1 and lipL32 genes; and ii) determine the interest of these genes in laboratory diagnosis of leptospirosis.
So, we selected Leptospira isolates (N=100) maintained to -80ºC. Fourteen of them were cultered and grew well in selective EMJH medium. In order to classify these isolates at the phenotypic and molecular level, were performed two growth tests [at 13ºC and with 8-azaguanine (225µg/ml) addition], and a morphological test using NaCl (1M) added to culture medium. The serogroup/serovar (sv) of each isolate was identified using monoclonal antibodies (mAbs). Leptospiral DNA of isolates (N=32) was extracted and later amplified using two conventional PCR protocols, optimized as part of this work, with primers targeting lipL32 and ompL1 genes (both present only in pathogenic Leptospira species). The protocol for lipL32 gene was also used in human patient samples (N=127) [urine (nu=99) and serum (ns=28)]. and finally, few DNA products (isolates and human samples) were sequenced.
Phenotypic assessment revealed the pathogenic status of isolates, and mAbs included them in two distinct serogroups: Ballum (sv. Arborea) and Icterohaemorrhagiae (sv. Copenhageni). Leptospira DNA was found in all isolates tested (N=32) by ‘lipL32’ protocol, while the other protocol 'ompL1' detected specific DNA in (n=27) of the same. Both protocols showed high specificity, but only the first showed sensitivity up to 1 bact/mL. The sequencing results showed that the phenotypic and molecular classification was overlapping.
The results demonstrated that the PCR protocol based on lipL32 gene is a useful tool for detecting pathogenic Leptospira DNA either for epidemiological studies (in reservoirs) or used in clinical samples, significantly improving the current molecular diagnosis of leptospirosis.

P227. Molecular and proteomic identification of *Neoscytalidium dimidiatum* as onychomycosis and dermatomycosis etiologic agent

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The Botryosphaeriaceae family includes genera frequently related with plant diseases. Nevertheless, human infections caused by fungal plant pathogens are becoming more frequent. *Neoscytalidium* species are mainly phytopathogenic but cases of phaeohyphomycosis have been reported. Within this genus, four species are currently recognized: *N. dimidiatum*, *N. orchidacearum*, *N. novaehollandiae*, and *N. oculus*. Although *N. dimidiatum* is frequently found in soil, it has been identified as an etiologic agent of onychomycosis or dermatomycosis. On the other hand, *N. oculus* was isolated and identified as an etiologic agent of an ocular lesion. All these species are very similar with regard to their macroscopic and microscopic traits, which lead to complex and imprecise identification based on morphological traits alone.

In this study, 34 isolates of *Neoscytalidium* spp. collected from onychomycosis or dermatomycosis cases in Medellin (Colombia) were identified at the species level using sequencing of the ITS nuclear rDNA region and MALDI-TOF mass spectrometry (MS). Molecular based phylogenetic results indicate that all isolates were grouped with *N. dimidiatum* reference strains. A reference *N. dimidiatum* spectrum, in the in-house library, was constructed and validated to identify the clinical isolates by MALDI-TOF MS. Four groups were observed in the dendrogram obtained from the proteins of each isolate profile, with an isolate (Plab-077) being placed alone in a single proteomic group. In conclusion, MALDI-TOF MS and ITS sequencing results are concordant and all isolates so far were identified as *N. dimidiatum*. Notwithstanding this, Plab-077 isolate is now under further study using LSU and TEF1-α regions in order to clarify if it represents a putative cryptic species or an atypical phenotype.

P228. Antibacterial activity of *Corema album* extracts: the first study against clinical isolates

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Corema album is one of the two species of the *Corema* genre, endemic in the Iberian Peninsula's Atlantic coast. This plant has been used in traditional medicine for years as an antipyretic, and recent studies report that's it's leafs and berries possess antioxidant and antitumoral activity due to its phenolic and flavonoid composition. In studies related to these compounds, they were associated with antibacterial potential.
This study investigated the antibacterial potential and the cytotoxic effect of leafs (before and after fire), berries, and berry pulp of *C. album* extracts.
The solubilized in ultrapure water extracts were evaluated for their antibacterial capacity towards drug susceptible strains such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Klebsiella oxytoca* and towards multiple drug resistant strains such as extended spectrum β-lactamase-producing E. coli (ESβL), methicillin resistant S. aureus (MRSA) and carbapenemase-producing K. pneumoniae (KPC). Assay methods included the broth microdilution method with extract concentrations from 0.0244 to 50mg/mL. Bacterial growth was evaluated by the colorimetric method with Iodonitrotetrazolium chloride 95%, in order to determine the minimum inhibitory concentrations (MIC) and the cytotoxic effect of the extracts.
Berry and pulp extracts presented antibacterial potential, being the only extracts to inhibit all strains tested. These extract's MIC values vary between 3.125mg/mL and 12.5mgmL, except for *K. oxytoca* and the resistant strain ESβL, corresponding to 25 and 50mg/mL, respectively. The extract's activity appears to be independent from the strain's resistant characteristics, demonstrating more efficiency against Enterococcus, at the lowest MIC observed, and less efficiency against ESβL, at the highest MIC.
The extracts of berry and pulp presented antibacterial activity and according to the potential observed we considered it to be related to its phenolic and flavonoid composition. *C. album*'s berries were characterized in previous studies and showed high levels of hydroxycinnamic acid, vanillic acid and quercetin. *C. album* extracts also demonstrated antibacterial activity against *P. aeruginosa*, in lowest level of MIC comparing to a close species as *L. salicaria*. As future perspectives, it would be relevant to study the fruits chemical characteristics in more detail, in order to determine its cytotoxicity and bacteria's cellular damage cause.

P229. RNAi as a tool to inhibit the angiogenic potential of human Mesenchymal Stem/Stromal Cells in malignancy

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Tumour angiogenesis is one of the hallmarks of cancer progression. Angiogenesis depends on a complex and dynamic interaction between different types of cells and many signalling molecules. Mesenchymal Stem/Stromal Cells (MSCs) have an active role in supporting the maintenance of a dynamic and homeostatic tissue microenvironment, by secretion of a broad range of biologically active molecules. Upon interaction with cancer cells, MSCs become active participants in tumour development, namely by secreting pro-angiogenic molecules, such as vascular endothelial growth factor (VEGF). Blocking the expression of VEGF at post-transcriptional level, using RNA interference (RNAi) technology, might be a promising strategy to slow down tumour growth.
This project involved the transfection of bone marrow-derived MSCs (BM-MSCs) with small-interfering RNAs (siRNAs), either chemically synthetized or expressed as a short-hairpin RNAs (shRNAs) from a minicircle (MC) vector, that target VEGF expression, using Lipofectamine 2000. VEGF expression was evaluated both by RT-qPCR and ELISA, 48 hours post-transfection. To evaluate the angiogenic potential of the engineered BM-MSCs, an *in vitro* endothelial cell tube formation assay using human umbilical vein endothelial cells (HUVECs) was performed using the conditioned medium (CM) collected 72 hours post-transfection of BM-MSCs.
Overall, RT-qPCR results revealed a VEGF-mRNA knockdown of approximately 50% after synthetic siRNA transfection of BM-MSCs. Similarly, ELISA results indicated a 40% decrease in VEGF secretion by transfected cells. CM retrieved from BM-MSCs transfected with the siRNA showed a decreased potential to induce tube formation by HUVECs, suggesting a reduced angiogenic potential compared to control (non-transfected) cells. Regarding BM-MSCs transfection results with the MC vector, although similar results were expected, increased VEGF expression was found, exhibiting a ~3-fold increase in the number of VEGF mRNA copies and a ~4-fold increase in protein production, in comparison with the control cells. Although further studies are required, these discrepancies appear to be related to the incorrect processing of the transcribed shRNA into a functional RNAi molecule.
In conclusion, this study provides important insights regarding the implementation of an RNAi-based system that targets VEGF expression in BM-MSCs, consequently diminishing their pro-angiogenic potential, aiming at slowing down tumour angiogenesis.

P230. Evolution of CTX-M-type extended-spectrum ß-lactamases toward carbapenemase activity; CTX-M-33, a CTX-M-15 derivative in a Portuguese *Klebsiella pneumoniae* isolate

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Objective: Since the first reports on CTX-M-type enzymes in the late 1980s, they have become the most prevalent extended-spectrum ß-lactamases (ESBLs) worldwide. Substitutions of amino acid residues within the CTX-M sequences may lead to significant modifications of their hydrolytic properties, such as CTX-M-15 hydrolyzing ceftazidime and differing from CTX-M-3 (sparing ceftazidime) by only a single amino acid (Asp 240 Gly). Nevertheless, most CTX-M enzymes share the same hydrolytic profile, and all do not possess any carbapenemase activity. Here we analyzed the hydrolytic properties of a novel CTX-M-type ESBL.
Methods: PCR and sequencing were used to screen for ESBL genes. The ESBL encoding gene was cloned and transformed into chemically competent *Escherichia coli* TOP10 (wild-type) and HB4 (porin deficient). Minimal Inhibitory Concentration (MIC) were performed by microdilution onto Mueller-Hinton agar plates. Specific hydrolytic assays were performed using crude extracts from *E. coli* TOP10 clones by UV spectrophotometry. Mutant prevention concentration (MPC) assays were also conducted.
Results: *K. pneumoniae* strain LX1 was recovered from a patient hospitalized in Lisbon, Portugal. It was resistant to broad-spectrum ß-lactams, and of reduced susceptibility to meropenem, while remaining fully susceptible to imipenem. It produced CTX-M-33, a CTX-M variant with a single amino acid substitution (Asp to Ser at Ambler position 109) compared to CTX-M-15. The $bla_{\text{CTX-M-33}}$ and $bla_{\text{CTX-M-15}}$ genes were cloned and expressed in *E. coli*. Comparative hydrolytic activity assays showed that CTX-M-15 and CTX-M-33 hydrolyzed ceftazidime and cefotaxime similarly. However, we observed that CTX-M-33, by contrast to CTX-M-15, significantly hydrolysed meropenem, although imipenem and ertapenem were spared. Furthermore, CTX-M-33 showed higher MPC values and wider mutant selection window in presence of meropenem, in accordance with the observed hydrolytic properties.
Conclusions: We identified here the very first CTX-M enzyme possessing carbapenemase activity. We showed that CTX-M-33 possessed a single amino acid substitution mutation (N109S) compared to CTX-M-15, which broadened its susbtrate range toward meropenem. We showed that production of CTX-M-33 enhanced the occurrence of meropenem-resistance mutant upon selective pressure. We identified here an emerging threat corresponding to a CTX-M-15 variant compromising the activity of meropenem, a last-resort antibiotic.

P231. *Raoultella ornithinolytica*: an opportunistic pathogen in the oral cavity of chronic kidney disease patients

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Raoultella ornithinolytica, formerly *Klebsiella ornithinolytica*, is a histamine-producing enterobacteria commonly isolated from fish and insects. Despite being often misidentified as *Klebsiella* sp. and consequently underreported, this species is emerging as a virulent pathogen of both nosocomial and community-acquired infections. The compromised immunity associated with the uremic state experienced in chronic kidney disease (CKD) makes patients more prone to infection than other populations. For this reason, colonization by clinically relevant Enterobacteriaceae, major agents of both nosocomial and dialysis-associated infections, may constitute a serious risk. As so, this work aimed to assess the prevalence of clinically relevant enterobacteria in the oral cavity of CKD patients.

Saliva samples were collected from 44 CKD patients undergoing peritoneal dialysis (CKD-PD) and from 37 healthy volunteers and were cultured in MacConkey Agar up to 3 hours after collection. After 48 hours of growth at 37ºC, all distinct-looking colonies were reisolated and were then identified using MALDI-TOF MS. The prevalence of the identified species was then assessed per participant.

In comparison to healthy controls, CKD-PD patients exhibited a much higher prevalence (43.2% vs. 10.8%, p=0.003) and diversity (7 vs. 3 isolated species) of Enterobacteriaceae in the oral cavity. Out of all the species isolated, only the prevalence of *Raoultella ornithinolytica* varied significantly between groups (p= 0.001). Approximately 30% of CKD-PD patients were colonized by *R. ornithinolytica* in the oral cavity, while this species was completely absent from the saliva of healthy controls.

These results suggest that CKD may induce a dysbiosis of the oral microbiome, leading to the proliferation of clinically relevant Enterobacteriaceae, such as *R. ornithinolytica*. This species has been reported as an agent of peritonitis and its presence in the oral cavity of immunocompromised CKD patients might constitute a risk for their well-being.

P232. Cyanobacterial outer membrane vesicles as antigen delivery-platforms for modulating fish immunology

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Mycobacterium marinum is the causative agent of Mycobacteriosis, a fish bacterial disease characterized by granulomatous inflammation in multiple organs, with a mortality rate that can range between 30-100%. Mycobacteriosis outbreaks have important effects on commercial fish production, in particular that of European seabass (Dicentrarchus labrax), a fish species of high economic interest. Mycobacteriosis is also an important infectious disease in zebrafish (Danio rerio), associated with severe losses in research facilities. Because no satisfactory vaccine or treatment is available, once a population of fish is infected, the most likely scenario is euthanasia of the entire group. Thus, it is imperative to develop an efficient strategy to prevent mycobacteriosis infections. One possibility is the use of bacterial outer membrane vesicles (OMVs), which are spherical bilayered structures naturally liberated from the outer membrane of Gram-negative bacteria that have been progressively used as carriers of immunogenic antigens. An example is the commercially-available OMV-based vaccine Bexsero® against *Neisseria meningitidis* serogroup-B15 in humans. In this sense, this work explores the tolerance of zebrafish larvae to OMVs derived from non-pathogenic and environmentally friendly bacteria (cyanobacteria).

First, OMVs derived from an hyper-vesiculating cyanobacterial strain (*Synechocystis* sp. PCC 6803 (ΔtolC)) and ranging between 50 and 500 µg LPS/ml, were tested for their effects on 3 days post fertilization (3 dpf) zebrafish larvae survival. LPS isolated from the same OMVs was used for comparison while commercial LPS from *Pseudomonas aeruginosa* (known to induce zebrafish mortality) was used as control. Next, the expression of pro-inflammatory cytokines, responsible for the acute-phase reaction (e.g., IL-1β, TNF-α, IL-6) was determined by RT-qPCR. All treatments were tested six-fold, in 6 well plates containing 20 larvae per well (pooled for total RNA extraction).

Synechocystis sp. PCC 6803 (ΔtolC) OMVs do not affect zebrafish larvae survival nor induce a significant inflammatory response, while purified LPS from this strain slightly reduce larvae survival. Overall, cyanobacterial OMVs seem good candidates to be used as carriers of immunogenic antigens in zebrafish. Future work will engineer cyanobacterial OMVs, packaged with heterologous *M. marinum* antigens, and evaluate these as antigen-delivery vehicles to immunize zebrafish and European seabass against *M. marinum*.

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P233. Unveiling novel mechanisms of evolution towards azole resistance in *Candida glabrata*: using directed evolution and genome-wide approaches

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Candida glabrata is a fungal pathogen that exhibits increased prevalence, mostly due to its ability to develop azole resistance. Although gain-of-function (GOF) mutations in the transcription factor CgPdr1, leading to drug efflux pump overexpression, are identified as the main mechanism of azole resistance in clinical isolates, a considerable number of azole resistant isolates does not display any such mutations. This highlights the need to unravel all other unknown molecular mechanisms that are also responsible for clinical azole resistance acquisition in *C. glabrata*.

In this study, directed evolution experiments were conducted using prolonged exposure of *C. glabrata* clinical isolates to serum-like concentrations of azole drugs. The evolved populations were then compared to the initial isolates using transcriptomics and genomics approaches. In a first case, a transcriptomics analysis of the *in vitro* evolution of an azole susceptible *C. glabrata* clinical isolate (044) towards the acquisition of resistance to posaconazole (21st day, strain 044Posa21), to clotrimazole (31st day, strain 044Clotri31) and to voriconazole and fluconazole (45th day, strain 044Fluco45), induced by longstanding incubation with fluconazole, was carried out [1]. This analysis led to the characterization of novel mechanisms of azole resistance involving the *CgEpa3* adhesin. Additionally, this step-wise analysis enabled us to suggest that prolonged fluconazole exposure progressively selects the subpopulations that exhibit higher resistance at a lower cost, eventual reaching the best solution which appears, indeed, to be the acquisition of GOF mutations in the *CgPdr1* transcription factor. More recent studies involving evolution towards resistance to other azole drugs are underway and are enabling the identification of novel unforeseen mechanisms of azole susceptibility. The evaluation of the clinical significance of these findings is also ongoing, through the analysis of *C. glabrata* isolates that have acquired azole resistance in the clinical setting.

Overall, these results provide a glimpse of the genome-wide evolution of *C. glabrata* populations toward multiazole resistance, revealing the multifactorial nature of this phenomenon.

[1] Cavalheiro et al, AAC, 63: e00995-18, 2019.

P234. Resistance to biocides and antibiotics in *Staphylococcus aureus* associated with SSTIs in ambulatory patients

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Staphylococcus aureus is major human pathogen responsible for a wide range of infections, including skin and soft tissue infections (SSTIs). It is known that the misuse of antibiotics leads to increased antibiotic resistance, but recent studies showed that biocides can also select for antibiotic resistant strains.
The aim of this work was to perform a phenotypic and molecular characterization of a collection of 34 *S. aureus* isolated from SSTIs in ambulatory patients and to assess their susceptibility to the main antibiotics and biocides used in clinical practice.
Antibiotic susceptibility testing by disk diffusion detected resistance to penicillin (33 isolates, 97%), cefoxitin (15, 44.1%), fluoroquinolones (17, 50%), macrolides and lincosamides (15, 44.1%), aminoglycosides (6, 17.6%) and fusidic acid (2, 5.9%). Fifteen isolates (44.1%) were multidrug resistant (MDR). Several antibiotic resistance genes (*mec*$_A$, *bla*$_Z$, *erm*(A), *erm*(C), *msr*(A), *mph*(C), *aac*A-*aph*D, *aad*$_D$, *aph*(3')-IIIa and *fus*$_C$) were identified in resistant isolates. Minimum inhibitory concentrations were determined for several biocides and topical antibiotics and used to estimate epidemiological cut- off (ECOFF) values, which revealed non-wild type isolates to bacitracin (9, 26.5%) and neomycin (5, 14.7%) which, in the case of neomycin were associated to carriage of *aad*$_D$ or *aph*(3')-IIIa. Non-wild type isolates were also identified towards cadmium (4, 11.8%) and arsenate (3, 8.8%), mainly associated with carriage of *cad*$_A$ or *cad*$_D$ and *ars*$_B$ resistance genes, respectively.
This work highlights high levels of antibiotic resistance among *S. aureus* causing infections in patientstreated outside the hospital environment, which included MRSA and MDR strains. Resistance to topical antibiotics was observed mainly for macrolides and lincosamides. The determination of ECOFFs allowed to detect non-wild type populations towards several topical antibiotics and biocides suggesting the dissemination of resistance determinants among strains associated with SSTIs in the community.

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P235. Virulence quantification of KPC- and OXA-48-producing *Klebsiella pneumoniae* isolates in a *Galleria mellonella* model: Towards a novel therapeutic approach using linear cationic polymers

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Klebsiella pneumoniae, one of the most common pathogens found in hospital-acquired infections, is often resistant to multiple antibiotics. In fact, multidrug-resistant (MDR *K. pneumoniae* producing KPC or OXA-48-like carbapenemases are recognized as a serious global health problem.
In this study we evaluated the virulence of *K. pneumoniae* aiming potential antimicrobial therapeutics. To achieve this goal we focused on a linear cationic polymer using *Galleria mellonella* larvae an *in vivo* model. KPC-2 and OXA-48 strains were obtained from patients treated in medical intensive care units in Lisbon, Portugal. *G. mellonella* were inoculated using KPC(+) and OXA-48(+) isolates from these patients. In the *G. mellonella* model at 48-72 hours, the KPC(+) *K. pneumoniae* isolates were more virulent than the OXA-48(+) *K. pneumoniae* isolates. Virulence was attenuated when low bacterial inoculum (one magnitude lower) were injected in *G. mellonella*. In addition, we also report for the first time the use of a cationic linear synthetic polymer (L-OEI) for the treatment of KPC- and OXA-48- producing *K. pneumoniae* isolates. This polymer has a broad spectrum antibacterial activity and exerts a fast bactericidal activity, by depolarizing the cytoplasmic membrane, against both Gram-negative (including *K. pneumoniae* isolates) and Gram-positive bacteria. Importantly, under the therapeutic window, the polymer does not show toxicity both *in vitro* (mammalian cell lines) and *in vivo* (larvae model). Given its almost negligible toxicity, these novel therapeutics may constitute a promising approach for the treatment of MDR *K. pneumoniae* infections.

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P236. IBER-XYFAS – Ibero-American network for the surveillance of *Xylella fastidiosa*

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Xylella fastidiosa is the causal agent of various plant diseases that continuously challenge agroforestry production causing significant losses to European and American countries. IBER-XYFAS is an international network of research groups, agrofood companies and regional governments, financed by CYTED that aims gather all available data on the bacterium, on its vectors, on the crops affected in Ibero-American countries and on the prevention and control activities that are being carried out. The specific objectives of the network will focus on the following work packages: P1) Information on the bacterium; P2) Information on transmission vectors; P3) Information on the interaction of the bacteria with the plant; P4) Information on therapies; P5) Information on remote sensing methodologies; P6) Information on the environmental, social and economic impact of diseases and control measures. The purpose of the information exchange is to generate knowledge that will contribute to the development of a technological alert and surveillance system that will enable local or national governments to take the necessary measures to continue, contain and ultimately eradicate the disease. The main outputs of IBER-XYFAS are: to promote the scientific and technological integration of the Ibero-American region and the effective transfer of knowledge and technologies; to encourage the participation of researchers from the Ibero-American region in other programs and the active participation of different actors in the control of the disease; to promote the development of effective transfer activities to

small producers and vulnerable sectors; to contribute to the improvement of the social perception of R+D+I and its results; to promote the improvement of production through the integration of technological and economic aspects; to design technological protocols for the control of the disease.

P237. Antibiotic resistance in *Staphylococcus pseudintermedius* associated with skin and soft tissue infections in companion animals

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Antimicrobial resistance (AMR) in staphylococci causing skin and soft tissue infections (SSTIs) in pets is a growing public health concern. In this study we characterized a collection of *S. pseudintermedius* causing SSTIs in pets to document the susceptibility profile to antibiotics and the presence of resistance genes.

The collection comprised 163 S. pseudintermedius isolates associated with SSTIs in dogs and cats collected between 2014 and 2018 at two laboratories in Lisbon (Portugal). Identification was confirmed by amplification of the sps_J gene and the susceptibility profile determined by Kirby-Bauer. The results were interpreted according to the VET08 CLSI recommendations (2018) or alternative guidelines when necessary. The bla_Z and mec_A genes were screened for all isolates. Other resistance genes (e.g. *erm*, *tet*, aad_D, *vga*(C), dfr_A(S1)) were searched only for those isolates showing a resistance phenotype.

Among the 163 S. pseudintermedius tested, 136 (83.4%) were resistant to penicillin and all of them carried the blaZ gene. Methicillin resistance (mecA+, MRSP) was detected in 51 isolates (31.3%), 48 of which presented a multidrug resistance (MDR) phenotype. The most common MDR pattern included resistance to beta-lactams, aminoglycosides, macrolides and lincosamides. Resistance to tetracyclines was found in 55.2% of the isolates, mostly related to tetM gene. Resistance to aminoglycosides and macrolides/lincosamides was detected in 39.3% and 37.4% of the isolates, respectively; in this last case, mostly associated with *erm*(B) gene. We also identified resistance to trimethoprim- sulfamethoxazole (30.7%), fluoroquinolones (25.8%), chloramphenicol (14.7%) and fusidic acid (4.9%). No resistance was observed for linezolid, tigecycline or quinupristin-dalfopristin.

This undergoing study revealed an elevated frequency of MDR and, in comparison with previous studies [1], an increasing trend of antibiotic-resistant *S. pseudintermedius* associated with SSTIs in pets. The close contact of these animals with humans may be a possible source for transmission of antibiotic resistant staphylococci, reinforcing the need of a One Health perspective in their study. These results also highlight relevant therapeutic limitations for the treatment of SSTIs in pets, which already include critically important antibiotics.

[1] Couto et al. JAC (2016) 71: 1479–87.

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P238. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae*: seasonal and spatial population dynamics

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Pseudomonas syringae pv. actinidiae (Psa) is a gram-negative bacterium responsible for the bacterial canker in *Actinidia chinensis* var. deliciosa and *A. chinensis* var. chinensis, a quarantine disease threatening the kiwifruit industry sustainability. The population genetic structure was characterized from 600 Psa isolates obtained from four Portuguese orchards with distinct abiotic conditions. The same kiwifruit plants were examined in two consecutive seasons to assess the diversity of endophytic and epiphytic Psa populations. Based on BOX-PCR fingerprinting analysis and MLVA, we determined that Psa population was highly heterogeneous with several co-existing populations. Evident changes occurred in the population structure between seasons translated in a notable decrease in Psa diversity in autumn. Moreover, differences between the epiphytic and endophyte population were also observed in samples collected simultaneously. Psa strains were identified has biovar 3 but our phylogenetic analysis revealed an unreported and highly polymorphic lineage. In this context, Psa populations seem to be selected over time from a diverse genetic pool according to their fitness. This perspective is important for the understanding of kiwifruit bacterial canker disease occurrence and Psa evolution and it is also relevant when adopting strategies for epidemics management.

P239. Enhancing phage endolysins enzybiotic properties through their combined action with antimicrobial peptides

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The uncontrolled dissemination of antibiotic resistance among bacterial pathogens has been calling for alternative antimicrobials, preferentially with new modes of action that minimize resistance development. Antimicrobial peptides (AMPs) and endolysins have been proposed as such alternatives to replace or complement conventional antibiotics when fighting drug-resistant bacteria. However, research in pre-clinical and/or clinical settings has uncovered some hurdles in the development of these agents as effective antimicrobials. Endolysins are bacteriophage-encoded enzymes (enzybiotics) and their antibacterial character derives from their degrading activity towards peptidoglycan, an essential component of the bacterial cell wall (CW). It has been shown that bacteria actively dividing in complex media may be able to counteract the lytic activity of endolysins, depending on the target bacterial cell and concentration of the lytic enzyme. The mechanisms by which bacterial cells restrain enzybiotic activity are still unknown, but they were shown to be highly dependent on the integrity of the cytoplasmic membrane (CM) proton-motive force (PMF). In fact, during the natural course of phage infection endolysins full activity require the PMF-dissipating action of another phage-encoded function, the holin. Holins are membrane proteins that insert in the CM to form holes that collapse the PMF. Interestingly, AMPs are natural short peptides that make part of the antibacterial defence mechanisms of most living organisms, and frequently their mode of action involves damage of the bacterial CM (often with PMF disruption). We have therefore envisaged that the PMF-perturbing action of selected AMPs could be used to boost the bactericidal activity of endolysins. By using model bacteria, which include staphylococcal and enterococcal species, we show that the combined action of AMPs and endolysins results in superior lytic activity. In addition, we demonstrate that fusion of AMPs to endolysins may generate single agents with enhanced bactericidal activity when compared to the unmodified enzymes. These results open good perspectives for the generation of a new class of potent antibacterial agents, that we named AMPLys, which combine in a single molecule CW-degrading and PMF-perturbing activities.

P240. Antimicrobial resistance in *Escherichia coli* from dogs never exposed to antibiotics: relevance to public health

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Antimicrobial resistance is a major public health concern, requiring an One Health approach. In veterinary medicine, bacteria resistant to antibiotics critically important for humans are often isolated from dogs. However, studies focusing on the epidemiology of resistant *Escherichia coli* from healthy dogs without previous exposure to antibiotics are scarce.
E. coli isolation from 91 fecal swabs from healthy dogs with no prior exposure to antibiotics was performed by conventional bacteriological methods and isolates' resistance profile assessed by disk diffusion according to CLSI using 13 antibiotics.
From the 91 samples, 62 were positive for *E coli*. Four isolates were selected per positive sample, rendering a total of 248 isolates, from which 71.4% were multidrug-resistant, 23.8% extensively drug- resistant and 12.1% pandrug-resistant. Cephalexin (83.1%), nalidixic acid (62.1%) and tetracycline (60.1%) were the antibiotics for which a higher prevalence of resistant isolates was observed.
Results show that dogs never exposed to antibiotics are relevant vehicles for resistant *E. coli* dissemination in the environment. In the future, identification of risk factors related to antibiotic resistance is essential for implementing antimicrobial stewardship programs in veterinary medicine.

P241. Can canine oral environment affect nisin antimicrobial activity regarding periodontal enterococci?

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Periodontal disease (PD) is one of the most frequent inflammatory diseases in dogs, being caused by a microbial biofilm on the dental surface and a consequent host inflammatory response. A possible approach for PD control involves the use of the antimicrobial peptide nisin. A previous study performed by our team reported that a guar-gum gel used as delivery system for nisin (nisin-biogel) exhibits antibacterial activity against enterococci biofilms obtained from the canine dental plaque. However, *in vivo* conditions may influence the nisin-biogel activity. For example, canine saliva comprises several components that may interfere with nisin activity. Our goal was to evaluate if canine saliva impairs the antimicrobial activity of the nisin-biogel.

A collection of 20 oral enterococci obtained from dogs with PD were used as bacterial models. Saliva samples were collected from healthy dogs, filtered and diluted in a 1:1 proportion in nisin or in nisin- biogel (0.75% w/v), to achieve nisin concentrations of 12.5, 25, 50 and 100 µg/mL. The antimicrobial activity of all suspensions was evaluated using a spot-on-lawn assay, followed by incubation at 37°C for 24 hours and inhibiton halos measurement. All assays were performed in triplicate on independent days.

In the presence of canine saliva, nisin and nisin-biogel at 12.5 and 25 µg/mL have no antimicrobial activity. However, at 50 µg/mL nisin was able to inhibit one isolate, and the nisin-biogel three. At 100µg/mL, nisin and nisin-biogel presented inhibitory activity against 95% (19/20) and 85% (17/20) of the isolates, respectively.

Considering the minimum inhibitory and bactericidal concentrations of nisin and nisin-biogel previously determined, saliva presence increased the required concentration for nisin's inhibitory activity. However, results suggest that the biogel acts by stabilizing nisin diffusion and promoting more consistent inhibition halos, being a suitable delivery system for nisin topical application.

In conclusion, saliva did not block the antimicrobial effect of nisin against canine PD enterococci, confirming the its potential for PD control.

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P242. *Gardnerella* spp. pre-conditioned vs competitive multi-species biofilm growth and the impact on the tridimensional biofilm structure

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Background
Bacterial vaginosis (BV) is one of the most common bacterial vaginal disorders among women of reproductive age. The hallmark of BV is the presence of a multi-species biofilm, formed primarily by *Gardnerella* spp., in a minor part by *Atopobium vaginae*, and also other anaerobic species. While a few studies have demonstrated that some BV-related species establish synergistic interactions with *Gardnerella* spp. *in vitro* dual-species biofilms, little is known regarding bacterial interactions in triple- species BV-associated biofilms. We evaluated the interactions and spatial distribution of *Gardnerella* spp., A. vaginae and a third BV-associated species, such as *Enterococcus faecalis*, *Lactobacillus iners*, *Mobiluncus curtisii*, *Peptostreptococcus anaerobius*, *Prevotella bivia*, and *Staphylococcus hominis*, using two distinct *in vitro* biofilm formation models.

Method
We analyzed the synergistic or antagonistic interactions in triple-species biofilms formed by two distinct experimental designs: one model mimicked the hypothesis that *Gardnerella* spp. is the early colonizer and allowed a mono-species *Gardnerella* spp. biofilm to be formed for 24 h before inoculating the other bacterial species; alternatively, in the second model, all three bacterial species were inoculated simultaneously, i.e. in a competitive way, and incubated for 24 h. Fluorescence in situ hybridization with specific peptide nucleic acid probes for *Gardnerella* spp. and *A. vaginae*, and DAPI staining were used to characterize the established biofilms. In addition, quantification of the biofilm mass by crystal violet was performed.

Results and Conclusion
Confocal microscopy data revealed distinct morphologies, but also some similarities, between the triple-species biofilms formed in both models, with a bigger diversity of ecotypes in the top layers of the biofilms, while the bottom layers were more conserved. However, biofilm quantification showed almost similar total biofilm biomass for both models, except for the consortium of *Gardnerella* spp., *A. vaginae* and *S. hominis* which presented higher biomass in the competition model.

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P243. *Cryptosporidium* and *Giardia* in Oysters: detection by Nested-PCR and Sequence Analysis

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Introduction: Giardia and Cryptosporidium are two protozoan parasites that infect man and animals and have been detected with frequency in shellfish mollusk. Both parasites have adequate life cycles to be transmitted by water and food. Giardia cysts and Cryptosporidium oocysts are the infective forms and are very resistant to environmental factors as well as to chemical treatments applied to water for human consumption. In Portugal, the research of these protozoa in food is scarce. Thus, the present work had as objective the detect Giardia and Cryptosporidium in oysters applying techniques of molecular biology to study the level frequency of contamination in these products and to make a food risk analysis associated with the human mollusks consumption.
Material and methods: Oysters (n=190) of three different species, European oyster, *Ostrea edulis*, Pacific oyster, *Crassostrea gigas* and Portuguese oyster, *Crassostrea angulata*, were collected in different areas of Portugal (North, Center and South) by Portuguese Institute of the Sea and the Atmosphere (IPMA) between 2011 and 2017. Oysters gills DNA was extracted by DNeasy Blood & Tissue kit (Qiagen) and the amplification of Cryptosporidium and Giardia small-subunit ribosomal RNA (ssu rRNA) was performed by nested-PCR. The species/genotypes identification was performed by DNA sequencing.
Results: The locus of the Giardia ssu rRNA gene was amplified by nested-PCR in 29 oyster samples (15.3%). The DNA sequencing identifies *Giardia lamblia* in 21 nested-PCR positive samples and the assemblage A in ten samples. *Cryptosporidium* sp. was not identified in any sample.
Conclusion: The high detection of *G. lamblia* assemblage A in the oyster samples is potentially alarming since this assemblage is usually associated with human infections worldwide. The use of prevention and control methods for parasites in the production of shellfish mollusk must be implemented to reduce the risk of food contamination.

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P244. Fusidic acid resistance among *Staphylococcus aureus* in Portugal

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Fusidic acid (FD) is a first-line antibiotic widely used topically for the treatment of *Staphylococcus aureus* skin and soft tissue (SSTI) and eye infections, as well as a systemic alternative for conventional antibiotherapy in bone and joint infections and septicemia. Resistance to FD is mainly due to the acquisition of either resistance genes (*fus*$_B$, *fus*$_C$ and *fus*$_D$) or mutations in the *fus*$_A$ gene, which encodes the FD target protein. Around 10% of infection isolates in Europe, were reported to be resistant to FD. In Portugal, although FD is commonly prescribed and available over the counter, there was no data regarding prevalence and resistance determinants in *S. aureus*.

To fill this gap, *S. aureus* strains previously isolated from carriage and infection in Portugal, were screened for FD resistance: (a) a collection of 204 methicillin resistant *S. aureus* representative of the major clones responsible for infection in Portuguese hospitals between 1985 and 2016; (b) 32 *S. aureus* (including three MRSA) associated to skin and soft tissue infections in pediatric patients and (c) 153 nasal carriage strains from two community populations, homeless individuals (n=44) and nursing students (n=109). FD resistance was confirmed by disc diffusion and by the determination of the minimum inhibitory concentration (MIC). Resistance genes were detected by PCR and fusA mutations were identified by DNA sequencing.

The global prevalence of FD resistance was 5.1% (20 out of 389 strains), but increased to 6.25% considering SSTI strains only. Resistance was more prevalent among infection strains (7.3%) comparing to carriage (2%)(p=0,032). The gene *fus*$_C$ was the prevalent determinant, detected in 80% of the resistant strains associated to higher MICs (8-16 µg/mL). Two strains showed mutations in the *fus*$_A$ gene, a single strain carried *fus*$_B$. The majority (69%) of fusC strains belonged to the Pediatric clone (ST5-IV-t311), whereas fusB was associated to the European clone (ST80-IV-t044).

In conclusion, in Portugal, resistance to FD in S. aureus remains low, but highly associated to specific clones and mostly due to the presence of *fus*$_C$. A continuous monitoring of resistance is warranted to ensure the efficacy of FD in the antibiotherapy of SSTI.

P245. Growth of *Candida albican* in natural products traditionally used as personal lubricants

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The yeast *Candida albicans* can be found in the vaginal mucosa and other mucous membranes. Imbalances between the yeast and the host can occur and an infection can be established that can progress to chronicity. Several factors are identified as responsible for changes in balance such as diet, taking antibiotics and using lubricants. In this study we selected some personal lubricants, namely olive oil, petroleum jelly, castor oil, sweet almond oil and coconut oil. The different lubricants were inoculated, with *C. albicans* ATCC10231 strain at a concentration of 6x104 CFU/mL and incubated at 37ºC. After 24 and 48 hours, serial dilutions of the mixture were made and the resulting number of CFU/ml was estimated by incorporating an aliquot in solid culture media. Of the lubricants tested, olive oil and sweet almond oil were the only ones that caused logarithmic reduction of *C. albicans* cell number; castor oil and paraffin had no significant action and finally the coconut oil caused proliferation of the yeast. To understand the differences in the results of the oils, we also measure water activity, pH and glucose content, but no results were found that could justify the differences.

In conclusion, special care should be taken in the selection of personal lubricants, because some of it may has a promoting action on the growth of *C. albicans* and may be associated with chronicity of some infections, such as coconut oil. However, we should know more about coconut oil in order to understand why it causes yeast to proliferate.

In the case of olive oil, the results indicate the premise of a good lubricant and arouse the interest in the introduction of this ingredient in commercial lubricant formulations.

P246. Evaluation of competition between substrates of the efflux pump system AcrAB-TolC of *Escherichia coli* using Red Nile

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The AcrAB-TolC efflux pump (EP) belongs to the resistance-nodulation-division (RND) family and acts as the major contributor to the intrinsic multidrug resistance (MDR) in *Escherichia coli*. While AcrAB-TolC structural models are quite advanced, the phenotypic characterization of this pump is mainly based on comparisons between MIC values of the wild type and AcrAB-TolC mutant strains. Another approach has been to examine the efflux activity of AcrAB-TolC against the intracellular accumulation/efflux of dyes as ethidium bromide. Previous work performed by our group showed that substrate competition through ethidium bromide accumulation assays it is difficult to observe.
To study the existence of substrate competition, we optimize of a real-time accumulation assay using a high-throughput format with 96-well microplates for Synergy HT microplate reader, using Red Nile as fluorophore/competitor. As substrates, we tested ciprofloxacin, gentamicin, oxacillin, doxorrubicin, chloramphenicol, erythromycin, doxycycline, minocicline, tetracyclin, tetraphenylphosphonium bromide, deoxycholic acid, acriflavine and safranin. The efflux inhibitors tested were carbonyl cyanide-m-chlorophenylhydrazone (CCCP), chlorpromazine (CPZ) and phe-arg-β-naphthylamide antibiotics (PAβN).
Preliminary results showed that efflux of Red Nile was affected by tetracycline and safranin in the presence of CPZ and CCCP but not in presence of PAβN. The EIs had no effect on in the acrAB- deficient AG100A but were able to inhibit efflux in the acrAB overexpressing strain. We also noted that CPZ and CCCP inhibit Red Nile efflux in a dose dependent manner. Further work includes the testing of the reaming compounds in presence and absence of the efflux inhibitors.
So far, we can conclude that tetracycline and safranin competes with efflux of Red Nile and that CPZ and CCCP are inhibitors of Red Nile efflux. We have optimized a protocol to study competition between substrates and validated a new fluorescent molecule to quantify efflux activity by binding to a different site on AcrAB efflux pump. This work has implications for the study of efflux pumps and for drug discovery.

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P247. Application of superparamagnetic nanoparticles for the early diagnosis of tuberculosis and HIV co-infection directly from respiratory samples

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The current diagnosis of HIV infection is based on invasive methods as blood samples. Non- invasive methods for collection of biological samples is an attractive alternative, as they are safer, less expensive and better tolerated by the patients. Since sputum is the main biological product for tuberculosis diagnosis, it is our hypothesis that sputum can be used for simultaneous diagnosis of HIV infection from the same sample. Here, we aim to develop a non-invasive methodology for simultaneous detection of *Mycobacterium tuberculosis* (Mtb) and HIV antibodies in sputum samples of individuals suspected of co-infection.
We selected superparamagnetic nanoparticles (MNPs) as a new approach for capturing the target, requiring smaller sample volumes and less preparation time. Therefore, Au-MNPs were designed to capture Mtb and anti-HIV antibodies from the same sample. The main steps involved the preparation and characterization of Au-MNPs combined with specific antigen or antibody; MNP ability to capture the target in "spiked in" sputum samples; DNA extraction method selection (for Mtb) and validation with clinical samples.
The results obtained showed that the method is rapid and efficient for the detection of tuberculosis and HIV infection from respiratory samples using Au-MNPs as solid support with high sensitivity and recoverability. This methodology can be easily transformed into an *in vitro* diagnostic system with the additional benefit of increased early detection sensitivity related to current diagnostic methodologies using non-invasive procedures. For tuberculosis diagnosis in HIV+ patients, this technique will allow the capture of a greater number of bacteria present in the sample, improving the detection capacity from clinical samples of current approved systems (e.g. Genotype MTBDR*plus*, Hain), also allowing the subsequent study of the mutations associated with resistance to the two main first-line antituberculosis agents. This technique will allow the detection of specific antibodies for HIV diagnosis, in the same sample used for the diagnosis of TB, in a non-invasive manner. It is based on an alternative biological product for blood using a methodology already approved for this diagnosis (OraQuick ADVANCE HIV 1/2, OraSure Technologies), but increasing its sensitivity, specificity and response time.

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P248. Antimicrobial potential of essential oils of Portuguese autochthonous Lavandula against resistant bacteria

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Nosocomial infections and multidrug resistant bacteria are a challenge in clinical practice and are responsible for high mortality and morbidity. The treatment of these infections is often difficult or even impossible with conventional therapies. Essential oils (EOs), complex mixtures rich in terpenoid and phenolic compounds, are presented as an alternative to synthetic drugs in the fight against multidrug resistance bacteria.
The objective of this study was to evaluate the intra and inter-species variability of essential oils of *L. luisieri*, *L. pedunculata* and *L. viridis* autochthonous from Alentejo, and the potential of their essential oils of and mixtures, against drug resistant Gram-positive and Gram-negative bacteria.
EOs were extracted by hydrodistillation from aerial parts of flowering plants, and their chemical composition was evaluated by GC-FID. EOs *in vitro* antioxidant potential of was evaluated by radical DPPH, total reducing power and β-carotene linoleic acid methods. Antimicrobial activity was assessed by solid diffusion disk assays and minimal inhibitory concentration. Moreover, the synergistic potential of mixtures of these EOs was evaluated by checkerboard method and the cellular viability was assessed by flow cytometry and by epifluorescence microscopy.
EOs chemical composition showed a high content in oxygenated monoterpenes (79-85 %), with a high content in irregular necrodyl derivates and 1,8-cineol for *L. lusieri* EO, fenchone and camphor for *L. pedunculata* EO and 1,8-cineol, camphor and linalool for *L. viridis* EO. Screening of antioxidant properties revealed capacity of EOs by the three different mechanisms studied, with high potential to protect the lipid substrate oxidation. Lavandula EOs were effective against the most drug resistant bacterial strains, showing a broad antibacterial spectrum. Furthermore, it was observed a synergistic effect between essential oil mixtures of *L. pedunculata* and *L. viridis*, and flow cytometry studies showed total cellular apoptosis for doses higher than minimal bactericide concentration.
Results highlight the potential use of essential oils of *Lavandula* spp. and their mixtures as natural antimicrobial agents, in food and pharmaceutical industries.

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P249. Risk human ST69 *Escherichia coli* clone producing KPC-3 from lemurs (*Varecia variegata*) in Portugal

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Objective The spread of carbapenemase-producing Enterobacteriaceae (CPE) is a great problem of healthcare worldwide. *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant with significant morbidity and mortality. The aim of this study was investigate the presence of CPE in captive black-and-white ruffed healthy lemurs (*Varecia variegata*).
Material and methods Rectal swabs were collected from 9 lemurs from a zoo in the Oporto region, Portugal. Positive carbapenem-resistant isolates were plated on MacConkey agar plates supplemented with meropenem (1 mg/L). Isolates were identified by 16S rRNA sequencing. Antimicrobial susceptibility testing was performed by the disc diffusion method and/or minimum inhibitory concentrations. CLSI VET08 clinical breakpoints were applied. Carbapenemase resistance genes were characterized by PCR.
Results Fecal samples from two heathy lemurs were positive for a single *E. coli* (ST 69) and two *K. pneumoniae* (ST147 and ST1079) producing KPC-3 carbapenemase. These isolates were resistant to ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime/clavulanic acid, ertapenem, imipenem, and piperacillin/tazobactam. They were also resistant to sulfamethoxazole /trimethoprim. One *K. pneumoniae* isolate was additionally resistant to ciprofloxacin, levofloxacin, norfloxacin, tetracycline and colistin (MIC at 4µg/mL), presented a multidrug resistance (MDR) phenotype. PCR and sequencing identified the KPC-3 carbapenemase encoding gene in all three isolates.
Conclusions To our best knowledge, this study reports for the first time the occurrence of KPC-3- producing bacteria in captivity animals in Portugal. These animals may represent an underestimated reservoir of carbapenemase genes. Epidemiological surveys are further needed to understand the process of acquisition of such threatening resistance determinants in captive animals.

Acnowledgements
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P250. Influence of surface properties on the antimicrobial effect of polymer-coated surfaces

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Introduction: Human environments provide excellent ecosystems for the proliferation of microorganisms and are potential sources of exposure to pathogenic agents [1]. Strategies available to minimize the colonization with microorganisms include inhibiting its adhesion, inhibiting the bacterial growth, or even killing them, thus avoiding the contamination of surfaces. The surface topography and its chemistry are key parameters that might influence bacterial attachment and consequently biofilm formation. Considering this, by manipulating the surface topography of material, surface-microorganism interactions can be affected, resulting in an alteration in viability of the adhered cells [2].
In this work, polymer-coated surfaces with different topography (smooth and rough materials) were tested and their effects on bacterial proliferation was evaluated.
Methods: Changes in topographic surfaces was induced by an embossing process. For the antibacterial assay, ISO 21196:2011 was implemented. Briefly, bacterial suspensions of either *Escherichia coli* or *Staphylococcus aureus* were inoculated onto the material surface for 24 hours at 35ºC. The antibacterial activity (R) was calculated by comparing the number of viable bacteria recovered after incubation on smooth and rough materials.
Results: Samples inoculated with *E. coli* showed a R=-1.8 which indicates that the surfaces used in this study do not have an antibacterial effect over this gram-negative bacteria. In fact, comparing the number of colony-forming unit (CFUs) recovered from the samples, rough surfaces shown an increment of 1.76x105 cells/cm2 (1.5x) in comparison with smooth samples.
For samples inoculated with *S. aureus* the R value was near zero, since the number of CFUs collected from rough and smooth surfaces, after 24h incubation was similar to those collected at time zero. This indicates that those surfaces seem to inhibit bacterial proliferation, which could be related not only to the topography but also to the chemical composition of material.
For future work, different embossing patterns and different material compositions will be analysed, to get a deeper insight on the effect of surface material properties over bacterial growth.

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P251. Antiproliferative potential of Alentejo *Lavandula* spp., Origanum virens and *Thymus mastichina* extracts

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The search for new drugs with antitumoral potential is a challenge to find new strategies to reduce the dose and systemic toxicity effects of chemotherapeutic compounds.
Extracts from aromatic plants are rich in secondary metabolites, such as polyphenols that have important antioxidant, anti-inflammatory and antiproliferative proprieties. Dietary health habits, rich in these natural compounds can provide the development of several chronic diseases, including cancer and other oxidative stress disorders. Several studies reported the human health benefits of flavonoids compound in diet or in supplementary foods.
The aim of this study was to evaluate the antiproliferative effect aqueous extracts of flavouring herbs against MDA-MB-231 breast cancer cells and correlate that with their phenolic content and antioxidant potential. For this purpose, four wild growth Lamiaceae species, *Lavandula luisieri*, *Lavandula pedunculata*, *Origanum virens* and *Thymus mastichicina* were selected. Aqueous extracts were prepared from decoction waters obtained by hydrodistillation of fresh parts of plants. Chemical composition was evaluated based on total phenols, flavonoids and tannins content. In-vitro antioxidant potential was screened by three mechanisms, DPPH radical scavenging capacity, β-carotene/linoleic acid system and iron reducing ability. Antiproliferative activity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against MDA-MB-231 breast cancer cells.
Studied aqueous extracts presented high amount of total phenols, flavonoids and tannins compounds. Results revealed the antioxidant properties of extracts, with high ability to act as radical scavengers, protectors of lipid substrate and iron reducing ability, as well as the antiproliferative potential to inhibit the proliferation of tumoral MDA-MB-231 cells (0,3 <IC50< 1,2 mg/mL).
Results point out the antioxidant and antiproliferative potential of these autochthonous flavouring herbs extracts in nutraceutical or pharmaceutical applications against oxidative stress disorders and cancer diseases.

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P252. Co-Occurrence and spread of mcr-1 and extended spectrum beta-lactamase genes from *Escherichia coli* and *Salmonella enterica* isolated from food producing animals

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Background: The treatment of infections due to multidrug resistant bacteria, such as carbapenem-resistant Enterobacteriaceae is a real challenge. The void of effective antibiotics led to the recent use of an old antibiotic, colistin, as one of the last therapeutic options. However, the emerging and rapid dissemination of the plasmid-mediated colistin resistance (mcr) genes represents a public health threat. The aim of this study was to assess the prevalence and dissemination by horizontal gene transfer of mcr and Extended Spectrum Beta-Lactamases (ESBLs) genes in *Escherichia coli* and *Salmonella* spp. isolated from intensive farming animals.

Materials/methods: Phenotypic detection of ESBLs in 98 *E. coli* and 144 *Salmonella enterica* was performed by diffusion method. bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes were screened by PCR. Colistin-resistant isolates were screened by observing growth in EMB Agar with 3,5mg/L of colistin after 24 and 48h of incubation. Colistin MICs of donors and transconjugants were evaluated by microdilution method. mcr-1 to -5 genes were screened by multiplex PCR. Sequencing confirmed the genes identification. Conjugation assays were performed using *E. coli* J53 as a recipient. mcr-1borne- plasmids were identified by PCR the replicon type method .

Results: Fifteen E. coli (15,3%) carried mcr-1; additionally, two also carried bla_{TEM-1}, one bla_{CTX-M1}, and one both bla_{TEM-1} and $bla_{CTX-M-15}$. Only bla_{TEM-1} was identified in four *S. enterica* isolates. mcr-1 positive strains were collected mostly from pigs (60%). All these isolates were classified as resistant with vMICs ranging from 4-32 mg/L. mcr-1 gene was successfully transferred by conjugation at a frequency of 10-7 to 10-2 cells per recipient; bla_{TEM-1} and $bla_{CTX-M-1}$ were also co-transferred. These genes were located on IncHI2, IncHI1 and IncN plasmids.

Conclusions: Only mcr-1 was identified in *E.coli*, mostly from pigs' samples. Nonetheless, mcr-1 is ubiquitously spread independently of the animal species. ESBL was also co-transferred. The genetic platform was variable and conjugation frequency data showed the high ability of dissemination of these plasmids. The results highlight the need of surveillance and implementation of antibiotic stewardship in animal production since it promotes the emergence of high resistant bacteria that can enter in food- chain.

P253. Antimicrobial activity and biofilm inhibition activity of clean label extracts of Brewer's spent grain

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Brewer's spent grain (BSG) is one of the most abundant by-products of the brewing industry and nowadays one of the most searched by-products. The amount of BSG wasted may represent a great valorization opportunity, since it has been described for its biological, nutritional and functional activities. Such biological activities have been associated with the presence of several important molecules, e.g. dietary fiber, proteins and phenolics, which show to have a great potential for application.
In this work, hydroethanolic extraction (Solid Liquid Extraction) was applied to BSG and the resulting extracts were studied in terms of antimicrobial activity and biofilm inhibition activity. Inhibition curves were performed as described by Silva et al., (2015) and the assay was evaluated upon five different microorganisms: Methicillin-sensitive *Staphylococcus aureus* (MSSA), *Salmonella enteritidis*, *Escherichia coli*, *Bacillus cereus* and *Listeria monocytogenes*, by the application of different extracts at different concentrations (5, 2.5, 1.25, 0.625 and 0.625 mg/mL). On the other hand, biofilm formation assay was carried out by adapting the protocol described by Costa et al. (2014).
The results of antimicrobial activity showed that all the extracts (for almost all concentrations) had inhibitory effect only against *L. monocytogenes*. The aqueous extract presented the lowest inhibition percentage against all tested microorganisms and it only inhibited *L. monocytogenes* at higher concentrations (5 and 2.5 mg/mL). The ethanolic extract presented the best behaviour among all tested extracts as it showed inhibitory effect against MSSA (5 mg/mL), *B. cereus* (5 and 2.5 mg/mL) and *L. monocytogenes* at all concentrations.
For biofilm inhibition assay results showed that extracts had activity against the formation of biofilm by the microorganisms. The difference from these results compared to the results observed in the inhibition curve assay might be due to the fact that the extracts have a better behaviour against the biofilm vitality/viability due to its intrinsic composition.
Therefore, the present results showed a great potential of hydroethanolic extracts of BSG, in order to extract valuable molecules with antimicrobial activity and biofilm inhibition from a abundant by product from food industry.

P254. The dark-side of drug resistance in *Escherichia coli*: efflux pumps and β-lactamase producing strains

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Escherichia coli is responsible for community and hospital-acquired infections. β-lactams are the first-line drugs for the treatment of these infections. The emergence of β-lactam resistance has become a major threat and one of the most important challenges to global health. There are several mechanisms by which *E. coli* can acquire resistance to β-lactams. Among these, β-lactamase production is the most common and studied. Here, we aimed to access the role of efflux on β-lactam resistance in 26 *E. coli* clinical isolates from different health care units. Strains were typed by ERIC-PCR and the phylogenetic groups determined using the Clermont method. Susceptibility testing was done by disc diffusion for antibiotics used in therapy. Genes associated with β-lactam resistance were screened by PCR and DNA sequencing when necessary. MICs for β-lactams and the contribution of efflux to this resistance was studied by synergism assays with efflux inhibitors (EIs) and ethidium bromide (EtBr) real-time efflux evaluation. As controls, we used the ATCC25922 and three *E. coli* isogenic strains with well- characterized efflux activity: wild-type AG100, AcrAB pump-deficient AG100A, AcrAB overexpressing AG100tet.
Strain typing showed diversity of ERIC patterns. Three strains belong to group A and one belong to group B1 (commensal); nineteen belong to group B2 (virulent extra-intestinal). Seven strains could not be differentiated. 12 strains were MDR, six non-MDR and eight susceptible. 18 were resistant to β- lactams, but only one was resistant to extended-spectrum cephalosporins. MDR strains presented resistance to quinolones, chloramphenicol, tetracyclines and/or aminoglycosides and all were susceptible to carbapemems and tigecycline. β-lactamase production was detected in all β-lactam resistant strains and included the presence of TEM in combination with OXA-1, SHV or CTX-M with AmpC overexpression and/or plasmid-mediated AmpC production. The results showed the existence of synergistic interactions between EIs and β-lactams and EtBr efflux. The real-time efflux assays demonstrated that increased efflux activity could be inhibited by EIs.
Our results demonstrated that β-lactam resistance in *E. coli* is multifactorial involving β- lactamase production and active efflux. For that reason, the use EIs as future adjuvant therapeutic approach to combat β-lactam resistance will be of utmost importance.

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P255. VSkin - A synthetic plant-derived skin desquamation agent for cosmetics and therapeutics applications

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The human epidermis is composed of five layers of stratified epithelial cells and is an organ in constant renewal. New cells are formed in the basal layer, and after a differentiation process, the cells reach the outermost layer of the skin. Renewal and maintenance require the cell shedding of corneocytes from the stratum corneum (SC). Aging and certain skin diseases can disturb this process leading to a decrease in desquamation rate, resulting in an increase in the thickness of the SC and skin scales formation.
Skin desquamation is the shedding of corneocytes from the SC and is part of the self-renewal and maintenance of the skin. Desquamation or exfoliation of epidermal layers of human skin induces an increased rate of epidermal cell renewal. Several serine, cysteine, and aspartic proteases participate in this exfoliation process including cathepsin D, cathepsin E, and SASpase.Herein, we present the development of a new enzyme-based product of plant origin as a skin exfoliation agent - VSKin. The desquamation activity of VSkin was demonstrated in: (i) stratum plantarum; (ii) cellular cultures of corneocytes and keratinocytes; and (iii) 3D skin models. VSkin desquamation activity on 3D skin models was shown to be pH dependent and comparable (if not better) to human cathepsin D desquamation activity. The cellular viability of non-detached cells is maintained after incubation with VSkin. Moreover, VSkin showed a higher desquamation activity when compared with bromelain, papain, papain extract (commonly used in the cosmetic industry as exfoliating agents).VSkin emerges as an innovative ingredient to be used in cosmetic or therapeutic treatment to enhance epidermal exfoliation and/or enhance epidermal cell renewal, therefore, improving the appearance and/or texture of the skin.

United States Patent No. 9,890,372, 2018; European Patent No. EP 2 981 278 B1, 2018

256. Production of a protein hydrolysate of *Pleurotus ostreatus* mushroom

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Fast demographic growth has led to a growing interest in low-cost alternative protein sources to meet demands. Consequently, the attention of many researchers has focused on finding under- exploited sources of proteins, alternatives to those of animal origin, usually plant proteins have been used for this purpose. However, fungal proteins can be of a higher quality than those from many plants and may have certain economic advantages. Edible mushrooms can be processed to obtain protein concentrates, isolates or hydrolysates with improved functional properties, these in turn can be used in the formulation of functional foods or dietary supplements giving them an added value. The aim of this work was to determine the optimal conditions to obtain a protein concentrate from *Pleurotus ostreatus* mushroom powder: pH of extraction, precipitation, flour-solvent (1:5, 1:10, 1:20 w/v) ratio and amount of extractions, and then hydrolyze it with papain to obtain an extensive protein hydrolysate (degree of hydrolysis 10%).

It was found that at low pH protein solubility is lower than at high pH, reaching its minimum point of solubility at pH 4 (32 ppm), which is considered the isoelectric point. The maximum point of solubility was reached at pH 12 (502 ppm).

A positive linear correlation was found that by increasing the proportion of solvent, a greater amount of protein is solubilized (5.3, 8.1 and 9.6 mg/ml respectively), and the yield is higher. As for necessary times of extraction, it was determined that the amount of soluble protein decrease significantly with each extraction (from 6.3 to 0.9 mg/ml). In a second extraction it was obtained a significant amount of protein (2.1 mg/ml).

It was obtained a mushroom protein hydrolysate with hydrolysis degree of 24.6%. Regarding to its proximate composition compared with the unprocessed mushroom powder it can be noted that the lipid content increases almost fourfold (from 1.85 to 7.13), this may be due to a binding mechanism between protein and lipids. On the other hand, protein content is almost doubled (from 32.18 to 56.99), carbohydrate content decreases almost by half (from 50.47 to 25.95) and fiber is practically removed.

III6. Health Microbiology and Biotechnology

P257. Bacterial detection through a modified staphylococcal amidase

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Background: The peptidoglycan molecule is the major component of the cell wall of bacteria and a unique molecule to the bacterial kingdom, making it an ideal target for many antibiotics in clinical use. Furthermore, during growth, bacteria shed parts of their cell wall into the external milieu, and so the presence of muropeptides – peptidoglycan fragments–in sterile body fluids can be used as a surrogate marker for infection. We are thus using these properties to develop a biosensor to detect bacterial infections through the presence of peptidoglycan fragments, using the binding ability of an amidase enzyme (AM) of the pathogen *S. aureus*. Although AM has higher specificity towards *S. aureus* peptidoglycan, it also binds and hydrolyzes peptidoglycan from several other bacterial species. The structure of AM in complex with a peptidoglycan ligand identified the main amino acid residues important for PG recognition and hydrolysis (Buttner F. M. et al., (2014) J Biol Chem). In this study, we used genetic and biochemical approaches to optimize AM binding and hydrolytic activities.
Methods: To increase peptidoglycan binding affinity, broaden the range of binding specificity, and alter PG hydrolytic activity, different recombinant versions of AM were constructed, and specific amino acid substitutions were performed by site-directed mutagenesis. The recombinant proteins were overexpressed in *E. coli*, purified by affinity chromatography, and their hydrolytic activities and PG binding affinities towards different bacterial species were assessed.
Results: Site-directed mutagenesis lead to recombinant proteins which kept or lost their peptidoglycan hydrolytic activity according to the different amino acid substitutions. The muropeptide-binding ability of the recombinant proteins was tested, showing that the altered proteins had different affinities towards diverse peptidoglycan types.
Conclusions: The AM protein has been modified for optimal results to detect muropeptides, by removing hydrolytic activity and increasing the binding capability to different types of peptidoglycan. An energy independent biosensor device that could distinguish between bacterial species would be a major development for the early detection of bacterial infections.

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P258. Effect of *Pleurotus ostreatus* powder on the nutritional characteristics of bread

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Pleurotus ostreatus is one of the most produced edible mushrooms worldwide. It is a source of high quality proteins, rich in fiber, vitamins, minerals and essential amino acids. Several bioactive compounds have been identified in P. ostreatus, such as β-glucans, with immunosuppressive and immunomodulatory activities, hypoglycemic and prebiotic effects. Aguamiel, obtained from Agave Salmiana, is a sweet-tasting liquid that can be used as a sugar replacer. Prebiotics such as fructo- oligosaccharides were found in its composition. Together, *P. ostreatus* and aguamiel, may be incorporated into food formulations increasing nutritional and functionality of final products. In this work, *P. ostreatus* powder (PO) and aguamiel (used as sucrose substitute) were incorporated into a bread. The work aimed to characterize physicochemically the bread obtained.

Five bread formulations were produced to partially replace the wheat flour by the PO, as following – 100:0, 95:5, 90:10, 85:15, 80:20 (wheat flour:PO, respectively). Aguamiel was incorporated as a sucrose substitute in all bread formulations. The proximate composition of the bread formulations and the PO itself was determined by the conventional method of AOAC (2000), including moisture, total ash, fat, protein and carbohydrate. Bromatological and physical analysis of the breads were evaluated, the hardness, specific volume and color of the different products were identified.

Results showed that there was no significant difference neither in hardness nor in the moisture (25% - 32%) determined between formulations. The specific volume decreased, and the color saturation increased, by increasing the amount of PO incorporated in the bread. The nutritional value of the bread increased significantly ($p > 0.05$) with the addition of PO. The protein content increased from 1.1% to 1.5% ± 0.2. Also, crude fiber increased from 0.04% to 0.65% ± 0.25 and ashes from 2.5% to 3.8% ±0.6. The fat composition of the samples increased from 5% to 7% ± 1 and there was no significant difference in the content of carbohydrates (55% to 65% ± 5). PO used was a rich source of protein (22.3%), fiber (5.5%) and minerals (9.9%).

P. ostreatus and aguamiel showed great potential to increase nutritional value of traditional bread.

III6. Health Microbiology and Biotechnology

P259. Antibiotic-resistant bacteria in physiotherapy - an exploratory study

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Antimicrobial resistance has gained global importance as one of the most serious threats to public health. Spread of antimicrobial resistant isolates is a challenge in many niches of health care.
The developed project focuses on the dissemination of bacteria resistant to antibiotics in physiotherapy.
In practice, hands contact between the physiotherapist and the patient is thebasic tool of physiotherapy, being involved in a wide range of therapies and treatments. Therefore, the goal of this project was to understand if this direct contact established during this practice can make the physiotherapist, as well as the community they serve, vehicles of the dissemination of antimicrobial resistance. In this project, from the 29 samples of 3 distinct units. Resistance was found in 36 isolates, being Amoxicillin (AML) and Amoxicillin + Clavulanic Acid (AMC) resistance the most relevant. *Acinetobacter* spp. was the most prevalent in this project.
The developed project showed the importance of hand hygiene practices in the prevention and control of antibiotic resistant infections and colonization.

P260. Antibiotic resistance in home care

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With the increase in the average life expectancy, long-term home health care is a real need. Domiciliary patient care practices can promote the spread of resistant bacteria.
The aim of this work was to detect, identify and characterize multi-resistant bacteria present in the nails of home health care providers with the purpose of evaluating whether this type of care can be a focal point for the dissemination of resistant bacteria. In this study, detection of Gram-negative bacteria producing ESBL, AmpC and carbapenemases were the main objective.
Samples were obtained from the nails of home care providers. Isolates were selected in MacConkey agar and MacConkey agar with adequate antibiotics. Colonies were randomly selected to perform susceptibility tests, using agar diffusion method, according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Chromagar Orientation, ID 32 GN, API 20 E and biochemical tests were used to perform presumptive and confirmatory identification.
In the samples of home care providers, twenty-four isolates were obtained from fifteen samples. Of these isolates, nine were multiresistant (MDR), of which four are ESBL producers, two are AMPC producers and all three are resistant to fluoroquinolones.
These results show that home healthcare might create focal points of dissemination of bacteria resistant to antibiotics.

P261. Decreased echinocandin susceptibility of *Aspergillus fumigatus* following anidulafungin exposure

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Invasive aspergillosis (IA) is a potentially lethal infection that affects mostly immunocompromised patients caused by *Aspergillus fumigatus*. Echinocandins are a second-line therapy against IA. Caspofungin (CSF), anidulafungin (ANF) and micafungin (MCF) are used in salvage therapy for IA as well as for empirical or prophylactic therapy. Echinocandins have been shown to cause lysis of growing hyphal tips but are considered fungistatic against molds. *In vivo* echinocandins resistance is uncommon, however its wide clinical use could shortly lead to the emergence of *A. fumigatus* resistance.

The aims of the present work was to assess the development of reduced echinocandins susceptibility phenotype by an *A. fumigatus* strain and to unveil the molecular mechanism underlying such phenotype.

A. fumigatus clinical isolate (AFS strain) was incubated overnight at 35ºC with sub-Minimal Effective Concentration (sub-MEC) concentration (0.06 µg/mL) of ANF. The ANF concentration was augmented to double when fungi growth was prominent, reaching the final concentration of 8 µg/mL. *In vitro* induction experiment took 30 days. Every 5 days, MEC values of echinocandins were determined according to the broth dilution antifungal susceptibility testing from CLSI. Although clinical breakpoints are not established, a MEC value ≥ 1 µg/mL was considered resistant. After 30 days of ANF exposure (AFR0 strain), cross-resistance to echinocandins was developed. In order to assess the stability of the echinocandins MEC values increments, the induced strain was sub-cultured for an additional 30 days in the absence of the drug and MEC values re-determined and the resistant pattern remained stable (AFR1 strain). A point mutation was found in *AFR0* and *AFR1* strains, corresponding to an amino acid replacement of glutamine by glutamate at position 671 of Fks1p (E671Q).

Herein, a mutation in *A. fumigatus* FKS1 gene with the potential to reduce susceptibility to echinocandin is described. These data suggest that modification of Fks1p in *A. fumigatus* might confer resistance to echinocandins. Given the emerging importance of this mechanism for clinical resistance in pathogenic fungi, it would be prudent to be alert to the potential evolution of this resistant mechanism in *Aspergillus* spp infecting patients under echinocandins therapeutics.

P262. Extraction and evaluation of the biological activities of compounds from red seaweed *Porphyra* sp.

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The marine environment is a source of multiple organisms with unique biological properties which have been sparsely exploited. These biological activities include anticancer, antioxidant, anti- inflammatory, anti-diabetes, anti-obesity, among others. Marine algae belong to those organisms and have deserved particular attention due to their diversity, availability and ability to adjust to environment changes. However, within the same algae species the level of these compounds present some variability depending on the environmental growth conditions. The extraction conditions used also influences the efficiency and type of the bioactive compounds obtained. Thus, the objective of the current work was to evaluate the biological activities (antioxidant, anti-diabetic and anti-hypertensive) of *Porphyra* sp. extracts prepared by enzymatic and chemical methods. The enzymatic method involved the utilization of Alcalase, Viscozyme or Cellulase and hot water (50ºC, stirring for 24h), ethanol (room temperature, stirring for 24h) and bead milling/water (30Hz, 1h) extraction was used.
The lowest yield extraction was achieved with ethanol (<10%) and the yield of water extraction by both methods was around 30%. Enzymes had a significant enhancing effect on the extraction yield (47-67%), particularly Alcalase. Some differences in the antioxidant activities evaluated by several methods (DPPH, ABTS, reducing power and copper and iron ion-chelating activities) were obtained but a clear trend was not observed. The total phenolic content of the different extracts was very similar and no correlation between the total phenolic content and antioxidant activities were observed. Extracts obtained by enzymatic methods exhibited higher α-amylase and α-glucosidase inhibition activities. All extracts presented angiotensin converting enzyme inhibitory activity but the highest activity was obtained in the Alcalase extract. These results suggest that *Porphyra* sp. enzymatic extracts exhibited the highest biological activities making them potentially useful ingredient in functional foods and nutraceuticals.

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P263. Molecular detection of *Helicobacter pylori* antibiotic resistance

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Introduction and aims:
Helicobacter pylori is a bacteria that colonize the gastric epithelium of half of the world population leading ultimately to gastric cancer. Over the years, efficiency of treatment has decreased due to an increase in the antibiotic resistance among *H. pylori*.
The aim of this study was to characterize the mutation profile of genes involved in *H. pylori* resistance to the main antibiotics used for eradication therapy, namely clarithromycin, fluoroquinolones and metronidazole.

Methods:
Helicobacter pylori infection was assessed in gastric biopsies from 33 dyspeptic patients by real-time PCR. *H. pylori* positive samples were further studied for detection and characterization of the genes involved in antibiotic resistance: 23S rRNA, *gyr*A and *rdx*A by real-time PCR followed by amplicon sequencing.

Results:
H. pylori was detected in 22 patients (66.7%; n=22/33). Concerning clarithromycin resistance, the analysis of 23S rRNA gene reveled mutations in 17.6% of the patients with the identification of a mutation associated with clarithromycin resistance (A2144T), but also, with the identification of novel mutations in the positions 2324, 2344, 2365 and 2379. For fluoroquinolones the analysis of *gyr*A gene showed mutations corresponding to Asn-87 and Asp-91, together with novel mutations. Regarding metronidazole, the sequencing of *rdx*A gene unveil several mutations, some of which are largely associated with metronidazole resistance.

Conclusion:
Antibiotic resistance of *Helicobacter pylori* is on rising and is affecting the efficacy of current used therapeutic regimens.
Based on our results, it can be concluded, that infection with mutated *H. pylori* strains is considerably high (81.8%; n=18/22). Mutations described in the literature that are resistance-related were detected as well as novel mutations. Therefore, future studies should be conducted to understand the role of these new mutations in *H. pylor*i antibiotic resistance.

P264. Sodium bicarbonate has a negative impact in vulvovaginal *Candida albicans´* virulence

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Candida albicans is an opportunistic pathogen involved in many infectious diseases, including vulvovaginal candidosis (VVC). VVC is the second most frequent vaginal infection. About 5
-8% of all VVC cases reoccur, which is a condition that has been associated with a negative impact in the quality of life of women worldwide. Downregulating *C. albicans*' virulence in the vaginal tract could be a promising strategy to control recurrence. In this study we aim to evaluate the potential of using sodium bicarbonate in the control of VVC recurrence.
The expression of phenotypic traits associated with recurrence has been assessed in the presence of increasing amounts of sodium bicarbonate. Specifically, biofilm formation, formation of the germinative tube (hypha) and in-vitro growth rates have been determined for a range of sodium bicarbonate concentrations, using a panel of 25 clinical vulvovaginal *C. albicans* isolates. The minimum inhibitory concentration (MIC) of sodium bicarbonate was determined for all strains by microdilution assay, for comparison. In addition, cell morphology when exposed to sodium bicarbonate was observed using scanning electron microscopy.
We found that when *C. albicans* cells were exposed to sodium bicarbonate (at least two times the MIC), there was a reduction of 1.5 times of the normal growth rate, and a reduction of 93% in the cells producing hyphae, when compared with cells not exposed to sodium bicarbonate. With SEM, yeast cells seemed unaffected, but destruction of hyphal-cells were observed. In addition, there was a 50% reduction in biofilm mass, when *C.albicans* cells were exposed to 15 times the MIC.
We conclude that *C. albicans*'s virulence is attenuated in the presence of sodium bicarbonate, and that this compound might be an excellent adjuvant for therapy targeting control of recurrence of VVC.

P265. Cinnamaldehyde modulates efflux of antimicrobial resistant *Staphylococcus aureus*

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Bacterial resistance to antimicrobials has become one of the most important concerns worldwide as it results in severe infection rates and has a high economic impact. Some global resistance mechanisms have emerged probably following the misuse of the available antimicrobials (biocides and antibiotics). One predominant mechanism is the overexpression of efflux pumps that when inhibited should result in decreased antimicrobial resistance. This work aims to assess the potential of cinnamaldehyde, a natural-based product, as an efflux pump modulator. A number of antibiotic and biocide resistant *Staphylococcus aureus* strains were used to evaluate the extrusion of ethidium bromide (EB), as a measure of efflux pump activity. *S. aureus* SA1199b, XU212 and RN4220: pUL5054 strains overexpressing specific efflux pumps. *S. aureus* NCTC 10788 was used for comparison - as an antimicrobial susceptible strain. Cinnamaldehyde effect on these strains was studied by its addition to a bacterial suspension containing EB and fluorescence was followed over time. Reserpine, a known efflux pump inhibitor, was used as positive control. Its use caused EB accumulation in SA1199b strain. This effect was also observed when cinnamaldehyde was added to SA1199b, XU212 and NCTC 10788 strains. In order to further explore cinnamaldehyde effect on efflux pump inhibition other antimicrobial resistant strains were used: *S. aureus* ATCC25923 (susceptible strain), ATCC25923_EB (overexpression of norA), SM39 (antiseptic resistance efflux pump gene qac_A) and SM52 (antiseptic resistance efflux pump gene *smr*). Verapamil and thioridazine were also included as positive controls of additional efflux pump inhibition mechanisms. Cinnamaldehyde promoted EB accumulation in *S. aureus* ATCC25923 and *S. aureus* SM52 (antiseptic resistance efflux pump gene *smr*). These results highlight the great potential of natural products, in particular cinnamaldehyde, to be used – alone or combined - on the reversal of antimicrobial resistance mechanisms.

P266. Thermodynamic study of BSA and BHb adsorption onto a strong anion-exchanger at different salt conditions

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For the prediction and optimization of a chromatographic process, a comprehensive understanding of the relationship between single and multicomponent adsorption is of great importance. Many factors may influence ion-exchange (IEC) adsorption of proteins. These factors include adsorbents, chromatographic ligands and proteins properties as well as experimental conditions. In addition, the adsorption of target protein may also be regulated by adsorption sequence and by the initial concentration of target and co-adsorbing proteins. Hence, the understanding of multi proteins adsorption on IEC adsorbents is extremely complicated, depending on the studied system. In this study, single and bi-component adsorption equilibrium isotherms of Bovine Serum Albumin (BSA) and Bovine hemoglobin (BHb) have been measured on a strong anion-exchange chromatographic support (Toyopearl GigaCap Q-650M) at pH 9 with different salt conditions. For systematically assessment of the binary adsorption competitive mechanism, the influence of initial protein concentration ratios, and adsorption sequence were studied in detail. In addition to this, flow microcalorimetric experiments were also performed to better understand the adsorption mechanism of BSA and BHb.

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P267. IgG purification with modified carbon nanotubes

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Antibodies (Ab's) are proteins with high specificity for binding and inactivation of antigens, such as bacteria and viruses. Ab's, such as immunoglobulin G (IgG)s have been the subject of extensive research for the treatment of infectious diseases, immunodeficiencies and as new therapies for cancer. Currently, IgG is the most commercially available Ab type being usually obtained from small mammals or by cell cultures. However, Ab's are among the most expensive therapeutic options because of the lack of a cost-effective and efficient purification method. The most common method for the purification of IgG uses protein A affinity chromatography, which is expensive. Thus, there is an urgent need for new purification technologies for IgG production. In this work, the main objective was to determine the best conditions for purifying IgG from rabbit serum. The selective extraction of IgG was carried out using functionalized carbon nanotubes (CNTs). Experimental conditions, such as pH and material/serum ratio and IgG concentration were optimized. All materials were characterized by TEM, FTIR and N2 adsorption-desorption isotherms at 77 K.

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P268. Tackling bacterial resistance using antibiotics as Ionic Liquids and Organic Salts

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Bacterial resistance to current antibiotics has a major impact on worldwide human health, leading to 700K deaths every year. The development of novel antibiotics did not present significant progress, namely regarding clinical trials, over the last years, due to low returns. Thus, innovative alternatives must be devised to tackle the continuous rise of antimicrobial resistance.
Ionic Liquids and Organic Salts from Active Pharmaceutical Ingredients (API-OSILs) have risen in academia for over 10 years as an efficient formulation for drugs with low bioavailability and permeability, as well as reduction or elimination of polymorphism, thereby potentially enhancing their pharmaceutical efficiency[1-3]. To the best of our knowledge, our group is the first to perform research on the development of API-OSILs from antibiotics as a way to improve their efficiency. More specifically, we have successfully combined ampicillin[4], penicillin and amoxicillin[5], as well as fluoroquinolones (ciprofloxacin and norfloxacin)[6,7], carbapenems (meropenem) and cephalosporins (cefuroxime) (Figure 1) as anions with biocompatible organic cations such as choline, alkylpyridiniums and alkylimidazoliums.
In this communication, we present our latest developments in the *in vitro* antimicrobial activity of the Ionic Liquids and Organic Salts from these antibiotics, in particular towards Methicillin Resistant *Staphylococcus aureus* and also multi-resistant *Escherichia coli*, in addition to sensitive strains of gram- positive and gram-negative bacteria.

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P269. Peptide chimeras with collagen-boosting effects, antibacterial, and antibiofilm activity on Gram-negative bacteria

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Complicated skin and soft-tissue infections (cSSTI), such as, e.g., diabetic foot ulcers, exhibit signs and symptoms that are consistent with localized bacterial biofilms that contribute to tissue destruction, delayed wound-healing and other serious complications. Such infections culminate in hospitalization for adequate medical intervention[1] and their severity may be exacerbated by hospital acquired infections, often associated with multi-drug resistant bacteria from the so-called “ESKAPE” (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) group.[2] Gram-negative pathogens from this group are the most worrisome, and often isolated from cSSTI. New options for management and treatment of cSSTI are urgently needed and current biomedical approaches aim at providing antimicrobial protection to the open wound together with a matrix scaffold, often collagen-based, to boost reestablishment of a healthy skin.[3]

We present a group of peptide chimeras encompassing antimicrobial and collagenesis-inducing motifs. The best construct displays minimal inhibitory concentrations (MIC) as low as 1.0 M and 2.1 M against *Escherichia coli* and *P. aeruginosa*. This peptide was also active against multidrug-resistant clinical isolates of *Klebsiella pneumoniae*, *E. coli* and *P. aeruginosa* and hampered the formation of/disaggregated *K. pneumoniae* biofilms of resistant clinical isolates. In conclusion, this peptide is a highly promising lead towards development of a novel topical treatment for cSSTI.

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P270. Impact of pneumococcal conjugate vaccine (PCV13) on pneumococcal carriage among portuguese children after introduction in the National Immunization Plan

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Streptococcus pneumoniae is a leading cause of several infectious diseases worldwide such as otitis media, pneumonia, bacteremia and meningitis. This human pathogen colonizes asymptomatically the nasopharynx, and children attending day-care centers are the major reservoirs contributing significantly to its transmission in the community. The polysaccharide capsule represents the major virulence factor of this pathobiont. To date, 98 capsular types (serotypes) have been described. In 2001, the seven-valent pneumococcal conjugate vaccine (PCV7, targeting serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) became available in Portugal. In 2010, PCV7 was replaced by a 13- valent vaccine (PCV13, targeting PCV7-types and serotypes 1, 3, 5, 6A, 7F and 19A). PCV13 became available in the National Immunization Plan (NIP) in July 2015.

This study aimed to evaluate the impact of PCV13 on nasopharyngeal carriage of pneumococci among children following its introduction in the NIP.

In January 2019, a cross-sectional colonization study was carried out among children up to six years old attending day-care centers in Oeiras. Demographic data, vaccination history and antibiotic usage, as well as nasopharyngeal samples, were obtained from 464 children. Pneumococci were isolated, total DNA was extracted, whole genome sequencing was carried out, and capsular types were assigned using SeroBA software.

Vaccination rate was 100% among children younger than 2 years of age (average 94% among all carriers). Overall, 60.3% of the children carried pneumococci. The most abundant serotypes were non- vaccine types 11A (15.8%), 15B/C (14%), 35F (9%), 23A (8.6%), and serogroup 24 (7.5%). Vaccine types in circulation were 19F (2.9%), 6A (1.8%), 3 (1.1%), 14 (0.7%), 6B (0.4%) and 19A (0.4%). Among unvaccinated children 19.2% carried a vaccine type compared to 3.7% of vaccinated children.

The results indicate that introduction of PCV13 in the National Immunization Plan is impacting on the serotype distribution of pneumococci in circulation in the community. Non-vaccine types are now the most frequently found. Vaccine serotypes, although a minority, remain in circulation particularly among unvaccinated children highlighting the importance of achieving universal vaccination to achieve maximum benefits.

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P271. Isolation of new strain of *Bacillus subtilis* in Azores that produces a thrombolytic enzyme

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We screened twenty-four Bacillus isolates belonging to the CBA bacteria collection (Azores) for the production of fibrinolytic enzymes, using a fibrin agar plate. Based on the amount of activity produced, we selected the isolate S127e that was identified as a *B. subtilis* based in 16S rDNA and *aro*E gene sequences. Fibrinolytic enzymes with untapped properties are currently requested for thrombolytic therapy; therefore, we purified the produced enzyme, which was assigned with a molecular mass of 27.3 kDa, a predicted pI of 6.6 and optimal activity at 48 ºC and pH 7. Furthermore, it had maximal affinity for Ala-Ala-Pro-Phe and was almost completely inhibited by chymostatin. Using primers designed based on sequences identified by MS, we amplified an ORF with 825 bp that encodes for the purified enzyme. Encoring gene was submitted to Genbank (accession number MF281668) and with the designation of Apr_{E127}. Apr_{E127} encodes for an open reading frame of 272 amino acid residues. SMART assembly analyses allowed the identification of a subtilisin-like domain between 25 to 253 aa, with the active site residues Asp-32, His-64, Ile-107, Leu-126, Asn-155, and Ser-221. The order of catalytic residues Asp / His / Ser was well conserved indicating that Apr_{E127} belongs to clan Bs and the S8 subtilisin family of serine proteases. Functional assays allowed concluding that Apr_{E127} was a direct-acting thrombolytic enzyme. This enzyme increased by 3.7 % thromboplastin time, from 37.6 to 39 s, and prothrombin time by 3.2 %, from 12.6 to 13 s, both within normal ranges. In whole blood euglobulin assay, Apr_{E127} did not impair coagulation but reduced significantly the lysis time. In an *in vitro* assay, Apr_{E127} completely dissolved a thrombus of about 1 cc within 50 min. Moreover, in a *in vivo* assay Apr_{E127} reduced a thrombus prompted in a rat-tail by 11.4% compared to non-treated animals in 24 h.

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P272. Biomaterials-based therapeutic approach targeting *Mycobacterium ulcerans* infection

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Buruli ulcer (BU) is a necrotizing skin disease caused by *Mycobacterium ulcerans* infection, whose pathogenesis is associated with the secretion of a toxin – mycolactone, that leads to tissue damage and immunosuppression. BU is the third most common mycobacterial disease, after tuberculosis and leprosy, but there is currently no prevention strategy for the disease 1 . Initial lesions of BU present as nodules, papules, plaques or edemas, but due to their indolent nature, these lesions are often detected at later stages, after progressing to chronic ulcers with undermined edges or even to osteomyelitis 2 . Since 2004, the treatment guideline for BU comprises a daily systemic administration of rifampicin and streptomycin with a duration of 8 weeks, which contributed to the reduction of aggressive wide surgical procedures. Nonetheless, treatment of BU is still challenging, due to the occurrence of paradoxical reactions, the probability of recurrence and side-effects of antibiotics 1 . Thus, we propose an alternative treatment approach for BU based on a hybrid delivery system using poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) and gellan gum (GG). These biomaterials are very attractive for drug delivery and tissue engineering, given their good biocompatibility and biodegradability 3,4 and were herein combined with antibiotics to obtain a controlled delivery of antibiotics, for a topical and low invasive application. The ultimate goal is to circumvent the limitations of conventional antibiotherapy and to support wound healing. In this context, we produced GG spongy-like hydrogels incorporating streptomycin and rifampicin-loaded PHBV microparticles, that were further characterized regarding physical, chemical and biological properties. Therapeutic efficacy of hybrid biomaterial delivery system was assessed *in vitro* through the broth microdilution test, and biocompatibility was demonstrated *in vitro* using L929 mouse fibroblasts.

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P273. Antimicrobial Activity of New Silver (Thio)Semicarbazone Derivatives

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Background

Among the bioactive metals, silver exhibits the highest toxicity to bacteria, viruses, and other eukaryotic microorganisms. A large number of bioactive Ag(I) coordination compounds with different ligand environment have been reported and, in particular, the derivatives containing weak Ag−O and/or Ag−N bonds can exhibit a high bioactivity, lower light stability, and inferior solubility in water than the compounds with rather strong Ag−S or Ag−P bonds. Herein we describe the synthesis and antimicrobial activity of two new silver(I) compounds that feature the {AgNO} or {AgSO} environments and were derived from semicarbazone and thiosemicarbazone type ligands.

Methods

Organic ligands HL1 [1, 1-((4-nitrophenyl)(phenyl)methylene)-semicarbazone] and HL2 [2, 1-((4-nitrophenyl)(phenyl)methylene)-thiosemicarbazone] were prepared from 4-nitrobenzophenone via a one-step procedure from semicarbazide or thiosemicarbazide. Discrete silver(I) complexes [Ag(HL1) (NO3)] (3) and [{Ag(HL2)(NO3)}6] (4) were obtained in the reaction of AgNO3 with 1 or 2 in acetonitrile- acetone mixture. The Minimum inhibitory and minimum lethal concentration of the organic ligands were tested against Gram positive *S. epidermids* and *S. aureus* and Gram negative *P. aeruginosa* and *E. coli*. The ability to inhibit biofilm formation was also determined.

Results and conclusion

Two new silver(I) coordination compounds were prepared and fully characterized by standard methods (FTIR, NMR, ESI-MS, elemental analysis) as well as single-crystal X-ray diffraction. Of the 4 tested compounds, only 4 presented antimicrobial activity, including the ability to impair biofilm formation. However, the antimicrobial activity was only observed for the gram-positive bacteria. Further research on the design of other silver(I) coordination compounds and exploration of their antimicrobial potential is currently in progress in our laboratories.

Acknowledgments

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P274. Exploring Japanese quail immune repertoires for antibody discovery

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Avian hosts are widely used for generation of antibodies and enable scalable and cost- effective production in eggs. Notably, hens also enable accelerating monoclonal antibody (mAb) discovery in biopharmaceutical industry since: 1) their phylogenetic distance from mammals ensures generation of robust and specific antibodies against conserved mammalian proteins and 2) the preparation of combinatorial phage-display libraries from chicken sources is simplified over mammalian systems.

We have been exploring the potential of Japanese quail (*Coturnix japonica*) immune repertoires for mAb development. In the present work, multiple sequence search and analysis tools we used to revisit *C. japonica* genome (available since 2013) and identify/characterize genetic regions encoding for thelight (LC) and heavy chain (HC) of quail Ig, namely using chicken data as reference.

We were able to identify two main regions, corresponding to genes encoding quail Ig LC and HC. These supported the design of specific oligonucleotides that were subsequently used in PCR amplification of antibody fragments from a spleen cDNA library, previously obtained inhouse from hyperimmune birds. Preliminary NextGen sequencing (NGS) analysis of the fragments confirmed the expected distribution of conserved and variable regions. Finally and supporting the genetic analysis, protein structural homology model predictions also allowed to identify folding conservation within certain domains, when comparing quail and chicken Igs.

This work has enabled the design of optimal oligonucleotide sets to generate combinatorial phage- display libraries from quail immune repertoires. Notably, these sets are distinct from the ones reported in the literature for chickens. Moreover, we have successfully implemented NGS methodologies to analyze and characterize quail antibody repertoires in terms of size and diversity. Together, these assets open new possibilities to explore quails as source of antibodies for diagnostics and therapy.

P275. Occurrence of *mcr-1* in *Escherichia coli* from rabbits of intensive farming

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Background: The emergence of mobile colistin resistance genes (mcr) is yet another challenge in the fight against antimicrobial resistance, with reports proving the dissemination of these genes in different countries and different environments, including food producing animals. Rabbit intensive farming is highly dependent on the use of antibiotics, making this animal species relevant in the epidemiology of antimicrobial resistance.
Objectives: Three colistin-resistant *E. coli* strains recovered from intestinal content of necropsied meat rabbits reared in two intensive production systems in Portugal were characterized. The isolates were recovered in 2015 and 2016 during routine diagnostic.
Methods: Antibiotic susceptibility profiles were determined by disk-diffusion or reference broth microdilution method (colistin). The isolates were screened for *mcr*-(1-5) genes by PCR and sequencing. Plasmid characterization (PCR-PBRT/pMLST/sequencing) and location (S1-PFGE- hybridization) were performed. Isolates were subjected to conjugation assays using the recipient strain *E. coli* HB101. Clonal relatedness was assessed by MLST.
Results: These isolates were retrieved from three samples received in our laboratory in 2015 and 2016, amongst a total of 13 samples collected from five commercial farms. Two isolates were recovered from samples of kits fed with a commercial diet medicated with colistin, yet, one isolate was from a rabbit not exposed to colistin. All isolates were multidrug-resistant and presented colistin minimal inhibitory concentrations ranging from 4 to 8 µg/mL. They belonged to different clonal lineages and *mcr*-1 was carried in IncHI2/ST2 (clones ST1589 in farm I and ST206 in farm II) or IncHI2/ST4 (clone ST1431 in farm II) plasmids, non-transferable by conjugation assays.

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P276. Bio-hybrid magnetic nanoparticles for the purification of immunoglobulin G

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Immunoglobulin G (IgG) is the most widely used antibody (Ab) for biomedical applications such as treatment of Immunoglobulin G (IgG) is the most widely used antibody (Ab) for biomedical applications such as treatment of cancer, carriers of toxins, and in the treatment of autoimmune diseases [1]. Currently there are several conventional methods available for the purification of Abs, being the most used protein A affinity chromatography [2]. However, the preparation of highly purified Ab is still considered a difficult task, hampering their widespread implementation due to the high manufacturing associated costs.
In this work, hybrid magnetic nanoparticles comprising a magnetite (Fe_3O_4) core encapsulated with a hybrid material of silica and the polysaccharide k-carrageenan (Fe_3O_4@SiO_2/SiCRG) were investigated for the extraction and purification of IgG from serum rabbit samples using simple magnetically assisted separation. The particles were synthetized and characterised by FTIR spectroscopy and transmission electron microscopy. The conditions for Ab purification, namely contact time, serum concentration and pH were evaluated. At the optimised conditions a purity of 74.9 % and a yield of 54% was observed. The increase in the IgG purity obtained shows the good performance of these new particles to separate target proteins from complex media, for which IgG from serum samples was here used as a proof of concept.

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P277. Using carbon nanotubes for antileukemic drugs purification

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Nowadays, the average life expectancy is progressively increasing. Nevertheless, there are still several fatal diseases generally associated with aging, such as cancer, heart and neurological diseases. Biopharmaceuticals as nucleic-acid-based products, antibodies and recombinant proteins and enzymes are applied in order to overcome these age-related diseases.
L-asparaginase (LA) is one of the most broadly used therapeutic enzymes, efficient for the treatment of acute and chronic lymphoblastic leukemia, Hodgkin's disease and different types of melanomas [1]. The main problem related to the therapeutic use of LA is the difficulty in its production and purification. LA is produced via fermentation and its purification is usually comprised of several steps, which can include precipitation, liquid-liquid extraction and chromatography techniques [2]. High yield and purity demand long processing times, followed by the increase of process costs. So, the development of a cost-effective production/purification process is of emerging concern.
In this work, reusable functionalized nanomaterials, namely CNTs, were studied as a cost-effective support to purify LA. Commercial LA was used for preliminary tests. Experimental conditions, such as pH, contact time and material/LA mass ratio were optimized. LA activity was assessed by Nessler reaction, which quantifies the amount of ammonium released after the enzymatic reaction [3].

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P278. *In vitro* evaluation of short antifungal peptides on pathogenic *Candida* spp. and commensal vaginal lactobacilli

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Vulvovaginal candidiasis (VVC) and its recurrent form (RVVC) are infectious diseases produced by yeasts of the genus *Candida*, affecting three quarters of women worldwide at least once in their lifetime. The chemically-synthesized killer peptide (KP) has been demonstrated to be an effective therapeutic molecule against vulvovaginal candidiasis (VVC) in murine models. Nevertheless, its effects on members of the commensal vaginal microbiota or on potential hosts that could be used to facilitate recombinant expression, have not yet been investigated. In this study, we evaluated *in vitro* the effect of KP, two new antifungal peptides (P-A and P-B) and the KP-derivative P6 on six pathogenic strains of *Candida* spp. strains, six commensal vaginal lactobacilli (CVL) and potential expression host strains, *Escherichia coli* BL21 [DE3], four *Lactococcus lactis* strains and *Saccharomyces cerevisiae* P351. The peptides were synthesized by Fmoc and optimal time, pH and temperature for dimerization (active form) were evaluated. Optimal dimerization for all peptides was obtained at pH 7.4, 40 ºC, 16 h incubation. Interestingly, P-A and P-B displayed remarkable microbicidal activity 4 h after dissolution in dH2O at room temperature compared to KP and P6, which exerted no inhibition. The inhibitory activity over a range of peptide concentrations was evaluated in agar plates after incubation (4h, 37°C) with 103-104 cfu/mL of the microorganisms in dH2O. *Candida* spp. and potential hosts were sensitive to all peptides. Further investigations are needed to determine how best to heterologously produce these peptides. Importantly, with respect to vaginal health-related applications, CVL were resistant to all peptides. P-A and P-B exhibited lower minimal microbicidal concentrations than KP and P6, but the relative potency among peptides depended on the indicator strain. Surprisingly, 100 mM peptides had less activity than lower concentrations (0.1-10 mM). In conclusion, we designed new antifungal peptides with enhanced antimicrobial activity which, along with KP and P6, did not inhibit CVL. Studies on vaginal epithelial cell cultures and mice need to be performed to confirm these results prior to human testing.

P279. Comparative *In vivo* evaluation of phycocyanin-based hydrogel for anti-bacterial and burn wound healing

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Severe burn injuries are major global public health crisis that can lead to severe mental and emotional distress as well as leading to significant morbidity and mortality in low- to middle-income regions worldwide. In this regard, pain, infection, low healing rate, and hypertrophic scarring of the burn wounds in nearly every organ system still remain a major challenge in burn injury research and management. Several technological advances have aimed to reduce the limitations of conventional treatments for burned skin contractures and scars by providing a safer and feasible approach, leading to a permanent solution for regeneration of damaged tissue. loading agents in the 3D formed hydrogels have been reported to promote wound healing due to their hygroscopic nature and mimicking native extracellular matrix (ECM) microenvironments. This study was performed to assess the burn wound- healing efficacy of phycocyanin hydrogel by employing deep second-degree burns in a Wistar rat model mainly due to their anti-bacterial effects and accelerating mesenchymal stem cells (MSCs) repair in tissue recovery. This study consisted five groups including just dropping liquid phycocyanin, alginate hydrogel, phycocyanin-loaded alginate hydrogel (with or without MSCs) and control. Our study showed that after 7 days, necrotic tissue, inflammation and collagen deposition decreased in the phycocyanin 3D loaded hydrogel group compared with the other groups. *In vivo* study showed that phycocyanin- loaded alginate hydrogel with MSCs had significant wound healing activity in rats with complete re- epithelization, in reorganization of the dermis with significantly increased epidermal and vascularization markers expression. Further assessment of dermo-epidermal junction, leukocyte infiltration and collagen deposition, in addition to Real-Time RT-PCR and immunohistochemical staining was performed for studying the healing mechanism. According to the results, phycocyanin had the potential to be a feasible and safe agent for enhance cutaneous burn wound healing and reduce scar formation in rats, which might be related to TGF-β1 signaling.

P280. Anti-cancer activity of the green tea leaves extract ZnO nanoparticles on human epidermoid carcinoma cells

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Green ZnO nanoparticles (NPs) from natural sources have been recently attracted high attention for biomedical applications mainly because of their prominent biological characteristics such as their excellent biocompatibility, low cost, and efficient toxicity. The present study aimed to elucidate the anticancer activity of green tea leaves extract (GTE)/ZnO nanoparticles (NPs) complex against human cancer cell lines compared to their toxicity on healthy cell lines. For this purpose, green tea leaves extract (GTE)/ZnO NPs was first were synthesized with the mean diameter of ten nanometers and characterized by X-ray Diffraction (XRD), Fourier-infrared scanning electron microscopy (Fe-SEM), UV-visible spectroscopy, Brunaure-Emmett-Teller (BET), Fourier transformed infrared spectroscopy (FTIR). The cellular uptake and cytotoxicity of ZnO NPs in various concentrations (5-150 ppm) were assessed in A-431(human epidermoid carcinoma cell line), HEK 293T (Human embryonic kidney 293 cell line) and HFF normal human fibroblasts cell line using the 3-(4,5-dimethylthiazol-yl)-5(3- carboxymethoxyphenyl)-2Htetrazolium (MTT) assay for the different period (24, 48 and 72 h). Our findings clarified the efficacy of ZnO NPs/GTE complex as an effective anti-cancer agent in a size dependent manner in the removal of cancer cells with a positive correlation with reduced toxicity on normal cells confirmed. In contrast, we found that the synthetic nanoparticles no significant cytotoxicity on normal cells in concentration of 50 ppm over the time ($p < 0.05$), they were able to destruct cancerous cells ($p < 0.05$). Altogether, we conclude that green tea leaves extract (GTE)/ZnO NPs may pose various potential biomedical applications including safe and natural anti-cancer agent against various cancer cells as well as being suitable candidates for bioimaging.

P281. Evolution of the oral microbiome throughout pregnancy – a systematic review

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During pregnancy, a panoply of dynamic changes in the anatomy, physiology and immunity of women happen, which can impact the oral microbial ecosystem. These shifts in the oral microbiome can predispose women to oral diseases and, according to several epidemiological studies, might even influence the systemic health of the pregnant women.
With the purpose of attaining a deeper comprehension of the evolution of the oral microbiome during pregnancy, a search was performed in PubMed using the terms (oral microbiome OR oral microorganisms OR oral microbiota OR oral ecology OR oral microflora OR oral flora) AND (pregnancy OR pregnant women). Inclusion criteria comprised longitudinal studies performed in humans and reviews. Cross-sectional studies, studies unrelated to oral microbiome or studies that tested the use of pro- and prebiotics were excluded. From the 440 papers retrieved from the query, only 8 were included in this review.
In the selected papers, the samples collected were mainly subgingival plaque (n=5) and saliva (n=4), and the timing of the sampling included the three trimesters of pregnancy (n=7) and post-partum period (n=4). In saliva, the average taxonomic composition and abundance remains stable throughout pregnancy and in the post-partum period. Conversely, in the subgingival plaque, the microbial biomass increases in early pregnancy and significantly shifts, with periodontopathogenic bacteria thriving during pregnancy. This tendency is reverted after labor, when health-associated bacteria repopulate the subgingival plaque. As for the mycobiome, studies (n=2) reported a significant increase in oral yeast counts from the first to the third trimester of pregnancy and a significantly higher prevalence of oral Candida species in the second and third trimesters of pregnancy compared to the non-pregnant women.
Lastly, literature describes that, while the salivary microbiome remains apparently stable throughout pregnancy, the subgingival microbiome seems to shift according to the trimester of gestation. Nevertheless, there is an evident lack of investigation in this field and, therefore, more longitudinal studies with a larger number of participants and molecular identification methods are required to fully understand the complex relations among oral microorganisms, their microbial shifts and their role during pregnancy and in the post-partum period.

P282. Antimicrobial activity of *Satureja montana* by products' essential oils as possible feed ingredients

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A wide range of antibiotics are used to sustain worldwide poultry industry large productions. The drawback of their dietary extensive use is the promotion of antibiotic resistance . To overcome this threat, it is urgent to look for alternative substances that benefit health and animal growth[1]. Possible alternatives are the phytobiotics, compounds with antimicrobial, antioxidant and/or immunomodulatory properties and naturally available in plants[1].

Satureja montana is an aromatic plant whose leaves are marketed as spice by food industry, generating a large amount of stems as byproducts which are a possible source of antimicrobial compounds, as observed for the leaves. The present work evaluated the antimicrobial activity of *S. montana* byproducts' essential oils(EOs) against pathogenic species with economic impact in poultry industry[2].

EOs were obtained using hydrodistillation or solvent-free microwave extraction with similar yields and chemical composition, being carvacrol the most abundant component (825–950 µg/mg). These EOs exhibited antimicrobial activity against *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus* using an agar disc diffusion method and broth microdilution assay. The *in vitro* minimal inhibitory concentrations of these EOs (225, 250, and 150 µg/mL) were similar to the ones reported for carvacrol against the same or related strains. These results allowed to conclude that carvacrol is the active compound in *S. montana* byproducts.

So far, this study points that *S. montana* byproducts have potential to be exploited within the poultry industry as possible active feed ingredient used as an alternative to the antibiotics that are used prophylactically.

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P283. Ionic-liquid-based aqueous biphasic systems as alternative platforms for the extraction of bovine serum albumin

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Aqueous biphasic systems (ABS) fall within liquid-liquid separation techniques, being usually studied in the extraction and purification of various biomolecules, such as proteins. However, most of these studies have been carried out with ABS formed by two polymers or a polymer and a salt. However, the less studied ionic-liquid-(IL)-based ABS offer several advantages over the widely studied conventional polymer-based systems, such as low viscosity, high selectivity and high extraction efficiencies and yields. In this work, novel IL-based ABS were investigated for the extraction and purification of bovine serum albumin (BSA) from bovine serum. Imidazolium-, phosphonium- and ammonium-based ILs combined with the anions acetate, arginate and good buffers were synthesized, characterized, and applied in the development of new ABS with K2HPO4/KH2PO4 at pH 7. The respective ABS ternary phase diagrams were determined at 25°C, allowing to address the influence of the IL cation and anion on the ABS formation and phase-forming compositions required to carry out the extraction studies. Initial assays carried out with commercial BSA revealed a preferential migration of the protein to the IL-rich phase, with 100% of extraction efficiencies achieved in a single-step. However, depending on the IL applied, the BSA recovery yields range between 30% and 100%. When using the real sample, bovine serum, a maximum recovery yield of 80% and an improvement in the purity by 22% were obtained in one-step. Therefore, the new ABS here proposed may be used as an alternative platform for the extraction and purification of BSA.

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P284. Determination of recombination efficiency by real-time PCR – a strategy to evaluate minicircle production

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Minicircles (MCs) are plasmid-like DNA molecules comprised only by an eukaryotic expression cassette that have been shown to allow higher transfection efficiency and more persistent transgene expression in cells than regular plasmid DNA vectors. MC production is typically performed in *Escherichia coli* by replicating a parental plasmid (PP) and promoting its intramolecular recombination via induction of recombinase expression *in vivo*. In this process, the prokaryotic backbone and eukaryotic cassette of the PP are separated, originating two distinct circular molecules - the miniplasmid (MP) and MC, respectively. A critical aspect of MC production is thus the recombination of PP and the evaluation of this process efficiency.
This work focuses on the development of a real-time PCR (rt-PCR) based method that relies on the specific identification of each DNA species – PP, MP or MC – by using pairs of primers that, under qPCR conditions, are expected to only amplify a region in their designed target molecule. The method was firstly evaluated using artificial mixtures of (i) PP and MP, (ii) PP and MC and (iii) MP and MC with known concentrations and which were probed for all three DNA molecules. The results show that the method is able to determine the percentage of target number of molecules in solution, with a standard deviation smaller than 10% relatively to the expected percentage of target in the sample, being observed an inverse correlation between the deviation and real percentage. The method was then used to analyze complex samples either by using cells or pre-purified DNA samples obtained at different recombination induction stages during cellular growth. Although the recombination efficiencies obtained for cell samples indicated that further optimization is needed to perform this type of analysis, the results with pre-purified DNA samples collected from 3 independent cell growths were closely correlated to the recombination efficiencies determined by densitometry analysis of agarose gels, showing also a smaller standard deviation (up to 2%) between analyses.
In conclusion, the method developed in this work is suitable at this point to analyze complex pre- purified samples, resulting in more consistent results than obtained by densitometry analysis.

P285. Detection of cell wall targeting antibiotics in *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive pathogen. The so-called methicillin-resistant *S. aureus* (MRSA) strains are of special concern, due to their ability to resist beta-lactam treatment. Beta- lactams, along with other antibiotics, target the cell wall, an essential bacterial structure comprised of a large polysaccharide matrix, called peptidoglycan. One strategy used by *S. aureus* to face the effects of cell wall targeting antibiotics requires the activation of the Cell Wall Stress Stimulon (CWSS). CWSS allows a coordinated response of multiple genes, that depends on the triggering of the three-component regulatory system VraTSR. VraT, whose topology remains unclear, is believed to be the system activator, sensing a still unknown stimulus. VraT is proposed to promote the auto-phosphorylation of the histidine kinase VraS, which then transfers the phosphate group to the response regulator VraR. In this work, we show that the CWSS activation is heterogeneous in isogenic populations and that both VraT and VraS localize at the membrane. We also demonstrate that the C-terminal domain of VraT, thought to be the sensory region of the protein, is located on the outside of the membrane. Taken together, these results help to elucidate the role of this three-component system, required for full expression of resistance against cell wall targeting compounds.

P286. Susceptibility to antibiotic and biocides in *Staphylococcus schleiferi* associated with canine SSTIs

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This work aims to study the antimicrobial susceptibility profile of a collection of *Staphylococcus schleiferi* associated with skin and soft tissue infections (SSTIs) in dogs. The initial collection comprised all isolates (n=89) identified as *S. schleiferi* or *Staphylococcus spp.* in two veterinary laboratories in Lisbon, between 1999 and 2018.
The identification of the isolates at the species level was carried out by a species specific *nuc* gene PCR, and at the subspecies level by urease production. Antibiotic susceptibility was evaluated by disk diffusion. The presence of antibiotic resistance genes (*mecA*, *blaZ*, *msrA*, *mph*(C), *vga*(A), *vga*(C), *fusB*, *erm* and *tet* genes) and biocide resistance genes (*qacA*/*B*, *smr*) was screened by PCR. The level of susceptibility to biocides (including quaternary ammonium compounds, chlorhexidine and octenidine) was evaluated by determination of minimum inhibitory concentrations (MIC) by broth microdilution. The MIC distributions were used to estimate epidemiological cut-off (ECOFF) values.
Of the 89 isolates, 28 were confirmed as *S. schleiferi*, corresponding to 27 *S. schleiferi subsp. coagulans* and one *S. schleiferi subsp. schleiferi*. Two isolates (2/28, 7.1%) were methicillin-resistant (MRSS, *mecA*+). Five isolates (5/28, 17.8%) displayed a multidrug resistance (MDR) phenotype. Penicillin resistance was detected in more than half of the isolates (64.3%) and 57.1% carried *blaZ*. We also observed resistance to fluoroquinolones (12/28, 42.9%), to erythromycin and clindamycin (3/28, 10.7%), to fusidic acid (3/28, 10.7%) and to tetracycline (1/28, 3.6%). Of the remaining antimicrobial resistance genes screened, we only detected *erm*(B) in one isolate resistant to erythromycin and clindamycin. The estimated ECOFF values revealed no non-wild-type isolates towards the several biocides tested.
This study provides one of the few reports on the characterization of *S. schleiferi* causing infection in dogs in Portugal. To the best of our knowledge, this is the first report of the occurrence of MDR, including MRSS strains and *S. schleiferi subsp. schleiferi* associated with canine infections in our country. These findings highlight the emergence of antimicrobial resistance in *S. schleiferi*, which is the second most frequent staphylococcal species associated with SSTIs in dogs.

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P287. Tackling bacterial infections by eliminating persister cells with efflux inhibitors

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Bacterial persisters are an important source of chronic and recurrent infections. Recent studies hypothesized that persisters employ a defense mechanism via enhanced efflux to expel toxic compounds allowing persister formation before entering dormancy. We investigated the contribution of efflux to the emergence of drug-tolerant persisters using *Escherichia coli* as a model.
E. coli ATCC25922 and five clinical strains were exposed to rifampicin to induce tolerance. Induced persisters were selected with ampicillin. The non-hereditable drug-tolerant phenotype was confirmed treating the cells with three consecutive cycles with and without ampicillin. The presence of active efflux was studied by MIC determination in the presence of efflux inhibitors (EIs), real-time fluorometry for efflux activity evaluation and analysis of mRNA transcriptional levels of selected efflux pump (EP) genes. As controls, we used three isogenic strains with well-characterized efflux activity: wild-type *E. coli* AG100, AcrAB pump-deficient AG100A and EP overexpressing AG100tet.
The results showed that persisters displayed the typical biphasic killing curve, confirming the development of tolerant persister phenotypes. Persisters could be selected with ampicillin after induction with rifampicin and their antibiotic tolerant phenotype was non-hereditable. Persister cells were as susceptible as the parental strains (after 18h exposure - long-term response). However, real- time fluorometry assays showed the existence of increased efflux activity, compared to the parental strains (30min exposure - short-term response), which could be inhibited by EIs. The EP genes *acrA*, *acrB*, *tolC*, *acrD*, *acrF*, *emrA*, *emrB*, and *marA* were found overexpressed in all selected persisters.
We demonstrate the existence of active efflux systems in persister cells. These results support the hypothesis that the activity of EPs allow the maintenance of a drug-tolerant population in a sub- optimal treated bacterial population from which genetically resistant mutants emerge. EPs not only confer resistance to antibiotics used in therapy but also play a crucial role in the emergence of bacterial persistence mounting an active response via activation of EPs. The therapeutic utility of compounds that have the capacity to inhibit EPs in persister cells, potentiating the effect of antibiotics, will increase the effectiveness of antibiotics currently used for the treatment of persistent infections.

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P289. Inhibition of *Clostridioides difficile* by lactic acid bacteria

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Clostridioides difficile infections have been associated with the antibiotics use (altering the intestinal microbiota, enabling the proliferation and toxins segregation by *C. difficile*). Moreover, the increase of antibiotic-resistance of hypervirulent strains turns imperative finding alternative therapeutics with preventive and/or prophylactic effects, as the use of probiotics. Lactic acid bacteria are considered as a major group of probiotic bacteria and their ingestion has been pointed out to confer a range of health benefits. There are several mechanisms by which probiotic strains may exert their inhibitory activity towards undesired enteropathogens: secretion of different metabolites as lactic acid, hydrogen peroxide, short-chain fatty acids, bacteriocins, proteases, etc. The objective of this study was to test the anticlostridial activity of 450 lactic acid bacteria (isolated from various food products) against 5 *C. difficile* strains. For those lactic acid bacteria demonstrating inhibitory activity, the mechanisms of inhibition was investigated.
Suspensions of each *C. difficile* strain were incorporated onto Brain Heart Infusion soft agar with 0.1% sodium thaurocolate and 10 µl drops of each lactic acid bacteria and their supernatants were spotted on the agar plate with the target organism. Clear supernatants of anticlostridial-LAB were sterilized, the pH adjusted, treated with catalase and proteinase K and the procedure was repeated for each treated supernatant.
Of the 450 lactic acid bacteria tested, only 77 were able to inhibit at least one *C. difficile* strain as a result of competition between cells. When the cells were centrifuged and the supernatant was used, 26 maintained their anticlostridial activity apparently: by the action of lactic acid (15), by the production of hydrogen peroxide (3), by the presence of proteinaceous compounds (6) and by the action of other nature compounds (5).
Although further in vitro tests are still needed, such as the ability of selected LAB to inhibit the invasion of *C. difficile* into intestinal Caco-2 cells, these LAB isolates may be potential anti-*C. difficile* strategies as alternative to antibiotics or as preventive of *C. difficile* infections.

P290. Biocide and antibiotic resistance in *Staphylococcus aureus* causing skin and soft tissue infections in companion animals

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In veterinary medicine, skin and soft tissue infections (SSTIs) are among the most frequent pathologies, often caused by staphylococci, including *Staphylococcus aureus*. Over the past decades, the increasing prevalence of methicillin-resistant *S. aureus* (MRSA) strains in animals became an emerging problem. Additionally, the misuse of biocides is suggested as a potential selective force for the emergence and dissemination of antimicrobial resistant bacteria.
This work aimed to study a collection of 55 *S. aureus* associated with SSTIs in companion animals (mainly dogs and cats) from two laboratories in Lisbon, collected between 1999 and 2018. Identification was confirmed by a species-specific *nuc* gene PCR. The antibiotic and biocide (antiseptics and heavy- metals) susceptibility profiles of the isolates were determined by disk diffusion and MIC determination, respectively. The distributions of inhibition halos and MICs were used to estimate epidemiological cut-off (ECOFF) values. The presence of antibiotic and biocide resistance determinants was screened by PCR. Efflux activity was evaluated by the ethidium bromide (EtBr)-agar Cartwheel Method and EtBr MIC determination.
Among these 55 SSTIs-related *S. aureus*, 56.4% (31/55) were MRSA. Over 85% of the isolates were resistant to at least one antibiotic class and nearly 13% showed a multidrug resistance (MDR) phenotype. Resistance was most frequent for beta-lactams (81.8%), fluoroquinolones (56.4%) and macrolides/lincosamides (14.5%), Resistance to beta-lactams and macrolides/lincosamides was mostly related to carriage of *blaZ* and/or *mecA*; *erm*(A) or *erm*(C). We also detected resistance to fusidic acid, florfenicol and gentamicin, related to *fusC*, *fexA*, and *aadD*, respectively and to bacitracin. Biocide ECOFFs allowed the identification of several non-wild type (NWT) isolates for tetraphenylphosphonium, arsenate, cadmium and zinc, mainly associated with genes for several efflux pumps.
Overall, our findings demonstrate a high prevalence of antimicrobial resistant *S. aureus* strains associated with SSTIs in pets, particularly MRSA and MDR, evidencing a growing limitation of available therapeutic options for the management of these infections. These results reinforce the need of a One Health approach on the study of bacteria causing infections in animals.

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III6. Health Microbiology and Biotechnology

P291. Immobilized microbial lipase enhanced the enantioselectivity for resolution of profen esters

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Background: Lipases are most dynamic for industrial applications, as they possess properties such as chemo-, regio- & enantioselectivity, but their commercial application is hindered due to their high cost and poor stability. To overcome these problems, enzymes may be recommended for immobilization study. It has been observed from earlier studies that immobilized enzyme can be reused for many reaction cycles, easy recovery from product/reaction mixture, which ultimately reduced the cost of product. Enhancement in stability of enzyme, usually reported in the literature.
Introduction: In the present study, chemically modified silica was used as a potential support for lipase immobilization, due to its properties such as inertness, inexpensive, thermally and mechanically stable and readily availability. enzyme Lipase from *Bacillus subtilis* strain (BSK-L) was covalently immobilised on 3-Aminopropyl tri ethoxy-silane (3-ATPES) modified silica gel along with Glutaraldehyde (GA) as a cross-linker.
Results: Glutaraldehyde mediated the efficient immobilization of lipase on modified silica and the resulting immobilized enzyme shown good lipase activity. In a harsh environment such as pH, temperature, organic solvents and metal ions, immobilized lipase (BSK-L) displayed an improved stability in comparison to free lipase. Moreover, a noticeable reusability of the immobilized enzyme, i.e. 85% of residual activity was observed even after reuse for 10 reaction cycles, which makes the process economical and more effective. Immobilized BSK-L revealed 87% and 75% of enantiomeric excess for ketoprofen and flurbiprofen, respectively during hydrolysis of their butyl esters, which is higher compared to the enantiomeric excess obtained using free lipase and *Candida antarctica* Lipase-B (commercially procured).
Conclusion: This methodology exploited a Glutaraldehyde to mediate effectively the rapid and simple immobilization of lipase on silica beads. A conceptual application of immobilized lipase was validated for hydrolysing profen ester. It was concluded that covalently immobilized BSK-L could be employed for resolution of chiral drugs/ drug intermediates with high enantioselectivity and for production of high added value compounds.

P292. Epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital in Lisbon over a 6-year period (2013-2018)

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Carbapenemase-producing *Klebsiella pneumoniae* are increasingly reported worldwide and represent a major health threat. The aim of the present study was to provide updated epidemiological data on clinical *K. pneumoniae* carbapenemase-producers in Portugal.
All carbapenemase-producing *K. pneumoniae* isolates (n=46) recovered between 2013 and 2018 from a 123-bed hospital in Lisbon, Portugal, were included in this study. They were all carbapenem non- susceptible, and were further characterized by antimicrobial susceptibility, identification of resistance determinants, pulsed-field gel electrophoresis (PFGE) analysis, multilocus sequence typing (MLST), and plasmid analysis. The most common carbapenemase identified was KPC-3 (n=36; 78%), followed by OXA-181 (n=9; 20%), and GES-5 (n=8; 17%). Interestingly, co-production of KPC-3 and GES-5 carbapenemases was observed in 7 isolates. The *blaOXA-9* gene encoding a narrow-spectrum class D beta-lactamase was identified in 31 isolates, all being KPC-3 producers. A single GES-5-producing isolate co-produced the extended-spectrum beta-lactamase BEL-1, both corresponding genes being co-located on the same ColE1-like plasmid. The *blaOXA-181* gene was always located onto an IncX3- type plasmid, whereas the *blaKPC-3* gene was carried onto IncN, IncFII, IncFIB, and IncFIIA plasmid types. Although the 46 isolates were distributed into 13 PFGE types and 9 STs, 72% belonged to 5 major clones: PFGE A-ST147 (KPC-3/GES-5; 15%), B-ST147 (KPC-3); D-ST13 (KPC-3; 15%), G-ST348 (KPC-3; 15%), and J-ST17 (OXA-181; 13%). A large proportion of isolates showed reduced susceptibility to fosfomycin (96%), trimethoprim-sulfamethoxazole (93%), ciprofloxacin (85%), gentamicin (76%), and amikacin (63%). In addition, non-susceptibility to tigecycline was found in 28% of the isolates and resistance to colistin was observed in one isolate (negative for *mcr*-type genes). All isolates remained susceptible to ceftazidime/avibactam (MIC values ranging from 0.125 to 3 mg/L), with some exhibiting a worrying reduced susceptibility (resistance breakpoint being >8 mg/L). Notably, isolates co-possessing *blaKPC-3* and *blaGES-5* genes showed higher MICs for ceftazidime/avibactam (>=1.5 mg/L; p=0.014), while isolates producing carbapenemase OXA-181 presented lower values (<=0.25 mg/L; p<0.001).
Considering the increasing occurrence of carbapenemase-producing *K. pneumoniae* in this hospital, systematic screening is highly recommended in other Portuguese clinical settings to limit any further spread of such multidrug-resistant strains, and therefore tend to prevent endemic levels in Portugal.

P293. Prospective evaluation of intestinal carriers of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae at admission in a Portuguese hospital

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Among Gram-negative bacteria, the wide spread of extended-spectrum beta-lactamases (ESBL) producing isolates is considered as a global threat. This study aimed to prospectively evaluate the prevalence of ESBL-producing Enterobacteriaceae fecal carriers at admission in a Portuguese hospital and to determine the epidemiology and antimicrobial resistance pattern of ESBL-producing isolates.
Between December 1st, 2018 and February 2nd, 2019, rectal swabs were collected within the first 48h from 151 patients admitted to the hospital. In addition, a total of 48 rectal swabs were obtained from weekly screenings of 37 patients hospitalized for more than 48h. All ESBL- and/or carbapenemase- producing enterobacterial isolates were tested for antimicrobial susceptibility, and characterised by PFGE and MLST.
The prevalence of ESBL producers at hospital admission was 17% and 24% among patients hospitalized for >48h, while the prevalence of carbapenemase producers was 3% in both cases. Most of the isolates were *Escherichia coli* (54%) and *Klebsiella pneumoniae* (41%).The most common ESBL identified was CTX-M-15 (n=17/34; 50%), followed by CTX-M-14 (n=10; 29%), CTX-M-33 (n=4; 12%), SHV-12 (n=2), and CTX-M-55 (n=1). The 20 *E.coli* isolates were distributed into 16 pulsotypes and nine sequence types (ST), out of which ST131 included 60% of the isolates. The 15 *K. pneumoniae* were grouped in 12 PFGE types and nine STs, out of which three (ST17, ST449, and ST147) included 60% of the isolates. A high proportion of isolates showed resistance to ciprofloxacin (86%), SXT (68%), tobramycin (57%), and gentamicin (43%). All isolates remained susceptible to fosfomycin.
In conclusion, a high prevalence of ESBL-producing Enterobacteriaceae was found at hospital admission and more than 50% of the isolates showed resistance to first-line antibiotics for the treatment of uncomplicated lower urinary tract infections. The choice of empiric drugs in the community should be cautious, leaving fosfomycin as a safe alternative.

P294. Extraction of L-asparaginase through supported ionic liquid materials based on silica

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Acute lymphoblastic leukemia (ALL) accounts with approximately 5000 new cases in the United States each year. Oncaspar, the first-line biopharmaceutical used to treat ALL, is based on L- asparaginase (LA), with annual sales of approximately USD $100 million [1]. Its purification accounts for up to 80% of total production cost [2], and so, it is key to discover new LA purification strategies to decrease its current cost, in order to allow its routinely use by a widespread population.
Supported ionic liquid materials (SILs) based on silica have been previously reported in the literature for the separation of natural compounds from vegetable biomass [3]. Nevertheless, other applications have been scarcely taken into account [4]. In this work, the study of specific interactions between SILs and LA was evaluated, allowing further purification from the fermentation broth in which it is produced. Initially, commercial LA was used to understand the adsorption performance of the enzyme on SILs. Experimental conditions, such as pH, contact time and SILs/LA ratio were assessed and optimized regarding the LA yield and recovery activity. The results show the ideal conditions for LA are pH 8 and contact time with SILs of 60 min. Through the envisioned purification strategy, process costs, energy consumption, and waste produced, might be considerably decreased, which may lead to the LA cost-cut and wider application.

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P295. Exploring the contribution of efflux on the resistance to antibiotics, biocides and dyes in clinical isolates of *Escherichia coli*

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The emergence of multidrug resistant (MDR) bacteria represents a global threat to human health and is a top priority of health organizations and infection control programs. Particularly, MDR *Escherichia coli* requires close attention since is rising worldwide. Here, we investigate the contribution of efflux and expression of efflux pumps (EPs) to the MDR phenotype in a collection of 27 clinical *E. coli* isolates from different Portuguese health care units.
Antibiotic susceptibility testing was done by disc diffusion. Screening of drug resistance- associated genes was done by PCR. The contribution of efflux mechanisms to antimicrobial resistance was studied by MIC determination of antibiotics, biocides and dyes, in the presence and absence of efflux inhibitors (EIs). Quantification of efflux activity was evaluated by measuring the accumulation and efflux of the bisbenzimide Hoechst 33258 and the analysis of mRNA transcriptional levels of selected EP genes. As controls, we used the ATCC25922 and three *E. coli* isogenic strains with well- characterized efflux activity: wild-type AG100, the AcrAB pump-deficient AG100A, the AcrAB overexpressing AG100tet.
From the 27 *E. coli* isolates, 13 were MDR and 11 non-MDR. Amongst the reference strains, only AG100tet was MDR. All clinical strains, except one, were resistant to β-lactams, but susceptible to extended-spectrum cephalosporins. MDR strains presented resistance to quinolones, chloramphenicol, tetracyclines and/or aminoglycosides and all were susceptible to carbapemems and tigecycline. The results showed significant reductions in the MICs of the antibiotics, biocides and dyes, including Hoechst 33258. Efflux assays demonstrated increased efflux activity in these strains, which could be inhibited by EIs. mRNA transcriptional levels showed overexpression of one or more EP genes: *acrA*, *acrB*, *tolC*, *acrD*, *acrF*, *emrA*, *emrB*, *emrE*, and *marA*.
This work supports efflux as one important mechanism towards the acquisition of MDR in *E. coli* clinical isolates, complementing other resistance mechanisms, highlighting the relevance of combined therapy to tackle these infections. Moreover, the use of new fluorescent molecules as Hoechst 33258 will allow increasing our knowledge about the activity of efflux systems as well as identifying new substrates and inhibitors adding in the development and implementation of new therapeutic strategies.

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P296. Occurrence of carbapenemase-producing Enterobacteriaceae in a Portuguese river: *blaNDM, blaKPC* and *blaGES* among the detected genes

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Carbapenems are last-resort drugs used to treat severe infections. Despite the increasing number of reports of carbapenem-resistant Enterobacteriaceae (CRE), there is still limited information on their distribution in the environment. Here we report the characterization of CRE isolates from a polluted river.

Water samples were collected from Lis River (Portugal) and filtered through sterile 0.45-µm membranes. Filters were placed on Chromocult agar supplemented with 4µg/ml of imipenem. Isolates were typed by BOX-PCR and PFGE. Phylogenetic affiliation was assessed by 16S rDNA sequencing and antimicrobial susceptibility was determined against 16 antibiotics. CRE isolates were screened by PCR for *blaKPC*, *blaNDM*, *blaVIM*, *blaGES* and *blaOXA-48*. Conjugation assays were performed for isolates carrying carbapenemase genes and MICs were determined for donors and transconjugants. Four CRE isolates were chosen for whole genome sequencing (WGS).

Of the 24 CRE isolates, 13 representatives were identified as *Klebsiella pneumoniae* (n=9), *Enterobacter* (n=3) and *Citrobacter* (n=1). Besides carbapenems, isolates were resistant to aminoglycosides, quinolones, sulfamethoxazole/trimethoprim and tetracyclines. All isolates harboured carbapenemase-encoding genes: *blaGES-5* (*Citrobacter*), *blaNDM-1* (*Enterobacter*) and *blaKPC* (*K. pneumoniae*). Transconjugants were obtained from 2 *K. pneumoniae* isolates. The carbapenems MICs for the transconjugants were 21 to 750 times higher than the ones determined for the recipient strain. WGS analysis of K. pneumoniae isolates CR12 and CR20 revealed a *blaKPC-3* located in an IncFIA- pBK30683-like plasmid that, in isolate CR12, also carried *blaTEM-1*, *blaOXA-9*, *aacA4*, *aadA1*, *strB*, *sul2* and *dfrA14*. These isolates belonged to ST147 and ST231, respectively, which have been associated with carbapenem resistance worldwide and were often linked to community invasive infections and hospital outbreaks. *blaGES-5* was found downstream an intI3 gene in the *C. freundii* isolate (CR16), being associated to an IncQ2/pQ7-like plasmid. Other resistance determinants were found in CR16, including *blaCMY-2*, an Int1-qnrB4-ampC (*blaDHA-1*) region. *blaNDM-1* was found in *Enterobacter roggenkampii* (CR11) located on an IncA/C2 plasmid carrying also *dfrA12*, *sul1* and *aadA*. Overall, our results portrait Lis river as an important reservoir of CRE and highlight the propagation of acquired carbapenemase genes outside the clinic and in river environments.

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P297. Evaluation of the potency of antibiotic formulations in the Egyptian market

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Interest in searching and developing new antimicrobial agents to combat microbial resistance has been growing recently. Therefore, a greater attention has been paid to both screening and evaluation methods of antibiotics activity. The present study aimed to evaluate the potency of some antibiotics containing pharmaceutical products of some Egyptian market companies using microbiological assay based on agar diffusion method and using standard strains in order to determine their therapeutic efficacy.
These antibiotics such as gentamicin, ciprofloxacin, doxycycline, amoxicillin and ceftriaxone were purchased from local pharmacies and evaluated in the current study.
The results of this study showed the relative potency of gentamicin was 41.4%-120% and 28%-41% for ciprofloxacin. While for doxycycline relative potency was 26%-72.6% and 16%-88% for Amoxicillin. As well as ceftriaxone potency was ranged between 48%-97.4%. One product of ceftriaxone, two products from gentamicin and two from amoxicillin were estimated to be within the acceptable range of bioequivalence (80%-120%), while the other products showed unacceptable relative potency. A complaint reporting system about quality and effectiveness problems needs to be considered as a priority source of such information to inform decision-makers.

P298. Detection and inhibition of β-lactamase activity in clinical isolates of *Escherichia coli* combining β-lactamase and efflux inhibitors using an MTT-based method: phenotypic and genotypic validation

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Resistance to antimicrobials is a naturally occurring process, consequence of evolution, to all bacteria, of which β-lactamases are a way to neutralize the lethal effects that antibiotics can present. Recent work done by our group demonstrates that efflux activity contributes to β-lactam resistance in *Escherichia coli*.
Here, we aim to establish a cost-effective colorimetric method for the fast screening of β-lactamases AmpC, extended spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs) carbapenemases and the simultaneous presence of increased efflux activity, in E. coli, for routine use.
26 *E. coli* strains were typed by ERIC-PCR. Drug susceptibility was assessed by disc diffusion. MICs of β-lactams were performed in presence and absence the β-lactamase-specific inhibitors: cloxacillin for AmpC; clavulanate for ESBLs; EDTA for MBLs and boronic acid for carbapenemases. MIC of β-lactams were also performed in presence and absence the efflux inhibitors chlorpromazine and PAβN in combination with the β-lactamase-specific inhibitors using a 96-well broth microdilution MTT-based method. Results were validated using the phenotypic standard methods for β-lactamases detection by disc diffusion and the screening of genes encoding chromosomal and plasmid-encoded β-lactamases by PCR. E. *coli* ATCC25922 was used as a quality control in all assays.
β-lactamase production was detected in all clinical strains and included the presence of AmpC TEM with OXA-1, SHV, or CTX-M or AmpC overexpression and/or plasmid-mediated AmpC production. Carbapenemases and MBLs were not detected in the panel of strains tested. We were able to reduce the MICS of β-lactams in presence the efflux inhibitors chlorpromazine and PAβN in combination with the β-lactamase-specific inhibitors demonstrating that despite the presence β-lactamases, the MICs of β-lactams can be reduced in the presence of efflux inhibitors.
In this work we optimize a cost-effective method for the fast screening of β-lactamases AmpC, ESBL, MBL, carbapenemases and simultaneous efflux activity, which could be used in low-income countries.

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P299. Antimicrobial activity and mechanism of action of novel carbon monoxide releasing molecules

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Carbon monoxide-releasing molecules (CORMs) were developed for the controlled delivery of CO to biological targets, and were developed to treat hypertension through vasodilatation. However, they also have bactericidal activity as first shown for *E. coli* and *S. aureus*, and later for several other pathogens.
In this work, new metal carbonyl CORMs were synthesised, and their bactericidal activity tested against Gram positive and Gram negative bacteria.
We also demonstrate that CO is released intracellularly in bacteria, using the turn-on fluorescent probe COP-1. Remarkably, we also show that these molecules remain stable in the bloodstream, making them good candidates as antibiotics for targeted deliver of CO to tissues without the risks of blood poisoning.

P300. Preliminary studies on the role of *mazEF* in *S. epidermidis* biofilms dormancy

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Background
Staphylococcus epidermidis has been recognized as one of the main causes of nosocomial infections, mainly due to its ubiquitous presence on human skin and mucous membranes and capacity to form biofilms on the surface of indwelling medical devices. *S. epidermidis* biofilm-associated infections are a major concern since biofilms present higher antimicrobial tolerance and ability to evade host immune defenses, often resulting in recurrent and relapsing infections. Importantly, some bacteria within biofilms have been found to enter a dormancy state, presenting less sensitivity to host immune response and antimicrobial therapy. Moreover, it was earlier found that *mazE*, a gene encoding a protein of the *mazEF* complex, was only expressed in situations where dormancy was induced. Thus, the aim of the study was to analyze the role of *mazEF* in *S. epidermidis* biofilm dormancy.

Method
First, *S. epidermidis* 1457 biofilms were studied to ensure this strain would fit the model that was previously developed to study dormancy: biofilms were grown in excess of glucose to induce dormancy and to prevent dormancy $MgCl_2$ was added to the medium. Then, this strain was used to construct a mutant for the genes *mazEF*, and its complemented strain. Wild type, mutant and the complemented strains were used to form biofilms and assess the number of viable and cultivable cells, respectively, by flow cytometry and CFU, as well as the biomass of the biofilms, quantified by optical density.

Results and Conclusion
S. epidermidis 1457 biofilms entered a dormant state when grown in glucose enriched medium, presenting lower ratios of cultivable/live cells comparing to biofilms grown in the presence of $MgCl_2$, especially in the case of the mutant strain. Interestingly, all biofilms grown under dormancy-induced condition showed a decrease in the number of cultivable cells, but *mazEF* mutant strain showed the most significant difference be-tween induced and prevented dormancy conditions. Overall these preliminary results suggest that *mazEF* complex has a role in dormancy.

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P301. The effect of culture media on in vitro growth and biofilm formation of Bacterial vaginosis (BV)-associated pathogens

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Background
Bacterial vaginosis (BV) is an important vaginal bacterial disorder worldwide, being characterized by a change in the vaginal microbial composition from a health-associated microbiota to a dense polymicrobial biofilm. Although this biofilm plays an important role in BV progress and recurrence, very little information exists regarding its in vitro formation. The current study aimed to investigate the influence of different culture media on the planktonic growth and biofilm formation ability of BV- associated microorganisms, namely *Gardnerella sp*., *Atopobium vaginae*, *Lactobacillus iners*, *Mobiluncus curtisii*, *Peptostreptococcus anaerobius* and *Prevotella bivia*.

Method
Five different media including Brain heart infusion broth supplemented with yeast extract, starch and gelatin (sBHI), Brucella broth supplemented with hemin and vitamin K1 (BHV), New York City broth supplemented with 10% inactivated horse serum (NYC), Schaedler broth (SB), and a medium simulating genital tract secretions (mGTS) were used with the mentioned composition, but also supplemented with ascorbic acid, excepting mGTS which already has this component in its composition. Optical density measurement and crystal violet staining were performed to characterize the planktonic and biofilm bacterial growth.

Results and Conclusion
Significant planktonic growth was observed in NYC and NYC supplemented with ascorbic acid for *Gardnerella sp*., *A. vaginae* and *L. iners*. Conversely, *M. curtisii* showed an insignificant growth in all media tested. *P. bivia* presented higher growth in sBHI with ascorbic acid, followed by NYC, while *P. anaerobius* had better growth in SB and SB with ascorbic acid, also followed by NYC. Biofilm quantification showed high in vitro biofilm growth for *Gardnerella sp.*, *P. anaerobius* and *P. bivia* in almost all culture media excluding BHV and BHV with ascorbic acid. Contrary, only NYC was able to promote biofilm formation for *A. vaginae*, *L. iners* and *M. curtisii*. Taken together, this suggests that NYC is an optimal medium for in vitro growth and development of BV-associated biofilms.

References & Acknowledgments
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P302. Checkerboard assay method highlights synergistic effects between Carvacrol, α- Terpinene, γ-Terpinene, ρ-Cymene and Linalool

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Background
Essential oils (EOs) are complex mixtures composed of different constituents with low molecular weight. The biological activities of EOs can be closely related to the activity of the main components but can also result from interactions between major and minor constituents. The use of EOs as therapeutic alternatives has increased due to their good antimicrobial activities. However, one of the disadvantages of using EOs is the possible variability in their compositions between collections of the same essential oil, which can compromise some effects. The aim of this study was to assess the interactions between five components from EOs.

Methods
Interactions between five commercial compounds, carvacrol, α-terpinene, γ-terpinene, ρ-cymene and linalool, were determined by the checkerboard method against *Gardnerella* spp. To each combination, the value of Fractional Inhibitory Concentration (FIC) was determined by FIC= FIC A + FIC B, where FIC A= (MIC of A in combination)/(MIC of A alone) and FIC B= (MIC of B in combination)/(MIC of B alone). The effect was considered synergistic when FIC was ≤0.5, partial synergistic when FIC was >0.5 and 1 and ≤4 and antagonistic when FIC was >4. In each assay, the lowest value of FIC was selected.

Results and conclusion
All tested compounds demonstrated antimicrobial activity against *Gardnerella* spp. Different interactions were observed between the mixtures of components. Combinations with carvacrol led to interactions with low values of FIC, namely a synergistic effect in the combination with ρ-cymene. Furthermore, the combination of α-terpinene and ρ-cymene also resulted in a partial synergistic/synergistic effect. All the other tested combinations resulted either in a partial synergism or additive effect. Interestingly, none of the combinations resulted in an antagonism. These findings highlighted the interactions that can occur between different components within EOs, as well as the potential antimicrobial activities that can be achieved by such interactions.

References & acknowledgments
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P303. The *Staphylococcus epidermidis* biofilm matrix confers protection against a phage that is highly active against dormant cells

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The complex biofilm structure confers to bacteria a key survival strategy. Biofilms are microbial communities that can attach to both abiotic and biotic surfaces and are frequently associated with the development of several nosocomial infections. The increasing need for innovative and efficient treatments to target these complex structures has led to an increasing interest on phages as a strategy for biofilm control and prevention. Theoretically, due to the closeness of cells, phage infection of biofilms is expected to be very efficient. However, several reports on phage-biofilm interactions had demonstrated a poor efficacy. In fact, the biofilm phenotype protects cells against phage predation due to several factors such as the dense involving matrix, cells with low metabolic rate and the quick development/multiplication of phage resistant variants. Staphylococci are amongst the most prevalent genera isolated from different types of infection. They usually form thick biofilms and are very difficult to target with antibiotics being, consequently, a useful model to study phage-biofilm interactions. Several staphylococcal phages were isolated and tested against biofilms. Although some studies have demonstrated the efficacy of phage against biofilms, only a few were successful against staphylococcal biofilms.

In this work we isolated a novel *Staphylococcus epidermidis*-specific phage, named SEP1. SEP1 has a broad lytic spectrum of activity and the rare ability to infect stationary phase cells. Indeed, phage-host interactions were analyzed by flow cytometry that showed that stationary-phase cells responded immediately to SEP1 addition. Moreover, quantitative PCR experiments revealed that phage genes were already being expressed after 5 minutes of contact with stationary phase cells.

However, SEP1 was inefficient against *S. epidermidis* biofilms. To understand the underlying factors impairing SEP1 inefficacy, this phage was tested against distinct biofilm-derived bacterial populations. Interestingly, SEP1 was able to lyse both active and dormant biofilm cells, suggesting that the inefficacy on biofilm control resulted from biofilm composition and architecture. To demonstrate this hypothesis, SEP1 was tested in scraped biofilms resulting in a 2-log reduction in the number of culturable cells, after six hours of infection. Our results provide compelling evidence indicating that the biofilm matrix can work as a decoy, hindering phage infection.

III6. Health Microbiology and Biotechnology

P304. Graphene oxides as *Pseudomonas aeruginosa* inhibitors

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Most strains of *Pseudomonads aeruginosa* are opportunistic pathogens. *P. aeruginosa* can cause very serious infections with often fatal consequences in immunocompromised or cystic fibrosis patients. Another serious complication is resistance of pathogenic microorganisms to antibiotics. It should be added that the resistance of microbial populations growing in biofilms is often up to 1000 times higher compared to planktonic cells. Most strains of *Pseudomonads aeruginosa* easily form very stable biofilms on various types of biological and abiotic surfaces. Biofilm formation is thus considered to be one of the major virulence factors. There are a number of approaches to suppress biofilm phenotype and thereby significantly reduce pathogen resistance. For this purpose, a number of natural substances or physical methods have been applied with partial success. Recently, attention is also paid to the appropriate application of nanoparticles of various materials, whereas relatively new are graphene-based nanoparticles. Differently modified graphene oxides as potential inhibitors of growth and biofilm formation of several *P. aeruginosa* strains from diverse environments were tested in this work. The cultivations were carried out in 100-well microtiter plates using Bioscreen C analyzer. The cell adhesion and biofilm stability under different concentrations of graphene oxides were quantified by crystal violet assay. Metabolic activity of the cells in biofilm was measured using MTT (3-[4,5- dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric test based on the reduction of MTT into formazan by the dehydrogenase system of living cells. Applied graphene oxides did not show significant toxicity to suspension or biofilm populations of *P. aeruginosa* strains up to a concentration of about 2500 mg/ l. However, totally different results were obtained when the graphene oxides were decorated with metals. Ag-decorated graphene oxide at 100 mg/ l reduced *P. aeruginosa* biofilm to 1/4 and metabolic activity cells in biofilm reached only 20% compared to control cultivation. At concentration 200mg/ l practically all cells were metabolically inactive. Likewise, the graphene oxide decorated with cadmium proved inhibitory effect on metabolic activity of *P. aeruginosa* biofilm, but at a concentration of about 1000mg / l.

P305. Inhibition of *Candida albicans* biofilms by extracts from *Vitis vinifera*

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Candida albicans is an opportunistic pathogen and in immunocompromised individuals may cause mucosal and dermal Infections but also hematogenously spreading infection with high mortality. The ability of colonization both biological and abiotic surfaces and the formation of stable and resistant biofilms is important virulence factor of this yeast. The transition between planktonic and biofilm cell phenotypes is a complex process which is largely controlled by the production of signal molecules. Cell attachment and biofilm formation is mainly associated with tyrosol production, on the contrary, continuous farnesol production during growth of *C. albicans* prevents cell adhesion and formation of biofilm structures. Substances interfering with the function of signal molecules or affecting their biosynthetic pathways can significantly suppress this virulence factor - the formation of a resistant biofilm. This ability has been manifested by creation of a number of secondary metabolites of plant, where polyphenols are particularly important. Plant tissue extracts containing mixtures of natural substances are more effective in this respect.
Suspension and biofilm cultivations of applied strains of *C. albicans* were performed in microtiter plates. Crystal violet staining was used for the quantification of total biofilm biomass. Metabolic activity of the cells in biofilm was measured using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric test based on the reduction of MTT into formazan by the dehydrogenase system of living cells.
Vitis vinifera is a plant known for producing of stilbenoids (resveratrol etc.) and a number of other polyphenols. Different extracts from blue grapes and grape cane were used to study inhibition of C. albicans biofilm formation. Pure substances resveratrol and 3-phenyl acetic contained in extracts were used as control. These substances decreased biofilm formation but their effect on *C. albicans* biofilm was less significant. On the other hand, used extracts of V. vinifera inhibited both biofilm metabolic activity (by more than 70% and 60%) and biofilm structure, even when were applied in lower concentrations.

I7. Biological Resource Centers and Portuguese Network

P306. Microbial Culture Collections Hosted by INIAV

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The National Institute for Agrarian and Veterinarian Research I. P. (INIAV) is the State Laboratory of the Ministry of Agriculture, Forests and Rural Development. It carries out research activities in agricultural and veterinary fields and research to support public policies while defending national interests and deepening Europe's common policies. INIAV's responsibilities include the preservation and management of plant and animal germplasm databases, and national collections of reference and harbours valuable microorganisms collections.
In the area of animal health and zoonoses, the department UEISPSA – LNRSA has several biological collections that are mainly constituted by animal pathogenic agents.
In the area of plant health, the UEIS-SAFSV holds three collections:
The fungus culture collection (MEAN) had its origin by the middle of 1940 in the Estação Agronómica Nacional and includes filamentous fungus associated with plants, either pathogens or saprophytes and also mycorrizas and edible mushrooms.
The Plant pathogenic bacterial collection (CPBF) was established in the mid ninety's and holds panoply of cryoconserved strains from regulated and quality organisms collected from cultivated and spontaneous hosts in Portugal and foreign countries.
The collection of nitrogen fixing bacteria is a very specific collection (started in 1970). Is constituted mostly by autochthones bacteria, isolated from different legumes, belonging to various species and is kept by the Soil Microbiology laboratory. These bacteria are used for application in agriculture as biofertilizers (inoculants).
The EVN Collection has began in 1973, in what is currently the Oenology Laboratory of INIAV-Dois Portos and includes bacteria, filamentous fungi and more than one thousand yeast strains (over 50 genera) mostly related to vine and wine environments.
The Department of Technology and Innovation (UEIS – UTI) holds the LMAI collection of microorganisms. This collection began in the 1960s and includes bacteria, yeasts and filamentous fungi isolated from waters & environment and food and feed products. Some of these strains were isolated from traditional Portuguese fermented food products and are studied about theirs technological properties and probiotic potential.
The participation of INIAV in the Portuguese microBiological Resources Centres Network (Pt-mBRCN) will certainly be an opportunity to evolve and improve Portuguese microbiological resources.

I7. Biological Resource Centers and Portuguese Network

P307. Underpinning CIMO's Culture Collection – bring together in-house repositories of relevant microorganisms into a mBRC

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Microbial Biological Resource Centers (mBRCs) are quality-managed culture collections that ensure the ex situ preservation of microorganisms, while providing public access to their microbial diversity (i.e. to live strains or to genomic DNA from these strains), to relevant data related to it (e.g. taxonomic identification, culture conditions, ecophysiological features, etc), and also to expertise services such as training or consulting.
The main mission of the Mountain Research Centre (CIMO), at Bragança, is to develop sustainable land systems, improving endogenous research competencies, and linking research and stakeholders in mountain areas. CIMO currently host in-house microbial culture collections relevant for agriculture and food industry. These repositories are comprised by bacterial and fungi strains (> 1500 isolates), mainly obtained from plants and food from NE Portugal.
In this work, we describe procedures to be followed throughout the establishment of the CIMO Culture Collection, while presenting the phylogenetic diversity of bacteria and fungi strains already characterized and to be included in the future mBRC.

I7. Biological Resource Centers and Portuguese Network

P308. Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC): A whole new world to explore and discover

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Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biological resource centre located at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), that comprises more than 1000 different cyanobacterial and microalgae strains. Isolates have been obtained since 1991, from samples collected in different environments and locations. LEGE-CC strains were mainly isolated from Portuguese ecosystems (including Madeira and Azores Islands) but also from other countries worldwide (e.g. Australia, Brazil, Colombia, Morocco, Mexico, Dominican Republic, Cape Verde). The fact that most part of our strains were obtained from Portugal associated with rich phylogenetic diversity among cyanobacteria and microalgae groups confers uniqueness to LEGE-CC. In the last decades, an increasing attention has been directed towards cyanobacterial and microalgae due to the fact that some of these microorganisms have demonstrated the ability to produce a myriad of chemical compounds, including toxins or other bioactive molecules with potential biotechnological application. LEGE-CC is the basis for several projects and different scientific studies. Our strains are being screened over the years for a diverse range of purposes and targeting different applications and activities (e.g. anti-cancer, anti-biofouling, anti-microbial, anti-biofilm, anti-obesogenic and related diseases, cosmetics, food, etc.). Indeed, several studies demonstrated that our LEGE-CC stains have potential and effective capacity to produce a diverse array of metabolites, including toxins (e.g. Microcystins, Anatoxin, Cylindrospermopsin and BMAA) or newly discovered molecules (Portoamides, Hierridin B and C, Bartolosides, Nocuolin A, etc). LEGE-CC is member of World Federation for Culture Collections (WFCC), European Culture Collections Organisation (ECCO) and it is also part of the Research Infrastructure EMBRC.PT. As other biological resource centres worldwide, LEGE-CC seeks to provide several services (e.g. provision of starter cultures for diverse purposes, isolation and identification of strains, cyanotoxins analysis, cryopreservation, training courses, etc.). Until today only a small percentage of LEGE-CC strains have been explored and based on potential that was shown in previous studies we believe that our collection is a great source to solve some actual problems such as the lack of new antibiotics, incorporation of new eco-friendly compounds on anti-biofouling paints or discover of new drugs against cancer, obesity and related diseases among other applications.

I7. Biological Resource Centers and Portuguese Network

P309. Biotropical Resources: The leading national tropical resource centre from Global Health and Tropical Medicine (GHTM/ IHMT NOVA) A Portuguese microBiological Resource Center Network (PT-mBRCN) Member

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The Global Health and Tropical Medicine from the Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (GHTM/ IHMT NOVA), has remarkable and probably unique national collections resultant from the longstanding research collaborations developed in the Portuguese speaking endemic countries.
In the late 2016, the Biotropical Resources was created with the purpose of organizing and systematizing these collections, as well as the integration of new biological resources. This new infrastructure was designed to work as a strategic tool for research development on health sciences, mainly on infectious diseases and tropical medicine, through the access to singular and diverse collections of non-human origin (e.g. bacteria, virus, parasites, vectors or reservoirs as mosquitoes and gastropods), as well as of human origin (e.g. blood, serum, urine). Presently, the infrastructure integrates nearly 4,500 samples that resulted from three institutional collaborations: two on fascioliasis and leptospirosis in Portugal (continental and Azores) and one regarding paediatric microbiome in Guinea Bissau.
Samples and data are collected under strict protocols following the ISBER, OECD Best Practices, the Nagoya Protocol and European Union General Regulation for Data Protection (GRDP) with data protection practices approved by NOVA Data Protection Office.
The Biotropical Resources is a member of the recently created Portuguese microBiological Resource Center Network (PT-mBRCN), an initiative that will strength and consolidate not only our collections, but also other national resources as a public service for the scientific community.
In the near future, we will focus our strategic initiatives in networking improvement and resources consolidation (through continuous integration of the pre-existing institutional and new collections).

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I7. Biological Resource Centers and Portuguese Network

P310. Azores Regional Veterinary Laboratory - Fungi Collection - Portuguese Microbiological Resource Center Network (Pt-Mbrcn)

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The official Laboratory of Azores for the animal health and Veterinary Public Hygiene hosts a Fungi Collection. The collection is affiliate to the newly founded Portuguese Microbiological Resource Centre.
The collection has its origin in 1995 to accommodate the strains isolated at the Mycology department of the Laboratory, mainly dermatophytes. Besides dermatophytes from animal infections from the Azores islands and abroad, it hosts many fungal strains from food and feeds, pathogenic and not pathogenic yeasts. Currently with 150 strains, and increasing every day, the collection is in a restructured process. The fungal strains are maintained in ultralow temperature, freeze-drying or on agar slants.
All strains were identified based on morphological and biochemical characteristics, 72 strains have been characterized by sequencing in collaboration with MUM – Micoteca da Universidade do Minho.
The collection provides species for various research projects, mainly supporting the University of the Azores, recently for the project “Phytoplankton-bacteria interactions: from coexistence to coevolution in a changing ocean - PhyBa_CO2”- Post Doctorate thesis and “Study of antibacterial and antifungal activity of lactic acid bacteria isolated from São Jorge cheese”- Master's thesis.
The basic goal is to certify according to ISO 20387:2018 and be a reservoir of isolated fungi in the Azores islands.

III9. School Science and Science Communication

FP311. Bioinformatics in secondary education: from wishful thinking to reality

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A panoply of initiatives has been promoted in several countries to integrate bioinformatics in secondary education. These enterprises have been used as case studies to understand how bioinformatics may foster secondary students' scientific and digital literacy, their interest and attitudes, and identify the main constrains that are preventing teachers to implement bioinformatics exercises in the classroom. In our group a research is being carried out aimed to select, adapt, implement and evaluate a set of bioinformatics-based activities. Framed in this research, a portfolio of bioinformatics-based exercises was created and implemented in 14 classrooms, suitably supported by training courses for teachers and a webpage to provide teachers with didactic instruments and a communication channel. Students' assessment was carried out by a quasi-experimental study based on a pre-/post-test design, applying specifically designed surveys. A mixed methods approach was followed. Parametric tests for data assessment were complemented with non-parametric tests. For qualitative data, a content analysis was executed. Concerning teachers' assessment, informal observations were carried out in addition to a survey. Non-parametric tests and content analysis were performed. Insights on the data collected revealed that students are curious about bioinformatics and that there is an enhancement of their perception about the importance of bioinformatics to biological research. In addition, students' self-confidence to further explore bioinformatics resources is raised, and they admit that these activities improve their understanding of researchers' job, which emphasizes the citizenship education component of the activities. On their side, teachers revealed to be interested in bioinformatics, and recognize its importance and adequacy to the curricula. However, teachers pointed out reasons for not including bioinformatics in their educational practices, namely the scarce offer of training for teachers in this scientific topic, the lack of resources in schools, and the poor offer of bioinformatics activities curricular framed. Ultimately, by stressing the importance of basic bioinformatics learning to uphold secondary students as informed citizens in an increasingly digital society, this research is a wake-up call for educational stakeholders regarding the need to boost teachers' confidence in this subject and to provide the resources required to update their pedagogical practices.

III9. School Science and Science Communication

P312. Science Comics as a tool to improve bioscience literacy

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One of the major challenges in contemporary science is to develop innovative ways to engage society in scientific topics and in research. Using artistic languages to spread scientific messages and to arise the curiosity could be an important contribute to increase scientific literacy and also the interest in science. We also believe that the best way to improve scientific literacy doesn't necessarily involve the production of more information about science, but rather more suitable communication approaches that might be better understood by target audiences.
According this mindset, at CNC research center, a multidisciplinary team of scientists took the challenge of develop several comics about scientific topics in order to aware Portuguese population about scientific issues with high impact in society.
The comics have the power of the image and the color to transmit a message in a soft and informal way. The process of creation of science-related comics could be divided in six critical phases: 1) rigorous definition of what we want to transmit; 2) characterization the target audience(s); 3) respect the space; 4) identification the graphical and narrative style; 5) balance between drawings and information; 6) evaluation of the impact.
The 2-page comics were published in one of most prestigious newspaper in Portugal – Jornal Púbico. Since the begging of this partnership between scientists (CNC) and journalists (Público) we already published six comics about different scientific themes: diabetes, brain, sleep, infertility, sports and its impacts in health and tuberculosis. Overall the comics targeted about 180 000 people (according the Público´s daily circulation number) with an advertising value of 60 000 euros. We believe that this strategy could be an important and creative way to promote the scientific literacy and improve the engagement of society in science.

P313. Implementation of Micromundo@Uporto: an educacional project based on service- learning promoting antimicrobial resistance awareness among university and school students

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Background: Small World Initiative (originally from USA) is a global citizen science project implemented in Spain (2016/2017) with a Service-Learning strategy involving two educational levels (university+school-students). It aims to contribute to solving the societal challenge of Antimicrobial Resistance (AMR) across clinical-food-environmental areas ("One Health"). The experimental challenge proposed to school students is the discovery of microorganisms producing new antibiotics while exploring the soil microbial diversity.
Objectives: To implement this project in Portugal with MicroMundo@UPorto designation through two Curricular Units-UCs (Bacteriology-Pharmacy Faculty and Microbiology-Nutrition and Food Science Faculty) of Porto University and to estimate its impact on the improvement of university students' academic performance, acquisition of social/soft skills and AMR awareness as well as school students' interest in science.
Methods: University-students tutored by university professor/researcher were responsible for the organization/teaching of 4 sessions (2h/each-4 weeks) to Basic/Secondary school-students. After MicroMundo@UPorto announcement, 41 university-students volunteered to participate in 8 teams (5-6 university-students+1-2 supervisors; 3 schools-140 students) and to be responsible for a class (20-25 school-students). After training (theoretical/laboratory classes) in the University, students worked as a team and met with their tutor for school' sessions preparation. Post-survey-based evaluation (Enalyser online survey) of the project was applied to university-students, school students and teachers.
Results/Conclusions: School sessions (February-March/2019) involved 4 sessions: S1-project explanation+AMR+biodiversity and soil collection (total-n=80 from 8 districts); S2-soil weighing+dilution +plating; S3-colonies identification+selection (total-n=800 isolates) for the antibiosis assays (n=50 positive results); S4-Results interpretation+discussion. Survey evaluation revealed a high level of satisfaction among both university and school students towards the acquisition of competencies for scientific and soft skills. School teachers were particularly enthusiastic throughout the project and strongly recommended it to other schools. Besides AMR awareness, we could observe an improvement in university-students' perception related to the two Microbiology-UCs effects on professional practice and an enrichment in autonomy, responsibility/commitment, planning, public communication, teamwork, improvisation and empathy, essential skills for better prepared future health professionals. The success of the pilot experience motivated us to extend the initiative to other Universities. For that, we have successfully organized the first MicroMundo@Porto Workshop (July-2019) attended by 12 future tutors from different Portuguese/Spanish Universities.

P314. Evaluation of the resistance profiles in bacteria isolated from companion animals – 2016 to 2018

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The antibiotics discovery was a big advance in the treatment of bacterial infections, in human and in veterinary medicine, being the basis of modern medicine.
Antibiotics are widely used in veterinary clinics and hospitals in its daily routine and their excessive or inadequate use in animals has led to the appearance of resistant bacteria. This phenomenon is nowadays a worldwide concern due to its impact in animal health as well as in human health.
The main objective of this study was to analyse and compare antibiotics resistance profiles in bacteria isolated from animal samples attended at the Hospital Veterinário Universitário de Coimbra, from March 2016 to December 2018.
This study was conducted in two steps. Firstly, a descriptive characterization of the 206 samples corresponding to the results obtained from antimicrobial susceptibility tests in bacteria isolated from dogs and cats, was performed. Secondly, and after considering the defined inclusion criteria, an analysis was developed using only cases of single bacterial infections by the evaluation of its resistance profile among the three years of study.
So, during the study period, the resistance profiles in isolates obtained in urine samples (n=54) showed an increase in amikacin resistance in 2018, when compared to the previous two years (p=0,02).
Bacterial agents isolated from cutaneous swab (n=13), evidenced an increase in the resistance to trimethoprim/sulfamethoxazole (p=0,04) during the three years of study. A higher percentage of isolates belonged to *Escherichia coli* (61%) and *Staphylococcus pseudintermedius* (27%) species and considering *E. coli* a significant difference in the resistance profile for tetracycline (p=0.002) was observed from 2016 to 2018. Also, regarding the same bacterium, it was observed a tendency (p=0,06) for an increase of the resistance profile in amikacin and chloramphenicol.
This study demonstrated an increase in the resistance profiles in isolates obtained from companion animals such as cats and dogs, especially in infections caused by *E. coli*. These data are in accordance to other national and international studies recently published.
Thus, an awareness of healthcare professionals and of the general population is crucial for the conscient and prudent prescription and use of antibiotics.

III9. School Science and Science Communication

P315. Science engagement through vídeos

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It is undeniable that science communication plays a crucial role in the establishment of relationships between science and society. Nonetheless, the rapid evolution of scientific knowledge and popular cultures, with a high digital engagement, demands the development of more and better digital strategies to engage society in scientific topics.

In order to address this challenge, at CNC we have been developing science communication videos focusing in different research topics: the video lines Selfie Science and Always Seeking Knowledge (ASK) Researchers. Selfie Science episodes aim to schematically explain scientific research projects to a non-academic publics. ASK Researchers series promotes the online interaction with society - everyone can submit their questions to the featured researcher to answer in an interview format. We launched the project in January 2019 and since then we explored several scientific topics. Particularly, in June we launched videos regarding biotechnology, focusing cell reprogramming and antibody purification, and in July, the videos featured the microbiology field, as they both discussed research on *Candida albicans* and mycobacteria.

We believe that this strategy promoted better dissemination of the microbiology and biotechnology fields in online platforms. We observed these videos reached around an average of 2000 people, and viewed an average of 2500 minutes total. With a Google Trends analysis in the Coimbra region, the terms “dendritic cell”, “antibody”, “microbiology” and “fungi” were more searched after the videos were published.
Overall, video seems to meet the digital demand for quick and visual information. Next steps to promote a better dissemination of the videos and consequently a bigger impact in scientific culture include the dissemination of these video lines in schools and the development of deeper impact evaluation studies.

Links for videos (Portuguese):

Biotechnology:
- Selfie Science (Luís Oliveira, Fibroblasts reprogramming into dendritic cells): https://www.facebook.com/CNC.UC/videos/1204038466442482/
- ASK Researchers (Ricardo Vieira-Pires, Quail Eggs to Produce Drugs?): https://www.facebook.com/CNC.UC/videos/606645699825146/

Microbiology:
- Selfie Science (Lisa Rodrigues, Candida albicans and A2A receptors): https://www.facebook.com/CNC.UC/videos/481061399322398/
- ASK Researchers (Susana Alarico, Mycobacteria: Naturally Resistant to Antibiotics?): https://www.facebook.com/CNC.UC/videos/2616869015206724/

FP316. Sequentially Combination of moderated High Pressure followed by Heat treatment for Egg White improved Pasteurization

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Egg is a highly perishable product, being often associated with salmonellosis. To avoid this problem, thermal pasteurization (TP) is usually applied, but this treatment causes changes in functional properties. High pressure (HP) is increasingly used commercially to pasteurize foods at 500-600 MPa, but applied for egg products causes protein denaturation. At lower pressures, HP causes sub-lethal damages on microorganisms, weakening them and with potential to decrease microorganisms thermal resistance, allowing a subsequent less intense thermal pasteurization.
The aim of this study was so to evaluate the performance of HP pre-treatments (50–200 MPa/5–20 min) followed by a less intense TP (55 °C/3 min) on egg white (EW) and compare it with the commercial TP procedure (55.6 °C/6.2 min) for inactivation of inoculated *Salmonella senftenberg* 775W, as well as the impact on functional and physicochemical properties of EW.
The results showed that, generally, the increment of pressure and holding time led to an increase of *S. senftenberg* 775W inactivation (0.13–2.39 log CFU/mL). When the combined treatment (HP+TP) was applied, pressures above 90 MPa caused higher inactivation occurred to below the quantification limit, thus enhancing the thermal inactivation effect. Instead, the commercial TP reduced *S. senftenberg* 775W below the detection limit, while TP alone only reduced about 4.00 log CFU/mL. Concerning the functional and physicochemical properties, parameters such pH, total soluble solids and water holding capacity, no changes were observed regardless the applied treatment. Nevertheless, instrumentally measured colour changes were observed, despite being undetectable by the naked eye ($\Delta E^*<5$). Turbidity and surface hydrophobicity increased slightly after processing, possibly due to some denaturation and formation of insoluble aggregates, which were accompanied by a decrease in soluble protein and total SH groups. Foaming capacity (FC) also decreased slightly after HP+HT processing, but foaming stability (FS) remained similar to that of the raw EW. Industrial TP caused a sharp reduction in FC, however, the FS was improved compared to raw EW.
These results hint the possibility to use sequentially combined treatments (HP followed by TP) to enhance inactivation of *S. senftenberg* 775W using less intense TP treatments and also to improve the functionality of EW.

I10. Industrial and Food Microbiology and Biotechnology

FP317. The growth medium: a critical parameter for lactic acid bacteria survival to freeze-drying

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The industrial use of lactic acid bacteria for the food industry requires preservation technologies that ensure the long-term maintenance of the viability and functionality of bacteria. Freeze-drying, commonly used for this purpose, can cause cytoplasmic membrane damages due to the freezing step and water removal (membrane deformation, inhomogeneous phospholipid phase transition, membrane permeabilization…) (For a review,see: Santivarangkna, Kulozik and Foerst, 2008). Fluidity and fatty acid composition of membranes, directly related to the growth medium composition, have been identified to influence bacteria survival to freezing (For a review, see: Carvalho et al., 2004). However, study of the effect of the growth medium composition on the bacterial survival to the freeze-drying process has been little studied. In this study, we investigated the effects of two commonly used growth media (MRS and M17) and their components (yeast extract, glucose and tween 80) on Lactococcus lactis NZ9000 survival to freeze-drying. Changes in L. lactis morphological and physiological characteristics were explored by determination of membrane fluidity and membrane fatty acid composition. A significant better survival to the process of L. lactis grown in MRS (96%) than grown in M17 (69%) was noticed ($p = 4.10\text{-}5$). To investigate which components of MRS media were responsible for the better L. lactis survival to the process, yeast extract, glucose and tween 80 were added to the M17 media. Growth in M17 supplemented with glucose (final concentration similar to MRS) increased significantly ($p = 0.03$) L. lactis survival to freeze-drying (91%). A clear relationship was observed between carbon limiting condition (M17) and bacteria low survival to freeze-drying. Moreover, fluorescence anisotropy values indicated an increase in L. lactis membrane rigidity after a culture in MRS or M17 supplemented with glucose compared to culture in M17. These increases in membrane rigidity can also explain the previous results. In conclusion, the survival of lactic acid bacteria to freeze-drying was closely related to the composition of the growth medium and especially to the carbon concentration. The growth medium can significantly change structure and dynamic properties of cytoplasmic membrane, altering the resistance of bacteria to technological stresses.

I10. Industrial and Food Microbiology and Biotechnology

FP318. Mannosylerythritol Lipids (MEL) bioprocesses development: Substrate selection and feeding regimes towards a high biosurfactant titres

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Mannosylerythritol Lipids (MELs) are glycolipid biosurfactants that exhibit excellent interfacial activity and biochemical properties. These microbial produced surface-active molecules have several advantages over synthetic surfactants, as lower toxicity, biodegradability and environmentally friendly production. Such characteristics makes them attractive for a wide range of potential industrial applications in bioremediation, biomedical, petrochemical and food processing. Their directed applicability is mainly dependent on an efficient production strategy, which is sustainable and economically attractive for industrial implementation. Previous strategies for MEL production by yeast strains, from lignocellulosic materials-based sugars alone attained high purity level while low titre (1,2). On the other hand, the use of high concentration of oil-based feedstocks as microbial substrates, resulted in high MEL titre, although with high unconsumed residual lipidic content (3). Therefore, there is a call for an industrial scalable bioprocess combining the use of cost-efficient and renewable raw materials, the ability of the *Moeziomyces* spp., which are unconventional yeasts, and specific carbon source feeding regimes to reach high microbial surfactant yields. Here are explored strategies for MEL production, by *Moeziomyces* spp., combining hydrophilic and hydrophobic carbon sources and alternative fed-batch regimes, to reach high MEL titres and purity. Namely, this study screens the combination of glycerol (a biodiesel by-product) or sugar-based substrates with vegetable or waste vegetable oils. Glycerol or sugar-based substrates are supplied initially to the yeast culture for cellular growth and combined with further addition of low amounts of oils in pulsed feeds, to foster MEL production. Waste vegetable oil pulsed feeds improved MEL titres around 1.5-fold compared to the sole vegetable oil regimes, under equimolar carbon utilization (4). Also the pulsed strategy allowed to achieve high purities, as the residual lipids present at the end of the fermentation are lower than the ones obtained on typical cultivations using vegetable oil as sole carbon source (3). A preliminary economic analysis of the process suggested is presented.

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I10. Industrial and Food Microbiology and Biotechnology

FP319. *Bacillus subtilis* endospores' germination control by hyperbaric storage and the effect of nutrients-availability

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Pasteurized low-acidic and high water activity products are more prone to endospore development, thus needing refrigeration (RF) or antimicrobials to hurdle endospore development. Hyperbaric storage (HS) is a new food preservation methodology stating storage pressure control, inasmuch temperature control, to hinder microbial development and several biochemical/chemical degradative reactions. As it can be performed at uncontrolled room temperature (RT), energy is only needed during the short compression/decompression phases of the pressure vessel, allowing considerable energetic savings, being a possible alternative to the conventional RF processes.
The feasibility of HS at uncontrolled RT to control *Bacillus subtilis* endospores' development was evaluated, along with the impact of the nutritional composition of the inoculation matrix on the endospores' behavior under HS. To do so, McIlvaine buffer (a nutrient-free matrix) carrot juice (highly perishable juice) and brain-heart infusion broth (BHI-broth, the optimal growth media for *B. subtilis*), all at pH 6.0, were inoculated with *B. subtilis* endospores and stored under HS conditions (25, 50 and 100 MPa), up to 60 days, at RT (18-23°C). Control samples were kept at atmospheric pressure (AP) under RF (4 °C) and RT.
The results revealed a nutrient-dependence in the inactivation rates of endospores while under HS. At 50 and 100 MPa, approximately 1.5 and 2.0 log units' reductions were observed in McIlvaine buffer after 60 days of storage, while samples kept at AP at RT and RF remained unchanged throughout storage. Regarding carrot juice and BHI-broth, at 50 and 100 MPa, the endospore loads were reduced below the quantification limit (2.0 logCFU/mL, initial load of 6.2-7.6 logCFU/mL) after 30 and 60 days of storage, respectively, with the inactivation rates being higher at 100 MPa, while for RF was observed endospore development after 60 days. At 25 MPa, endospore germination was accelerated when compared to samples kept at AP/RT, possibly due to a combined effect of pressure and nutrients to induce endospore germination.
These results hint HS at uncontrolled RT to preclude *B. subtilis* endospores' development in a highly perishable juice, opening the possibility of HS as an alternative to the conventional RF processes to control endospores growth in foods.

I10. Industrial and Food Microbiology and Biotechnology

P320. *L. monocytogenes* predictive growth modelling in a ready-to-eat food as a function of temperature

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Listeriosis is a severe foodborne disease, with low incidence and high fatality rate, peaking at 20% in risk groups - the highest for any foodborne pathogen. Unlike the majority of foodborne pathogens, *Listeria monocytogenes* is able to grow at refrigeration temperatures. Inconsistent temperatures during food production and distribution, as well as at the consumer's household, may gather the conditions for the pathogen to thrive, reaching unsafe concentrations.
Many ready-to-eat processed foods present an increased health risk for the consumer, because of the extended refrigerated shelf-life and not being subjected to a heat-treatment before consumption.
L. monocytogenes growth behavior in ready-to-eat (RTE) chicken salads as a function of temperature was studied to develop predictive growth models, taking into account the processing and storage conditions, and the foreseen shelf-life.
A 3-strain mix (serogroups IIa, IIb and IVb) of cold-adapted *L. monocytogenes* (4 log cfu/ g) was homogeneously inoculated into the RTE chicken salads that were stored at 4, 12 and 16°C, for 8 days. At appropriate time intervals, a series of decimal dilutions were prepared from each sample and plated onto ALOA agar, after which the plates were incubated at 37ºC for 48 h, under aerobic conditions, and *L. monocytogenes* were enumerated. The four-factor modified logistic growth model described by Baranyi & Roberts (1994) was fitted to the derived microbiological data for the estimation of the pathogen's growth kinetic parameters: lag time (λ), maximum growth rate (μmax), and maximum population density (Nmax). The predicted μmax varied from 0.021±0.008, to 0.052±0.024 and to 0.066
±0.009 log cfu/g/h at 4ºC, 12ºC, and 16ºC, respectively. The considered storage temperatures significantly influenced *L. monocytogenes* μmax ($p<0.05$). Finally, the square-root-type model proposed by Ratkowsky et al. (1982) was used to describe *L. monocytogenes* growth rate as a function of temperature.
The present study provides important initial information on predictive growth modeling for *L. monocytogenes* in RTE chicken salads, which can be used in subsequent quantitative microbial risk assessments.

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I10. Industrial and Food Microbiology and Biotechnology

P321. The use of genetic characterization to assess *L. monocytogenes* persistence in a delicatessen food producing industry

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L. monocytogenes in processed ready-to-eat foods is usually linked to post-processing contamination from the food-producing environment. Certain *L. monocytogenes* subtypes are known to persist in food contact surfaces for years. This work aimed to investigate the genetic relatedness of *L. monocytogenes* isolates collected in a delicatessen food-producing industry in Alentejo with a history of persistent contamination.
A total of 90 samples (19 raw materials, 11 intermediate products, 23 final products, and 37 equipment swabs samples) were collected during a 6-year period. *L. monocytogenes* detection was performed according to ISO11290-1. Presumptive isolates were confirmed and serogrouped by multiplex PCR (Kérouanton et al., 2010) and subjected to pulsed-field gel electrophoresis using the PulseNet procedure (Graves & Swaminathan, 2001) with *Apa*I and *Asc*I enzymes. A dendrogram was built in BioNumerics software (v.6.10) with a 1.5% optimization setting and band-position tolerance of 1.5%. Cluster analysis considered the unweighted pair group method with arithmetic averages and band- based Dice correlation coefficient. Pulsotypes were considered clones when they had at least 90% of similarity.
Using conventional microbiological methods, twenty presumptive isolates were obtained, while only 17 were confirmed as *L. monocytogenes* using PCR. Four different serogroups were found: IIa (35%), IIb (6%), IIc (53%) and IVb (6%). *L. monocytogenes* confirmed isolates presented 6 PFGE types and isolates with identical restriction patterns were recovered from raw materials, intermediate and final products, and equipment at different times. The presence of genetically related *L. monocytogenes* strains throughout the studied time-frame emphasizes the possibility of a common source. Results underline cross-contamination as a potential way of disseminating *L. monocytogenes* within the assessed food industry. The food safety system should be addressed, more specifically supplier selection and sanitizing methods, as it might favor the adaptation of some isolates to the existing food- processing environment conditions.
Strain persistence in food processing facilities contributes to the transfer of *L. monocytogenes* to foods, augmenting consumer´s exposure to the pathogen.

I10. Industrial and Food Microbiology and Biotechnology

P322. Cleaning validation in a food industry - Monitoring the efficacy of disinfecting agents for food equipment by ATP methodology

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Food environment plays a major role in the transmission of microorganisms and, therefore is a major issue concerning Food Safety. In the food industry, chemicals are routinely used to sanitize and disinfect product contact surfaces. Those chemicals provide a necessary and required step to ensure that the foods produced and consumed are as free as possible from microorganisms that can cause foodborne illness.
The overall study objective was to validate a new set of detergents/disinfectants, by assessing the efficiency of a cleaning/disinfection program of surfaces and equipment in the slicing operational unity of a meat processing plant using Adenosine triphosphate (ATP) methodology.
The study was conducted for 11 weeks comprising 256 samples, all collected in the slicing unit equipment. Three different chemical disinfectants were used: A- Detergent/disinfectant (foam, sprayed) Sodium hydroxide + amines; B- Disinfectant (foam, sprayed) Amines (N-(3-aminopropyl)-N- dodecylpropane-1,3-diamine); C- Disinfectant (liquid spray for operator's hands and conveyor side frames) Ethanol (ethyl alcohol) and 2-propanol (isopropyl alcohol).
Samples were collected by swabbing a 10X10 cm surface of the different equipment before production starting: 1st sampling points: product entry, slicing area, tongs, blade, conveyor belt, conveyor pulley; 2nd sampling point- conveyor bed; 3rd sampling point- conveyor side frames.
The ATP readings are displayed in a proprietary measurement scale referred to as zones of cleaning where readings of: 2.9 Zones = FAIL, indicating the surface is considered dirty and should be re- cleaned and re-tested (non-conformity).
An overall reduction of 5% on non-conformities occurred along the surveillance period compared to previous period (with former chemicals) and at the end of this study (week 11) none non-conformities were detected. When a non-conformity was detected extra cleaning and disinfection moments were immediately introduced; alerts implies only a disinfection procedure.
Thus, the efficiency of the cleaning/disinfection program of surfaces and equipment in the slicing operation unit, using the new detergents/disinfectants was validated, once the introduced strategies were effective on the reduction of organic matter residues in cleaned surfaces.

I10. Industrial and Food Microbiology and Biotechnology

P323. Unveiling the role of the elusive HgAATs in ester production by *Hanseniaspora guilliermondii*

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The wine yeast *Hanseniaspora guilliermondii* (Hg) has the ability to produce aromatic compounds which greatly contribute to the fruity and floral aroma of alcoholic beverages. Recently, through the reconstruction of the metabolic network of *Hg* UTAD222 we identified a set of genes predicted to be involved in aroma formation, representing the Hg "flavorome". Notably, within this cohort of proteins we could not identify homologues for known acetyl transferases (AATs), involved in formation of acetate esters, contrasting with the reported high production of these compounds. A deeper analysis of the Hg UTAD222 ORFeome led us to identify four proteins (HGUI_006997, HGUI_00952, HGUI_01907 and HGUI_01910) that harbor motifs conserved within the AATs enzyme family, these proteins only having orthologues in other *Hanseniaspora* species.
The present work intends to establish a relationship between ester formation with expression levels of these putative alcohol acetyl transferase coding genes.
This data will pave the way for a better elucidation of the putative role of these proteins in acetate ester formation, which in turn will accelerate research focused on its more rational utilization by the wine industry, and also by other bio-industries where they could be explored as cell factories for the production of biobased acetate esters.

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I10. Industrial and Food Microbiology and Biotechnology

P324. Exploring the aquaculture resistome

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The use of antibiotics in aquaculture has resulted in the emergence of reservoirs of antibiotic resistant bacteria in farmed fish and other animals, as well as in the aquatic environment. The aim of this study was to analyse the whole genome sequence (WGS) and resistome of a selection of isolates collected from different bacterial and animal species combination that reported non-susceptibility to at least one of the tested antibiotics, including 20 antimicrobial agents from 9 classes (β-lactams, aminoglycosides, folate pathway antagonists, fusidic acid, glycopeptides, mupirocin acid, phenicols, quinolones, and tetracyclines).
In this study, 15 Enterobacteriaceae isolates (3 *Citrobacter freundii*, 4 *Enterobacter* spp., 2 *Escherichia coli*, 1 *Klebsiella pneumonia*, 1 *Klebsiella* spp., 1 *Kluyvera cryocrescens*, 1 *Leclercia adecarboxylata*, 1 *Raoultella ornithinolytica* and 1 *Raoultella planticola*) were analysed, collected among samples of *Sparus aurata* (from an aquaculture tank and from a market), and one sample of *Mytilus galloprovincialis* (from a market).
WGS was performed on a MiSeq Illumina platform. INNUca was used for quality control of reads, de novo assembly and contigs quality assessment. Prokka and ABRicate were used for genome annotation and screening for antibiotic resistance and/or virulence factors-encoding genes, respectively. Freeware web-based resources (e.g., PathogenFinder, ResFinder, CARD, VirulenceFinder, PlasmidFinder, PHAST) was also used to estimate pathogenicity determinants, antibiotic resistance and/or virulence factors-encoding genes, plasmids, and prophage sequences.
Results obtained allowed the identification of not only antibiotic resistance genes, but also virulence factors, efflux pumps and phages. We highlight the presence of the *qnrB19* gene, which confers resistance to quinolones, in a *Leclercia adecarboxylata* strain (from a skin sample of *Sparus aurata*, from an aquaculture tank) and in an *Escherichia coli* (from an intestine sample, from *Sparus aurata* obtained in a market).
In conclusion, this work highlights the resistance mechanisms that are being promoted in aquaculture environments, thereby allowing antibiotic resistance to develop and spread via food fish and the environment, which will result in significant human health threats.

P325. Multielement analysis as a tool to trace authenticity of PGI Alcobaça apple cake fillings

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The designation of a food product as Protected Geographical Indication (PGI), legally framed by the European Union, is a guaranty of authenticity, traceability and sustainability. One of the many PGI products attributed to Portugal is Alcobaça apple. This fruit has its consumption in fresh, as well as in apple fillings, industrially processed, as constituents of fruit cake fillings.
Although fresh fruits are easy to identify, their industrially processed products are more difficult, requiring the identification of unique characteristics of the target products, which may serve as markers of authenticity.
It is possible that the fruits of a region possess characteristics associated to the environment, conferring them these required unique characteristics for their identification, even when processed.
In this study, the mineral profiles of Alcobaça PGI Apple var. Golden Delicious and Armamar, not included in the PGI (fresh fruit and fruit fillings), were identified to evaluate the applicability of multielement data on the determination of geographical origin and authenticity. Seventeen elements (B, Al, P, Cr, Mn, Fe, Cu, Zn, Rb, Cs, Ba, La, Ce, Tl, Pb, Sr, Mg) were analysed by ICP-MS and ICP-OES after microwave digestion. The results show that all PGI apples had lower caesium (Cs) and rubidium (Rb) concentrations than apples from Armamar 0.028 and 6.3 µg/g for Alcobaça PGI apples and 1.097 and 10.9 µg/g for Armamar apples, for Cs and Rb, respectively. The same trend was observed for the fruit fillings, 0.010 and 1.7 µg/g for PGI Alcobaça, and 0.030 and 2.8 µg/g for Armamar, for Cs and Rb, respectively. These differences can be explained by the schist soil characteristic of Armamar.
The present study shows that multielement analysis combined with the appropriate statistical tools can be a valuable contribution for the identification and authenticity of the geographical origin of apple fillings, even if industrially processed.

I10. Industrial and Food Microbiology and Biotechnology

P326. Cell-surface display engineering of industrial *Saccharomyces cerevisiae* for hemicellulosic-to-ethanol consolidated bioprocesses

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The utilization of lignocellulosic biomass to produce biofuels and commodity chemicals has appeared as a solution to alleviate the envisioned depletion of fossil resources. Nevertheless, the attainment of economically viable lignocellulosic-based processes requires an effective utilization of the hemicellulosic fraction, which may comprise up to 40% of the total biomass[1]. This represents a major bottleneck, mainly due to the requirement of chemical/enzymatic treatments for the hydrolysis of hemicellulose into fermentable sugars, and the fact that hemicellulose is mainly composed of xylose, a sugar that is not readily consumed by *Saccharomyces cerevisiae*–the most used organism in industrial biotechnology. In this context, consolidated bioprocessing, which combines saccharolytic and fermentative abilities in a single microorganism/consortium, appears as a solution to decrease environmental and economic costs in lignocellulosic biorefineries. Therefore, in this work, hemicellulolytic enzymes were displayed on the cell surface of robust industrial *S. cerevisiae* strains with advantageous traits (e.g. thermotolerance and inhibitor tolerance). These strains were also engineered for xylose consumption with both the isomerase and the oxidoreductase pathways, which was previously optimized for fermentation of inhibitor-containing hydrolysates[2]. The combination of these modifications allowed the direct production of 11.1 g/L of ethanol from non-detoxified hemicellulosic liquor obtained by hydrothermal pretreatment of corn cob, representing an ethanol yield of 0.327 g/g of potential xylose/glucose. To the extent of our knowledge, this is the highest ethanol concentration reported from direct conversion of a lignocellulosic-derived hemicellulose by *S. cerevisiae* without the addition of external hydrolytic catalysts. Additionally, the cell-surface display of hemicellulases presented a fermentative advantage in simultaneous saccharification and fermentation of corn cob hemicellulosic fraction, greatly decreasing the necessity for commercial enzymes. These results prove the value of industrial *S. cerevisiae* strains as hosts for the construction of whole-cell biocatalysts for hemicellulosic-based processes, without the need for expensive exogenous enzymes, chemical catalysts or laborious detoxification steps.

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I10. Industrial and Food Microbiology and Biotechnology

P327. Arabitol production from lignocellulosic biomass through GRE3-overexpressing industrial *Saccharomyces cerevisiae* strains

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Arabitol is a five carbon sugar alcohol that belongs to the pentitol family, the same of xylitol and ribitol, being one of the top 12 biomass-derivable building block chemicals. Due to its sweetness similar to glucose and low caloric content (0.2 kcal/g) it is used as an alternative sweetener in food industry [1].
The concerns about the depletion of fossil fuel reserves and the economic and environmental problems associated with their use have led to the search of renewable energy sources. Lignocellulosic biomass emerged as sustainable alternative for the production of value-added products, once lignocellulose is one the most abundantly renewable biomass available on earth. Thus, the development of a lignocellulose-based bioeconomy must compromise the valorisation of lignocellulosic biomass for the production of value-added products [2].
Currently, arabitol is industrially produced by chemical reduction of lactones [3]. However, bioconversion of sugars present in lignocellulosic biomass to arabitol could be a viable alternative to chemical production. An endogenous aldose reductase from *Saccharomyces cerevisiae*, with a broad substrate specificity, was previously reported to be able to convert xylose and arabinose to xylitol and arabitol, respectively [4,5].
In here, we demonstrate the feasibility of using an engineered yeast strain overexpressing an aldose reductase gene for the conversion of arabinose to arabitol. Due to the unspecificity of the enzyme, arabinose and xylose could be simultaneously converted to arabitol and xylitol, respectively, which can lead to the development of a multi-chemical yeast production platform, contributing to the establishment of a lignocellulose-based bioeconomy.

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I10. Industrial and Food Microbiology and Biotechnology

P328. Voltammetric monitoring of the mead fermentation process

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The present work involved the monitoring of fermentative processes for the production of mead by the HPLC (chromatographic method) and cyclic voltammetry (electrochemical method). The objective was to cross the analytical information between the two methodologies to verify if the application of cyclic voltammetry allows a fast and in-situ monitoring of the fermentative process. The fermentations were carried out on a solution of heather honey with Brix value of 26º (approximately, 900 g of heather honey and 1.26 g of nutrients ENOVIT diluted up to the volume of 2.4 L with mineral water; glucose and fructose initial levels of 92 g and 136 g, respectively). Yeast species used to perform the fermentation process was *Saccharomyces cerevisiae*. Samples were collected between 0 to 192 hours of fermentation and prepared for HPLC analysis and cyclic voltammetry. In the HPLC analysis the levels of glucose, fructose, acetic acid, glycerol and ethanol were quantified using an Aminex HPX-87H column (BIO-RAD) and a Varian ProStar 220 chromatography system with Varian RI-4 detector. The analysis was carried out using an eluent of 0.004M sulphuric acid with a flux of 0.6 mL/min. The electrochemical system consisted of a platinum working electrode, a platinum auxiliary electrode and an Ag/AgCl reference electrode. The cyclic voltammograms were obtained using a potential scan between -1.5 to +2.0 V and a scan rate of 0.25 V/s. HPLC results showed that in the fermentations performed, the glucose and fructose levels were reduced to concentrations below 10 g/L and the levels of acetic acid, glycerol and ethanol are close to 1 g/L, 8 g/L and 14%, respectively. Preliminary results of cyclic voltammetry are presented, which, combined with multivariate statistical techniques, show its potential in monitoring of the fermentation process.

P329. The L-arabinose isomerase from the food grade *Bacillus subtilis* for the production of tagatose: a natural sweetener

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The increasing concern about adverse health impacts from excessive sugar consumption is the main driving force for the replacement of simple sugar by natural sweeteners (1). Tagatose is a hexose monosaccharide rarely found in nature, namely in some fruits and dairy products. This rare sugar represents a promising sweetener due its low calorie content (1.5 – 2.5 kcal/g), sweetness profile similar to sucrose and prebiotic and anticariogenic properties (2-3).
Biotechnological production of tagatose by enzymatic isomerization arises as alternative to chemical processes. L-arabinose isomerase (L-AI, EC 5.3.1.4) catalyses the conversion of arabinose into ribulose as well as galactose into tagatose. Several L-AIs from different microorganisms have been proposed for bioproduction of tagatose, however the L-AI from *Bacillus subtilis* (BS-L-AI) was reported with unique substrate specificity for L-arabinose and therefore unable to produce tagatose (4).
In this work the *araA* gene encoding L-AI from the food-grade *Bacillus subtilis* (BS-L-AI) was cloned and overexpressed in *Escherichia coli*. The recombinant enzyme was purified and characterized. BS- LAI exhibited maximal activity at 42 °C and pH 7.5 and showed superior thermostability at 32 °C. The enzyme (7mg/mL) was able to convert galactose into tagatose with a maximum conversion yield of 55%. Interestingly, and in contrast with previous studies, our results demonstrated the BS-L-AI production capacity and its potential as biocatalysts for the natural sweetener production.

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I10. Industrial and Food Microbiology and Biotechnology

P330. Evaluation of good practices of handling in food establishments of Florianópolis – Brazil

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Foodborne diseases is a public health problem affecting thousands of people every year. Good Handling Practices (GHP) covers the minimum sanitary and hygiene practices for food processors. These standards must be used in order to ensure that food is safe and suitable for human consumption and to avoid that the microbial risks rises to a level that could be harmful to human health. There are determinant factors in all steps of food processing such as: buildings struture, water reservoirs sanitation, quality of ingredients, training of personnel, and diligence before, during and after food processing. The present study evaluated thirty four (34) food service establishments in Florianópolis (SC – Brazil), as restaurants, take aways, bars and coffee places, using the check-list adapted from RDC 216/2004. Establishments were classified as Excelent, Good, Regular and Poor according to their scores based on the conformity with GHP standards. The results shown that 82.3% (28) did not reach 60 points and were considered as poor quality, four (11,8%) of them as regular, only two establishments (11.9%) were classified as good and no one was rated as excellent. The highest score was reached by R8, totaling 85.9 points, and the lowest by R6, with 32.5 points. The conservation conditions of ingredients and ready-to-eat foods were the most critical evaluated parameter for the low scores. Most of the food services were in conformity with the criteria conserning staff higienic practices as wearing clean uniforms and tidiness. The results point to the need for reinforcement of GHPs training, awareness for the correct professional performance of food handlers and technical responsables and more rigorous inspection by the competent authorities.

I10. Industrial and Food Microbiology and Biotechnology

P331. Occurrence of pathogenic microorganisms after evisceration in two slaughterhouses

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The monitorization of the microbial contamination of carcasses surfaces throughout the slaughter line, enables the verification of the slaughter hygiene operations, as well as good production practices, which allows us to define new methodologies for quality control and food safety. The aim of this study was to achieve data about the occurrence of potentially pathogenic bacteria at different stages in two Portuguese slaughterhouses. Two hundred fifty-nine swab gauze carcasses surface samples (ISO non-destructive method) delimited with sterilized templates (100 cm^2) from 2 slaughterhouses were taken after evisceration, immediately before refrigeration, from the external surface of the thigh, neck, abdominal and lumbar dorsal areas and internal surface of the carcass in the pelvic, abdominal and thoracic areas. All samples were analysed according to ISO procedures for detection and enumeration of *Salmonella* spp., *Staphylococcus* aureus and *Listeria monocytogenes* and enumeration of Enterobacteriaceae. *Salmonella* spp., and *Staphylococcus* aureus were detected in 21 samples (8,1%) and 90 samples (34.7%), respectively. *Listeria monocytogenes* was not detected. A higher percentage of *Staphylococcus aureus* was detected on the abdominal area in the external (11 samples) and internal surfaces (13 samples). *Salmonella* spp. was mostly detected on the external surface, mainly on the neck (6 samples) and abdominal area (4 samples). On average, for Enterobacteriaceae, 0.96 and 0.63 log CFU/cm2 were enumerated in external and internal surfaces, respectively. Higher counts were observed on the abdominal area in the external (1.23 log CFU/cm2) and internal surfaces (0.87 log CFU/cm2). Higher levels of Enterobacteriaceae contamination and detection rates of *S. aureus* were obtained on the abdominal area in the external and internal surfaces. The contamination observed in this particular area may be due to the proximity of the organs removed by the evisceration process. The detection of *Salmonella* spp. on the neck may indicate that the washing process can contribute to microbial redistribution throughout the carcass. Non-effective singeing and carcass manipulation after evisceration are other factors that may contribute to carcass contamination. These results show the imperative requirement for continuous improvement of slaughter operations and good production practices in order to ensure food safety.

I10. Industrial and Food Microbiology and Biotechnology

P332. Detection and genetic diversity study of *Salmonella* spp. in a Portuguese slaughterhouse

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Salmonella is an Enterobacteriaceae and there are about 2300 serotypes identified. It's the second largest cause of food-borne diseases and it still causes outbreaks and even death. Infected animals and food are excluded from the market and represent losses for the distribution companies. Pork is one of the major transmission vehicles of this bacteria and it was the source of this study samples once the samples was collected in a Portuguese slaughterhouse.

Salmonella spp. detection was carried out according to ISO procedures and the cultures were stored at -20ºC at BHI and 45 % (V/V) of glycerol. Sixteen cultures were picked to BHI and incubated 24 hours at 37ºC from which we proceeded to DNA extraction. DNA from *S.* typhimurium and *S.* enteritidis, used as positive controls, was also obtained.

Firstly, the identification of *Salmonella* spp. using specific primers was done, followed by a specific identification of the serotype using a Multiplex-PCR that targeted specific genes of *S.* enteritidis and *S.* typhimurium, the serotypes most prevalent in pigs, and in clinical analysis of *Salmonella* food poisoning patients. After the identification, the genetic diversity of the samples was evaluated using ISSR markers.

Salmonella spp. was confirmed in the sixteen tested samples, nine of which were *S.* typhimurium and seven were *S.* enteritidis. Polymorphism analysis allowed the evaluation of the genetic diversity, in *Salmonella* spp., and the detection of the differences among the collected samples. With the ten primers we obtained 104 markers, 82 of which were polymorphic corresponding to a polymorphism rate of 78.84%. The dendrogram obtained revealed five different groups, two of them were formed exclusively by each serotype, and the respective positive control. The different groups observed were also related with the different pig's source. The results shows differences between the samples, which may indicate that there are different phenotypes and genotypes among the same serotype.

I10. Industrial and Food Microbiology and Biotechnology

P333. Optimization of fermentation conditions of *Rhodotorula glutinis* yeast to produce high- added value carotenoids

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Rhodotorula glutinis is a yeast capable to synthesize several valuable compounds with a wide industrial/commercial use, particularly, high-added value microbial carotenoids, such as, β- carotene, torulene and torularhodin. Frequently the production of microbial carotenoids using *R. glutinis* yeast cells only uses nitrogen from inorganic sources. Therefore, it is necessary to evaluate the impact of organic sources in their production. A two-step optimization strategy using a statistical experimental design was employed to enhance the production of microbial carotenoids from *R. glutinis* cells. A 22 central factorial design was used to optimize the impact of different c concentrations of carbon source and nitrogen from organic source. The results revealed that both factors have significant influence on biomass and carotenoids production. After optimization, the microbial carotenoids were then produced in a 5 L bioreactor, with pH variation. The production was increased approximately of 20%, 25% and 23% (m/v) for β-carotene, torularhodin and torulene, respectively. The proposed methodology proved to be quite adequate for the design and improvement of the bioprocess. The multifactorial statistical approach, that considers the interaction of independent variables, is a good approach for the select the most appropriate conditions of a further scale up of the process.

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I10. Industrial and Food Microbiology and Biotechnology

P334. The effect of the plant hormone ethylene on somatic embryogenesis of tamarillo (*Solanum betaceum* Cav.)

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Somatic embryogenesis (SE) is either a high-value biotechnological tool applied to multiple plant species for large-scale cloning and also an interesting model to study processes that occur during embryo development. This developmental pathway has been studied in our laboratory and applied to several plant species including *Solanum betaceum* Cav., commonly known as tamarillo. This species is a small solanaceous tree, which is cultivated mainly for its edible fruits due to their great nutritional profile. The conditions to achieve SE in tamarillo are well optimized, with several studies already published. Despite the knowledge already acquired regarding SE in this species, several molecular mechanisms are still poorly understood, in particular, the effect of ethylene on plant tissues during each SE stage. Taking into account the lack of information of the ethylene effect on SE and knowing its role in processes related to morphogenesis and stress responses, the effect of this hormone on SE induction and on somatic embryo development was evaluated. Thus, different tamarillo explants were culture in MS medium containing different ethylene modulators, 50 µM silver nitrate ($AgNO_3$), 10 µM aminoethoxyvinylglycine (AVG) and 20 µM ethephon (ETH).
The leaf explants exposed to $AgNO_3$ and AVG have produced mainly non-embryogenic callus (non-EC). Nevertheless, in presence of ETH, the formation of somatic embryos was observed in the induction medium after 10 weeks and an increase in induction of EC was also observed. At the development stage, the treatment with $AgNO_3$ and AVG has enhanced the number of somatic embryos developed from EC while the presence of ETH inhibited their formation.
Although the molecular mechanisms of ethylene action in this study remain unknown, taken together the results suggest that ethylene can be both stimulatory and inhibitory depending on each specific stage. In this regard, ethylene seems to play a specific role in each SE stage. This study is a first approach not only to understand the possible effect of this hormone on SE, but also a starting point for characterize other molecular aspects that had not been questioned before, contributing to the optimization of this process.

I10. Industrial and Food Microbiology and Biotechnology

P335. Inactivation of *Alicyclobacillus* spp. in fruit: the potential of medium pressure mercury lamps and light emitting diodes as a first-stage disinfection process in the fruit juice industry

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Alicyclobacillus spp. (ACB) are non-pathogenic, thermo-acidophilic, spore-forming bacteria that have become an increasing threat to the fruit juice industry. Several ACB isolates are able to produce phenolic compounds that lead to organoleptic modifications (odour/flavour) and alter the quality of these fruit-based products. Therefore, spoilage caused by ACB, can result in product recalls, thus affecting consumer confidence and causing significant economic losses for the industry. To overcome this issue, alternative disinfection processes need to be developed since conventional ones aren't effective against these resilient bacteria.
Ultraviolet (UV) radiation from mercury lamps has already been described as extremely effective in inactivating a wide range of microorganisms. Also, light-emitting diodes (LEDs) have shown to be a promising alternative to disinfection.
In this study, the taint-producing *A. acidoterrestris* strain DSM 3922T, in its vegetative and sporulated forms, was subject to UV radiation from a medium pressure mercury lamp and from LEDs emitting at 265 nm and at 255 nm. All UV inactivation experiments were performed on peach (*Prunus persica* L.) peels, which are representative of a more challenging fruit matrix since the outer surface is not smooth due to the presence of fuzz (hairiness).
Moreover, the internalization of ACB in the fruit was also evaluated after spiking the peach peel and submitting a peach to 4oC to simulate transport under refrigerated cargo conditions and another to meteorological conditions. Both conditions led to the internalization of about 0.1% of ACB. The inactivation treatments were therefore tested with ACB spiked below the peel and, as expected, the peel will scavenge the light and protect ACB from inactivation.
This work shows that medium pressure mercury lamps and LEDs emitting at 265 nm can be extremely effective processes to inactivate ACB when present in the fruit peel (99 % and 91% inactivation percentages, respectively). This inactivation was observed for both the vegetative and sporulated ACB forms. Higher inactivation percentages can easily be attained by increasing the number of lamps or LEDs, increasing the intensity of the emitted radiation, and/or decreasing the distance between the target microorganisms and the UV radiation emitting devices.

I10. Industrial and Food Microbiology and Biotechnology

P336. Discrimination of three bacteria species using a potentiometric electronic tongue

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The detection, monitoring and/or prevention of microorganism growing is of utmost relevance in several research fields, from food to environmental areas, being an important topic either from an academic or an industrial point of view. Conventional methods like plating techniques are the most widely used, being needed novel and faster screening methodologies like electronic noses, electronic tongues (E-tongues) and impedance based methods. In the present work, a potentiometric E-tongue (Fig. 1), comprising 40 lipid polymeric sensor membranes with cross-sensitivity, was used to identify and discriminate three bacteria (*Enterococcus faecalis* ATCC29212; *Staphylococcus aureus* ATCC653 and *Escherichia coli* ATCC29998) at two concentration levels (low and high). Brain Heart Infusion Broth medium was used for cultivating each of the three microorganisms, which were then individually inoculated into 1 L Erlenmeyer flasks (working volume of 600 mL) and incubated overnight (batch mode) at 35°C, on a rotary shaker (90 rpm). After incubation, the biomass was spectrophotometrically determined, being measured the optical density at 550 nm. The cultures were split in volumes of 50 mL. The cells of each sample were harvested (centrifugation at 9000 rpm for 10 min), after discarding the supernatant, washed with distilled water and re-centrifuged (9000 rpm for 10 min) and stored (20 ºC). The obtained biomass was dried overnight at 30°C, and stored at 20 ºC. Before E-tongue analysis, the cells were rehydrated with 20 ml of deionized water for 30 minutes at room temperature and aqueous sample solutions with different cells concentrations were obtained. Each E-tongue analysis took five minutes, enabling establishing a pseudo-equilibrium between the samples and the sensors' membranes, being the signals potentiometric profiles recorded. The classification performance of the E- tongue was assessed by applying a linear discriminant analysis (LDA) coupled with the meta-heuristic simulated annealing (SA) variable selection algorithm. The preliminary results showed that an E- tongue-LDA-SA predicting model could be established, based on the information gathered by a sub-set of 15 sensors, allowing to correctly classify 100% (Fig. 2) and 85% (original and leave-one-out cross- validation procedure, respectively) of the samples according to the microorganism and respective concentration level.

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P337. Activity of *Dekkera bruxellensis* and the effect on the aroma profile of monovarietal wines

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The problem of wine contamination by *Dekkera bruxellensis* is mainly due to the production of flavours associated with vinylphenols and especially ethylphenols. But this yeast can also produce several other metabolites such as acetic acid, isovaleric acid, 2-methylbutyric acid and isobutyric acid which can influence the quality of the wine and the perception of volatile phenols. Substrates other than hydroxycinnamic acids, such as amino acids, can be used to make a wide variety of other aroma- active compounds contributing to the complexity of the "Brett character". This work aimed at characterizing the metabolic profile of two *D. bruxellensis* strains in wines of two grape varieties (Touriga Nacional and Cabernet Sauvignon) focusing on families of aroma compounds such as esters, higher alcohols and volatile fatty acids. The two strains of *D. bruxellensis* involved showed different capacities to produce volatile phenols. Different volatile phenols profiles were obtained depending on the wine and on the yeast strain. The concentration of ethyl esters, including ethyl acetate, was higher in the inoculated wines of both grape varieties than in the control wines. On the contrary, isoamyl acetate was lower in most of the treated wines. The fatty acids isovaleric acid and caprylic acid increased in the inoculated wines, specially on Cabernet Sauvignon wines, and, as also happened for the esters, *D. bruxellensis* PYCC4801 produced higher values than the wine isolated strain 21. The concentration of the three monoterpenes analysed increased in the Cabernet Sauvignon wines but not in Touriga Nacional wines. The grape variety effect observed can be related with different compositions of glycosidically bound terpenes subjected to the ß-glycosidase activity of *D. bruxellensis*. Besides the production of volatile phenols, *D. bruxellensis* was able to modify the composition of wines through the production of metabolites of other chemical families.

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P338. Screening of bacteria able to produce eps using xylose as a carbon source

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Exopolysaccharides (EPS) are macromolecule compounds of monosaccharides. They can be used in the food industry to improve the texture, sensorial qualities, nutritional properties and the stability of fermented products and also can be widely used in the cosmetic and pharmaceutical industries as bioflocculants. This study aimed to compare EPS production of 22 bacterial strains using xylose as the sole carbon source for cost reduction of the production. The strains were reactivated in MRS medium and for EPS production, they were inoculated in SDM medium at 36° C, under stirring for 20 h. Samples were withdrawn twice, at T0 and after 20 h of fermentation, for EPS extraction and pH monitoring. Crude EPS was precipitated from the supernatant by adding 2 volumes of cold ethanol stored at 4° C for 24 h and then it was collected by centrifugation at 10,000 ×g for 20 min, being the pellet dried at 40° C. From EPS were quantified total sugar (by phenol sulfuric method at 495 nm) and dry weigh (g L-1). After 20 h of fermentation *Lactobacillus casei* (Ke 8), *Sporolactobacillus nakayamae* (Vini 6) and 2 strains of *Leuconostoc mesenteroides* (CH25 and B512) showed a decrease in the pH, probably due to the fact that these are lactic acid producing bacteria, while *Pediococcus pentosaceus* (CCC 3), *L. rhamnosus* (B103), and 2 strains of *L casei* (Ke 2 and Ke 11) showed higher pH values. The highest EPS yield was obtained with *Weissella paramesenteroides* (CH 24) which showed an increase of 120%, followed by *L. paracasei* (Ke 7) with an increase of about 60% and *Bacillus coagulans* (Ale 3) with an increase of 33.33%. The B103 and another strain of *Weissella paramesenteroides* (CC 28) maintained production, while the other 16 isolates presented a decrease in production. The highest amount of total sugar in EPS was obtained with *Weissella paramesenteroides* (strain CC 29) followed by B103 and *Pediococcus pentosaceus* (BSLM 9). For the calculations we adopted as a reference the initial value (T0). Thus, this study showed an important contribution regarding EPS production, exploring new carbon sources as xylose for scaling-up production.

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P339. Chemical and microbiological characterization of Portuguese “innovative” alheiras

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Alheira is a well-known Portuguese fermented sausage traditionally made with pork and/or poultry meats. Recently, other varieties of alheiras (codfish, mushrooms, tofu, soy and vegetables) have been appeared in the market to meet different preferences of consumers. Therefore, the main objective of this study was to characterize these new products, regarding their microbiological and chemical characteristics.
For fourteen different products, microbiological characterization included enumeration of several indicator organisms and detection of important pathogens as *Listeria monocytogenes*, *Salmonella* spp. and sulphite reducing *Clostridium* spores. Chemical characteristics determined were water activity, pH, nitrite, nitrate, biogenic amines and acid organic content.
Despite water activity and pH levels were insufficient to assure microbiological safety of the analysed alheiras, nitrites, nitrates, biogenic amines and organic acids content were found to be within accepted limits for this kind of products. Also foodborne pathogens like *L. monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and sulphite reducing *Clostridium* spores were not found in any sample.
At our knowledge, this is the first study reporting characterization of this Portuguese “innovative” alheiras. What is now important to understand is why, for the same producers, traditional are more contaminated than “innovative” alheiras, since they are produced in the same facilities and, eventually, under the same conditions.

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340. Autochthonous Microbiome of Azeitão Protected Designation of Origin cheeses

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Portuguese traditional cheeses, many of which with Protected Designation of Origin (PDO) status, are manufactured carrying out artisanal ways. The present study analyzed Portuguese traditional PDO ewes' cheeses from the Azeitão region, collected over a three years period (2016, 2017 and 2018), at five dairies. In the selected case study, and according to PDO status, no starter cultures are added, therefore the autochthonous microbiome of these cheeses is responsible for the fermentation processes required during cheese manufacture and maturation thus, for the organoleptic features of each product.

Aiming to characterize the microbiome present in each cheese sample, molecular microbiology, including a metagenomic approach (sequencing of the V1-V3 region of the 16S RNA), were used.

Although results obtained identified *Lactococcus* and *Leuconostoc* genera as the most abundant among cheese samples, despite the year of production or dairy; data gathered also showed significant variations on the microbial diversity between dairies and in the course of time.

Overall, this study showed the complexity of the autochthone microbiome of traditional PDO cheeses, highlighting the need for future investigations.

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P341. Active whey protein edible films and coatings incorporating lactic acid bacteria for fungi control in cheese

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Moulds are the main spoilage organisms in dairy products. Fungal contamination despite causing discolouration, off-flavours, alterations in texture and appearance, which leads to waste and thus economic losses, can also be responsible for health issues. Additionally, some of the spoilage fungi can produce toxic secondary compounds designated by mycotoxins. The use of lactic acid bacteria (LAB) in a perspective of biopreservation can be considered as an alternative for fungal control. The incorporation of LAB into edible films and coatings can be an effective strategy to improve the quality and shelf life of some food products.
In this work, the incorporation of *Lactobacillus buchneri* UTAD104 into whey protein-based films and coatings were tested for the control of *Penicillium nordicum* in a cheese matrix. The addition of cells to the films and coatings formulation resulted in thicker films with less luminosity and significantly different colour than control films (without cells). Nevertheless, cells inclusion did not alter moisture content, water vapour permeability, mechanical properties (tensile strength and elongation at break) and hydrophobicity of the films. FTIR and XRD data suggest that cells did not modify the films' chemical structure or crystallinity. Bioactive films were able to maintain approximately 1×105 CFU/mL during the first 30 days of storage at 25 ºC. When applied in cheese, films and coatings with *L. buchneri* UTAD104 prevented fungal contamination for at least 30 days. The mycotoxin ochratoxin A was not found in cheeses treated with films and coatings containing *L. buchneri* UTAD104. Results showed that the inclusion of LAB with antifungal properties in edible films and coatings could help to reduce or eliminate fungal contamination in cheeses.

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P342. Effect of high pressure on surfactin production by *Bacillus subtilis*: implications for its application by the oil industry

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Surfactin, a lipopeptide biosurfactant produced by *Bacillus subtilis* strains, exhibits extraordinary surface active properties, as well as stability at a wide range of temperatures and salinities, making it useful to replace the chemical surfactants in many industrial applications. The oil industry can take advantage of its application to increase the productivity of oil reservoirs, through a technology known as microbial enhanced oil recovery (MEOR). However, in order to make this technology advantageous from an economic point of view, the surfactin-producing strains must be able of growing and producing the biosurfactant inside the oil reservoirs. In this work, *B. subtilis* #573, isolated from an oil reservoir, was evaluated regarding its ability of producing surfactin under oxygen limited conditions at high pressure. A central composite design (CCD) was used to model the effect of pressure (3.8-46.2 bar) and temperature (35.3-46.7°C) on surfactin production. The results obtained demonstrated that pressure (in the range studied) did not exhibit a negative effect on surfactin production by this isolate, whereas temperatures higher than 45°C reduced its production. For most of the different combinations of pressure and temperature assayed, surfactin production was observed after 24 h, and the surface tension was reduced to values bellow 26.5 mN/m. At 41°C and 47 bar, *B. subtilis* #573 produced 31 ± 2 mg of surfactin per liter after 24 h, reducing the surface tension to 25.6 ±0.6 mN/m. These results were similar to those achieved at the same temperature at atmospheric pressure (26.0 ± 0.3 mN/m and 27 ± 3 mg surfactin/L). The surfactin produced in both cases exhibited a critical micelle concentration value around 15 mg/L, and the chemical characterization (through UHPLC-MS) demonstrated the production of similar percentages of the different surfactin isoforms (C12-, C13-, C14-, C15- and C16-surfactin) in both conditions. Finally, the applicability of *B. subtilis* #573 in MEOR was studied in sand-pack columns. In assays performed at 41°C and 47 bar, additional oil recoveries around 14% were obtained after 14 days in in situ assays. These results demonstrate the applicability of *B. subtilis* #573 in in situ oil recovery processes.

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P343. Development of a low-cost culture medium for biopolymer production by *Rhizobium viscosum* CECT 908 and its potential application in Microbial Enhanced Oil Recovery

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Polymers are a versatile class of compounds that play an essential role in our society, being their production estimated in more than 180 million tons per year. Nowadays, the world market is dominated by synthetic and plant-derived polymers. Biopolymers of microbial origin are characterized by their better environmental compatibility and biodegradability when compared with the synthetic ones, and their production is faster than those obtained from plants. Microbial biopolymers usually exhibit excellent rheological properties, stability at a wide range of temperatures, salinities and pH values, as well as a broad variety of chemical structures, which results in different physicochemical and rheological properties. However, despite their outstanding properties, their application is still limited by their high production costs. In this work, an alternative low-cost culture medium was developed for biopolymer production by *Rhizobium viscosum* CECT 908, containing sugarcane molasses (60 g/L) and corn steep liquor (1%, v/v) as carbon and nitrogen sources, respectively. Using this low-cost medium, higher biopolymer production and apparent viscosity values (5.2 g/L and 6700 mPa s, respectively) were obtained comparing with the synthetic medium (2.3 g/L and 1100 mPa s), which contained glucose and yeast extract. As a result, the cost of the culture medium necessary to produce 1 Kg of biopolymer was reduced more than 20 times. The biopolymer produced in the alternative low-cost medium exhibited better rheological properties as compared to xanthan gum, including higher viscosity at the same concentration. Furthermore, it was found to be stable at temperatures up to 80ºC, NaCl concentrations as high as 200 g/L, and high shear rates (300 s-1). Polymers are widely used by the oil industry to increase the oil reservoirs productivity during the tertiary oil recovery processes. In sand-pack column assays performed using a heavy crude oil ($\eta_{40ºC}$= 170 mPa s), this biopolymer produced using the low-cost medium demonstrated a better performance than xanthan gum, recovering almost 50% of the entrapped oil. Results herein obtained highlight that the *R. viscosum* biopolymer is a promising candidate for application in MEOR as an alternative to the conventional microbial and synthetic polymers currently used.

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P344. The use of the Collander equation as solute partition-predictive model

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Efficient and economical downstream processing of biological products has been one of the main challenges of biotechnology industry. Aqueous two-phase systems (ATPSs) are long known to be a promising separation technique and a valuable alternative to the conventional approaches.
Since their first application, ATPSs have been used for a wide range of applications, mainly in the separation and recovery of bioproducts. Their clear advantageous features and their potential as extraction technique has been demonstrated through the years.
Regardless of their potential, only recently the use of ATPSs by the industry sector has received relevant interest. Yet, for their successful use it is important to study systems' properties at molecular level and understand the mechanisms of solute partitioning.
The Collander equation was proposed to describe solute partition in water-organic solvent systems, but this model has been effectively extended to correlate partition of unrelated compounds in two (or more) different ATPSs, supporting the idea that this model can be used to predict partitioning in ATPSs.
So far, the use of the Collander equation to describe and predict the partition of solutes present in complex mixtures, in ATPSs, was never reported. Thus, we attempted to apply this empirical model to a real case scenario of ATPS partitioning, aiming the recovery of three natural pigments obtained by submerged fermentation of a Penicillium strain.

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P345. Fermentability of fructo-oligosaccharides produced by *Aspergillus ibericus* by human gut microflora

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Fructo-oligosaccharides (FOS) are a well-known class of prebiotics which selectively stimulate the growth of bifidobacteria in the gut. Although FOS occurs naturally in many fruits and vegetables, its content is low and are season-limited. As an alternative, we have identified a new isolated strain of *Aspergillus ibericus* as a good FOS producer. To increase FOS content in the mixture and decrease the amount of non-prebiotic sugars released during fermentation, FOS were produced using an integrated fermentation strategy. A co-culture of *A. ibericus* with a *Saccharomyces cerevisiae* YIL162 W was used, for simultaneous FOS production and purification by each strain, respectively. In the present work, the functionality of the FOS produced by *A. ibericus* as a prebiotic was assessed. FOS prebiotic potential was evaluated in anaerobic batch cultures for 24 h. Human faeces from 5 healthy volunteer individuals were used. With the faecal inoculum, several carbon sources were tested, namely a commercial FOS sample derived from inulin - Raftilose® P95 from Beneo-Orafti, Belgium and the FOS samples produced by the aforementioned *A. ibericus*. The dynamic bacterial populations changes were assessed by PCR-real time, as well as the production of short chain fatty acids (SCFA) and lactate – quantified through analytical methods (HPLC). Both carbon sources were compared for their prebiotic potential. A bifidogenic effect was observed for both microbial and commercial FOS. The growth of lactobacilli probiotic strains was similar for both FOS substrates. Thus, the microbial FOS triggered a beneficial effect on gut microbiota composition. SCFA – including succinate, acetate, propionate and valerate - were produced by the five faecal inoculum tested, at high concentrations using both substrates. Lower amount of formate and butyrate were also produced. Despite similar trends between both FOS substrates, a tendency for an earlier increase on SCFA concentrations in the culture was found for the microbial FOS, potentially indicating a faster metabolization rate. Nonetheless, microbial FOS seems to have similar prebiotic potential when compared to commercial FOS samples, potentially indicating a feasible route for bio-based FOS production. In conclusion, microbial FOS exhibited promising potential as nutraceutical ingredients for gut microbiota modulation with likely prebiotic features.

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P346. Valorization of fish by-products: physico-chemical properties of skin gelatins

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In the fish processing industry an important fraction of the enormous amount of wastes produced are fish skins, part of which may be valorized through the extraction of gelatins. This research work exploited the extraction and characterization of gelatins from the skin of two seawater fish species, namely codfish and salmon. Characterization was performed by rheology, in which phase angle and, elastic and viscous moduli (G' and G'') were determined throughout temperature ramps of 4-40°C and 40-4°C, which allowed to assess melting and gelling temperatures, respectively. Texture analysis, in which Bloom, rupture strength, brittleness and adhesiveness were measured, allowed comparison of the physical properties of the gelatins. Rheology results showed that, in codfish gelatins, melting temperature decreased from 18.5 to 14.5°C when extraction temperature increased from 20 to 50°C and gelling temperatures were around 5°C for codfish gelatins. Elastic and viscous moduli varied with extraction temperature; higher for codfish gelatin extracted at milder temperatures. Lower gelling temperatures may be due to lower content of proline/hydroxyproline in fish gelatin. Melting temperatures for salmon gelatins were similar between all tested gelatins (17-18°C), while gelling temperatures varied between 8.4-4.7°C. Regarding G' and G'', behaviors varied between temperature ramps. Texture analysis, assessing gelatins physical properties, concerning Bloom and rupture strength, showed that, in general, salmon gelatins presented higher values than codfish counterparts. Overall, results revealed that the species from which the gelatin was extracted, as well as the extraction process used, were key parameters in order to obtain a final product with specific properties. Such achievements are important to the food industry, by paving the way to the introduction in the market of gelatins with distinct rheological and textural properties, which enables them to enlarge their range of applications.

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P347. Application of new starters with oenological potential in immobilization systems

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Cellular immobilization is a technology that is already used in several fermentative processes. In winemaking, microbial immobilization systems can be used in both alcoholic and malolactic fermentation. Currently, the inoculation of must with starters of active dry yeasts to promote alcoholic fermentation is a common practice in wineries. The use of immobilized yeasts in alcoholic fermentation has several economic and technological advantages compared to the inoculation of free yeasts. In the immobilization of yeast for wine production, a very important criterion to consider is the selection of the immobilization support.
The aim of this work was the production of new solutions of immobilized yeasts with oenological potential.
A set of four lyophilized *Saccharomyces cerevisiae* yeasts strains and two commercial active dry yeasts were immobilized in calcium alginate and in two different porous supports with natural origin: volcanic tuff and Luffa. The immobilized systems were tested in microvinification assays with synthetic and white grape must. The alcoholic fermentations were monitored during 7 days by consumption of sugars and ethanol production and the efficiency of the immobilization systems tested was compared with the performance of the free yeasts. The results showed that immobilized yeasts converted completely the sugars with glucose consumption and ethanol production rates similar to the free yeasts. After this period the stability of the matrixes and the presence of yeast cells on the immobilized system were confirmed by Scanning Electron Microscopy (SEM). Dynamic of microbial communities in microvinification assays performed with white grape must were monitored by high-throughput sequencing (NGS), displaying consistent results for immobilized systems tested when compared with free starters and a different profile of the spontaneous fermentations.
These results proved that tested supports with immobilized yeasts revealed high efficiency in alcoholic fermentation, displaying the potential use of these matrices systems in oenological context, simplifying the clarification process by eliminating some steps and reducing production costs.

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P348. Cheese ripening chambers and cheese browning contamination

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In this work a study was carried out on the environment of the three ripening chambers (C1, C2, C3) of a cheesemaking factory in the Castelo Branco region to study the role of environmental contamination in the appearance of color defects in cheese surface. This factory process raw milk of sheep and goat and produces soft or semi-hard cheeses with about 45 days of ripening. Along this period, cheeses are located in a first ripening chamber, C1 (first fifteen days, 95-97% RH and 8-90C), followed by the second ripening chamber, C2 (the remaining days to complete 45 days, 87-90% RH and 12-14.50C) and the conservation chamber, C3 (conservation before sale, 91% RH and 140C). To know the role of environmental contamination of cheese ripening chambers in the appearance of color defects, particularly brownish color defects, Petri dish with adequate culture media were open in four different sites of each chamber, for a period of four hours. Nutrient Agar (AN) for enumeration and isolation of psychrotrophic microorganisms, Potato Dextrose Agar (PDA) for enumeration and isolation of molds and yeasts, and Pseudomonas Base Agar (PAB) for enumeration and isolation of *Pseudomonas* spp. were used, with the conditions of incubation of 10 days at 6.5°C for psychrotrophs, 48 hours at 25°C for *Pseudomonas* spp. and 5 to 7 days at 25°C for molds and yeasts. There was a higher microbial load in chamber C3 than in C1 and C2, with an uncountable number of colonies for molds and psychrotrophic microorganisms. Strains of *Kytococcus sedentarius*, *Pseudomonas putida* and *Pseudomonas fluorescens* were isolated and tested positively for pigment production using an adequate culture media that simulate the cheese surface, named Cheese Agar (with and without 1% tyrosine). Contamination of ripening chambers is thus an important source of contamination to take into account in the appearance of color defects on the cheese surface.

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P349. In vitro cloning of *Actinidia deliciosa* through axillary shoot proliferation and organogenesis

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In vitro cloning (micropropagation) can be achieved through different approaches such as axillary shoot proliferation, organogenesis and somatic embryogenesis. In this work the first two approaches were applied to clone selected Portuguese plants of kiwi - *Actinidia deliciosa* (A.Chev.) C.
F. Liang & A. R. Ferguson - of the female cultivars Hayward and Tsechelidis, and the male cultivar Tomuri. Seedlings from controlled pollinations were also used as explants to obtain different lines that will be tested in the field to determine their production potential. In the case of seedlings, shoot apices were cultured on MS medium containing different concentrations of the benzyladenin (BA, 0–5.0 mg/l). To propagate adult plants, cuttings (20–30 cm) from field-growing vines were washed with sterilized water, treated with a fungicide (Mancozebe, Sapec, 1g/l) during 10 min. and then washed again. Following this treatment the cuttings were sprayed with another fungicide (Aliete, Bayer, 1g/l) and placed in jars containing water, in a greenhouse, at 25 ºC, under a photoperiod of 16h light/8h dark. When sprouts reached a length of 3 cm, nodal segments were cut and placed on MS medium containing different concentrations of BA (0–5.0 mg/l). Nodal segments and apices from established shoots (2-3 cm) were subcultured in the same medium for multiplication. Organogenesis was induced on leaves of proliferating shoots on media containing different combinations of BA (0,2–2.0 mg/l) and NAA (1-naphthaleneaxcetic acid, 0,1 or 0,5 mg/l). Rooting was induced in vitro or ex vitro. In vitro, shoots were treated with the auxin IBA (indole-3-butyric acid) for 2-4 weeks and transferred to a medium without auxin. Ex vitro rooting was achieved following a 1 min. dipping in an IBA solution (1g/l IBA) and transfer to a substrate (peat/perlite). When root induction was induced in vitro, plantlets were transferred to the same substrate when root length was circa 1 cm and shoot length between 2–3 cm. Using this procedure, several genotypes of the cultivar Hayward and one genotype of the cultivar Tsechelidis were micropropagated.

Ação 1.1 Grupos Operacionais: I9K – Promoted by PDR2020 and co-funded by FEADER, Portugal2020

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P350. Tools to modulate heterologous gene expression: RNA stability determinants

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Prokaryotic mRNAs have short half-lives what is consistent with the need for rapid adaptation of the pattern of protein synthesis to environmental changes. RNA half-lives are frequently perceived as depending on too many variables, however, transcript stability is generally missed as a checkpoint amenable to manipulation in synthetic designs. Previous studies showed that the 5' untranslated region (5'UTR) can function as a good mRNA stabilizer and we inspected the contribution of 5'UTR composition on transcript stability and consequently protein production levels.
For that, we constructed combinatorial libraries of genetic sequence elements, like promoters and 5' UTRs, that were merged to a reporter gene (superfolderGFP). Our approach was done in *E. coli* MG1655, a well-studied model organism. Reporter output (fluorescence microscopy, spectrofluorimetry and flow cytometry) and Northern blot-based measurements of absolute mRNA half-lives, revealed that such UTRs were found to keep intact their ability to modulate transcript stability when excised from their natural context and placed as the upstream region of the reporter gene. By keeping transcription fixed and combining different UTRs with a constant ribosomal binding site we showed that mRNA decay can be made the limiting constituent of the overall gene expression flow. The data indicated that manipulating mRNA stability had little effect on expression noise in the corresponding population. Moreover, increased heterologous expression brought about by mRNA stability did not make cells more vulnerable to resource-consuming stresses.
Ultimately, this work resulted in a collection of well-characterized mRNA-stabilizing sequences that can be composed along with other expression signals in any construct following the assembly rules of the Standard European Vector Architecture (SEVA) format.

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P351. Table salt as vehicle for *Campylobacter* spp. cross-contamination

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Campylobacter is the major cause of human gastroenteritis (campylobacteriosis) caused by bacteria. Campylobacteriosis is frequently associated with consumption of raw or undercooked poultry meat or via cross-contamination to ready to-eat food during meal preparation. The aim of this study was to investigate the potential for cross-contamination of lettuce via table salt during handling raw poultry. The survival of *Campylobacter* spp. on artificially inoculated table salt was also investigated.
A cocktail of eight strains of *Campylobacter jejuni* and *Campylobacter coli* was used to inoculate chicken skin, to contamination levels ranging from 101 to 105 colony forming units (CFU)/g. Transfer experiments (n=3) were performed by handling the contaminated skin and then stirring 5 g of table salt placed in a sterile petri dish. Salt was homogenised with a sterile spatula and 150 mg were then transferred to lettuce. Campylobacter spp. survival in table salt was evaluated at determined time intervals during 180 min after inoculation of 5 g of table salt with the *Campylobacter* cocktail at final concentration of 105 CFU/g salt. Enumeration of *Campylobacter* spp. was performed following the ISO 10272-2:2017.
Campylobacter was detected in lettuce samples when the chicken skin was contaminated with levels above 102 CFU/g. A reduction of 1.7 log10 CFU/g of *Campylobacter* was obtained after 30 min of exposure to salt. After 60 min a reduction of 2.4 was observed and kept up to 90 min. After 180 min the levels were below the detection limit. The detection of *Campylobacter* in lettuce coming from a low levels of chicken contamination through the table salt and survival over a period that allows meal preparation, including handling raw chicken and preparing salad for a meal, indicates a high risk for *Campylobacter* foodborne illness. Therefore, cross-contamination via table salt could potentially be a kitchen route for exposure of humans to *Campylobacter*.

I10. Industrial and Food Microbiology and Biotechnology

P352. Cloning and expression of several Interleukin and cytokine genes in *E. coli*

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Cytokines are a large category of small peptides, such as interleukins, that are involved in several signalization and communication processes of immune response. They play various functions from inflammation response to cancer development and sepsis. Over-expression or problems in production control of this small peptides can lead to the development of various diseases, including autoimmune diseases, such as rheumatoid arthritis. Inhibition of some of these proteins can result in a therapy that can ease certain symptoms of cases of immunologic diseases. In order to search for solutions that ease the symptoms of these diseases it is important to study various cytokines and try to develop mechanisms that attenuate their function when expressed in high levels. Taking this into account, this work consists in cloning and expressing seven truncated proteins of interleukins IL-1α, IL-1β, IL-17A, MIF, TNFSF11, CD20 and TNF-α. The synthetic genes, that encode for the proteins in study, were inserted in the cloning vector pNZY29 were cloned using the strain INVαF' of *E. coli* as a host cell. These genes were isolated by amplification by PCR method and purified. Then inserted in the expression vector pLATE31. This one was cloned in the strains INVαF' or TOP10F' of *E. coli* and isolated and purified. These purified vectors were used to transform the following *E. coli* expression strains BL21 (DE3), BL21-Gold (DE3), BL21-CondonPlus RIPL (DE3), BL21- Gold (DE3) pLysS, BL21Star (DE3), BL21 SHuffle, BL21 SHuffle LysY and BL21 XJB (DE3) and expressed and detected by SDS-PAGE. The polyacrylamide gel allowed the detection of the strains with high levels of over- expression of the recombinant proteins in study.

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I10. Industrial and Food Microbiology and Biotechnology

P353. Valorisation of marine peptones as alternative growth substrates for lactic acid bacteria

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The increase in discards and by-products resulting from unsustainable fishing practices is a pressing issue, mainly due to the waste of resources it presents. The implementation of measures to valorise these resources, addressed through the development of novel methods for their conversion into value-added products, has thus become a necessity. Fish peptones obtained via hydrolysis of these materials possess well-documented potential, including their use in microbial growth media. They acquire significant importance as alternative growth sources, since growth substrates account for the majority of production costs of microbial cells and fermentation bioproducts. In this work, peptones obtained from ten species of fish discards (divided in three fractions: skin and bones, heads, whole fish) were tested against MRS media in the growth of *Lactobacillus rhamnosus* R11 and *Lactobacillus acidophilus* Ki to ascertain their efficacy as sources of carbon and nitrogen, among other parameters. Growth curves were performed in triplicate, with two aliquots collected at predetermined times. One was used for microbial enumeration, with viable cell numbers determined by the plate count technique (log (CFU)/mL). The other allowed for assessment of metabolic activity, with pH measured before centrifugation and the supernatant used in the quantification of protein (Lowry method) reducing sugars (DNS method) and glucose and organic acids (HPLC). Media containing marine peptones were observed to be more effective than controls for most of the parameters studied. In particular, they showed greater efficacy as sources of carbon and in promotion of microbial growth (supported by 0.2 units lower pH, higher optical density and CFU – one log cycle higher, in some cases) when compared to commercially available MRS medium. It can therefore be concluded that these peptones have potential as alternatives to commercial growth media for lactic acid bacteria.

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I10. Industrial and Food Microbiology and Biotechnology

P354. Neuroprotective activity of aqueous extracts of *Calamintha nepeta*, *Mentha* spp. from Alentejo: in vitro acetyl and butyrylcholinesterase inhibition studies

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Alzheimer´s disease is characterised for the loss of cholinergic neurons with progressive decrease of acetylcholine with loss of cognitive function. The increase of oxidative damage with aging process is often related with the development of neurodegenerative diseases. Commercialized drugs for Alzheimer treatment have a lot of side effects being important the search of new drugs with greater specificity, more efficient and with less toxicity.

Mediterranean diet promotes the use of many aromatic plants as condiments, very rich in antioxidants, that may be an important factor in promoting active aging. Their extracts are rich in polyphenols that have important antioxidant potential due to their ability to capture free radicals or to break down peroxides. Some extracts from Lamiaceae family are rich in phenolic compounds with ability against therapeutic multitargets. This allows the inhibition of cholinesterase activities and promote the decrease of oxidative stress and consequently can be promising prevention and therapy of neurodegenerative disorders.

For this study, three autochthonous flavouring herbs, from Lamiaceae family, *Calamintha nepeta* subsp. *nepeta*, *Mentha spicata* and *Mentha pulegium* were selected. Decoction water extracts were prepared by hydrodestilation of fresh aerial part of plants in order to evaluate their chemical composition and antioxidant and neuroprotective properties. The chemical composition was carried out by quantification of total phenols, flavonoids and tannins. Antioxidant ability was evaluated by inhibition of catalase and glutathione peroxidase activities and neuroprotective activity was acessed by inhibition of acetyl and butyryl-cholinesterase activities. Aqueous extracts of selected flavouring herbs showed high content of phenols, flavonoids and tannins and high ability to inhibit catalase activity (460 <IC50< 900 mg/L) and glutathione peroxidase activity (73<IC50< 245 mg/L). Moreover, extracts presented high ability to inhibit acetylcholinesterase (1200<IC50< 3400 mg/L) and butyrylcholinesterase activity (1240 <IC50< 2600 mg/L).

Results point out the potential use of these extracts as nutraceutical or pharmaceutical preparations in the prevention or treatment of oxidative stress and neurodegenerative pathologies.

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I10. Industrial and Food Microbiology and Biotechnology

P355. Lamiaceae essential oils as a food preservative to control the growth of mycotoxin- producing fungi

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Fungi belonging to the genera *Aspergillus*, *Fusarium* and *Penicillium* are frequently mycotoxin producers. Mycotoxins are toxic to humans and animals, even ingested in small quantities, with carcinogenic, mutagenic, cytotoxic or neurotoxic properties. Essential oils (EOs) from aromatic and medicinal plants have important antimicrobial properties and nowadays are an alternative to synthetic fungicides, with multiple benefits including low toxicity and antioxidant properties in foods, preserving their phenol constituents.
The aim of this study was to evaluate the antifungal potential of essential oils against some mycotoxin-producing fungal strains. For this propose, wild growth *Lavandula luisieri*, *Lavandula pedunculata*, *Lavandula viridis*, *Calamintha nepeta*, *Mentha pulegium*, *Mentha spicata*, *Origanum virens* and *Thymus mastichina*, often used as flavors in food, was selected. Essential oils were extracted from aerial parts of plants by hydrodistillation, and chemical composition of EOs was evaluated by GC-FID. Antifungal activity was assessed by solid diffusion disk assays and minimal inhibitory concentration. Antioxidant properties of most effective EOs were studied by DPPH radical scavenging method, β-carotene linoleic acid system and inhibition of lipoxygenase activity.
EOs were very rich in oxygenated monoterpenes but O. vulgare EO showed similar content in oxygenated monoterpenes and monoterpene hydrocarbons. Essential oils showed a large spectrum of antifungal activity. Fungal strains from genera Aspergillus showed high sensitivity to *L. luisieri*, *L. viridis*, *M. spicata* and *O. virens* EOs. Moreover, EOs of *L. luisieri*, *L. viridis*, *M. spicata* and *O. virens* showed total growth inhibition of *F. oxysporum* strains. *L. luisieri*, *M. spicata* and *O. virens* EOs were also very active against *Penicillium* strains, with total inhibition of growth. The most effective EOs showed also antioxidant scavenging potential, high ability to protect the lipid substrate oxidation and capacity to inhibit lipoxygenase activity, preventing also the food oxidation.
Results suggest the potential use of these flavouring herbs essential oils as food additives, preserving food and preventing their contamination by mycotoxin-producing fungi.

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I10. Industrial and Food Microbiology and Biotechnology

P356. UV pulse light application for *Listeria monocytogenes* control in a traditional dry- fermented smoked meat sausage

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Salpicão is a ready-to-eat traditional dry-fermented smoked meat sausages produced in a northern region of Portugal. Consumers search for food convenience and traditional products like salpicão accomplish this requirement. This is reflected in the growing of packaged sliced salpicão trade with the consumers being at a greater risk for listeria infection due to potential contamination during sausage slicing without posterior listericidal treatment. The objective of this work was to study the effect of pulsed light (including UV, representing 10-15% of total spectra, UVPL) treatment on salpicão to control *Listeria* spp. Salpicão was purchased in a local producer and maintained in refrigeration. Sausages were sliced with 3-4mm thickness and cut in squares 4x4cm; two groups of samples were prepared: inoculated with Listeria and not inoculated. Slices were inoculated with approximately 7log cfu/g of a culture of *Listeria monocytogenes* strains. All samples were vacuum packaged before being submitted to UVPL using an apparatus PLTecum Unit (Claranor, France). Number of pulses (9 pulses of 300 µs/each) and frequency (1Hz) were considered fixed effects. A Central composite design was applied regarding the factors voltage (1828 to 3000V) and distance to the UV lamp (2.6 to 5 cm). The fluence was measured by a laser power detector (JoulmeterUP17P connected to MAESTRO-monitor, Gentec-EO, Canada), and ranged between 4.57 and 17.08J/cm2. Colour was performed using CIE- L*a*b* coordinates system (Minolta colorimeterCR-300). Samples temperature was measured before and after the UVPL with an IR-Thermometer (Testo, Spain). *Listeria monocytogenes*, lactic acid bacteria (LAB), negative coagulase staphylococci (CNS) and Enterobacteriacea counts were carried out according to ISO Standards. Statistical treatment of data was performed. Before UV treatment, salpicão samples presented 8.41log cfu/g, 0.89log cfu/g and 0.33log cfu/g for total LAB, CNS and Enterobacteriacea respectively. Samples inoculated with *L. monocytogenes* presented counts of 6.95 ± 0.08log cfu/g before UVPL. For a fluence of 15.87J/cm2, the maximum of 1.48log cfu/g reduction was obtained for L. monocytogenes. The UVPL treatment did not influence the sample colour. LAB, CNS and Enterobacteriacea counts were not significantly affected by UVPL treatment. UVPL treatment may be used for decontaminating salpicão slices and reduce the viability of *L. monocytogenes*.

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P357. Assays of *in vitro* pollen germination of *Actinidia deliciosa* (A.Chev.) C. F. Liang & A. R. Ferguson

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Actinidia deliciosa (kiwi), is a woody wine largely cultivated in Portugal due to its edible fruits, the kiwifruit or Chinese gooseberry. Being a dioecious species, pollination occurs naturally when male plants are mixed among the female ones or when artificial pollination is carried out. In this last situation, farmers buy the pollen or use pollen themselves collect. Pollen quality must be evaluated and effective methods of pollination and conservation developed. A simple way to assess pollen effectiveness is to perform in vitro germination. In this work, the ability of pollen from different origins to germinate was tested and different ways to conserve it analysed. Thus, pollen from three companies, here named (A, B and C) as well as pollen from Portuguese orchards was placed in a minimal medium containing 15 mg/l $CaCl_2$, 10 mg/l KNO_3, 5 mg/l H_3BO_3 and different sucrose concentrations (0 – 24%). In the field, artificial pollination can be achieved through mechanical pollen dispersion or pulverization of a pollen- containing solution. In the first case, pollen is usually mixed with other substances to assure a correct flow, such as *Lycopodium* sp. spores. When a liquid medium is used, different compounds can be added to promote pollen germination such as sucrose and/or hormones. In our assays, mixtures of pollen with *Lycopodium* spores, microcellulose, pectin, and carrageenan, at different proportions were tested. When pollen was tested in a solution, different growth regulators, such as gibberellic acid (GA3), auxins and ascorbic acid were tested. The results showed that pollen from different origins germinated at rates higher than 90%. Only pollen from two orchards (Cantanhede and Amares) displayed lower germination percentages (around 60%). Pollen from the Tomuri cultivar gave better results than Chieftain. The results also showed that 6% sucrose and GA3 favor pollen germination. In vitro assays, in which kiwi pollen was mixed with *Lycopodium* spores, have not shown an increase on pollen germination. The results also showed that pollen could be kept at -20 ºC without loss of its germination potential.

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I10. Industrial and Food Microbiology and Biotechnology

P358. Microbial Shelf-life extension of high perishable liquid foods by hyperbaric storage at room temperature

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Highly perishable foods, i.e., foods with high water activity/low acidity, need an efficient preservation methodology to increase its shelf-life, usually refrigeration (RF), but RF is considered the third major source of CO_2, representing approximately 1% of the CO_2 emissions worldwide. Thus, environmentally friendlier food preservation methodologies are of upmost importance without compromising food safety and quality. Hyperbaric storage (HS) is a new concept of food storage, being the pressure applied during all the storage time, between 25-220 MPa, from a few days to some months. Recent results hint HS as the possibility to store foods at room temperature (RT) with reduced energetic costs at levels of 0.001 €/kg to HS instead of 0.026 €/kg to RF. In fact, energy is only necessary during compression/decompression phases, and not to keep it along storage.

So, in this work HS/RT feasibility was studied to evaluate the possibility of shelf-life extension of two highly perishable liquid foods (raw watermelon juice and high pressure pasteurized milk). For that, watermelon juice was maintained under HS at 75MPa/RT, up to 1 year. In addition, as a more complex food, milk was also studied, regarding microbial and physicochemical parameters, up to 40 days so far. For juice, the results of 75/RT showed lower microbial loads than the initial ones (~2.0 log CFU/mL) at 21st day, while the juice kept under RF was already spoiled (above 6.0 log CFU/mL) after 7 days. When the storage time was extended to 1 year, the results revealed that HS caused an additional reduction of total mesophiles/psychrophiles by at least more ~1.0 log CFU/mL (to below the detection limit), while Enterobacteriaceae/yeasts and moulds were kept below the detection limit (1 log CFU/mL). A similar behaviour was verified for milk, where the initial microbial loads were reduced under HS (75 and 100MPa) by ~2.0 log CFU/mL (to below the detection limit) for the same microorganisms. In addition, globally, HS/RT resulted in physicochemical parameters equal or better than RF to both food products.

In conclusion, HS/RT reduced the microbial loads and maintained the food fresh-like characteristics, while reducing the energy consumption and the environmental impact.

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P359. Hyperbaric storage of raw milk at room temperature

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Hyperbaric Storage (HS) is a novel preservation methodology that has resurged in the recent years, since it allows significant energetic costs reduction, as energy is only needed to generate the pressure and also no energy is needed to maintain the temperature. It acts similarly to refrigeration by microbial growth inhibition and in some cases, microbial inactivation may also occur.

In this work, raw, unpasteurized milk was stored under pressure (50/62/75/100 MPa) at variable uncontrolled room temperature (17-24°C) and compared with refrigeration (4°C) under atmospheric pressure. The evolution of the vegetative total aerobic mesophiles (TAM), lactic acid bacteria (LAB), Enterobacteriaceae (ENT), coliform bacteria (COL) and yeast and moulds (YM) counts was monitored throughout 60 days. Also, *Escherichia coli*, *Listeria innocua* and *Salmonella senftenberg* were inoculated to a final concentration of 5 logCFU/mL in another set of raw milk samples, and stored under 50/75/100 MPa.

Milk stored at 4°C showed clear signs of deterioration after 14 days of storage, with TAM and ENT counts reaching above 6 logCFU/mL. On the other hand, ENT and YM were very sensitive to all HS conditions, being reduced to counts below the detection limit (1 logCFU/mL) after 28 and 14 days of storage, respectively. TAM were more resistant to HS being reduced by around 1.13, 1.16, 1.27 and
1.83 logCFU/mL after 7 days under 50/62/75 and 100 MPa, respectively. However, after 28 day of storage at 50 MPa, TAM, LAB and COL were able to recover and grow to counts above 6 logCFU/mL. Samples under 62 and 75 MPa showed an overall microbial growth inactivation behaviour similar for all studied microbial groups, being this effect stronger for samples under 100 MPa. Regarding the inoculated microorganisms, *E. coli* was the most sensitive to pressure, with counts decreasing to below the detection limit even for the lower pressure (50 MPa after 21 days), followed by *S. senftenberg* (being reduced below 1 logCFU/mL only at 75 MPa after 10 days of storage) and then *L. innocua* (with counts reduced to below the detection limit under 75/100 MPa only after 21 days of storage).

P360. Sub-lethal high hydrostatic pressures to enhance fermentative processes: Yoghurt as case study

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Industrial yoghurt fermentation occurs under conditions favourable to the growth of lactic acid bacteria (LAB) at atmospheric pressure (AP), nevertheless, it is possible to perform fermentation processes under high hydrostatic pressures, as a sub-lethal stress to induce metabolic changes on LAB metabolism, to develop yoghurts with new/improved sensorial characteristics.
This work aimed to study refrigerated storage (4°C for 23 days) of yoghurt produced at 43 and 50°C under sub-lethal high pressure, at 10, 20, 30 and 40 MPa, in comparison with the fermentation process at AP (0.1 MPa). LAB (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus*) and quality parameters like pH and syneresis were evaluated, along with textural analyses to infer how pressure would impact the obtained yoghurt along storage.
For yoghurts fermented at 43ºC, higher fermentation pressures resulted in slightly lower LAB loads (a maximum of 1.01 Log CFU/mL) and increased the fermentation time (a maximum of 3 h 25 min), syneresis (a maximum of 44%), all for 40 MPa, and firmness (a maximum of 2.5-fold) for 30 MPa. Under refrigeration, LAB were more active during the first 15 days of storage in yoghurts fermented under pressure (increasing loads up to 0.54 Log CFU/mL). However, for yoghurts fermented at 50ºC, higher fermentation pressures also increased the fermentation time and syneresis rates, whereas lower fermentation pressures resulted in higher LAB loads. In fact, the only samples which could be considered as yoghurt were the ones fermented at 10 and 20 MPa. Yoghurts fermented at lower pressures turned out to be the stiffest too (firmness maximum of 1.2 N for 20 MPa). The pH was not affected by pressure or storage for all yoghurts (fermented at 43 and 50ºC), despite a small decrease along storage.
These results are promising regarding the use of sub-lethal high pressures to induce metabolic shifts on bacteria to obtain products with different characteristics. Further research is of interest to ascertain the biotechnological potential of fermentation processes under sub-lethal high pressure in general and in particular for yoghurt production.

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P361. Evaluation of *Aloe vera* juice as a substrate for *Enterococcus faecium*

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Fermented plant material beverages stand out from fermented food in general, since their consumption presents no risk of intolerance and allergies associated with dairy food. *Aloe vera* has been used to varied medical purposes due to their bioactive compounds. Lactic acid bacteria, generally used in fermented beverages, are known also for their potential health and nutritional benefits. This study aimed to evaluate *Aloe vera* juice grown in Mexico as a substrate for *Enterococus faecium* isolated from breastmilk. To obtain the juice, *Aloe vera* gel was treated enzymatically with cellulase. The juice was inoculated with *E. faecuim* at 37ºC and initial pH 4.7 ± 0.2. Biomass growth and pH were evaluated along the fermentation. Fermentation was also run with *Aloe vera* juice supplemented with 20 g/L of glucose. Glucose consumption and organic acids produced were analyzed by HPLC, and Total phenols were determined by Folin- Ciocalteu colorimetric method. Results obtained with *E. faecium* were compared with fermentations run with a commercial *Lactococus Lactis* strain. Glucose from the *Aloe vera* juice was consumed in 24 h by the *E. faecium*, with a production of 2.25 g/L of lactic acid. In the juice supplemented with glucose, *E. faecium* did not consumed all the glucose up to 60 h fermentation, and produced 7.3 g/L of lactic acid. The pH droped to 3 units in both treatments. There was a significant difference between the total phenols determined for both fermentations. A total of 192.50±0.04 mg GAE/L was found for the fermentation run with the *Aloe vera* juice and 196.53±0.06 mg GAE/L for fermentation with addition of glucose to the juice. For fermentations run with the commercial strain of *L. Lactis*, a higher amount of total phenols was determined, 222.30±0.05mg GAE/L and 226.90±0.02 mg GAE/L for treatment without and with supplementation of glucose. Other acids of interest were also produced by both bacteria such as succinate, format, acetate, propionate, and iso- butyrate. *Aloe vera* showed to be suitable for the growth of the probiotic *E. faecium* bacteria, boosting bioactive metabolites in the obtained juice, which after process optimization may result in a new functional beverage.

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P362. Cholinium-based ionic liquids as additives to enhance the biocatalytic activity of L-Asparaginase

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L-asparaginase (ASNase) is an enzyme of high value for pharmaceutical industries (e.g. in the treatment of pediatric acute lymphoblastic leukemia) and food industries (e.g. to mitigate acrylamide levels in processed food). Although, biocompatible ionic liquids (ILs) have been recognized as promising solvents, or additives, for stabilizing enzymes, ILs studies in the stabilization and activation of ASNase are scarce. To address this, the enzymatic activity of a commercial ASNase in aqueous ILs was evaluated. ASNase was exposed up to 24 h to aqueous solutions of cholinium ([Ch])-based ILs, at three temperatures (25, 37 and 50 °C), and the biocatalytic activity measured. At 25 ºC, the exposure to [Ch]-based ILs aqueous solutions enhanced the biocatalytic activity of ASNase, relative to ASNase in the presence of phosphate buffer solution (control). Most significantly, the exposure to cholinium chloride ([Ch]Cl) aqueous solution (at 0.050 mol[Ch]Cl moltotal-1) led to a large increase in the relative ASNase enzymatic activity (circa 250%). On the other hand, independently of the IL aqueous solution, increasing the temperature to 50 °C caused a negative effect in the catalytic behavior, leading, in most cases to ASNase inactivation. Spectroscopic (circular dichroism and fluorescence) and calorimetric analysis were performed to obtain further insights regarding the effect of [Ch]Cl on the ASNase structure, but no significant structural changes of the protein were observed. This work shows that the ASNase catalytic activity can be increased simply by exposing it to different [Ch]-based ILs solutions, a simple and useful approach for several ASNase biocatalytic applications, especially in food processes. Funding: FAPESP (2014/16424-7, 18/50009-8), CAPES (funding 001), CNPQ. A. Magri acknowledges the PhD grant (163292/2015-9) and financial support from CNPq. M. M. Pereira acknowledges the PhD grant (2740-13-3) and financial support from CAPES.

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P363. Diversity and antimicrobial susceptibility of bacteria from aquaculture species

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In aquaculture, a higher density of fish in a specific area is usually related with an increase in stress conditions, leading to a predisposition for infectious diseases and a higher antibiotic consumption. Hence, this study aimed to characterize antibiotic resistance (AR) in bacteria and understand the bacterial diversity in *Sparus aurata* (sea bream) and *Mytilus galloprovincialis* (mussels) collected from aquaculture.
During the years 2018 and 2019 we collected samples of *Sparus aurata* (n=12) and *Mytilus galloprovincialis* (n=1) from fish farms and markets. Sea bream and mussels' samples were homogenized and further diluted (each dilution plated in selective media). Colonies with different morphology were selected and DNA extracted. Strains were identified by MALDI-TOF and 16S rRNA gene amplification. Antimicrobial susceptibility was assessed by disc diffusion and MIC methods for 20 antibiotics from 9 classes. The investigation of clinically important AR-encoding genes was performed by PCR-amplification. The whole-genome-sequencing of an *Enterobacter cloacae* strain was also performed.
A total of 234 strains from *S. aurata* and 17 from *M. galloprovincialis* were isolated and identified. Aeromonadaceae, Bacillaceae, Enterobacteriaceae, Enterococcaceae, Hafniaceae and Shewanellaceae families were isolated in both species, while Comamonadaceae, Erwiniaceae, Erysipelotrichaceae, Micrococcaceae, Moraxellaceae, Pseudomonadaceae, Staphylococcaceae, Streptococcaceae and Yersiniaceae were only isolated in *S. aurata*; Morganellaceae and Vibrionaceae families were only found in *M. galloprovincialis*. Decreased susceptibilities to β-lactam, phenicols, tetracyclines, quinolones, glycopeptides, mupirocin, fusidic acid and trimethoprim/sulfamethoxazole antibiotics were found. A *qnrB*-type gene was detected in two Enterobacteriaceae strains, which conferred resistance to quinolones (one of them to flumequine, MIC=16mg/L). A *Klebsiella pneumoniae* strain with decreased susceptibility to ciprofloxacin revealed non-synonymous mutations in the *parC* gene. Genomic analysis of *E. cloacae* from ST190 lineage, allowed to identify β-lactam (blaACT-7-type) and phosphomycin (*fosA*) resistance genes, *iroN* virulence factor, several plasmids and phages.
We highlight the diversity of bacterial species from aquaculture samples and between farms and market. Some of these species and AR-encoding genes have already been detected in the human reservoirs. Thus, bacteria found in aquaculture might have the ability to acquire several AR-encoding genes and act as a reservoir of AR, which need to be monitored.

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P364. Improvement of Simultaneous Saccharification and Fermentation (SSF) of pretreated *Pinus pinaster* wood

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SSF of lignocellulosic biomass (softwood, hardwood and herbaceous) is an integrated process used to produce second-generation bioethanol. SSF of *Pinus pinaster* wood previously treated by autohydrolysis (AH) followed by soda ethanol organosolv pulping (SEOS) was performed. Two types of fermentable substrates were obtained: i) autohydrolysis liquors rich in hemicellulosic mono and oligosaccharides, which can potentially yield up to 2.40 $g.L^{-1}$ of glucose, 8.09 $g.L^{-1}$ of mannose, 3.31 $g.L^{-1}$ of xylose, 2.89 $g.L^{-1}$ of galactose and 1.39 g L-1 of arabinose; ii) cellulosic pulps composed by 90.56% of celulose, 3.75% of hemicelluloses, 4.49% of residual lignin and 0.95% of ash. SSF was applied to pine autohydrolysate with enzymatic loading of 15 FPU of cellulase Cellic® CTec 2 per gram of carbohydrate (CH) and *Saccharomyces cerevisiae* (ATCC® 26602)™ yeast. A working volume of 50 mL was used in Erlenmeyer flasks kept at 38ºC and orbital agitation for 72 h. Despite the availability of 18 $g.L^{-1}$ of monosaccharides, only 0.99 $g.L^{-1}$ of ethanol was produced with a conversion yield of 12%. SSF of pine pulp was carried out at 10 wt.% solids with the same microorganism and enzymatic loading. The influence of scale up (working volume of 50 and 350 mL) and operation mode (batch vs fed-batch, at 350 mL) on the SSF efficiency was evaluated. All experiments were maintained at 38ºC for 72 h. Primarily, SSF was carried out at 50 mL in Erlenmeyer flasks with orbital agitation and 39.8 g. L^{-1} of ethanol was achieved, at 70% conversion yield. When the working volume was increased to 350mL in stirred tank vessels (STV), SSF efficiency slightly decreased and 37.8 $g.L^{-1}$ of ethanol was obtained with a conversion yield of 66%. As the volume increased, the amount of substrate also increased, making mass transfer more difficult and, consequently, decreasing SSF performance. To overcome this problem, fed-batch was implemented, improving the process. For the same working volume of 350 mL in STV, fed-batch SSF led to ethanol concentrations up 55 $g.L^{-1}$, with a conversion yield of 98% and a productivity of 0.78 $g.L^{-1}.h^{-1}$.

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P365. Microbiological quality depreciation of raw sheep milk attributable to prolonged refrigeration

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Most of the microorganisms present in raw milk are contaminants of the outside that gain entrance into the milk from various sources. In cheese manufacturing, refrigeration of raw milk is a prerequisite to preserve quality, still it is crucial to consider how prolonged storage impairs its microbiological quality. The present study was carried out to evaluate the microbiological quality of raw sheep milk over the refrigeration period. Regarding this objective, samples obtained from bulk tanks of two dairy farms were analyzed for enumeration of mesophilic and psychrotrophic microorganisms, molds and yeasts, lactic bacteria and lactococci, on the collecting day and after one, two and four days of cold storage (4.6±2.2°C). Initially, mesophilic microorganisms (5.4 log CFU mL^{-1}), lactococci (5.1 log) and lactic acid bacteria (4.8 log) were the predominant microorganisms, followed by psychrotrophic microorganisms (4.4 log), molds and yeasts (3.5 log). A similar microbial quality was registered after two days of storage. However, a significant increase of mesophillic (6.8 log) and psychrotrophic (6.6 log) microbiota was found after four days, denoting a microbiological quality depreciation attributable to prolonged refrigeration.

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P366. Diversity, Distrubition and Conjugative Transfer of Plasmids in Multi-Drug Resistant *Salmonella Infantis* Isolates

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An increasing public health problem due to multidrug resistant foodborne pathogens such as *Salmonella* spp. has been encountered all around the world. Infantis, an emerging serovar of the *Salmonella*, became one of the most prevalent strain and showed resistance to multiple antimicrobials. Recent studies showed that, megaplasmids such as pESI and pESI-like plasmids, contributed to emergence of more fitted and resistant Infantis strains in broilers especially. Starting from this point, 70 drug resistant *Salmonella* Infantis isolates, isolated from raw broiler meat between years 2005 and 2015 in Turkey, were included to the study. By molecular methods, 8/70 Infantis isolates were detected to carry plasmids in sizes from 40 kb to 47 kb. Then, plasmids were purified and sequenced by Sanger Sequencing method. Sequencing revealed plasmids varies between 29 kb to 280 kb in size. Molecular methods and sequencing output different sized plasmids due to limitations of the methods. Comparative genomic analysis showed that 7 out of 8 isolates carried plasmids which are sharing common regions. The most comprehensive one, p50 (~280 kb), which comprises the others, was very similar to the plasmids detected in Hungary (pSI54/04), in Israel (pESI) and in the US (pFSIS1502169). p50 was detected to carry resistance genes *tetA* (tetracycline) and *aph(3')-Ic* (aminoglycoside) besides a class 1 integron with *aadA1* (aminoglycoside) and *sul1* (sulphonamide) genes. As a pESI-like plasmid, p50 was also a chimeric plasmid which was a IncP plasmid but carried IncI1 genes, and when IncI1 genes were examined an *E. coli* plasmid which was completely involved in the p50 was detected. The transfer of Infantis plasmids were also confirmed to commensal *E. coli* in this study. All of the findings revealed that, Infantis isolates from poultry in Turkey, carried plasmids which are genetically similar and contribute to the antimicrobial resistance. They can be conjugated to commensal *E. coli* and could be formed, developed and disseminated by the help of *E. coli*. This study helped us to see the similarity of the Infantis plasmids between the ones detected in different geographical regions and enlarge our knowledge of plasmid structure and possible threats causing from them.

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P367. Optimization of microsatellite fingerprinting technique for differentiating *Brettanomyces bruxellensis* wine isolates

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The ability of Brettanomyces bruxellensis to produce volatile phenolic compounds, imparting to the wine an unpleasant aroma known as “Brett flavour”, makes it one of the yeast species most feared by wine producers. Indeed, B. bruxellensis has an exceptional capacity to survive in minimal nutrient conditions, and can utilize alcohol as carbon source. It has been detected in wines that have been bottled for dozens of years. Microsatellite markers for genotyping B. bruxellensis strains have been designed, revealing a ploidy level related with the substrate of isolation and the geographical origin. The prediction of B. bruxellensis SO2 sensitivity using microsatellite genetic profiles has also been achieved. Other phenotypic characteristics related with B. bruxellensis spoilage capability must be evaluated, rendering these markers useful tools for winemakers.
In order to give B. bruxellensis microsatellite fingerprinting analysis faster and cheaper for routine laboratory analysis, the optimization of procedures was carried out. Colony and multiplex PCR were assayed as one step procedure and the results were compared with published results using the same culture collection strains. Isolates from wines from different Portuguese wineries were also characterised.
Comparing the results obtained with the one-step procedure, with the published results obtained using a multi-steps procedure, similar profiles were obtained. Additionally, identical results were obtained using individual microsatellite PCR or multiplex PCR, indicating that the optimization of the technique was adequate. When considering the culture collection strains and the wine isolates studied, several profiles were obtained. The collection strains presented 6 different profiles four of them shared with wine isolates. One profile was only presented by wine isolates, which were obtained from the same wine. The B. bruxellensis isolates from wines from one winery revealed three different profiles two in common with collection strains and the other one in common with most of the isolates studied, obtained from three other wineries, together with collection strains. An additional profile was also obtained for isolates from a different winery and a collection strain reinforcing the high discriminant power of this methodology.

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P368. H515, adjacent to the trinuclear Cu centre of *Aquifex aeolicus* McoA is a determinant of the enzyme hyperstability

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Multicopper oxidases couple the oxidation of substrates to the reduction of dioxygen to water. A conserved glutamate residue, within the entry solvent channel of MCOs and present in all well- characterized MCOs, is known to play a key role in steering the dioxygen to the trinuclear copper site, providing protons for its reduction and giving stability to this site. Unexpectedly, McoA from *Aquifex aeolicus*, a hypertermoactive (Top 75 C) and hyperthermostable (Tm 90 C) enzyme, has a His (H515) at that position. In this study, the role of H515 has been investigated using a combination of site-directed mutagenesis, kinetic, biochemical and biophysical methodologies. Biochemical analysis (copper content, T1 Cu redox potential, UV-visible and circular dichroism) of variants H515E and H515L revealed similar properties when compared to the wild-type. The replacement by a Leu leads to a decreased activity, showing that H515 has a role in dioxygen reactivity. Notably, the replacement of a His by a Glu leads to almost 2-orders of magnitude higher kcat/K_m for the ABTS oxidation, showing that the native His impairs the enzyme to achieve its maximal activity. With the purpose of understanding if a histidine in this position was “selected” in this specific enzyme as a determinant of its hyperstability, chemical denaturation was followed by fluorescence and revealed that G°, [GITC]50% and m are ~1.5 times higher for wild-type as compared with H515E. These results were corroborated by kinetic stability studies that show that the wild-type is more stable as compared with the H515E, with a half-life at 70°C of 10 and 2.5 h, respectively. Overall the present study suggests that hyperthermostable *A. aeolicus* McoA selected an His to play a role in dioxygen reduction, instead of a Glu, in order to maintain the structural stability required by the hyperthermophilic habitats where the native microorganism lives.

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P369. UV pulse light application for *Campylobacter* control in poultry meat

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Poultry meat is implicated as the main risk factor in human Campylobacteriosis. Thus to increase food safety, food industry and scientific community have promoted further research to find solutions to control *Campylobacter* spp. on poultry meat. The objective of this study was to assess the effect of pulsed light (including UV, representing 10-15% of total spectra, UVPL) to minimize *Campylobacter* on poultry meat. Breasts poultry meat were purchased at local supermarket, frozen and storage at -20ºC, 48h, to eliminate initial contamination of *Campylobacter*. Breasts were cut in squares 5x3cm, with 4-5mm thickness and two groups of samples were prepared: one inoculated 5log cfu/g of a pool of 3 *Campylobacter jejuni* strains and the other not inoculated. All samples were low-strength vacuum pack to simulate microaerophilic conditions and were submitted to different pulsed light energies in an apparatus PLTecum Unit (Claranor, Manosque, France). A Central composite design was applied regarding the factors Voltage (from 1828 to 3000V) and distance to the source UVlamp (from 2.6 to 5cm). Number of pulses (5 pulses of 300 µs/each) and frequency (1Hz) were considered fixed effects. The fluence (J/cm2) received by samples was measured by a laser power detector (JoulmeterUP17P connected to MAESTRO-monitor, Gentec-EO, Canada). Color was performed using CIE-L*a*b* Coordinates system (Minolta colorimeterCR-300). Temperature was measured by IR- Thermometer (Testo, Spain). Campylobacter, Enterobacteriaceae and total psychrotrophic bacterial counts were carried out according to ISO Standards. Statistical treatment of data was performed. Poultry samples presented counts of 2.05log cfu/g and 1.68log cfu/g for total psychrotrophic and Enterobacteriaceae, respectively. Samples inoculated with Campylobacter presented counts of 4.9±0.01log cfu/g. The binomial factorial treatment (voltage and distance) with UVPL induced different fluences received by samples ranging from 2.82 to 9.67J/cm^2. The samples treatment with UVPL aiming to reduce *Campylobacter* seemed to be not statistically significant compared to contaminated samples not treated; however a trend of a decrease of *Campylobacter* when the fluence increased could be observed. The Enterobacteriaceae counts increased with fluence and could be related to increased samples temperature. The UVPL treatment also influenced meat colour, with increased values of L*, a* and b* when fluence was higher.

P370. Production and characterization of isoeugenol monooxygenase from *Pseudomonas nitroreducens* and bioconversion into vanillin by whole cell reactions

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Vanillin is one of the most used compound in food, cosmetic and pharmaceuticals industries as flavour and flagrance. It is mostly produced by chemical synthesis, due to the high demand of such compound and the limited availability and high price of natural resources, such as vanilla pods. However, most chemical synthesis methods to obtain synthetic vanillin have many disadvantages such as low purity of final product, hazardous operation conditions and pollution producing during the process.
In recent years, many studies proposed alternative and eco-friendly methods for vanillin production from renewable and low-cost resources [1]. Isoeugenol is a widespread phenylpropanoid in essential oils of plants which can represent a low-cost precursor for vanillin biosynthesis.

In this study, we have successfully cloned and expressed in Escherichia coli an isoeugenol monooxygenase from *Pseudomonas nitroreducens*, which converts isoeugenol into vanillin in a coenzyme-independent manner. PnIEM was biochemically characterized, showing as optimal conditions of reaction 30°C and pH 9. Furthermore, the bioconversion of isoeugenol into vanillin was successfully achieved using free and immobilized cells overproducing PnIEM enzyme. Our results propose a new key enzyme for a green and cost-effective conversion of lignin bio-wastes into valuable natural vanillin.

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P371. Hyperthermophilic laccases for the XXI century biorefineries

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Biocatalysts from hyperthermophiles are promising for industrial applications due their high intrinsic stability, revealing the robustness required for harsh industrial processes. We have recently reported a laboratory evolution approach that lead to the improved efficiency of the *Aquifex aeolicus* McoA hyperthermophile bacterial metallo-oxidase for the typical laccase substrate ABTS (2,2'-azinobis-(3- ethyl-benzothiazoline-6-sulfonic acid)) while showing an enhanced kinetic and thermodynamic thermostability (Brissos et al. 2015 ACS Catalysis 5, 4932-4941). Herein we have used a combination of directed evolution (DNA-shuffling and error-prone PCR followed by high-throughput screening) and rational design (deletion of signal peptide and Met-loop deletion) to evolve the McoA 2B3 hit enzyme for 2,6-dimethoxyphenol (syringol), a lignin-related phenolic substrate. McoA 2B3 evolved variant although showing a 100-fold increased catalytic efficiency ($kcat/K_m$) for ABTS (a non-phenolic organic substrate) as compared to the wild-type showed a residual activity for lignin-related phenolic substrates. The concerted application of the aforementioned engineering methodologies allowed the identification of 23B3 variant showing 9 non-synonymous mutations, similar thermal stability properties as the wild-type (with a melting temperature around 100 C), 5-fold higher production yields and featuring 1500-fold higher kcat/Km for syringol. Currently we are investigating the catalytic potential of hit 23B3 using a set of representative lignin-related phenolic compounds including syringyl, guaiacyl and hydroxybenzene derivatives. These results will positively impact the utilization of enzymes for lignin depolymerization and conversion into valuable products and materials. Enzymatic depolymerization of lignin into phenolic platform chemicals is envisaged as one of the potential environmentally friendly breakthrough applications for the successful valorisation of lignin bio-wastes.

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I10. Industrial and Food Microbiology and Biotechnology

P372. New enzymes for biorefinery applications: towards the production of bio-resins

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Lignocellulose biorefineries are a promising alternative to the fossil fuels paradigm we live in today. Lignin is the most abundant aromatic polymer in Nature, however, due to its recalcitrance, its degradation requires the cooperative activity of a large repertoire of microbial enzymatic activities.
Dye-decolorizing peroxidases (DyPs) are a new family of microbial heme-containing peroxidases that show attractive properties for lignocellulose biorefineries due to their ability to oxidize lignin-related compounds.
In this study we are tailoring the *Bacillus subtilis* BsDyP, through the implementation of a Directed Evolution protocol, to improve the catalytic activity of this enzyme towards the conversion of sinapyl alcohol, a phenolic compound resulting from the lignin depolymerization, into syringaresinol, a valuable precursor of epoxy and polycarbonate resins. This study will include a set of strategies combining protein engineering, kinetic, biochemical and biophysical approaches to improve the efficiency of BsDyP towards this catalytic reaction.

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I10. Industrial and Food Microbiology and Biotechnology

P373. Redesigning the substrate specificity of an hyperthermostable metalloxidase

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Multicopper oxidases oxidise a range of aromatic substrates such as polyphenols, methoxy-substituted phenols and amines, concomitantly with the reduction of molecular dioxygen to water. One striking property is the activity that only some MCOs, the so called metallo-oxidases, exhibit towards metal ions, such as Cu(I) and Fe(II) as reducing substrate. In this work we have used directed evolution to change the substrate specificity of the metalloxidase McoP from the archaeon *Pyrobaculum aerophilum* for organic substrates. The aim is to contribute to answering the long-standing question in protein science of “how function and structure are related” and to investigate the molecular mechanisms of enzyme fitness evolution, critical for engineering new proteins, metabolic pathways and organisms for biotechnological applications. Directed evolution is a powerful protein engineering tool to tailor biocatalysts with improved features including stability, substrate specificity or product selectivity, and enantioselectivity, and simultaneously contribute to understanding determinants of structure-function relationships. Four rounds of random mutagenesis of the *mcop*-gene followed by high-throughput screening (≈ 60,000 clones) led to the identification of the 1B5 variant featuring a 1-order of magnitude higher efficiency than the wild-type enzyme for the organic substrate ABTS (2,2’-azinobis-(3-ethyl- benzothiazoline-6-sulfonic acid)). The higher efficiency (k_{cat}/K_m) relies in a 3-fold higher k_{cat} and importantly, in a 10-fold lower K_m value, showing that the affinity for the substrate increased steeply during evolution. Interestingly, 5 out of the 12 mutations were located closely to the substrate binding site. These most likely affect its overall conformation in order to accommodate larger substrates as compared with the small metallic ions used by the native McoP enzyme. DNA shuffling of wild type and 1B5 genes are currently being used to distinguish functional from non-functional (neutral) mutations. These studies will allow a better insight on the structure-function relationships within the mechanisms of multi-functionality with interest in the realm of biotechnology.

Acknowledgments: This work was supported by the project grant PTDC/BBB-EBB/0122/2014, PTDC/BII-BBF/29564/2017, GREEN-IT "Bioresources 4 Sustainability" UID/Multi/04551/2013 and Project LISBOA-01-0145-FEDER-007660 funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and FCT.

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P374. Table salt as vehicle for *Salmonella* spp. and *Listeria monocytogenes* cross-contamination

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Salmonella spp. and *Listeria monocytogenes* are foodborne pathogens that contaminate a variety of food products. Among various routes of transmission, consumer mishandling of foods at home plays a significant role in the occurrence of foodborne diseases. Of particular risk is the cross- contamination events that may occur in the kitchen during meal preparation when an ready-to-eat (RTE) food, that will not undergo a heating step prior to consumption (e.g. vegetable salads), comes in contact with a contaminated raw food or surface. In this study, the cross-contamination and transfer rates of *Salmonella* spp. and *L. monocytogenes* from chicken meat to lettuce, via cross-contamination of table salt, during simulated food-handling were determined. Additionally, the survival of both pathogens on artificially inoculated table salt was investigated.
Chicken meat samples (50 g) were inoculated with a mixed cocktail of *L. monocytogenes* (n=7) or *Salmonella* spp. (n=5) strains to a final contamination level ranging from 102 to 106 colony forming units (CFU)/g. The transfer experiments (n=4) were performed by one volunteer that touched the contaminated chicken and then of table salt, that was subsequently used to season lettuce samples. The survival of these pathogens in table salt was investigated by inoculation table salt with the mixed cocktail of each pathogen (ca. 106 CFU/g) and stored at room temperature (RT). At specific time intervals samples were taken and bacterial numbers determined following the ISO 6579:2002 and ISO 11290-1&2.
Salmonella spp. and *L. monocytogenes* was detected in cross-contaminated lettuce samples at all contamination levels tested. *Salmonella* spp. was able to survive for xx days, levels were below he detection limits after 86 days, while *L. monocytogenes* resisted for 120 days of exposure. This study showed the ability of Salmonella spp. and *L. monocytogenes* to survive for a long time on table salt at RT, and proven that these pathogens can be transferred from table salt to RTE food during handling. Thus, it is very important prevent the cross-contamination to ensure consumer safety and reduce outbreaks of salmonellosis and listeriosis.

I10. Industrial and Food Microbiology and Biotechnology

P375. Evaluation of *Salmonella* spp. and *Listeria monocytogenes* survival in table salt and cross-contamination effect

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Salmonella spp. and *Listeria monocytogenes* are foodborne pathogens that contaminate a variety of food products. Among various routes of transmission, consumer mishandling of foods at home plays a significant role in the occurrence of foodborne diseases. Of particular risk is the cross- contamination events that may occur in the kitchen during meal preparation when an ready-to-eat (RTE) food, that will not undergo a heating step prior to consumption (e.g. vegetable salads), comes in contact with a contaminated raw food or surface. In this study, the cross-contamination and transfer rates of *Salmonella* spp. and *L. monocytogenes* from chicken meat to lettuce, via cross-contamination of table salt, during simulated food-handling were determined. Additionally, the survival of both pathogens on artificially inoculated table salt was investigated. Chicken meat samples (50 g) were inoculated with a mixed cocktail of *L. monocytogenes* (n=7) or *Salmonella* spp. (n=5) strains to a final contamination level ranging from 102 to 106 colony forming units (CFU)/g. The transfer experiments (n=4) were performed by one volunteer that touched the contaminated chicken and then of table salt, that was subsequently used to season lettuce samples. The survival of these pathogens in table salt was investigated by inoculation table salt with the mixed cocktail of each pathogen (ca. 106 CFU/g) and stored at room temperature (RT). At specific time intervals samples were taken and bacterial numbers determined following the ISO 6579:2002 and ISO 11290-1&2. *Salmonella* spp. and *L. monocytogenes* was detected in cross-contaminated lettuce samples at all contamination levels tested. Salmonella spp. was able to survive for xx days, levels were below he detection limits after 86 days, while *L. monocytogenes* resisted for 120 days of exposure. This study showed the ability of *Salmonella* spp. and *L. monocytogenes* to survive for a long time on table salt at RT, and proven that these pathogens can be transferred from table salt to RTE food during handling. Thus, it is very important prevent the cross-contamination to ensure consumer safety and reduce outbreaks of salmonellosis and listeriosis.

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I10. Industrial and Food Microbiology and Biotechnology

P376. Probiotic potential of fructo-oligosaccharides produced by *Aspergillus ibericus*

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The gastrointestinal tract harbours a diverse and dynamic microbial community that directly impacts human health. Prebiotics, such as fructo-oligosaccharides (FOS), play a crucial role in the modulation of colonic microbiota, reducing pathophysiological disorders and associated chronic diseases.
The prebiotic potential of FOS produced by a newly isolated strain – *Aspergillus ibericus* was studied. FOS fermentability by the probiotic *Lactobacillus rhamnosus* was evaluated.
L. rhamnosus was grown in de Man-Rogosa-Sharpe (MRS) broth, with different carbon sources: glucose (positive control), no sugar (negative control), microbial-derived FOS from *A. ibericus* and Raftilose®P95, a non-microbial commercial FOS sample (from Beneo-Orafti, Belgium). A final concentration of 2 % (w/v) in sugar was used. Fermentation was carried out in a 96-well microplate and a shake flask, for 24 h, at 37 ºC, with an agitation of 120 rpm. Biomass growth was analysed by optical density at 620 nm. The consumption of sugars and the production of short chain fatty acids (SCFA) and lactate was quantified by HPLC.
Maximum cell growth was reached at approximately 12 h, for all carbon sources. The highest growth was achieved for glucose samples, followed by the microbial-derived FOS, then Raflitose and finally the negative control. Although the microbial-derived FOS promoted great cellular growth, only kestose (GF2), together with residual amounts of glucose and sucrose presented in the sample, were consumed. This may explain the two different slopes exhibited during the exponential phase growth. Most likely hypothesis is that probiotic bacteria was cleaving GF2 in the first hours of fermentation, using only the smallest sugars present for growing. And Nystose (GF3) and fructofuranosylnystose (GF4) were not consumed, even when prolonging the fermentation up to 48 h.
SCFA identified were valerate and propionate, as well as succinate, formate, acetate, iso-butyrate and n-butirate, although in lower amount. Higher amount of SCFA and lactate were determined while growing in the microbial-derived FOS, as compared to the commercial sample. Overall, lactate was the main metabolite produced during the fermentations.
In conclusion, the prebiotic potential of microbial-derived FOS synthesized by *A. ibericus* was demonstrated, providing promising indication of its usability as food ingredient with strong prebiotic features.

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P377. Tobacco cell suspension cultures as an alternative platform for the production of cardosins

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Cynara cardunculus L. or Cardoon is a plant used in traditional cheese manufacturing, in which an infusion of the flowers is added to the milk as coagulant agent. This activity is possible due to the presence of aspartic proteases (AP) known as cardosins and cyprosins. Due to its traditional nature, the infusion is variable and directly influences the final product. The production of these APs in alternative platforms such as bacteria and yeast has been challenging, thus hampering their industrial exploitation. In this project, we are studying alternative plant-based platforms for the production of APs from cardoon in order to obtain enzymes with normalized activity to be applied in cheese manufacturing. Tobacco BY2 cell suspension cultures were successfully used to produce cardosin B in its active form. The enzyme was not secreted but we were able to develop a purification process to obtain pure recombinant cardosin B using only one chromatographic step. The purified cardosin B presented optimal activity in similar conditions of temperature and pH as its natural counterpart. It also showed activity over caseins and was able to clot milk under standard conditions, displaying specific characteristics of aspartic proteases. We further explored the intracellular localization of cardosin B in BY2 cells using a fusion with red fluorescence protein. These cell lines showed a delay in cardosin B processing which allowed us to purify and further characterize the activation of the unprocessed form of cardosin B. This work paves the way for the use of tobacco BY2 cells as an appropriate system for the production of active cardosins and contributes to the development of new solutions for the production of APs from cardoon.

I10. Industrial and Food Microbiology and Biotechnology

P378. Improving a bacterial pyranose 2-oxidase through directed evolution for diagnosis biosensor applications

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Pyranose 2-oxidases (P2Oxs, E.C. 1.1.3.10) are enzymes that have a great potential to replace the typical glucose oxidases (GOxs) and glucose dehydrogenases (GDHs), specific for β-D- glucose anomer, in glycemia monitoring biosensors. P2Oxs are flavoenzymes mostly identified in Fungi that catalyzes the regioselective oxidation of C2 alcohol moiety of several aldopyranoses originating the correspondent keto-sugar with the concomitant reduction of O_2 to H_2O_2. The use of O_2 as cheap and clean oxidant and in particular the lack of D-glucose anomer preference represent very attractive points for the biotechnological application of P2Oxs. In this work, directed evolution (DE) methodologies were applied to improve the first identified bacterial P2Ox, from Pseudoarthrobacter siccitolerans, AsP2Ox, in its specificity and activity for D-glucose. 'Activity-on-plate' and 96-well plates screenings allowed analyze in a high-throughput mode, thousands of variants generated by error-prone PCR. The hit variant from the first-generation, 1A1, harbors one mutation, G366S, located close to the substrate binding site and biochemical and kinetic analysis showed a 2-fold increased k_{cat} and 2-fold higher protein production yields as compared with the wild-type. In a second round of directed evolution a new hit variant 5D5 was selected, carrying four additional mutations (S22S, A75T, A206T, Q295H). The pH profile of 5D5 revealed an optimum pH shifted 1 unit towards the alkaline range, a 6-fold higher k_{cat} and 3-fold production yields than the wild-type enzyme. The analysis of mutations using site-directed mutagenesis showed that both G366S and Q295H are key mutations, which under an epistatic effect contributed to the higher catalytic efficiency exhibited by 5D5 hit variant. The evolution of this enzyme stills in progress using 5D5 as the parent in new rounds of DE to achieve an improved variant exhibiting the properties that fit the desired application.

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I10. Industrial and Food Microbiology and Biotechnology

P379. Microbiological characterization of fresh cheeses made in Portugal from raw materials to final products

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Cheese is a dairy product obtained through processing techniques involving coagulation of the protein of milk, in particular, the casein portion. It is made by the action of rennet or other suitable agents, and by partially draining the whey from the coagulation, resulting in a concentration of milk protein. In Europe, the average cheese consumption per capita stands at 11.1 kg in 2019 and the market is expected to grow annually by 3.1%. Fresh cheese is ready for consumption shortly after manufacture, it is considered rich in proteins, vitamins, minerals and fatty acids, essential nutrients to a healthy diet. Due to its high water content, it is adequate for microbial growth; cheeses may be a vehicle of pathogens of importance for public health and they can spoil rapidly. This study aims to characterize microbiologically fresh cheeses made in Portugal from raw materials to final products. Samples of cow (12) and goat (12) pasteurized milks, of rennet (6) and of cow (12) and goat (12) fresh cheeses were analysed as per ISO Standards one day after cheese production during May and June of 2019. The results showed no significant differences amongst samples. For both final raw materials and final products, *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* were not detected or were below the detection limit of the enumeration technique. Nevertheless, goat cheese samples presented the highest counts of quality indicator microorganisms, reaching: *Staphylococcus* coagulase positive ($1.0x10^2$ CFU.g^{-1}), *Pseudomonas* sp. ($9.5x10^5$ CFU.g^{-1}) *Enterobacteriaceae* ($1.5x10^4$ CFU.g^{-1}), lactic acid bacteria ($1.7x10^5$ CFU.g^{-1}), moulds ($1.8\ x10^2$ CFU.g^{-1}), yeasts ($2.5x10^3$ CFU.g^{-1}), total microorganisms at 30 ºC ($5.5x10^5$ CFU.g^{-1}) and total microorganisms at 6.5 ºC ($1.8x10^5$ CFU.g^{-1}). In contrast, a lower microbial contamination was observed on the others samples. All of the samples were in accordance with Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

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P380. *Listeria monocytogenes* in a cheese factory: sources of contamination

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The main objective of this work was to study different sources of *L. monocytogenes* contamination of cheeses made with raw sheep's milk produced in a cheese factory in southern Portugal.
PFGE typing and molecular serotyping of 77 isolates of *L. monocytogenes* were performed and a set of eight selected isolates were typed by MLST and cgMLST. The bactericidal activity of chemical disinfectants used in the cheese industry according to EN 1040/2005 was also evaluated. *L. monocytogenes* isolates (n=77) were obtained from ripened sheep's cheese (n = 51), press-off sheep's cheese (n = 3), raw sheep's milk (n = 9), hand swabs (n=3) and factory surface swabs (n=11).
Molecular typing by PFGE resulted in 13 *Asc*I profiles and 10 *Apa*I profiles with the *Asc*I 007 / *Apa*I 002 pulsotype being the most representative (n = 32) of the *L. monocytogenes* isolates. Concerning all 77 isolates, 95% belonged to PCR serogroup IIa (n = 70) and 5% belonged to PCR serogroup IVb. Isolates from factory surface swabs with *Asc*I 007 / *Apa*I 002 pulsotype revealed tolerance to disinfectant III (2-5%, 15 minutes). All eight *L. monocytogenes* isolates selected for sequencing belong to CC7 (MLST), ST7 (MLST), SL7 (cgMLST) and lineage II. For the cgMLST type according to Institut Pasteur's scheme, four profiles were obtained (CT2915, CT2916, CT2917 and CT2918), which represents a better discriminatory level with respect to the two previously identified combined *Asc*I/*Apa*I profiles for these eight strains.
Molecular typing of these *L. monocytogenes* isolates revealed that raw sheep milk and the production environment are source of contamination and that some strains persist for at least two years in the environment of the processing plant.

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P381. Yeast study in *Arbutus unedo* fruits fermentations from the center of Portugal

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Strawberry tree (*Arbutus unedo* L.) is a forest species well adapted to Portuguese and south Europe climate and soils displaying potential to be successfully used as a businesslike culture, contributing to overcome the current challenges of the Portuguese agro-forestry sector. The fruits that are produced are collected when mature, normally in autumn, during a period of 2-3 months, being manely used for the production of a distilled beverage made from fermented fruits, named “Aguardente de Medronho”. Traditionally, fermentation occurs for several months, conducted by wild microbiota and at low temperature as no temperature control is usually settled. In the present work the microbiota from *A. unedo* fruits fermentation from two producers of the center of Portugal, namely Oleiros and Pampilhosa da Serra, was analysed and yeast isolates were studied.

Four samples of *Arbutus unedo* fruits fermentations were collected and placed in laboratory, at 16.5 ºC until the end of fermentation. Plate count analyses (total count, yeast and acetic bacteria counts) were performed at time of collection and after 2 months. Twenty yeast colonies were isolated from each sample at each sampling time and stored at -80 ºC in glycerol. Isolates were screened using *Saccharomyces cerevisiae* specific PCR amplification with SC1 primer pair. The isolates negative for SC1 were further identified by sequence analysis of the region D1/D2 of the 26S rRNA gene. To differentiate *S. cerevisiae* isolates, microsatellite analysis was performed.

The majority of the 160 isolates were positive for *S. cerevisiae*. Sequencing allowed the detection of additional six species namely *Saccharomyces bayanus*, *Lachancea cidri*, *L. thermotolerans*, *Zygosaccharomyces bailii*, *Sacharomycodes ludwigii* and *Brettanomyces bruxellensis*. The *S. cerevisiae* isolates were further characterized using SSR markers that revealed the diversity of wild strains present in *Arbutus unedo* fruits fermentations. Further investigation into *S. cerevisiae* isolates will be carried out to uncover domestication events that enabled to cope with the particular conditions of *Arbutus unedo* fermentations.

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P382. Assessment of different culture media for bacterial darkening associated with cheese color defects

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Portuguese cheese producers have faced, in recent years, with the appearance of brownish colorations on the surfaces of traditional cheeses resulting in great economic losses because the consumers rejected them. The objective of this work was evaluating the brown pigment production by the microorganisms isolated from different sources, in order to identify the microorganisms with the highest capacity to produce brownish pigment and to select the best culture medium to achieve this goal.
Fifteen bacterial isolates from dairy environment (n=5), cheese (n=2), milk (n=6) and water (n=2) were tested in seven different broth media (Müeller Hinton Broth-MHB, Luria Broth-LB with 0% and 1% tyrosine, Brain Heart Infusion-BHI, King B Broth-KBB, Nutrient Broth-NB and PPMD) and seven different solid culture media (Pseudomonas Agar Base-PAB, Müeller Hinton Agar-MHA, Nutrient Agar- NA, Cheese Agar-CA with 0% and 1% tyrosine, Luria Agar-LA and King B Agar-KBA) incubated at 28 ° C, 3 weeks.
About 86% of the strains tested produce brown pigment in PPMD and 31% and 33% produce brownish pigment in MHB and LB with 1% tyrosine, respectively. In solid culture media, the predominant pigmentation in all them was beige-brown (66% in MHA, 50% in NA, 40% in CA 0% tyrosine, 34% in CA with 1% tyrosine, 33% in PAB and 13% in KBA. Of all solid culture media, CA with 1% tyrosine had the highest percentage (33%) but the extraction of the pigment is difficult in this solid media. Thus, seven strains belonging to *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* were used to evaluate their capacity to produce brownish pigments in CB with 1% tyrosine, KBB and PPMD at 28 °C, 130 rpm for 1 week. Following this test, we observed that PPMD medium has natural brownish color and for this reason it does not allow a reliable visual assessment of brownish pigment production. In KBB medium, there was no brown pigment production. In CB with 1% tyrosine *P. putida* ESACB 191 was the only strain able to produce brown pigmentation under the conditions assayed. This strain was isolated from brownish cheese rind and was selected to proceed with brown pigment studies.

I10. Industrial and Food Microbiology and Biotechnology

P383. More sustainable dairy industries: selection of *Kluyveromyces* strains for direct conversion of concentrated waste streams into bioethanol

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The dairy industry is one of the most polluting industries in the food sector, with very high energy and water consumption demands. Dairy wastewater treatment is therefore mandatory to reduce its environmental impact and to allow water recycling and energy savings. In this work, retentates (concentrated lactose streams) obtained from dairy wastewater processed by nanofiltration or reverse osmosis were assessed for direct bioethanol production by lactose-fermenting yeasts.
Two of the tested *Kluyveromyces* strains efficiently fermented all available lactose, with ethanol yields higher than 90% of the theoretical maximum (>0.47 g/g yield). A *K. marxianus* strain was selected that under severe oxygen-limiting conditions was able to reach 70 g/L (8.9 % v/v) of ethanol, which is compatible with energy-efficient distillation processes.
The process here described for handling dairy wastewaters, making use of membrane technology, allows water recycling from the permeate, complying with legislation for drinking water, and simultaneous bioenergy (bioethanol) production from the retentate, therefore contributing to a more sustainable dairy industry.

I10. Industrial and Food Microbiology and Biotechnology

P384. Disinfectants efficiency against *Salmonella* spp. biofilms on stainless steel surface

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Salmonella spp. is a major cause of foodborne illness around the world. Its ability to produce biofilms contributes to an increased resistance to disinfectants and inefficient disinfection of food processing environments. This may be a challenge to reduce the prevalence of these bacteria in the food chain and contributes to salmonellosis cases in the population. The aim of this study was to evaluate the efficiency of three disinfectants applied over a biofim produced by *Salmonella* spp. isolated from a slaughter house. Biofilms were produced on stainless steel slices incubated in meat broth inoculated with *Salmonella* spp. during 48h to let the surface be covered by a biofilm layer (1.1 x 108 UFC/cm2). The slices surfaces were washed with sterilized tap water and the disinfectants efficacy were analysed in accordance with EN 1276 European Standard for evaluation of bactericidal efficacy of disinfecting liquids, analysis were done in triplicate. Tested substances were: ethanol:isopropanol: benzilic alcohol (46:27:1) [D1], hidrogen peroxide [D2] and benzalkonium chloride (0.5 e 2%) [D3] and the neutralizants were: polysorbate 80 (30 g/L), lecitin (3 g/L), saponin (30 g/L) [N1] and thiosulfate (10 g/L), polysorbate (50 g/L) and lecitin (3 g/L) [N2] . The slices were dip in disinfectant D1 followed by neutralizing N1 during 1, 15, 30 and 60 minutes in each solution and washed again prior to swab, dilution and plate the samples on TSA-YE. The procedure was repeated to D2-N2 and D3-N1. The results evidence that after 1 minute the average rates reduction were 5.0 log to D1, 5.5 log to D3 (0.5%) and 5.8 to D3 (2%). It was not possible to observed microbial growth after 1 min in contact with D2 and to bacteria on biofim after 15, 30 and 60 minutes in contact with tested disinfectants. Once the tested concentration were those recommended by disinfectants producers, we can conclude that it is necessary to keep these products in contact with the surfaces at least 1 minute to reach an appropriate microbial reduction.

I10. Industrial and Food Microbiology and Biotechnology

P385. Bioactive potential and antimicrobial activity of two pomegranate cv peel and seed extracts

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Pomegranate (*Punica granatum* L.) fruit is widely recognized for its high biological activity and benefits to human health. Nevertheless, pomegranate juice extraction generates considerable amounts of peel and seed by-products, because only half of the fruit represents the edible part. Although the juice itself possesses powerful biological capacities, such as antioxidant and antimicrobial activities, the by-products also possess equivalent or superior biological activities. On the other hand, the recovery and valorisation of these wastes will lead to the reduction of their environmental impact that is in line with the desirable models of a circular economy.

The present study aimed to access the antimicrobial activity of pomegranate peel and seed freeze- dried ethanolic extracts against different strains of pathogenic/contaminant and beneficial microorganisms. Two pomegranate cultivars (Wonderful and Acco) from Alentejo region (Portugal), were used as the vegetable material.

Extractions were made using mixtures of EtOH:H_2O (25:75, 50:50 and 75:25 v/v) from dried and ground vegetable material. After extraction, the characterization of the ethanolic extracts obtained from peels and seeds was performed in terms of total phenolic compounds, total flavonoids, antioxidant activity and acetylcholinesterase inhibition activity. Phenolic and flavonoid compounds were expressed as mg GAEq/mg of extract and mg CATEq/mg of extract, respectively. IC_{50} was used to express the results of antioxidant activity and acetylcholinesterase inhibition activity. The antimicrobial activity of the extracts that revealed the best bioactive potential, was accessed by the disc diffusion assay, the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC).

Peels of both varieties revealed the highest bioactive characteristics, with higher levels of antioxidant activity, phenolics and flavonoids. The solvent EtOH:H_2O 75:25 allowed obtaining extracts with the best correlation between extraction yield and antioxidant activity. All the pomegranate peel extracts showed selective antimicrobial activity against the tested microorganisms and differences in MIC and MBC. Further studies including cell toxicity assays are recommended, if the extracts are intended for food applications.

I10. Industrial and Food Microbiology and Biotechnology

P386. Bioreactor production of succinic acid from Kraft pulp hydrolysates

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Succinic acid is an important “building block” to the chemical and polymer industries, being the base molecule for the synthesis of a vast array of products Despite its biological origin, it was initially produced by the petrochemical industry, mainly through the oxidation and hydration of the maleic anhydride extracted from crude oil. However, there are disadvantages associated with this type of production, specially the high environmental impacts related to the use of crude oil, such as the emission of large quantities of CO_2. Nowadays, with the increasing development of biotechnology and due to environmental and economic concerns, the production of succinic acid shifted to the biobased industries.
The biological production of succinic acid includes the consumption of CO_2 via metabolic carboxylation reaction, thus contributing to balance emissions that resulted directly and indirectly by the petrochemical industry. Moreover, it is well documented the possibility of using agro-industrial wastes, such as lignocellulosics, as feedstocks the production of succinic acid. Some industrial wastes generated by the kraft pulping process can be considered suitable substrates for succinic acid. In fact, preliminary studies in anaerobic shaking flasks confirmed the feasibility of succinic acid production using hydrolyzed kraft pulps obtained from eucalyptus wood.
This study aimed to evaluate the production of succinic acid in a pH-controlled bioreactor with CO_2 sparging. Kraft wood pulps enzymatic hydrolysate obtained from *Eucalyptus globulus*, in collaboration with RAIZ (Instituto de Investigação da Floresta e Papel), were fermented by *Actinobacillus succinogenes* under different pre-selected experimental conditions. Regarding the experimental results, concentrations of succinic acid up to 20 g/L with a corresponding yields and productivities around 0.380 gsuccinic/gsugars and 0.650 g/L.h, respectively, were attained.

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I10. Industrial and Food Microbiology and Biotechnology

P387. Combinatorial biosynthesis of plant natural products in microorganisms

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Plant natural products (PNPs) are among the most significant compounds used in pharmaceutical and cosmetic industries. However, most PNPs accumulate at low quantities in plants and are difficult and expensive to isolate. Additionally, a high investment of land, water and time is required and pests and extreme weather cause insecurity in the supply chain. Moreover, their chemical synthesis is challenging due to their stereochemical complexity. Therefore, in the last decades, PNPs pathways have been engineered in microbial hosts using combinatorial biosynthesis. In this approach, genes from different species are assembled to construct complex biosynthetic pathways. Curcuminoids are PNPs whose biosynthetic pathways have been extensively explored in the last years due to their applications. They have been used in traditional food, cosmetic and medicine for centuries. Their therapeutic properties include anti-cancer, anti-inflammatory, anti-oxidant, anti-Alzheimer's, and anti- HIV, among others. Curcumin, the most promising curcuminoid, has a projected market size of USD
130 million by 2025. Herein, we propose an optimized artificial biosynthetic pathway to produce curcuminoids. This pathway involves 6 enzymes and produces ferulic acid as an intermediate using caffeic acid O-methyltransferase. Starting from tyrosine, 1325 μM of ferulic acid were obtained, comprising the first part of the pathway. Then, the second part of the pathway was also optimized. From ferulic acid we obtained the highest concentration of curcumin reported (1212.7 μM) so far, corresponding to a 26% increase [1]. Subsequently, curcumin was produced from tyrosine (the whole pathway) using a mono-culture. Production increased comparing to a previously reported pathway that used a caffeoyl-CoA O-methyltransferase [2]. Additionally, a co-culture strategy was evaluated to further improve the production by reducing cells metabolic burden. We used one *E. coli* strain able to convert tyrosine to hydroxycinnamic acids and another able to convert them to curcuminoids. Using CRISPR-Cas9 method we disrupted *lacZ* gene in one of the strains which allowed to follow co-culture population composition using the blue-white screening method. This co-culture strategy increased 8.8 times the curcuminoids production (126 μM) as compared to the mono-culture production. These results comprise a significant step towards the large-scale production of these valuable compounds.

I10. Industrial and Food Microbiology and Biotechnology

P388. Effect of Different Coagulants on the Amino acid Content of Soft cheese (wara) Produced from Sheep milk

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Soft cheese (wara) is an unripened cheese consumed in several parts of West Africa due to its various nutritional qualities. Soft cheese, a coagulated product of raw milk is usually produced from cow milk using *Calotropis procera*. Although soft cheese can also be produced from other animals using other coagulants. This study therefore sought to assess the effect of the different coagulants such as *Calotropis procera*, *Carica papaya*, lemon juice and steep water from cereals (maize, millet and sorghum) on the amino acid content of soft cheese produced from sheep milk. Raw milk sample was collected from sheep and processed into soft cheese by these coagulants and the amino acid composition of the sample was carried out using standard methods. The result revealed that *Calotropis procera* coagulated soft cheese has the highest essential amino acid content Leucine (10.21g/100g), while steep water from millet coagulated soft cheese has the lowest essential amino acid content methionine (0.72g/100g). However, lemon juice coagulated soft cheese has the highest non essential amino acid glutamic acid (16.27g/100g) in all the cheese samples. In conclusion, this study revealed that highly nutritious soft cheese can also be gotten from sheep milk other than the commonly used cow milk and other coagulants such as lemon juice can compete favorably well with *Calotropis procera* in production of highly nutritious soft cheese. It is therefore recommended that soft cheese produced from sheep milk coagulated by lemon juice should be incorporated into daily diet due to its highly nutritional content.

I10. Industrial and Food Microbiology and Biotechnology

P389. *Penicillium crustosum*: a threat to food safety?

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With the continuous growth of the world population, substantial increases in food production will be required as well as the need to guarantee its safety. Problems related to food/feed contamination have been frequently reported, including those related with fungi and their production of mycotoxins. Mycotoxins are fungal secondary metabolites that can cause severe health issues in either humans or domesticated animals when ingested, inhaled and/or absorbed. Ochratoxin A (OTA) is one of the most studied and poses a severe health risk. This toxin is present in a wide diversity of food and feed products with *Aspergillus* and *Penicillium* species being mainly associated with food spoilage and OTA contamination. The major OTA producers are *P. verrucosum*, *P. nordicum* and *A. carbonarius*. Recent studies have reported the presence of OTA in food matrices where known OTA producers are not present. Therefore, and based on previous evidences, other species, such as *P. crustosum*, are now being considered. The main goal of this work was to search for potential OTA producers among *P. crustosum* strains with different geographic origins and to search for potential genetic differences at the sub-species level.
A total of 44 strains of *P. crustosum* from different parts of the globe were studied. Mycotoxin production was analysed by HPLC-FL. In addition, genes associated with OTA production (ochratoxin polyketide synthase, ochratoxin non-ribosomal peptide synthetase and an ochratoxin transport protein) were tested. RAPD-PCR fingerprinting (M13 and GACA4) and beta-tubulin gene sequencing were used to perform a wide molecular characterisation.
Genetic differences between isolates were found allowing the clustering of strains from the same geographic region, except for isolates from Europe. Under the studied conditions, and with a HPLC-FL detection limit of 7.6 ng/ml, preliminary results showed that OTA was not detected for all studied strains. However, regarding the genes associated with OTA production, there were 4 positive strains for the 3 genes. Nevertheless, further studies with a broader array of conditions need to be considered.

I10. Industrial and Food Microbiology and Biotechnology

P390. Basidiomycete mushrooms isolated in Brazil: antioxidant power of *Trametes hirsuta* GMA-01 and *Lepista sordida* GMA-05

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Mushrooms have nutritional characteristics that make them dietary alternatives by their richness in protein, vitamins, minerals, essential amino acids, fibers and other nutrients. There are different varieties of mushrooms, many still unknown, each one with its characteristic flavor, color, odor and texture. Due to their particular properties, as great sources of molecules beneficial to human health, such macrofungi should be widely studied to understand better what biodiversity has to offer for human consumption. Thus, the objective of this study was to perform the isolation, molecular identification, aqueous and phenolic extractions from mycelium and the evaluation of antioxidant power from two native basidiomycete mushrooms, *Trametes hirsuta* and *Lepista sordida*. The microorganisms were isolated in Ribeirão Preto, São Paulo, Brazil and were identified by molecular markers. For growth, they were submitted to submerged fermentation at 28 °C and 120 rpm for 12 days in medium consisting of wheat bran extract for biomass production. Aqueous and phenolic extractions were performed. To measure antioxidant activity, four techniques were used: total polyphenols, ABTS, TEAC-ABTS and TEAC-DPPH. After isolation and molecular identification by ITS, the isolates were identified as *T. hirsuta* GMA-01 and *L. sordida* GMA-05. Regarding the antioxidant tests, *T. hirsuta* obtained the highest amount of phenolic compounds, 12.18 ± 0.39 and 1.64 ± 0.82 µg gallic acid equivalents (GAE) mg-1 extract in the aqueous and phenolic extractions, respectively. It was also the best in the ABTS and TEAC-ABTS tests, especially the aqueous extraction, which obtained EC_{50} values of 0.325 ± 0.01 mg mL-1 for ABTS and 1.40 ± 0.09 µM Trolox equivalents. (TE) mg-1 extract for TEAC-ABTS. While for TEAC-DPPH, *L. sordida* was the most concise, presenting 1.60 ± 0.04 and 3.63 ± 0.27 µM of TE mg-1 extract in aqueous and phenolic extractions, respectively. This study demonstrates the potential of mushroom biodiversity in producing antioxidant molecules, that could be used in many different areas, such as the food and pharmaceutical industry.

I10. Industrial and Food Microbiology and Biotechnology

P391. Optimization of fungal xyloglucanase and lichenase production by factory design

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Xyloglucanases are enzymes that cleave the xyloglucan polymer and have great utility in the degradation and conversion of lignocellulosic biomass, mainly due to their synergistic potential with cellulases in plant cell wall degradation. In addition, enzymes that degrading and/or modifying xyloglucan may find use in the production of novel oligoxyl glucan surfactants, the pharmaceutical, textile and paper industries. Lichenases (β-1,3; 1,4-glucanases), endo-glucanases, belong to the family 16 of glycosyl hydrolases (GH16) and hydrolyze the β-D-1,4 glycosidic bond adjacent to glucose residues 3-O-substituted, being inactive against β-1,4-glucans. The major products of β-glucan hydrolysis are: 3-O-β-cellobiosyl-D-glycopyranose trisaccharide and 3-O-β-cellotriosyl-D-glycopyranose tetrasaccharide. Its most important biotechnological applications are in the brewing aid and the feed industry. The aim of this study was to optimize the production of *Trichoderma koningii* LMBC 170 xyloglucanase and *Thermothelomyces thermophilus* LMBC 162 lichenase. A solution at a concentration of 107 spores/mL was inoculated into 125 mL Erlenmeyer flasks containing 25 mL Khanna medium supplemented with jatoba (*Hymenaea courbaril* L. (Leguminosae, Caesalpinioideae)) or tamarind (*Tamarindus indica*) seeds pretreated at different temperatures under static conditions or under agitation of 120 rpm for 96 h, with sampling every 24 h. Enzymatic activity was detected by the formation of reducing sugars by the 3,5-dinitrosalicylic acid (DNS) method. Factorial design was used to evaluate the influence of different variables on xyloglucanase and lichenase production and to obtain the best conditions. The best condition for xyloglucanase production was 72 h in agitated culture using 1.705% tamarind as carbon source at 30 ˚C, while the best lichenase production was in static cultivation also at 72 h using 1.5% tamarind as a carbon source at 50 ˚C. From the data it can be concluded that tamarind at higher concentrations induces a better production of these enzymes.

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P392. Assess of acid tolerance of non-typhoidal *Salmonella* and *Enterococcus faecium* from different epidemiological and genetic backgrounds

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Acid stress is one of the most frequently encountered hostile conditions that bacteria have to face (e.g. food-production-chain/feed/disinfectants/human and animal hosts). Nevertheless, acid tolerance profile of bacteria from diverse epidemiological and genetic backgrounds, including multidrug- resistant (MDR), is still poorly explored.

The aim of this study was to assess the susceptibility to acidic-pH of non-typhoidal *Salmonella* and *Enterococcus faecium* (Efm) from diverse origins. We included *Salmonella* (n=66; 23 serotypes) and Efm (n=74; clades A1/A2/B) recovered from human-n=54, food-animal production setting-n=20, food- n=56) and environment-n=10 (1997-2018; 6-countries). The minimum-growth-pH (growth-pHmin) was assessed by broth-microdilution using Mueller-Hinton-II adjusted with HCl (pH=2.0-6.5/16h-20h ±2h/37ºC) and the minimum-survival-pH (survival-pHmin) by plating the microdilution wells without visible growth in Brain-Heart-Infusion-agar (BHI) (24h-48h±2h/37ºC). An Acid-Tolerance-Response (ATR) assay was performed in 3 isolates of each genera (different growth/survival-pHmin), exposing bacteria in log-phase to an acid-shock-challenge (pH=3.0/15'-*Salmonella*/60'-Efm) or to a pre- adaptation to acidic-pH (pH=4.5/60' - for both bacteria) followed by an acid-shock-challenge (pH=3.0/15'-*Salmonella*/60'-Efm). After that a growth-pHmin and survival-pHmin assays were performed.

Most *Salmonella* showed a growth-pHmin of 4.0 (98%- n=65/66) and a survival-pHmin between 4.0 (52%-n=34/66) and 3.5 (48%-n=32/66). In Efm, the growth-pHmin ranged between 4.5 (65%-n=48/74) and 5.0 (35%-n=26/74) and the survival-pHmin between 3.0 (15%-n=11/74), 3.5 (43%- n=32/74) and 4.0 (42%-n=31/74). Only Efm isolates from food (39%-n=11/28), mostly from a poultry-processing-plant using peracetic acid as disinfectant (73%-n=8/11), presented the lowest survival-pHmin 3.0 (73%- MDR/82%-clade A2). Similar survival-pHmin=3.5-4.0 were observed for different Salmonella serotypes and Efm clades. However, a higher percentage of MDR-*Salmonella* (61%-n=27/44) were able to survive at pH=3.5 contrasting with non-MDR-*Salmonella* (23%-n=5/22) ($p<0,05$; Fisher-exact test). The ATR-assay (pre-adaptation acidic pH+acid shock challenge) enhanced survival-pHmin from 3 to 2.5 in 1-Efm (clade A2/MDR/from a poultry processing plant) and from 4 to 3.5 in 1-S. 4,[5],12:i:- (MDR/with mcr-1 gene/from pork meat).

Our data suggest that MDR-*Salmonella* and Efm with diverse epidemiological and genetic backgrounds can survive to low-pH values, although differences among clades/serotypes were not detected. MDR- *Salmonella* showed a better ability to survive to more acidic pH than non-MDR isolates. ATR-assays revealed strain-specific ability to survive under more acidic-pH after a pre-adaptation to middle acidic- pH.

P393. Effect of agitation, substrate consistency and degree of delignification on enzymatic hydrolysis of pretreated *Eucalyptus globulus* bark

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Eucalyptus globulus bark, a residue derived from Portuguese pulp industry, was subjected to a sequence of two treatment stages, to fractionate lignocellulosic components and increasing enzyme accessibility to cellulose. Bark chips were first extracted [1], followed by a kraft pulping[2] (152°C, 60– 75 min, active alkali 22%). The obtained pulp was hydrolyzed using a batch enzymatic hydrolysis with 20 FPU of Cellic® CTec2 per gram of carbohydrate (CH), at 50°C, to produce fermentable sugars (glucose and xylose). Several operation conditions were evaluated: agitation type (orbital and mechanical), impeller type (Rushton turbine, three radial glass blades, three axial glass blades and pitched blade turbine), different substrate consistency (6 and 9 wt%), degree of delignification (IK = 15.5 and 32) and scale-up (100, 350 and 1225 mL). The results showed that the residual lignin content influenced the sugar yields. Higher reducing sugars concentrations (92 mg/mL; hydrolysis yield 98%) were obtained in the assays with IK = 15.5, 350 mL and three radial blade glass impeller, compared to other tested operating conditions. Nevertheless, glass impeller with three axial blades test was better at the initial time-course of the reaction. For IK = 32 and 350 mL, the assays with 9 wt% consistency were unfeasible due to the higher residual lignin content, and homogenation difficulties.

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P394. Saccharification of eucalyptus bark pretreated by steam explosion: the effect of organosolv as a second pretreatment

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Eucalyptus globulus bark can be used as a raw material in a biorefinery, namely to produce simple sugars for fermentation. In this work, that residual biomass was submitted to i) a single steam explosion (SE) pretreatment, under three different conditions or ii) a sequence of SE with a second pretreatment, organosolv (70 wt% EtOH) (SE-OS). The pretreated bark samples were hydrolyzed using a batch enzymatic hydrolysis in 100 mL Erlenmeyer flasks, with orbital agitation (150 rpm) using Cellic® CTec2 at 50°C, varying the substrate consistency (6 and 9 wt%) and the enzymatic loading (20 and 40 FPU per gram carbohydrate, CH). In the performed tests with steam-exploded bark at 6 wt% of consistency and 40 FPU/gCH, the concentration of glucose (by HPLC) increases to 39 mg/mL, compared with other assays (6 wt% of consistency and 20 FPU/gCH or 9 wt% of consistency and 40 FPU/gCH). On the other hand, when the SE-OS pulps were tested, the highest concentration occurred at 9 wt% of consistency and 40 FPU/gCH, circa 28 mg/mL. Oven drying at 40°C was previously applied in the SE-OS pretreated biomass to eliminate residual ethanol (remaining from the OS treatment). The dried samples were directly used in the enzymatic hydrolysis stage, which could have been a reason for the decrease in glucose production, since wet substrates are more digestible in biological processes than dried ones. In conclusion, no improvement was observed in the saccharification yield of eucalyptus bark when organosolv was applied after steam explosion pretreatment.

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I10. Industrial and Food Microbiology and Biotechnology

P395. Cellulase modification for cellulosic biorefining improvement

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In recent years, the search for sustainable technologies and processes has been largely concerned. By that, the use of cellulase has a special role in many industry fields, namely in the pulp and paper process. In the present work, the enzymatic treatment has been studied as a pre-treatment to the mechanical refining, in order to decrease the energy consumption and to improve the paper characteristics. For that, modifications of the cellulase were made aiming at increasing its diameter and the cellulosic fiber's external fibrillation, improving the paper properties. A commercial cellulase (endo 1,4-β-D-glucanase) from *Trichoderma reesei* (EC 3.2.1.4) was physically and chemically (using carbodiiamide and glutaraldehyde) bounded into some macromolecules (gelatin, sodium alginate and polyacrylic acid), resulting in five modifications. Prior to any modification, the enzyme was characterized in terms of its enzymatic activity, protein content and the activity variation with temperature and pH. The enzyme activity, prior and after the modifications, was determined by CMC assay for endoglucanase, which consists in applying the DNS method for measuring the amount of glucose released during the hydrolysis at 50 ºC and pH 4.8. The higher cellulase activity was obtained at 60 ºC and pH 4.8. Under the operating conditions used in the industry, the enzyme reached less than 50% of its maximum catalytic potential. Throughout the modifications, those made by ionic interaction kept at least 80% of the enzymatic activity, being the gelatin and alginate the best bounded macromolecules. The chemical modification using glutaraldehyde showed a significant decrease in activity, probably due to the enzyme crosslinking. In summary, the modification methodologies need a more profound study, in order to be optimized and applied in the pulp refining step.

I10. Industrial and Food Microbiology and Biotechnology

P396. FT-IR spectroscopy: a tool to evaluate the impact of high-pressure processing treatments on molecular components of *Listeria monocytogenes*

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Background: High pressure processing (HPP) is an attractive alternative technology to conventional thermal treatments for inactivation of foodborne pathogenic bacteria. Despite its interest, the effect of HPP on bacterial cellular components is not well established, undermining the development of strategies for circumventing the emergence of HPP-tolerant bacteria. FT-IR spectroscopy, a low-cost and high-throughput methodology enable to detect small variations on bacterial macromolecular cellular components, has been used to elucidate cellular changes occurring in response to food-related stress conditions. However, FT-IR studies analysing HPP effects on molecular components of *Listeria monocytogenes*, an important food-borne pathogen, are few and limited to a reduced number of HPP conditions.

Objectives: To evaluate the impact of different HPP treatments on molecular components of *Listeria monocytogenes*.

Methods: Fourier-transform infrared with attenuated total reflectance (FTIR-ATR) spectra of two clinically relevant *L. monocytogenes* strains (RO15-serotype 4b/herring+spices/Romania/2013; ScottA - 1/2a/milk/France/1992) were acquired from stationary phase growth suspension cells, exposed to HPP treatments (300MPa-2/8/15'; 400MPa-2/8/15'; 600MPa-15') or not, using Nicolet iS50 FT-IR spectrometer (6 replicates/resolution of 4cm^{-1}/32 scan co-additions), and modelled with hierarchical cluster analysis (HCA) and partial least squares discriminant analysis (PLSDA).

Results: Strain specific spectra were observed before and after HPP treatments by HCA. *L. monocytogenes* cells submitted to HPP were clearly discriminated from non-treated cells by PLSDA, with variances occurring in all spectra. Three clusters were evidenced for each strain by HCA, corresponding to cells exposed to 300MPa-2', 400MPa-2' or 600MPa-15'. Additionally, all but two (RO15: 300MPa-8/15', ScottA: 400MPa-8/15') HPP treatments were discriminated by PLSDA, with multiple cell components being affected. Nevertheless, the main spectral variances were observed in proteins/amides I and II (1700-1500cm^{-1}) and in phospholipids/DNA/RNA (1500-1200cm^{-1}) regions.

Conclusions: Proteins and phospholipids of *L. monocytogenes* seems to be the main targets of HPP, which also are possibly differently affected with the different pressure conditions. More studies are needed to elucidate bacteria survival ability under these stresses and the critical targets associated with bacteria stress responses to enhance HPP treatment efficacy (e.g. development of specific food additives used along HPP treatment).

I10. Industrial and Food Microbiology and Biotechnology

P397. Microbial characterization of Serra da Estrela PDO Cheese

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The Serra da Estrela (SE) cheese is a famous traditional Portuguese cheese made from raw sheep milk. It has Protected Designation of Origin status since 1996 and its production represents one of the most important economic activities of the region.
The aim of this work was to characterize the microbial composition of SE cheese at different manufacturing periods within the same production season. For this, SE cheeses were collected from a local producer in January, March and June 2019. Cheese microbial load was analysed by culture plating and colony counting into several selective and differential media. The abundance data was analysed by one-way analysis of variance followed by post hoc Tukey test.
Overall, all cheese samples were absent of pathogenic bacteria. However, *Escherichia coli* was detected at a concentration of ≈2.5 Log CFU/g in January and March, decreasing slightly to 2.2 ± 0.1 Log CFU/g in June. Likewise, the Enterobacteriaceae content found was also relatively high, around 5.18 ± 0.09 Log CFU/g in January, decreasing significantly ($p<0.05$) in the months of March and June to ≈4.1 Log CFU/g. Lactic acid bacteria (LAB) were found at a concentration of ≈9.0 Log CFU/g regardless of the month of analysis. *Enterococcus* spp. was the lowest prevalent LAB genera, with ≈7.7 Log CFU/g, declining even further to 7.2 ± 0.1 Log CFU/g in June ($p<0.05$). On the contrary, *Lactobacillus* spp. presented significant increase in concentration of ≈0.5 Log ($p<0.05$) in June in comparison to January. *Streptococcus* spp. behaved similarly to *Lactobacillus* spp., however the observed increment was noticed immediately in March. Finally, *Leuconostoc* spp. abundance remain roughly unchanged throughout the season at ≈8.4 Log CFU/g.
Although the SE cheese manufacturing process uses raw sheep milk and no added starter cultures, our analysis shows that the tested samples presented acceptable microbiological quality. Across the production season, the LAB content remains roughly unchanged although some variations in specific genera were detected.
Characterization studies of traditional food products, especially Protected Designation of Origin and Protected Geographical Indication, are essential in the combat against food fraud.

I10. Industrial and Food Microbiology and Biotechnology

P398. New design of stirred reactors with high efficiency

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Research centres and biotechnology companies have developed in the last decades many new processes for complex compounds of industrial and economical interest. The key element in most of these processes are mixing tanks or stirred reactors. In contrast with the enormous effort that has been done to develop those new processes, little has been done to optimize stirred reactors used in biotechnology.
Computational Fluid Dynamics (CFD) has become an extremely useful tool for the design of chemical and biological reactors. We present experimental and numerical results of reactors that we have designed with the help of CFD showing an increase in efficiency in terms of:

1. Mass transfer
2. Mixing time
3. Reduction of shear stress suffered by the culture.

The design of the gas injection diffuser and the agitation blades are key factors to improve the mass transfer in gas-liquid reactors. Both elements are very important to get a uniform distribution and small bubble size inside the reactor and to avoid coalescence. An increment of the Kla coefficient of about 100% can be obtained with the aid of CFD. We have compared a standard stirred reactor design with a new design developed by D&BTech. We will show experimental results of an external test carried out in a 10 litres air-water stirred reactor.

The modification of the agitator design also allows to reduce the mixing time, which can be quantified by using the Uniformity Index (UI) over a fluid tracer. UI follows a first order dynamics in a stirred tank. Thus the mixing time can be computed as the dynamics' time constant and can be substantially reduced with the help of CFD. New designs show reductions of more than 75%.

Shear stress is a problem for delicate culture. Boundary layers and blades edges are zones of high shear. We have developed some techniques to minimise this problem in stirred tanks. In our numeric simulations we can reduce to less than 20% the maximum values and the size of regions of high shear stress.

I10. Industrial and Food Microbiology and Biotechnology

P399. Screening of the bioactive activities of various plant essential oils used in the Mediterranean diet

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Essential oils (EOs) are secondary metabolites from aromatic plants, which have been vastly used as perfume fragrances, in folk medicine, and also in culinary as flavouring agents. EOs have received especial attention as natural compounds presenting benefits for human health and food industry. In fact, EOs obtained from plants used in Mediterranean diet have demonstrated antimicrobial and antioxidant activities, which can be seen as an advantage for using as an alternative to synthetic preservatives for increase of shelf-life and safety of food products. Considering this, we selected five aromatic plants used in Mediterranean diet and traditional medicine and evaluated the bioactive properties of their different EOs (*Foeniculum officinalis*, *Melissa officinalis*, *Mentha pulegium*, *Thymus mastichina*, and *Thymus zyguis*). For this purpose, the antioxidant activity was evaluated by the 2,2- diphenyl-1-picrylhydrazyl (DPPH) method. The antimicrobial activity of the EOs was screened against several Gram-positive and Gram-negative bacteria and also yeasts, through disc diffusion, microdilution method and the vapor-phase antimicrobial activity determination. Amongst the tested EOs, *T. zyguis* presented the strongest antioxidant activity. The largest diameter of inhibition zone value was obtained for *M. officinalis* against *Candida albicans* (83.35±1.45 mm). *M. officinalis*, *M. pulegium*, *T. mastichina* and *T. zyguis* EOs showed the highest antimicrobial activity with MIC values between 0.25 and 32
µL/mL. Only the volatile compounds of *T. zyguis* and *M. officinalis* EOs demonstrated activity against bacteria and yeasts with inhibitory zones between 14.46±2.80 and 84.20±1.21 mm, with exception of some of the Gram-negative bacteria tested. These results describe the potential of these EOs as antioxidants, antibacterial and antifungal agents, pointing them as a good source of natural products with bioactive properties. Future research should explore these bioactivities and validate its application as potential antimicrobial.

I10. Industrial and Food Microbiology and Biotechnology

P400. Development of a Novel Fresh Cheese Incorporating Ripened Cheese Surpluses

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The global volume of food waste is estimated at 1.6 billion tons of “primary product equivalents”. Hence, avoiding such waste by valorization of the products, as encompassed by a circular economy, has gained much attention.
The current project aimed at the development of a novel fresh cheese product incorporating (ripened) cheese, which may come from the surpluses of the dairy industry, thus representing a mitigation of food waste. The added ripened cheese provide nutritional value to the fresh cheese, as well as a multitude of flavor compounds.
Ripened cheese can be dispersed into submillimeter particles when mixed in a hot paste of gelatinized starch, forming a melted cheese base (MCB). In this work, corn or waxy rice starch were dispersed in cold semi-skimmed HTST milk, and the mixture was heated for 5 minutes, with continuous stirring, until 85 ºC (for corn starch), or 90 ºC (for waxy rice starch). At this point, the gelatinization of the starch was noticeable and grated or finely cut cheese was added. Ewes’, goats’, Emmental and Cheddar cheeses were used in this stage. The mixture was removed from the hotplate and stirred until the cheese was fully dispersed, with no visible, macroscopic pieces. The MCBs were left to cool down to room temperature, and subsequently were diluted with milk, or milk enriched with skim milk powder (SMP), and renneted at 35 ºC with *Rhizomucor miehei* enzyme.
Several chemical and physical tests were then performed on the samples, such as macronutrient analyses, evaluation of syneresis, texture profile, and sensory analysis.
Results showed that gel formation of the mixture was hindered above a certain level of incorporation of ripened cheese, but this can be overcome by the addition of SMP to the preparation. Starch and SMP both reduced syneresis of the renneted gel. Starch seemed to decrease gel hardness, but addition of SMP had an opposite effect. The sensory attributes of the fresh cheeses could be modulated by varying the amount and type of ripened cheese, and of extra casein. The technical viability and consumer acceptability of these novel fresh cheeses were demonstrated.

I10. Industrial and Food Microbiology and Biotechnology

P401. Effect of Climate Change on Biofilm Forming Abilities of Pathogenic *Escherichia coli* Groups during the Production of Green Leafy Vegetables

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Recently, the demand for freshly consumed green leafy vegetables has increased with the changing eating habits. However, ready to eat salads prepared with these green leafy vegetables are common sources of foodborne outbreaks in the world. It is known that the spread, survival and growth of foodborne pathogens are affected by the changes in seasonality, which sets forth that climate change may have an effect on survival of foodborne pathogens. Therefore, this study aimed to investigate whether climate change increases the formation of biofilms in Enterohemorrhagic *Escherichia coli* (EHEC) and Enteroaggregative *E. coli* (EAEC) groups phenotypically. Preliminary, the effect of temperature on biofilm formation and survival of *E. coli* O157:H7, Shiga toxin producer O104: H4 and O78:H2 was analyzed on polystyrene 96-well plate. These bacteria were non-adherent to surface at 15°C. However, it was observed that biofilm forming abilities of EAEC O104:H4 and O78:H2 were weak and moderately adherent at 37°C, while EHEC O157:H7 was non-adherent at 37°C. This result suggests that temperature aspect of climate change may impact on biofilm formation and survival of EAEC.

I10. Industrial and Food Microbiology and Biotechnology

P402. Probiotic properties of lactic acid bacteria isolated from fermented foods

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Probiotic microorganisms are mainly ingested by the consumption of fermented foods. In general, for commercial purposes, and depending on the product, potential probiotics that are species and strain dependent should meet a number of requirements, as being safe, functional, beneficial and with technological and physiological properties. The aim of this study was to evaluate the probiotic potential of three lactic acid bacteria isolated from different fermented products.

Two isolates of *Enterococcus faecium* and one of *Leuconostoc lactis* were screened for the presence of virulence factors, virulence genes, antibiotic resistances, resistance to simulated gastrointestinal tract conditions in different food matrices and ability to adhere to human colon adenocarcinoma cell lines Caco-2. Only *L. lactis* was considered safe and selected for further tests since i) did not show any of the virulence factors tested (presence of haemolysis, production of hydrolytic enzymes Dnase and gelatinase and production of biogenic amines) as well as ii) any virulence gene with exception of *asa1* gene (aggregation substance protein), and iii) it was also susceptible to all antibiotics tested. *L. lactis* was able to resist during 4h in the presence of bile salts, but cells in acidic condition (pH 2.5) were reduced approximately 2 log cycles either in the presence or absence of pepsin. The sensitivity of *L. lactis* to acidic conditions led to the inability to survive through simulated gastrointestinal tract conditions. However, when incorporated into a complex food matrix only a reduction of approximately 2 log cycles occurred. *L. lactis* was also able to adhere in vitro to human colon adenocarcinoma cell lines Caco-2.

In conclusion, even though in vivo studies should be performed, *L. lactis* isolate seems to be a potential probiotic to be used in the food industry.

I10. Industrial and Food Microbiology and Biotechnology

P403. Enumeration and isolation of acid acetic bacteria in kombucha during fermentation

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Kombucha is a non-alcoholic fermented beverage. The fermentation is performed by a consortium of lactic acid bacteria, yeast, and acetic bacteria. The exact microbial composition is dependent on the source of the inoculum and the conditions of fermentation. However, the dominant bacteria in Kombucha tea culture are acetic acid bacteria (AAB). The main genera of AAB present in Kombucha are *Acetobacter* and *Gluconobacter*. These microorganisms are responsible cellulosic floating matrix producing on the surface of fermented tea. Considering the limitations of commercial culture medium for acetic bacteria enumeration and identification, this work aimed to evaluate formulated culture media describe in literature. The samples of Kombucha (water, sucrose [0.8g.L^{-1}], green tea [0.15g.L^{-1}]) were collected in different times of fermentation (0, 3, 7, 10 and 15 days) for AAB enumeration. A total of 8 culture medium: YGM, YG, R.A.E, MYP, AE, Suomaleinem, Moraes and GYC was used for AAB enumeration and plates were incubated at 30°C for 96 h. The BAA counts ranged to 4.16 (0 days) from 5.96 log10. mL^{-1}(15 days) in AE; in RAE the BAA count variated from 4.19 (0 days) from 5.4 log10. mL^{-1}(15 days); the results observed in GYC was 4.12 for 0 days and 6.84 log10. mL^{-1} for 15 days; in MYP, the counts ranged to 4.63 (0 days) from 7.20 log10. mL^{-1}(15 days); in Moraes, the count's recovery was 4.16 in 0 days and 5.96 log10. mL^{-1} in 15 days; in Soumaleinem the counts varied to 4.85 (0 days) from 6.78 log10. mL^{-1}(15 days); in Carr, the counts were 4.21 in 0 days and 6.87 log10. mL^{-1} in 15 days and DSM the AAB count was 3.21 (0 days) and 5.96 log10. mL^{-1}(15 days). In general, a higher count of AAB, during the fermentation, was the recovery in Sumomaleinem, except at the end of fermentation, the higher count was found in MYP. The lower microbial recovery was observed in DSM (3.62 log10. mL^{-1} [0 days] and 5.93 log10. mL-1[15 days]). Thus, the composition of culture media influenced by AAB recovery.

I10. Industrial and Food Microbiology and Biotechnology

P404. Analyses of microbial diversity of Kombucha during fermentation

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Kombucha is a traditional Asiatic beverage produced by fermentation of *Camelia sinensis* infusion. Kombucha fermentation is a symbiotic process by yeast, lactic acid bacteria, and acetic acid bacteria (AAB). In literature, there are few studies for microbial ecology of Kombucha. Therefore, this study aimed to assess microbial diversity of Kombucha fermented from black and green infusion. The infusions were fermented, separately, for 15 days at 28 °C. For microbial enumeration, the samples collected during the fermentation (0, 3, 7, 10 e 15 days). For AAB enumeration and identification, the samples were plated in four culture media: R.A.E, MYP, Suomaleinem, and Moraes. The plates were incubated at 28°C for 96 h, after incubation, ninety-four isolates identified by 16S sequencing. For yeast, the counts done in GYMP and Sabouraud supplemented with chloramphenicol (0,05g.L^{-1}) and incubated at 30 °C for 96 h. After incubation, thirty isolates identified by 16s sequencing. The AAB counts ranged to 4.75log10 UFC.mL^{-1} from 7.4log10 UFC.mL^{-1} in both teas. The yeast counts were 5.25log10 UFC.mL^{-1} at the beginning of fermentation, after 15 days the yeast population was 6.80log10 UFC.mL^{-1}. Two genera of yeast were detected, including *Candida* and *Scheffersomyces*. For AAB the genus identified was *Komagataeibacter*. Meanwhile, was possible identify 3 species: *Komagataeibacter hansenii* (n=42), *Komagataibacter europaeus* (n=20) e *Komagataeibacter xylinus* (n=19). The combination of cultivation methods and 16S sequencing was a useful approach for assess microbial diversity of Kombucha.

I10. Industrial and Food Microbiology and Biotechnology

P405. Effects of moderate electric fields on fungal load and shelf-life of chestnuts

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The chestnut (*Castanea sativa* Mill.) is a seasonal product that needs a post-harvest treatment to control fungal and insect worms development in order to extend its shelf-life. Studies have demonstrated the feasibility of ohmic heating (OH) and the effects of its moderate electric fields during microbiological inactivation treatments for food applications. This emerging thermal technology can be an alternative to chestnut conventional hydrothermal treatment (HT) or to its chemical fumigation. The aim of this study was to evaluate OH capacity to extend shelf-life of chestnuts fruits by eliminating its fungal load. Five treatments were carried out: I) unprocessed chestnuts (control), II) HT (50 ℃, 45 min), III) OH at 35 ºC, IV) OH at 45 ºC and V) OH at 55 °C. The ohmic processing was conducted in a 0.5 M NaCl solution at an electric field of 9 (V/cm) for 2.00, 2.83 and 3.33 min, respectively for each temperature. Then, the chestnuts were stored for 60 days under different atmospheric conditions: i) 25°C with 40% RH, ii) 25 °C with 90% RH, and iii) 5 °C with 70% RH. Once a week the chestnuts were visually inspected for fungal and insect larvae development. Additionally, fungal load of chestnuts (log CFU/g) was determined by plating serial dilutions in RBC agar. The results showed significant differences ($p>0.05$) in counts of fungi between unprocessed and treated samples, in particular when the storage of chestnuts was done at 5 ºC. The OH treatment conducted at 55 ºC showed the better results; at 5 ºC and 70% RH, no fungi could be detected in samples for 60 days; while at room temperature (25 ºC), no fungi were detected for 24 days. After this period, 3.8 to 5.1 log CFU/g were detected in fruits without any visible formation of molds. The HT performed better then OH at 35 ºC, but fungi could be detected after 24 days of storage at 5 ºC (3.8 to 4.9 log CFU/g). In conclusion, OH showed to be an effective alternative HT, allowing to reduce postharvest decay of chestnuts, increase its shelf-life, and thus meet food-safety international trade regulations.

I10. Industrial and Food Microbiology and Biotechnology

P406. Detection of Hepatitis A, Norovirus GI and GII genomes in wild and farmed fish captured along the Portuguese coast

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The presence of enteric viruses in water, food, and environment is a global concern, being norovirus (NoV) and Hepatitis A (HAV) the most frequently reported agents in foodborne outbreaks. These viruses can be transmitted primarily by person-to-person contact with infected individuals, or through the consumption of contaminated water and food, such as raw or undercooked seafood, frequently contaminated by exposure to urban wastewater. Usually, fish is not a concern regarding these viruses since it is mainly consumed cooked. Although in the last years, raw fish consumption is becoming more and more common among cuisine chefs, namely the use of fish like gilthead seabream, Atlantic mackerel and seabass in dishes like sushi, sashimi, poke and carpaccio.
In this study, a multiplex qPCR assay was developed for the simultaneous detection/quantification of HAV, NoV GI and GII genomes in fish tissue samples, using two types of templates (plasmid and in vitro transcribed RNA).
A total of 281 fish tissue were analyzed, mainly from four different species from the Atlantic coast: gilthead seabream and seabass, two of the most extensively farmed species in aquacultures and captured in the wild; and sardine and Atlantic horse mackerel, two of the most consumed fish species in Portugal.
As a result, our data revealed the presence of HAV, NoV GI and GII genomes in six pooled tissues, confirming that fish captured along the Portuguese coastal waters were exposed to these viruses at some point of their course, indicating the circulation of pathogenic enteric viruses in sea water or during fish transportation/handling. The monitoring of these pathogens in order to properly assess the risks associated with human health is crucial, since NoV and HAV has been determined to be of greatest concern from a food safety perspective based on their incidence, the severity of disease, including mortality, and their potential for transmission, making their control even more difficult.

I10. Industrial and Food Microbiology and Biotechnology

P407. Optically pure lactic acid from lignocellulosic feedstock - A study on glucose and mannose consumption

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Lactic acid (LA) is a chemical with a range of applications, and production demands have been increasing. The production of optically pure lactic acid based on renewable resources is of interest, since it gives possibility for tailor-made polylactic acid (PLA), with specific physical properties associated with different D- to L-LA ratios. This would enable highly functional and biodegradable polymers to be produced from biorenewable resources.
The wood sector is of large economic importance to Sweden, which one of the largest exporters of pulp, paper and sawn wood. Two-thirds of the territory of Sweden is covered by forest, most of which (>80%) is softwood (1). In comparison to hardwood and agricultural residues, which are rich in glucan and xylan, softwoods have a high content in mannan. For example, the typical dry mass composition of spruce is 43,8% glucan, 14,5% mannan and only 6,3% xylan (2), which means softwood hydrolyzates are rich in mannose.
In this work, two strains of the bacterium *Pediococcus acidilactici* engineered for the production of pure L- and D-LA isomers are investigated. The aim of the work is to assess fermentation performance of glucose and mannose in order to frame a study on softwood hydrolysate usage for optically pure lactic acid production.

I10. Industrial and Food Microbiology and Biotechnology

P408. Study of physiological and biochemical events leading to vitrification of *Arbutus unedo* L. cultured in vitro for the development of improved food biotechnology methods

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Arbutus unedo is an important agroeconomic species that has raised interest of the food industry to explore its pomological value, and promote the development of plant biotechnology methods for the propagation and improvement of this species. *In vitro* propagation of *A. unedo* in liquid media is an advantageous procedure that reduces the time for multiplication. However, tissue culture in liquid media often leads to the excessive accumulation of water in the apoplast that ends in shoots vitrification. Morphology and chemical-derived differences in the cell wall are pointed to have a major role in the fragile nature of vitrified plants. Fourier-Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR-ATR) was employed to assess the chemical-derived differences between vitrified and non-vitrified cell walls. Among the differences found, several spectral assignments were achieved for cellulose and matrix polysaccharides like pectin and hemicellulose. Concerning the spectral-related lignin differences, the FTIR-ATR analyses predicted that this polyphenol is present in leaves in higher amounts comparing to stems, and lignin measurements by the acetyl bromide procedure suggested the same pattern. However, further analyses following other procedures are required to better explain these unexpected results. Anatomical studies by scanning electron microscopy unveiled different outstanding epidermal patterns in the abaxial surfaces of vitrified leaves, when comparing with the normal and non- vitrified ones. These epidermal differences may be related with possible lack of epicuticular waxes and thinner cuticles in vitrified leaves, explaining the acclimatization difficulties reported by other authors. Regarding the many abnormal stomata exhibited by vitrified plants, this may justify their difficulty to balance absorption and evapotranspiration. Moreover, cross-sectioned leaves revealed broad lacunar spaces, as well cross-sectioned stems have spaced-hypertrophic cortical parenchyma cells. These apoplastic gaps were related with the accumulation of water responsible for vitrification. Finally, the characterization of vitrified tissues is considered an important contribution to uncover the mechanisms causing plants vitrification, not just by improving the methods developed for the micropropagation of this species, but also contributing to the valorization of this agroeconomic valuable species, in Portugal and in all the Mediterranean region.

I10. Industrial and Food Microbiology and Biotechnology

P409. Comparison of glucose release capacity in different rice products using a simplified *in vitro* strategy

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In general, rice is considered a high glycemic index (GI) food. Several factors may influence rice GI from food or meal components (, to processing methods (. *In vitro* estimation of the amounts of glucose released during gastrointestinal digestion of rice/ rice mixes can be useful to select those with lower GI for incorporation in a healthier diet. The aim of this work was to compare the GI of different rice products when submitted to simulated gastrointestinal conditions using a simple and low cost method (to estimate starch hydrolysis, and consequent glucose release. Cooked long-grain white rice and 4 pre-cooked brown rice samples (plain (PBR), with added mixture of 5 wholegrain cereals (BR5Mix), with added quinoa (BRQ) or with added mixture of vegetables and seeds (BRVS)) were submitted to simulated gastrointestinal digestion using a standardized *in vitro* digestion protocol according to Minekus *et al.* (2014). Samples were taken in duplicate after oral, gastric and intestinal phases and were analyzed using the glucose oxidase/peroxidase method and the DNS method to determine glucose release/hydrolyzed starch. Total starch was determined using Megazyme's "Total Starch" (amyloglucosidase/ α-amylase method - AOAC method 996.11). The four types of pre-cooked rice showed hydrolyzed starch rates and consequent glucose release ranging from 45% to 69%. BR5Mix revealed the highest percentage of hydrolyzed starch (69%), followed by BRVS (65%), BRQ (53%) and PBR (45%). Long grain white rice, contrary to expected, showed the lowest rate of hydrolyzed starch (38%). This low glucose percentage may be explained by factors such as the grain cooking time, the amount of water added at boiling, or the formation of resistant starch upon cooling. The fact that the pre-cooked brown rices have a higher percentage of starch hydrolysis may be related to the fact that these have a higher degree of gelatinization (due to pre-cooking) compared to white rice, which possibly allowed a higher digestibility of rice grains along the TGI.

References: Minekus *et al.* 2014. Food & function. 5:1113-1124.

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I10. Industrial and Food Microbiology and Biotechnology

P410. Production of an enzymatic cocktail by *Aspergillus awamori* grown on corn straw with stirred tank bioreactor

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Increased agroindustrial activity has led to the accumulation of large amounts of lignocellulosic material (LCM). LCM is nature's most abundant source of renewable carbon, representing a valuable industrial substrate with potential for many applications. Thus, the objective of this work was to screen for different holocellulases and to analyze the production of an *Aspergillus awamori* enzyme cocktail grown in corn straw (CS) using a stirred tank bioreactor. Screening was performed with *A. clavatus*, *A. flavus*, *A. terreus*, *A. niveus*, *A. awamori* and *A. brasiliensis* cultivated in minimal medium (MM), with 1% CS at 30 ºC or 37 ºC (for *A. niveus*), 120 rpm, for 5 days. Xylanase (XYN) and endoglucanase (EG) activities were evaluated by formation of reducing sugars using dinitrosalicylic acid (DNS). Cellobiohydrolase (CBH), β-glucosidase (BGL) and β-xylosidase (BXL) were determined by cleavage of PNP-β-D-cellobioside, PNP-β-D-glucopyranoside and PNP-β-D- xylopyranoside, respectively. After screening, a pre-inoculum was prepared with the best enzyme producer using a 500 mL MM flask and incubated at 30 °C, 120 rpm for 48 hours. The increase of enzyme production was performed in a Benchtop BioFlo 310 bioreactor, with 4.5 L of MM and 1% of CS, and was then inoculated the best enzyme producer. Cultivation was performed at 30 °C, pH 6.5, 275 rpm, air flow 2 v.v.m., for 5 days. During the screening, all fungi presented EG, CBH, BGL, XYN and BXL activities. However, *A. awamori* was chosen to continue the experiments because of its BXL activity which was 12.6 times higher than that produced by *A. niveus*. At the scale-up stage, XYN production (47.80 U/ mL) increased 4.1-fold compared to flask activity (11.52 U/mL). BXL also showed 1.6-times higher activity, as well as EG, CBH and BGL, which improved 2.3, 3.3 and 1.2 times their activities, respectively. It was concluded that the staggering of cocktail production improved the enzymatic activities and that corn straw is an excellent source of induction. Furthermore, this cocktail has the potential to be applied in the hydrolysis of different LCM due to the range of holocellulases present.

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I10. Industrial and Food Microbiology and Biotechnology

P411. Sensitivity of qPCR and classical culturable method in *Campylobacter* detection in poultry samples

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Campylobacter is the most frequent diagnosed bacterial cause of human gastroenteritis in UE and poultry are the main reservoir of this bacteria, especially of *C. jejuni* and *C. coli* due to the capability of this bacteria to adhere and colonize chicken intestine, asymptomatically. For protection of public health, food business operators and official authorities have an obligation to assure that foodstuff should not contain bacteria in quantities that could present an unacceptable risk for human health. These controls are based in microbiological parameters, stipulated in Commission Regulation (EC) 2073/2005.
It is well known that laboratory methods differ in specificity, sensitivity, time consuming and cost. In order to evaluate the accuracy of two laboratory methods to detect, enumerate and identify *C. coli* and *C. jejuni* species in poultry, 50 samples of feces and 50 samples of neck/skin swabs collected in 50 broiler flocks (2016/2018), were analyzed using classic microbiology and qPCR.
The overall prevalence of *Campylobacter* combining both analytical methods was high: 98.0 % in fecal samples; 96.8 % in neck/skin samples before slaughtering (NSBS); 78.9 % in neck/skin samples after slaughtering (NSAS). However, qPCR revealed a higher sensitivity, detecting *Campylobacter* in 96.0 %, 96.8 % and 57.9 % of fecal, NSBS and NSAS samples, respectively, when compared with the performance of culturable methods: 84.0 %, 87.1 % and 52.6 %.
Differences were also found regarding the quantitative assessment; the average number of genome copies/g (feces) and /cm^2 (skin) were 2.46×10^9 and $3{,}0\times10^4$, respectively while using classical microbiology were 3.47×10^7 UFC/g and 1.74×10^3 UFC/cm^2.
To conclude, this study demonstrates the importance of the conjugation of classic microbiology and qPCR to detect *Campylobacter* and define the “real” prevalence in poultry feces and skin. It also suggests the importance of re-think the methods applied by the regulation (EC) 2073/2005 on microbiological criteria for foodstuffs.

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P412. Peracetic Acid tolerance of MDR non-typhoidal *Salmonella* and *Enterococcus faecium* with diverse epidemiological and genetic background

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Bacteria face multiple stresses in different contexts and developed diverse mechanisms to overcome them individually or through events of cross-tolerance. Peracetic acid (PAA) is widely used in the food-chain as antiseptic/disinfectant (20-3000 mg/mL) and induces oxidative-stress in bacteria. However, data about bacterial tolerance to PAA (PAAT) and the conditions inducing such tolerance remain scarce.
Here we assess PAAT of non-typhoidal *Salmonella* and *Enterococcus faecium* from diverse epidemiological and genetic backgrounds and determine if induction with PAA and copper-Cu (also associated with oxidative-stress and widely used in food-animal production settings) increase PAAT. We included *Salmonella* (n=66; 23 serotypes) and *E. faecium* (n=74; clades A1/A2/B) recovered from human (n=54), food-animal production setting (n=20), food (n=56) and environment (n=10) (1997-2018; 6 countries). Most of the isolates were MDR (*E. faecium* 76%-n=56/74; *Salmonella* 67%-n=44/66). The MICPAA was performed by broth-microdilution (ISO20776-1:2006; range: 40-140mg/L) followed by MBCPAA determination (NCCLS:1999) (37ºC/48h; 2 replicas/isolate). Induction assays by PAA and by $CuSO_4$ were performed in 6 *Salmonella* and 6 *E. faecium* (human, food-animal production settings and food sources; with/without Cu tolerance genes: 3 *Salmonella* with *pcoD+silA* genes and 3 *E. faecium* with *tcrB+cueO* genes; diverse MIC/MBCPAA) by exposing bacteria (log-phase: 3-4h) to sub-inhibitory PAA or $CuSO_4$ concentrations (up to 10 and 100 times less the MICPAA/Cu) followed by MICPAA assay.
MICPAA= 40-60 mg/L and MBCPAA= 50-80 mg/L (MIC90= 60 mg/L; MBC90= 70 mg/L) were observed in *Salmonella*, and a MICPAA= 60-100 mg/L and MBCPAA= 80-140 mg/L (MIC90= 90 mg/L; MBC90= 140 mg/L) in *E. faecium*. No differences in MIC/MBCPAA were observed among serotypes/clades, sources or MDR/non-MDR bacteria. The induction with PAA or $CuSO_4$ did not affected the MIC/MBC of *Salmonella* and *E. faecium*.
Our data suggest that a high number of MDR *Salmonella* and *E. faecium* are able to survive to PAA concentrations used in the food-processing industries. Exposure to sub-inhibitory PAA and $CuSO_4$ concentrations, under the tested conditions, does not affect the ability to survive to PAA, in both bacteria. However, further studies are needed to better understand the environmental conditions that can challenge the efficacy of these and other antimicrobial compounds.

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P413. Impact of different viticulture and winemaking practices on microbial biodiversity during wine production

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Background: Portugal is recognized for the increasing quality and uniqueness of its wines, with great impact in the Portuguese economy. Over the last years, Portugal has been following the worldwide trend on development and adoption of innovative and sustainable processes for winemaking. One of the purposes of viticulture world is to emphasize the regionality of wine in order to obtain a distinctive high-quality wine that mirrors the singularity of the region. Wine regionality is known to be intrinsically associated with the concept of terroir and several studies have been carried out to develop vitivinicultural sustainable processes capable of enhance wine identity. Grape and wine must harbor an important and complex microbial population which, together with the remaining elements of the terroir, are directly related with the organoleptic properties of wine. Therefore, in order to produce unique wines, highlighting regional specificities, the microbial consortium present in grape, must and wine, as well as the chemical compounds produced during the winemaking process, must be identified.
Aim: This study aims to understand how changes in viticulture and winemaking practices impact grapevine biodiversity and wine microbiome, and thereby assess their actual consequences on the quality and singularity of the final product.
Experimental Design: For that purpose, a two-year study was carried. Two red grape varieties (Touriga Nacional and Aragonez) were produced in Herdade da Malhadinha Nova (Alentejo Appellation, Portugal) through two different production modes (integrated and organic) and the musts were fermented spontaneously and by the inoculum of commercial yeasts (indigenous yeasts versus commercial strains). The production and fermentation processes of the different eight wines were studied through classical microbiology and metagenomics tools.
Preliminary Results and Future Work: The first results of this ongoing study reveal alterations in biodiversity and microbiome composition and the production of different and unique wines. Future work will evaluate the contribution of the found communities to wine chemical composition and sensorial distinctness.

I10. Industrial and Food Microbiology and Biotechnology

P414. Biogas production through co-digestion of enzymatically pretreated corn bran and cow manure

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Biogas production from wastes is an alternative that contributes positively to the environment and minimize the dependence on fossil energy sources. Additionally, the reuse of biomasses helps to reduce the waste production, but a pretreatment is required to use it in the anaerobic digestion. Here biogas was produced through co-digestion of enzymatically pretreated corn bran and cow manure. Firstly, it was selected the most hydrolysable waste (barley bagasse, sugar cane bagasse, elephant grass, thick orange pie, average orange pie, wheat bran, coffee grounds, orange peel, white sludge, vinasse, corn bran, soy bran, soy peel, cotton bran, cassava husk, cassava flour, banana peel, corn bran, sorghum stem, sorghum seed, total sorghum and wet distiller grain) by the crude extracts containing amylase (secreted by *Aspergillus brasiliensis*), xylanase (*Aspergillus tamarii* Kita) and cellulase (*Trichoderma reesei*, Novozymes®). Later on, different mixtures of these enzymes were studied using simplex-centroid designs. The most hydrolyzed waste by each enzyme individually (measured by reducing sugar using dinitrosalicylic acid, DNS) at 50°C, 120 rpm and 24 h were corn bran, banana peel and sorghum seed. Then, the simplex-centroid designs resulted in model equations and respective response surface contours. Amylase extract had a significant positive influence on corn bran hydrolysis by maximizing the reducing sugar yield when it was used individually (35g/L of reducing sugar). After it, the pretreated corn bran and a cow manure (1:2 g of volatile solids) were employed for biogas production in batch assays. It was found a biogas accumulation of 326 mL in the 12nd day of anaerobic co-digestion, which were similar to the control (containing 35 g/L of glucose alone) and 53% higher than that found with corn bran without enzymatic pretreatment. In conclusion, it was observed that the crude extract optimized for amylase production affected significantly the corn bran hydrolyses and consequently the biogas production in a co-digestion with cow manure.

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P415. Effect of increased air pressure in gluconic acid production by *Aspergillus niger*

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Gluconic acid (GA) is an organic acid with many applications in food and chemical industries. GA is produced mainly by biotechnological processes and *Aspergillus niger* is the most used microorganism. This acid is obtained by the oxidation of glucose catalyzed by the enzyme glucose oxidase and this reaction has a high oxygen demand. Usually, oxygen is the major limiting factor of the process in common stirred tank bioreactors. This limitation can be overcome by increasing the total air pressure of the bioreactor. The goal of this work is the study of the improvement of oxygen mass transfer rate (OTR) from air to culture medium in the production of GA by *A. niger* 9213 using an hyperbaric bioreactor. Batch cultures were performed using glucose as substrate in the presence of calcium carbonate, in a stainless steel stirred tank bioreactor of 400 mL of working volume, at 1 vvm of aeration rate under 1 bar and 4 bar of total air pressure, corresponding to OTR values of 384 $mg \cdot L^{-1} \cdot h^{-1}$ and 768 $mg \cdot L^{-1} \cdot h^{-1}$, respectively.
The increase of air pressure led to a significant improvement of GA production. A 5-fold increase on GA productivity was reached (around 3 $g \cdot L^{-1} \cdot h^{-1}$) by the air pressure raise as well as on glucose uptake rate. The positive effect of pressure on GA production by *A. niger* was obtained using spores solution as inoculum or a pre-grown mycelium culture.
The use of air pressure increase is an effective alternative way of OTR improvement and this showed its applicability for GA production. It presents several advantages compared to the use of oxygen- enriched air that is costly and requires special handling.

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P416. Brewers' spent grain as substrates for production of cellulolytic and hemicellulolytic enzymes by different Aspergillus species

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Brewers' spent grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total by-products generated. It is a lignocellulosic material containing about 38% cellulose, 29% hemicellulose, chiefly arabinoxylans, and 13% lignin. The production of cellulolytic and hemicellulolytic enzymes using this material as substrate represent an eco-friendly strategy for the lignocellulosic biomass hydrolysis, generating fermentable sugars that can be converted into high- added value products, such as bioethanol, lactic acid, xylitol and others. Thus, this work aimed to evaluate the potential of cellulolytic and hemicellulolytic enzymes production by some *Aspergillus* species cultivated in BSG. Fungi were grown in minimum media, pH 6.5, with 1% BSG and inoculum was done with 107spores/mL, cultivated at 30°C, 120 rpm, for 5 days. Every 24 hours 2 mL of the samples were collected. The enzymatic activity was performed after the incubation of the crude extract with 1% Linear arabinan, Xylan from beechwood, Xyloglucan, Locust bean gum and CMC, at 50°C for 60 minutes and the reducing sugars were determined using dinitrosalicylic acid (DNS). Synthetic substrates (2 mM of PNP-α-L-arabinofuranoside, PNP-β-D-xylopyranoside, PNP-β-D-glucopyranoside and PNP-β-D-cellobioside) were also used at the same conditions. The extract from A. niveus showed the best arabinanase (0.284 U/mL) and β-glucosidase (0.126 U/mL) activities after 48 and 96 hours of cultivation, respectively. On the other hand, the extract from A. brasiliensis presented the best activities of α-L-arabinofuranosidase (0.129 U/mL), β-xylosidase (0.265 U/mL) and xylanase (2.15 U/mL) when cultivated for 48 hours. After 72 hours, this fungus also showed the best activities for xyloglucanase (1.06 U/mL), mannanase (0.617 U/mL) and endoglucanase (0.254 U/mL). The extract produced by *A. flavus* presented the best cellobiohydrolase activity with 0.113 U/mL after 120 hours of cultivation. It is important to mention that *A. awamori*, *A. clavatus* and *A. terreus* also showed good levels of different enzymes produced but they were not the best producers. These data suggest the great potential of different cellulolytic and hemicellulolytic enzymes production using BSG as substrate, which represents an eco-friendly destination for the residues and can generate high-added value products with great biotechnological application.

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P417. *Zymomonas mobilis* as a whole-cell biocatalyst for the production of prebiotics

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The increasing demand for “natural” labeled products and the adoption of a healthy life style drive the consumers preferences towards new foodstuffs that enhance the quality of life. Prebiotics belong to this group of products shown to improve well-being and general health. They are selectively fermented by the beneficial colonic bacteria improving the host health, being the fructooligosaccharides (FOS) one of the most commonly used prebiotics. FOS are non-digestible and calorie-free sweeteners, that can be obtained from sucrose using fructosyltransferase or β-fructofuranosidase enzymes from different microbial sources. Other important components of functional food include levan that is a fructan with prebiotic and antitumor activity; and sorbitol that is a non-cariogenic prebiotic sugar.

Zymomonas mobilis is an extensively studied bacteria for ethanol production, being also an interesting chassis to produce other added value products such as levan and sorbitol, since it contains native enzymes able to convert glucose and fructose into other sub-products. Although FOS production by different organisms has been widely reported, the use of *Z. mobilis* strains is poorly explored.

In this study, *Z. mobilis* ZM4 was evaluated as a producer of the described prebiotics. Shake flask experiments were performed at different temperatures (30 and 37°C) and substrate concentrations (100, 200 and 300 g/L). The initial sucrose concentration was found to have a significant influence on the production of all compounds. However, the shift of temperature did not affect significantly the production of sorbitol, FOS, as well as ethanol. The maximum concentration of levan (5.8 g/L) was obtained at 30°C. Overall, the results demonstrated that *Z. mobilis* ZM4 was able to produce a FOS content up to 30 g/L, from 300 g/L of sucrose, under static conditions, being FOS 1-kestose, nystose and 6-kestose the main FOS produced. Under these conditions, around 18 g/L of sorbitol, 5.8 g/L of levan and 50 g/L of ethanol were also produced.

This study demonstrated the potential of a faster and sustainable process for simultaneous production of FOS, sorbitol and levan using *Z. mobilis* ZM4 as a whole-cell biocatalyst.

FP418. On the old story of the accidental pathogen: can transcriptomics clarify how *S. epidermidis* becomes virulent?

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Background
Staphylococcus epidermidis is a commensal inhabitant of healthy human skin and mucosae that can originate important infections such as medical device-associated bloodstream infections, often associated to patients with impaired or undeveloped immune systems. Of concern, the current inability to discriminate between true bacteraemia caused by *S. epidermidis* and contaminated blood cultures often leads to misdiagnosis, resulting in a significant increase in patient morbidity and in healthcare costs. Until now, several genetic and phenotypic approaches were not able to identify any specific marker that clearly distinguishes commensal from isolates that cause infection. Hence, our goal was to identify possible RNA-based molecular markers for the diagnosis of *S. epidermidis* infections, a strategy never reported before.
Method
The transcriptome of three clinical and three commensal isolates exposed to human blood was sequenced using high-throughput RNA-sequencing (RNA-seq). A bioinformatics analysis was used to compare the 6 transcriptomes and to select potential markers that could be used to differentiate true *S. epidermidis* infections from laboratory contamination. Several approaches were performed and the obtained data was further confirmed by qPCR. Biological confirmation was then accomplished by qPCR in a representative worldwide collection of 70 *S. epidermidis* isolates.
Results and Conclusion
We identified and selected 5 genes that where able to discriminate between the 3 clinical from the 3 commensal isolates. However, when testing a wider range of isolates, the discriminative power of the selected genes was no longer observed. This suggested that, in fact, both clinical and commensal isolates are able to adapt to human blood and use similar strategies when causing infection. To demonstrate this principle, the survival rate of all 70 isolates was assessed after incubation with human blood. The results showed that both clinical and commensal isolates shared the same survival capability in human blood. Together, our data reinforces the idea that *S. epidermidis* is an accidental pathogen.

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II11. Bioinformatics, Omics and Bionanotechnologies

FP419. Proteomic to disentail the response to tellurite in highly metal resistant gram-positive bacteria

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Tellurium, a rare metalloid that has gained interest, is ever more present in specific environmental niches as a result of increased mining activity and the uncontrolled disposal of high-tech devices. Higher concentrations of Tellurium represent an increasing selective pressure for organisms present, like bacteria.
The effect of Tellurite [Te(IV)] in highly polymetallic resistant heterotrophic aerobic bacteria was analyzed by determining, in a group of selected strains, the growth variation, metal detoxification and metabolic change, all in the presence of Te(IV).
Bacteria isolated from mining sediments were tested for their minimal inhibitory concentration to several metals, including Te(IV), and growth rates were determined for selected strains. Testing of bacteria's ability to reduce Te(IV) to Te(0) was performed by following depletion of Te(IV) in growth supernatant and simultaneous observation of the formation of Te deposits. Lastly, the metabolic effects were evaluated by determining the differential proteomic, in the presence or absence of tellurite, using LC-MS of total proteins expressed in selected strains with, in house, sequenced genome.
The ability to resist to at least 1mM of Te(IV) is present in 19 strains out of 144 recovered. In a selected subset able to grow in liquid media, the growth rates decreased between 14% and 65% in the presence of Te(IV). The same is seen for the efficiency and rate of reduction of Te(IV) to Te(0), even between phylogenetically similar organisms, with reduction rates reaching values as high as 0.75 mg/OD/h. The presence of Te(IV) affected the general identified protein expression of *Paenibacillus* ALJ109b and *Bacillus* 3W19 strains. In *Paenibacillus* ALJ109b the regulation of 202 proteins, 2.8% of the proteome, were affected, while in *Bacillus* 3W19 the regulation of 393 proteins, 10% of proteome, were affected. The resulting modifications to metabolic pathways were similar despite the unique proteomic response by either strains.
In conclusion, resistance to higher concentrations of Te(IV) is present in a selected group of strains from a collection of polymetallic resistant bacterial. Moreover, the response to Te(IV) is organism-specific as demonstrated by different growth rates, reduction efficiencies, and rates and metabolism, viewed as a differential protein expression.

II11. Bioinformatics, Omics and Bionanotechnologies

FP420. Genome-wide diversification of *Mycobacterium bovis*

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Mycobacterium bovis is one of the members of *Mycobacterium tuberculosis* complex, a group of human and animal pathogens that cause human and animal tuberculosis (TB), respectively, and that exhibit a high level of homology at the nucleotide level. The low sequence diversity makes the study of the evolutionary history of these monomorphic bacteria a challenging assignment. This challenge can be overcome by whole genome sequence (WGS) analysis as the most informative method. Knowledge of the evolutionary patterns and genomic processes of *M. bovis* will help the understanding of TB mechanistic processes.

In this work, a total 44 *M. bovis* isolates recovered from cattle, red deer and wild boar, in a 12-year period (2003-2015) in Portugal, were analysed by WGS. The majority (n=34) were incorporated into European 2, the main clonal complex circulating in Iberian Peninsula.

The comparison of sequences with *M. bovis* reference genome AF2122/97 revealed more than 1.800 sites wherein at least one strain shows a single nucleotide polymorphism (SNP), with a mean of 623 alterations per genome. The majority of SNPs (86.4%) was located in coding regions, with a ratio of non-synonymous and synonymous alterations (dN/dS) superior to 1. The genes with SNPs were classified into functional categories and “cell wall and cell processes”, “intermediary metabolism and respiration” and “conserved hypotheticals” were the most frequent ontological categories, revealing the underlying importance in *M. bovis* evolution.

These results show the application of WGS as a high resolution tool for analysis of *M. bovis* genomic diversity and identification of specific signatures, thus contributing with reliable information concerning the evolutionary history of the pathogen, making possible to trace back past outbreaks in detail and to explore the hypothesis of wildlife as key reservoirs.

II11. Bioinformatics, Omics and Bionanotechnologies

FP421. An effective packaging cell line to highly produce Extracellular Vesicles for therapy of Machado-Joseph Disease

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Objectives:
Extracellular vesicles (EVs) are membrane-contained structures continuously produced by cells with capacity to carry small nucleic acids, particularly miRNAs, non-coding RNAs with around 22 nucleotides that regulate gene expression. Interestingly, miRNA enrichment into EVs has been suggested to have contribution of specific motifs, designated as ExoMotifs, upon interaction with ribonucleoproteins. Therefore, the aim of this work was to investigate whether ExoMotifs and ribonucleoproteins would promote packaging of synthetic miRNA-based silencing sequences into EVs, aiming to create an effective packaging cell line to highly produce therapeutic EVs to be used for therapy of Machado-Joseph disease /Spinocerebellar Ataxia type-3 (MJD/SCA3). MJD/SCA3 is a neurodegenerative disorder caused by abnormal over-repetition of a CAG tract within the ataxin-3 (ATXN3) gene, conferring toxic properties to the corresponding ATXN3 protein.
Methods:
To evaluate whether an Exomotif promotes packaging of miRNAs we first evaluated by qRT-PCR the levels of endogenous miRNAs containing the ExoMotif in EVs derived from human and mouse cell lines. We then associated ExoMotifs to silencing sequences targeting mutant ATXN3 (mutATXN3) and validated its silencing properties by Western blotting, and its packaging efficiency into EVs upon ribonucleoprotein overexpression, by qRT-PCR. Additionally, neuronal targeting proteins were expressed at EVs surface and their neuronal targeting efficiency was evaluated by immunocytochemistry and flow cytometry. For the purpose, we generated a packaging cell line by constitutively expressing these packaging elements with lentiviral vectors in order to overproduce therapeutic EVs that were collected by ultracentrifugation and then used to treat neuronal cells encoding mutATXN3. The treatment silencing efficiency was evaluated by a dual luciferase assay and qRT-PCR.
Results:
We found that endogenous miRNAs containing the ExoMotif were enriched in EVs when comparing to the cytoplasm of their precursor human and mouse cell lines. The silencing sequences with ExoMotifs retained the capacity to silence mutant ataxin-3 and were effectively incorporated into EVs. Furthermore, EV's packaging cell line produce bioengineered EVs that significantly decreased mutATXN3 mRNA levels in neurons.
Conclusions:
This study suggests that a packaging cell line can be an effective tool to highly produce therapeutic EVs, suggesting a novel promising class of vehicles to treat MJD.

P422. Phylogenomics of global linezolid-resistant *Enterococcus faecalis* strains: new insights on clonal and genetic hotspots for optrA acquisition

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Background: The number of linezolid-resistant *Enterococcus faecalis* (LREfs) carrying *optrA* is increasingly reported globally, but human and animal *optrA*-LREfs strains have not been compared in detail. We aimed to compare the genomic content of publicly available *optrA*-Efs genomes to give insights about the pathogenic potential of circulating clones and *optrA* mobilization.

Materials/methods: Twenty-four *optrA*-positive Efs genomes retrieved from GenBank (n=9 hospital; n=3 healthy humans; n=9 chicken; n=5 pigs; n=1 wastewaters; 6 countries) were analysed. Phylogeny was assessed using CSI Phylogeny (CGE: http://www.genomicepidemiology.org; iTOL). Genes encoding antibiotic resistance (ABR-Resfinder) and MLST were screened through CGE and in house databases with 57 virulence and 391 plasmid-replication genes were tested using MyDbFinder-CGE. PHASTER identified the presence of prophages. Genomic data for 23S rDNA and rplC/rplD/rplV mutations were compared to Efs-V583 (Geneious-Prime BLASTN). *optrA*-platforms were compared (Geneious;Easyfig- v2.2.2;VectorNTI-advance-v11) and gene functions annotated (eggNOG4.5.1, http://eggnogdb.embl. de/#/app/home).

Results: A great clonal diversity was observed (16 STs) between the 24 Efs, but two clusters comprising ST476-like [chicken/Tunisia;pigs/Malaysia;hospital/China (84-122-SNPs)] and ST21 [chicken-meat/Tunisia;hospital/China (113-SNPs)] strains were phylogenetically more related than the remaining (average 12.400-SNPs) and exhibited identical profiles of ABR/virulence. The ABR (1-15; average-11), virulence (37-50), plasmids (1-5;18-types) and prophages (1-3;14-types) gene sequences identified were related to a wide range of bacterial genera, mainly but not exclusive from Firmicutes. The location of *optrA* was known for 20/24 isolates (chromosome-n=8; 30-60 Kb plasmids-n=12). The same chromosomal 32kb-platform was identified in all but three LREfs (ST59/ST489-Colombia), all presenting a Δ*radC*-hotspot for *optrA* integration (Figure; 8 Efs had Tn*554::fexA-optrA* inserted here). A novel chimeric pheromone-responsive plasmid from a Tunisian LREfs (meat/ST859) was uncovered.

Conclusions: *optrA* was found in a diversity of strains and platforms, but preferential hotspot clones (ST476- and ST21-like) and the same chromosomal integration site were identified in strains from different sources (human/animal) and continents. The highly efficient site-specific integration-excision mechanism of Tn*554* and the finding of a common chromosomal hotspot for *optrA* integration and mobilization in a variety of clones and sources might explain the easy de novo generation of *optrA*- positive strains.

P423. A bioinformatic approach to understand antibiotic resistance due to small multidrug resistant (SMR)-type efflux pumps in *Acinetobacter baumannii* through whole genome analysis

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Efflux pumps (EPs) of the small multidrug resistant (SMR) family are poorly characterized in *Acinetobacter baumannii*. SMR EPs are implicated in the efflux of multiple organic cationic compounds. Here, we aimed to identify SMR-type EPs in *A. baumannii* through whole genome analysis and compare it with those of other bacterial species analysing their putative implications in antimicrobial resistance.
Bioinformatic analysis was performed using annotated genomes of *A. baumannii* retrieved from NCBI. Screening of candidate SMR proteins and the corresponding orthologous on other bacteria was done using NCBI and KEEG databases. BLASTP were conducted to compare predicted proteins and quantify the similarity between the amino acid sequences. BLAST Ring Image Generator was used to visualize the pairwise BLAST results of the SMR efflux transporters. The genomic arrangement of orthologue genes was analyzed using SyntTax. Subsystem functional categorization of the predicted CDS and visualization was done by using SEED viewer and multi-genome comparison was done with SEED using genome sequences available in the SEED repository.
We identified four chromosomally encoded SMR EPs - AbeS, SMR, EmrE, and SugE - and three SMR EPs encoded in plasmids or class 1 integrons - QacF, QacE and QacEΔ1. Of these, only AbeS, QacE and QacEΔ1 have been studied and associated with drug resistance in *A. baumannii*. We found that the chromosomal EPs are present in all strains whereas only some harbor SMR EPs encoded in mobile elements. We found high level of similarity (> 70%) at protein level with the same EPs in other bacterial species.
We concluded that the presence or absence of several SMR EPs in *A. baumannii* genome might explain the high predisposition this bacteria to resist to disinfectants, biocides and dyes. The finding of SMR orthologues in *A. baumannii* genome may suggest a similar role on drug resistance. This *in silico* approach will be helpful to predict important phenotypic characteristics, for antimicrobials other than antibiotics. In the future, clinical strains and mutants for these EPs will be used in order to demonstrate experimentally the role of these determinants in *A. baumannii*.

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P424. Towards the integration of regulatory networks with metabolic models

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Metabolism is present in every living being and for that reason understanding the mechanisms behind it is essential for comprehending the phenotypical behaviour of the organisms. Metabolic models reconstructed at genome scale level can provide in-depth comprehension of the metabolic processes within an organism, therefore emerging as one of the best strategies for studying these systems. Genome-scale metabolic models can be enriched with information from regulatory networks, such as gene expression data. Merging regulatory networks with metabolic models can help uncovering information that can be used for many applications, for example identifying potential drug targets.

In this work we propose developing bioinformatics tools capable of merging a genome-scale metabolic models with regulatory networks. A tool named CORAMI (Combination Of Regulatory And Metabolic Information) is being developed as a plugin for merlin software. A genome-scale metabolic model of *Saccharomyces cerevisiae* will be imported into merlin in order to collect the necessary metabolic data for CORAMI. Also, annotation data for metabolic genes will be integrated with regulatory genes in order to validate the integration and establish a correlation between these two models. Additionally, the regulatory network of *S. cerevisiae* chosen to be merged with the metabolic model was manual curated. Here we present the strategy for merging the two networks, which together can provide unique insights into *S. cerevisiae*'s phenotypical behaviour. In the future, several tools which will allow the user to explore the total potential of the plugin and retrieve more results for their studies will be developed.

P425. From common plastics to sustainable alternatives: A mechanistic study of a green catalyst

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Common plastics are causing too much damage to the environment and thus it is urgent to find sustainable alternatives [1]. Some biodegradable polymers (e.g. aliphatic polyesters) have been popularized and continuously studied due to their good mechanical properties and compatibility for biomedical applications [1,2]. Enzymatic polymerization is a promising green alternative for the synthesis of aliphatic polyesters [2]. However, wild-type enzymes are not always well suited to work efficiently in high temperatures and in presence of organic solvents [3], which prompts the use of modified enzymes capable to respond at industrial harsh conditions [4]. In this work, we compared and studied in detail the catalytic mechanism of two enzymes using Quantum Mechanics/Molecular Mechanics (QM/MM) Molecular Dynamic simulations. The first one, the *Candida antartica* lipase B (CalB), which is the most studied and used lipase as a catalyst in green synthesis of polyesters. The other one, a hyperthermophilic archaeon *Archaeoglobus fulgidus* carboxylesterase (AfEST), a promising candidate for potential industrial applications, because of its broad substrate specificity and high stability [5]. Our results show that chemical step for the hydrolysis with wild-type AfEST is already very efficient, with an overall free energy barrier of about 12.9 kcal/mol [6]. Also, the obtained results when comparing both enzymes [7], revealed that AfEST is a good starting point for protein engineering solutions that can contribute to solve the current plastic pollution problem.

II11. Bioinformatics, Omics and Bionanotechnologies

P426. Enzymatic transesterification of PCL-PEG co-polymers

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Aliphatic polyesters have attracted much attention in the biomedical field due to their excellent biodegradability, biocompatibility and drug permeability [1]. Polycaprolactone (PCL) and polyethylene glycol (PEG) are two examples of oligomers capable to form these aliphatic polyesters, in which it's slow degradation rate, and solubility with biological compatibility and non-toxicity, respectively, have been exploited in the pharmaceutical industry and biomedical applications, such as tissue engineering, surgical sutures, drug-delivery systems and scaffold fabrication technologies [2,3]. Enzymes can be employed in the synthesis of these polyesters, avoiding the use of metal catalysts which make the process eco-friendlier and its products are safe to human use. They are hydrolases for the carbonyl ester bond of hydrophobic substrates (e.g. triacylglycerols, phospholipids and other insoluble substrates) [4] acting in organic solvents - conditions with high interest for industrial applications [5]. In this work we investigated the full catalytic cycle of *Candida antarctica* lipase B (CalB) for the ring-opening polymerization (ROP) of ε-caprolactone and the transesterification of ethylene glycol in toluene, using Quantum Mechanical/Molecular Mechanical Molecular Dynamics (QM/MM MD) calculations [6].

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P427. Prediction of novel non-coding RNAs relevant for the growth of *Pseudomonas putida* in a bioreactor

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Pseudomonas putida is a microorganism with great potential for industry due to its stress- endurance traits and easy manipulation of the metabolism. However, optimization is still required to improve production yields. In the last years, manipulation of bacterial small non-coding RNAs (ncRNAs) has been recognized as an effective tool to improve the production of industrial compounds. So far, very few ncRNAs are annotated in *P. putida* beyond the generally conserved.
In the present study, *P. putida* was cultivated in a two-compartment scale-down bioreactor that simulates large-scale industrial bioreactors. We performed RNA-Seq of samples collected at distinct locations and time-points to predict novel and potentially important ncRNAs for the adaptation of *P. putida* to bioreactor stress conditions. Instead of using a purely genomic approach, we have rather identified regions of putative ncRNAs with high expression levels using two different programs. Only the regions identified with both approaches were considered for further analysis and, in total, 725 novel ncRNAs were predicted. We also found that their expression was not constant throughout the bioreactor, showing different patterns of expression with time and position. This is the first work focusing on the ncRNAs whose expression is triggered in a bioreactor environment. This information is of great importance for industry, since it provides possible targets to engineer more effective *P. putida* strains for large-scale production.

P428. *Torulaspora delbrueckii* genomic data: bioinformatic analysis towards elucidation of its fermentation capacity and application in wine industry

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The most widely used yeast in wine, beer and bread fermentation is *Saccharomyces cerevisiae*. However, recently, *Torulaspora delbrueckii* attracted interest due to its properties, from flavor and aroma-enhanced wine to the ability to be preserved longer in frozen dough. In this work, the *T. delbrueckii* genomic information publicly available was explored and the annotation of its genome was attempted for the first time. In a second phase, a comparative study was conducted between *T. delbrueckii* and *S. cerevisiae* at genomic level regarding fermentation related genes.
BLAST analysis was performed using the total data available regarding *T. delbrueckii* genomes. Upon deconstructing the output from YGAP (Yeast-Genome-Annotation-Pathway - http://wolfe.ucd. ie/annotation/), it was observed that of the 5063 putative sequences detected for *T. delbrueckii* COFT1 strain, 4525 had an homolog assigned in the *S. cerevisiae* reference strain. Upon applying a 70% similarity cutoff, the species with the highest frequency of hits were *Zygosaccharomyces parabailii* and *Zygosaccharomyces rouxii*. *S. cerevisiae*, although commonly known as the most similar species to *T. delbrueckii*, especially regarding biotechnological potential, appeared only as the third most frequent result. These results emphasize a potential use of the genus *Zygosaccharomyces* in biotechnological industries.
In a second phase, 43 genes were chosen as being involved in fermentative metabolism. Most of those genes revealed as having similarity with genes present in *Z. parabailii*. 19 proteins had no significant hit (considering 70% of similarity) in the BLAST analysis against *S. cerevisiae* wine strain EC1118. Among those, ATF1 and ATF2 popped as the most relevant ones, after literature comparison. These alcohol acetyltransferase genes have a major role in the synthesis of a broad range of volatile esters during winemaking. These genes could be the responsible for the differences found in *T. delbrueckii* fermentations, that distinguish them from the ones obtained with *S. cerevisiae*, specially regarding aroma compounds formation.

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II11. Bioinformatics, Omics and Bionanotechnologies

P429. YEASTRACT+: a new portal for genome-wide comparison of transcription regulation in yeasts

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The YEASTRACT+ information system (http://YEASTRACT-PLUS.org/) was just released [1] as a wide-scope tool for the analysis and prediction of transcription regulatory associations at the gene and genomic levels in yeasts of biotechnological or human health relevance. YEASTRACT+ integrates the previously existing YEASTRACT (http://www.yeastract.com/) [2] and PathoYeastract (http://pathoyeastract.org/) [3] databases and introduces the NCYeastract (Non-Conventional Yeastract) database (http://ncyeastract.org/), focused on the so-called non-conventional yeasts.
YEASTRACT+ includes currently updated information on *Saccharomyces cerevisiae*, *Candida albicans*, and *Candida glabrata*. PathoYeastract was extended to include two additional pathogenic yeast species: *Candida parapsilosis* and *Candida tropicalis*. Furthermore, the NCYeastract database was created, including five biotechnologically relevant yeast species: *Zygosaccharomyces baillii*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Yarrowia lipolytica*, and *Komagataella phaffii*. The YEASTRACT+ portal gathers 289 706 unique documented regulatory associations between transcription factors (TF) and target genes and 420 DNA binding sites, considering 247 TFs from 10 yeast species.
In this release, the pre-existing tools for the prediction of the TFs involved in the regulation of gene/genomic expression were upgraded to enable predictions based on orthologous regulatory associations described for other yeast species, including two new tools for cross-species transcription regulation comparison, based on the multi-species promoter and TF regulatory network analyses.

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P430. Bacteriophages content of *Serratia plymuthica* is independent of strains geographical location or environment

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Serratia plymuthica is a ubiquitous species and strains have been isolated worldwide. *Serratia* species are recognized to be involved in biological control, protecting plant host from the phytopathogenic organisms and to have ability to promote the plant growth. This study aims to evaluate the bacteriophages signatures present in *S. plymuthica* strains in order to understand if their number and type i) are dependent of habitats; ii) shared the same phylogenetic relationships as showed by pan- genome analysis; and/or iii) can be used as biological markers. The genome was analyzed of 14 strains isolated from pinewood nematode, plant leaves, soil, rapeseed roots, pasteurizer, and water; from eight different countries. Its pangenome was analyzed by using BPGA software. PHASTER and VIRFAM were used to identify and annotate the bacteriophages present in these genomes. Phylogeny was analyzed using: i) 16S rRNA gene; ii) core genome; iii) bacteriophages regions; iv) head-neck-tail modules of bacteriophages. Protein sequences were queried into the genomes that are involved in i) virulence and nematode, ii) nematicidal, iii) toxins of *Serratia plymuthica*, and iv) plant growth promotion. NMDS was calculated based on the four categories per si and all together. The genomes sizes ranged from 5.25 to 5.55 Mbp and from 50.1 to 56.2 G+C mol% and the ANI varies from 75.3 to 100. Core genome (2,244 genes) phylogeny analysis grouped the strains M24T3 and Leaf50 in one cluster, both related to plants, close with strain PRI-2C, and the remaining grouped in one cluster. This finding was also observed in all the NMDS analyses. Phylogenetic analysis of bacteriophage regions showed five clusters related to the viral family. This finding is also supported by head-neck-tail modules phylogenetic analysis and not related to the environment of the strain. The gene encoding for cI repressor protein was present in 12 strains and a similar strains-phylogenetic relationship was obtained when compared with the core genome analysis and might be used as biological marker. In conclusion, phylogeny of core genome and NMDS analysis showed the same relationship between strains and bacteriophages' regions grouped according to their family, and are not related to strains geographical location or environment.

P431. Recovering proteinaceous binders from Paint models of Medieval Paintings: A new Approach with Silica Nanoparticles and MCM41

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Medieval paintings have complex materials challenging to characterize & analyse. The components in paintings are pigments, varnishes and binders like oils, proteins, polysaccharides, etc. obtained from different biological sources. Animal glue, albumin, bovine bones (collagen), casein, gelatin, chitosan are some common binders found. The paint binders determine the state of the painting and degradation caused by other factors but, also gives important information for Historical Art, conservation and restoration processes. The origin of proteinaceous binders are particularly difficult to identify in a complex painting matrix. Immunodetection is one approach to determine the proteins in binders while being highly efficient and accurate. Several paint models with ovalbumin, casein and collagen were prepared using specific recipes for the purpose of mimicking original paint samples. Microsamples of paint models were taken and several extraction processes were employed to create protein extracts. Silica nanoparticles and MCM-41 was synthesized and combined with microsamples for protein recovery. In the case of Ovalbumin, the samples have an increase in recovered protein when combined with the Silica nanoparticles. For collagen, the recovered protein increases drastically when combined with MCM41. For casein, there is no significant difference with/without the silica nanoparticles or MCM41. The results show a good level of extraction, making it possible for subsequently identifying the proteins extracted from the microsamples by immunoassays.

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P432. Genome-wide identification of genes required for methanol tolerance in *Saccharomyces cerevisiae*

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Methanol is an interesting substrate for the production of added-value chemicals by methylotrophic yeasts and is present in pectin-rich agrofood residues hydrolysates. Genome-wide studies to unravel the molecular determinants of methanol toxicity and tolerance are scarce but essential to develop more robust strains.

Genes required for methanol tolerance in the eukaryotic model yeast *Saccharomyces cerevisiae* were identified in the present work by screening the Euroscarf haploid deletion mutant collection in YPD medium supplemented with 8%(v/v) of methanol at 35ºC. This chemogenomic analysis identified 407 determinants of methanol tolerance. Clustering of genes required for maximum tolerance revealed enriched functional categories that were not reported in previous chemogenomic analyses performed with ethanol, such as chromatin remodelling and DNA repair.

The expression of twelve transcription factors were found to be required for methanol tolerance, in general regulating a large percentage of the genes of the methanol tolerance dataset (up to 65%, registered for Sfp1 that regulates ribosomal protein and ribosome biogenesis gene expression in response to stress). The deletion of two transcription factors, Ixr1 and Opi1, led to total abrogation of growth upon methanol supplementation. Ixr1 is a negative regulator of hypoxic genes during normoxia and a reported regulator of more than 20% of the methanol tolerance genes. Opi1 is involved in phospholipid biosynthesis regulation and controls approximately 5% of the dataset. The networks regulated by these transcription factors suggest that regulation dependent on oxygen availability may play an important role during methanol exposure.

This study unveils the underlying methanol toxicity mechanisms, emphasizing the importance of DNA repair and membrane remodelling responses. Furthermore, this work provides valuable information on methanol tolerance determinants in the yeast model and on the regulatory networks involved in overcoming methanol toxicity. The obtained data contributes to guide the improvement of yeast tolerance to methanol.

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P433. Genome-scale metabolic model of the human pathogen *C. albicans*: aiming the identification of promising new drug targets

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Candida albicans is the most common cause of invasive candidiasis, partly due to its ability to acquire drug resistance. With the rise in frequency of multidrug resistant clinical isolates, therapeutic options are running low. The identification of new drug targets and new drugs is crucial to overcome the increase in therapeutic failure. Currently, genome-scale metabolic models can be considered established tools for drug targeting.
In this study, we propose the first genome-scale metabolic model for *Candida albicans*, iRV1930. The model consists of 1556 reactions, 1344 metabolites, 1053 genes, and 5 compartments. This model, currently under validation, proved accurate when predicting the capability of utilizing different carbon and nitrogen sources when compared to experimental data. This model was reconstructed using open source software tool, merlin 3.9.6, and is provided in the well-established systems biology markup language (SBML) format, thus, it can be used in most metabolic engineering platforms, such as OptFlux or Cobra.
Altogether, this model provides a promising platform for global elucidation of the metabolism of *C. albicans*, currently being used to guide the identification of new drug targets to tackle human candidiasis.

II11. Bioinformatics, Omics and Bionanotechnologies

P434. The ESKAPE pathogens mobilome: a systematic analysis

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The ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* sp. species) are the leading cause of multidrug-resistant infections worldwide. Although plasmids and integrative and conjugative elements (ICEs) typically harbor cargo genes that can confer a competitive advantage to the host (such as resistance to antimicrobials and the ability to metabolize multiple compounds and to colonize different hosts), a systematic analysis focusing on the mobilome of ESKAPE pathogens is missing. We sought to explore the mobile elements present in a collection of 1469 publicly available ESKAPE genomes. We identified a total of 2576 plasmids among 887 strains and 1082 ICEs in 674 strains, equivalent to 60.4% and 45.9% of the ESKAPE pathogens with at least one plasmid and/or ICE, respectively. Plasmids were highly frequent in *E. faecium*, *K. pneumoniae*, *A. baumannii* and *Enterobacter* sp. strains (93.1%, 85.2%, 72.9% and 63,9%, respectively) while ICEs were prevalent in *P. aeruginosa*, *K. pneumoniae* and *E. faecium* genomes (76.4%, 67.6% and 57.7%, respectively). We found a variety of known antibiotic resistance genes in plasmids, while virulence genes were mainly localized in ICEs. Polyketide synthase clusters responsible for the biosynthesis of bioactive natural products were prevalent in ICEs. Other secondary metabolites such as bacteriocins and siderophores were preferably localized in plasmids. Similar functions are characterized for the prevalent prophages in the ESKAPE pathogens. Understanding the contribution of mobile genetic elements for the promotion of resistance and virulence traits among the ESKAPE pathogens is critical for the development of novel defense strategies to fight this public health menace.

P435. Prediction and design of aptamer structures and their interaction with a target molecule

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Aptamers are short, single-stranded DNA or RNA molecules that can selectively bind to a specific target, with higher affinity. Their easy artificial synthesis and low production cost makes them exceptional molecules for diagnostic tools. They are selected from a large oligonucleotide library through an interactive process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). The success of SELEX selection and the affinity of the resulting aptamers depends to a large extent on the nature of an initial random nucleic acid library. In that regard, nucleic acid mimics (NAMs) (synthetic molecules that hybridize/mimic the natural nucleic acids) can give important contributions. They can either be used in 1) post-SELEX strategies to increase the overall affinity of aptamers towards the analyte as well as the nuclease resistance; or 2) in de novo selection processes to increase the diversity of the initial pool/library. Currently, we have developed efforts in the area mentioned in 1). The successfully post-SELEX strategies are dependents on adequate bioinformatics tools that can predict the folding and docking of aptamers with their target molecules, so that possible key locations can be identified for adding NAMs. As a case study, we have selected an aptamer already described for Staphylococcal enterotoxin type A (SEA), named A15. We used the following pipeline of bioinformatic tools -mFold/RNAstructure 6.1, Assemble2/Chimera and Visual Molecular Dynamics (VMD)- to predict the interaction between A15 and SEA. Based on preliminary results, we suggested modifications in the loop zones, assuming that predicted loops in secondary structures are essential for aptamer-toxin interaction. Future work will involve testing these modifications and check for improved affinity and resistance of A15.

III12. Microbial-Host Interactions

FP436. Insights on *Acinetobacter baumannii* heteroresistance to colistin: what is the fate?

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Staphylococcus epidermidis, a commensal bacterium of healthy human skin and mucosae, can cause serious bloodstream infections such as bacteremia and sepsis. These infections are very hard to cure with current antimicrobial strategies and, thus, it is urgent to find new treatment options. To do so, the study of *S. epidermidis* virulence factors is of utmost importance. Therefore, the *ex vivo* human blood model has gained special interest because it enables the study of *S. epidermidis* behavior in the context of a bloodstream infection. However, this model presents limitations, mainly related to the availability of donors, complicating its implementation in the academic context. To overcome this limitation, the possibility of replacing fresh human blood by commercial blood from other mammals was evaluated.

The survival of several *S. epidermidis* strains, the secretion of proteases and the level of transcription of the genes *sepA* and *hld* were determined after 4 hours of interaction with fresh human blood and commercial horse and sheep blood. The results obtained showed, in two the inocula tested (10e8 and 10e5 CFU/mL), that although in human blood the number of bacteria tended to decrease (4 to 6-fold) during period of incubation, in both horse and sheep blood a significant increase in the number of bacteria was observed (2 to 4-fold). Furthermore, the results obtained suggested that the replacement of human blood by horse or sheep blood did not cause significant alterations in the secretion of proteases. Finally, the transcription level of the gene *sepA* was similar in the 3 types of blood, but the transcription of the gene *hld* was significantly different among conditions, being 5 to 50-fold more expressed in human blood than in horse and sheep blood, respectively.

Overall, the results obtained show that depending on the parameters under analysis, fresh human blood may or not be replaced by commercial horse or sheep blood implicating, thus, previous evaluation of its substitution.

This study was supported by FCT through the funded project PTDC/BIA-MOL/29553/2017, under the scope of COMPETE2020 (POCI-01-0145-FEDER-029553) and by the strategic funding of unit UID/BIO/04469/2019.

III12. Microbial-Host Interactions

FP437. ColOsH - a dynamic simulator of the human Colon: concept and design

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The impact of functional food and pharmaceuticals on health is intrinsically related with the gastrointestinal (GI) system efficiency. GI simulators are key vehicles for researchers/developers to deliver trustable and reliable results on microbiome modulation and host-microbiota interaction. Changes in the GI microbiome have been largely associated to human diseases such as obesity, diabetes, inflammatory bowel, and cancer. Current dynamic in-vitro GI models allow the simulation of microbiota activity. However, none of them combines dynamic microbiota activity with peristaltic simulation, multi-colon zones and microbiota-host interaction in the same simulator. To address the limitations of current systems, our team is developing a new colon simulator within the ColOsH project (PTDC/BTM-SAL/30071/2017). ColOsH is a novel concept for a dynamic modular Colon system including peristalsis simulation by Oscillatory-flow and Colon-Host interaction.
The ColOsH consists of a Colon-Reactor (CR) representative of the three colon zones (ascending, transverse and descending), combined with an integrated Colon-Host (CH) simulator for interaction studies between microbiota and epithelial cells, mucus producing cells and immune system cells.
The CR is composed by three oscillatory-flow reactors (OFR) linked in series, carved in a plate. The OFR is a channel shape reactor with periodic sharp constrictions resembling the in-vivo colon villus. The OFR operates under oscillatory-flow mixing, intending to simulate peristalsis. The laminar-flow operation of the OFR will enable working with microbiota and cells in the same simulator. The fouling, generally obtained at low oscillatory condition, will favour bacteria deposition and metabolites exchange with cells. Also, the exceptional OFR mix efficiency is expected to avoid fluid phase separation from solids enabling work with high density immobilized microbiota. To design and setup the OFR operational conditions, a numerical model was developed. The numerical tool mimics flow-field and mix conditions induced by peristalsis on the microbiota. The CH module, consisting in a channel carved in a plate, holds a membrane for cells growth. The CH is installed in the lower part of the CR. The CH and the CR plates are separated by a semi-permeable membrane.
The ColOsH will be an important tool for health claims and food safety evaluation.

III12. Microbial-Host Interactions

FP438. The banana root endosphere microbiota is an important reservoir of potential biocontrol agents against *Fusarium oxysporum* f. sp. *cubense*

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Banana (*Musa acuminate* Colla) is a widely cultivated crop in (sub)tropical regions. *Fusarium* wilt of banana (FWB), caused by the soilborne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), poses a great risk to banana industry worldwide. Pesticides do not represent a sustainable option for its control and other alternatives, such as biological control agents (BCA) are gaining interest. The fact that beneficial endophytes can colonize the same niche than Foc favors them as potential control tools against FWB. We pursued two aims: the unravelling of the banana root endosphere microbiota, and the isolation, identification and characterization of culturable members of this specific niche as potential BCA within a FWB integrated management strategy. A thorough root surface sterilization protocol was implemented to ensure only the handling of banana root endophytes, originated from plants of different farms at Tenerife, La Palma and La Gomera islands. On the one hand, DNA from each sample was purified and the 16S rDNA gene (for bacteria) and the ITS2 region (for fungi) were amplified and sequenced by MySEQ. On the other hand, individual colonies (bacteria and fungi) from ground root tissues showing distinctive morphology when growing in different culturing media were isolated. A collection of indigenous endophytes (>1000) was thus generated (80% and 20% single/pure bacteria and fungi cultures, respectively). Subsequently, *in vitro* antagonism tests against different Foc races were conducted. More than 100 strains showing antagonism were molecularly identified and a phenotypic characterization was performed to identify traits associated to biocontrol and plant growth promotion. Based on these results the best isolates were selected. Both culturable and non-culturable approaches showed low microbial diversity, particularly within bacterial communities. Only few significant differences in alpha-diversity were found. Concerning beta-diversity, the most relevant significant differences were observed among farms regardless of the island from where they originated. Results point to the fact that banana roots are a good source of potential BCAs against FWB. Biocontrol assays using the selected native endophytes were successfully carried out and their results will be discussed.

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III12. Microbial-Host Interactions

FP439. Genomic features potentially involved in *Micromonospora*-legume interactions

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Micromonospora is a ubiquitous actinobacterium that has been isolated from many environments. This bacterium has also been retrieved from legume tissues where it can establish a close non-specific beneficial relationship with its host plant. Moreover, its presence has been confirmed in nitrogen fixing nodule cells, without affecting its functionality (Benito et al., 2017). In previous works, several commonly known plant-related features were located in the genome of the endophytic strain *Micromonospora lupini* Lupac 08 (Trujillo et al., 2014) but the exact role of *Micromonospora* in the symbiosis with leguminous the plant is still unknown. Sixteen strains from six different leguminous plants and different plant tissues (nodules and leaves) were isolated and their genomes sequenced. These genomes were included in a final database of 74 genomes, that comprised representatives of the main *Micromonospora* environments. This database was used in a comparative genomic analysis to search for genes potentially related to the bacterial-plant interaction. Our results revealed high correlation between the habitat and the genes found. Most of the genes labeled as differential were involved in carbohydrate metabolism, with an enhanced array of genes focused in the degradation and transport of simple sugars commonly found in the rhizosphere. Many genes involved in nitrogen metabolism, transporters of different compounds, degradation of sugars, production of organic acids, vitamins and hydrolytic enzymes were identified as differential traits, being overrepresented in most of the plant-related *Micromonospora* genomes. The study generated a database comprising 69,046 potentially plant-related bacterial genes, some of them with unknown function. The study of these genes can open a new hypothesis in the different stages of the relationship between *Micromonospora* and its host plant, from the soil environment to the colonization of the plant.

P440. Can commercial horse or sheep blood replace fresh human blood in an *ex vivo* model to study *S. epidermidis* virulence?

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Staphylococcus epidermidis, a commensal bacterium of healthy human skin and mucosae, can cause serious bloodstream infections such as bacteremia and sepsis. These infections are very hard to cure with current antimicrobial strategies and, thus, it is urgent to find new treatment options. To do so, the study of *S. epidermidis* virulence factors is of utmost importance. Therefore, the *ex vivo* human blood model has gained special interest because it enables the study of *S. epidermidis* behavior in the context of a bloodstream infection. However, this model presents limitations, mainly related to the availability of donors, complicating its implementation in the academic context. To overcome this limitation, the possibility of replacing fresh human blood by commercial blood from other mammals was evaluated.

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Overall, the results obtained show that depending on the parameters under analysis, fresh human blood may or not be replaced by commercial horse or sheep blood implicating, thus, previous evaluation of its substitution.

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P441. The *Chlamydia trachomatis* inclusion membrane protein IncM causes multinucleation in infected host cells

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Chlamydia trachomatis is a human bacterial pathogen causing genital and ocular infections of major clinical and public health impact. It resides and multiplies exclusively within host cells in a membrane bound vacuole, termed inclusion. During its life cycle, *Chlamydia* delivers several effector proteins into host cells that modulate multiple processes. Among these effectors, there is a major group of transmembrane proteins, termed Incs, which are inserted at the inclusion membrane.
C. trachomatis IncM was recently found to interact with a centrosomal human protein, CCDC146. This centrosomal protein was also recruited to the periphery of the inclusion membrane, upon infection with *C. trachomatis*. To understand the relevance of this interaction, a *C. trachomatis incM* mutant was constructed. This revealed that IncM was not necessary for CCDC146 accumulation around the inclusion. However, we observed that, at 18 h post-infection, inclusions containing *incM* mutant chlamydiae localized closer to the host cell centrosome. This suggested that IncM modulates the intracellular spatial localization of the inclusion.
As the centrosome plays important roles during the eukaryotic host cell cycle, we analyzed other processes that could also be potential targets of IncM. We found that cells infected by *C. trachomatis* lacking IncM showed lower levels of multinucleation than cells infected by the parental wild type strain. Multinucleation is a phenomenon commonly observed during *C. trachomatis* infection of tissue cultured cells, and results from an inhibition of host cell cytokinesis.
We are currently trying to understand how IncM is modulating the spatial localization of the inclusion and blocking cytokinesis in the host cells, and whether these two activities are correlated.

III12. Microbial-Host Interactions

P442. Identification and characterization of novel *Chlamydia trachomatis* virulence proteins

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Chlamydia trachomatis belongs to a large group of Gram-negative obligate intracellular bacteria that cause mainly ocular (trachoma) and genital infections in humans. These infections are an important public health and clinical burden worldwide. *C. trachomatis* is characterized by a developmental cycle involving the interconversion between an infectious but non-replicative form, and a non-infectious and replicative form. During this cycle, *C. trachomatis* remains within a vacuole (known as inclusion) and manipulates various host cell processes by the delivery of several type III secretion (T3S) effectors into host cells. While various *C. trachomatis* T3S effectors have been studied, certainly many others remain to be discovered as there are several identified candidates that have never been studied and numerous hypothetical proteins encoded in *C. trachomatis* genomes. In this work, we aim to identify novel *Chlamydia* effectors. For this we will focus on candidate effectors we previously identified and test them using a method based on a 13-residue phosphorylatable glycogen synthase kinase (GSK) tag. The presence of a GSK-tagged protein in a eukaryotic cell leads to host cell protein kinase-dependent phosphorylation of GSK. Therefore, by having plasmids encoding candidate effectors with a GSK tag introduced in *C. trachomatis*, the presence of the proteins in the cytoplasm of the host cells can be tested by immunoblotting using anti-phospho-GSK (anti-P-GSK) antibodies. We initiated this by constructing plasmids encoding *C. trachomatis* known effectors CT105 (expressed in mid- developmental cycle), CT694 (expressed late in the developmental cycle) and CT006 (early expressed inclusion membrane protein) with a GSK tag, which will be used as positive controls. We also generated plasmids encoding fusions of RplJ (chlamydial ribosomal protein), NrdB (chlamydial ribonucleoside-diphosphate reductase subunit beta) or EGFP to GSK, as negative controls. We already analysed mammalian cells infected by *C. trachomatis* strains expressing CT105-GSK and RplJ-GSK, which allowed to confirm the feasibility of the method. Experiments are in progress to generate *C. trachomatis* strains expressing the other positive and negative controls to further fine tune the method and screen for novel *C. trachomatis* effectors.

III12. Microbial-Host Interactions

P443. Botryosphaeriaceae diversity on forest hosts in Portugal

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The fungal family Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) is known to include several species of opportunistic pathogens or latent endophytes that affect many angiosperm and gymnosperm hosts worldwide. These fungi usually attack plants exposed to environmental stress like drought or plants that are already affected by other pathogens or pests. Diseases caused by these species result on fruit rots, leaf spots, wood necrosis and eventually tree death. Recent studies have identified the occurrence of four Botryosphaeriaceae genera in Portugal in different forest hosts. However, the diversity and distribution of these plant-pathogens in our country is still poorly understood. Several surveys were conducted across Portugal with aim to isolate and identify Botryosphaeriaceae- related diseases associated to the main forest tree species in Portugal (*Quercus suber*, *Eucalyptus globulus* and *Pinus pinaster*). In total, thirteen different Botryosphaeriaceae species were identified with twenty-three different plant-fungi interactions. We reported for the first time the occurrence of *Diplodia insularis* in Portugal and eight new plant-fungi interactions. These results show for the first time the Botryosphaeriaceae diversity and distribution across the country and suggest that pathogenicity trials should be conducted in order to understand the impact of the new reported species and the new fungi- host interactions.

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P444. Identification, characterization and involvement of specific *Pseudomonas simiae* PICF7 genes in belowground colonization and biocontrol of *Verticillium* wilt of olive

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Olive is the most iconic tree crop in the Mediterranean Basin. *Verticillium* wilt of olive (VWO), caused by *Verticillium dahliae*, is present in most of the olive growing areas. The disease is very difficult to control, posing a major threat for the olive oil industry. The use of beneficial microorganisms represents an excellent option to be used in combination with other control tools within an integrated disease management strategy. *Pseudomonas simiae* (fluorescens) PICF7 is an indigenous inhabitant of the olive rhizosphere/root endosphere and an effective biocontrol agent against VWO. Two objectives were pursued: the identification and characterization of genes involved in phenotypes such as rhizosphere/soil persistence (copper resistance), efficient root colonization (biofilm formation), plant growth promotion (phytase activity) and VWO biocontrol. An available Tn*5* random insertion mutant bank was screened (> 5,500 tetracycline-resistant colonies) to select mutants affected in one of the traits mentioned above. The identification of the disrupted genes was performed by nested-PCR and DNA sequencing. Mutants that showed disruption of putative genes coding for: (i) the transcriptional regulator CusR or the chemotaxis protein CheW, impairing PICF7's growth in medium supplemented with Cu^{2+}, (ii) a membrane protein or a flagellar regulatory protein, generating biofilm formation defective phenotypes, and (iii) histidinol-phosphate aminotransferase or hemolysin D that abolished phytase activity, were eventually selected. Biofilm-defective and copper-sensitive mutants displayed the same antagonistic effect against *V. dahliae* than the parental strain in different culturing media (Potato dextrose agar,[PDA], nutrient agar and Waksman's agar). In contrast, phytase-defective mutants lost this ability in PDA. In planta bioassays were conducted using the olive cultivar Picual. Root colonization ability of PICF7 mutants was assessed by confocal laser scanning microscope and fluorescently-labelled derivatives of each mutant. Colonization pattern of PICF7 and that of phytase and copper mutants were similar. Biofilm-defective mutants showed good olive rhizosphere/rhizoplane colonization, although no evidence of endophytic behaviour was detected in this case. Results from greenhouse biological control assays showed that all tested mutants displayed similar VWO control performance than strain PICF7. This indicates that the genes here studied are not involved in biocontrol of VWO.

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III12. Microbial-Host Interactions

P445. Iron acquisition influences the interaction between *Staphylococcus epidermidis* and the host innate immune system

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Staphylococcus epidermidis is one of the most important commensal microorganisms of human skin and mucosae, but additionally it is often the cause of potential life-threatening infections in immunocompromised patients. Unlike other bacterial species, including *Staphylococcus aureus*, the interaction of *S. epidermidis* with the host innate immune system has not been the focus of substantial research. On the other hand, we have recently demonstrated that *S. epidermidis* heavily relies on iron acquisition to develop biofilms, which is widely regarded as its major virulence factor. In order to study the interaction of *S. epidermidis* with the host innate immunity, and whether iron acquisition can modulate such process, we infected murine RAW 264.7 macrophages and human monocyte-derived macrophages (hMDMs) with *S. epidermidis* 1457 “wild type” and mutant strains defective for iron acquisition. We found out that the type of macrophages greatly impacts the infection outcome: while RAW 264.7 failed to control intracellular replication, hMDMs exhibited a pronounced bactericidal effect. The bacterial fate within hMDMs is also dependent on the macrophage phenotype, as M1-like macrophages did not allow intracellular replication, while M2-like macrophages failed to control bacterial replication during early time points of infection (0-2h). Lastly, the absence of certain iron acquisition systems, specially the lack of siderophore production, resulted in lower/ null replication rates within macrophages. We also demonstrated that the siderophore-deficient mutant strain exhibited higher susceptibility to hydrogen peroxide, which might explain its poorer survival in the harsh intracellular milieu of macrophages. Collectively, our results provide an analysis of the interaction between *S. epidermidis* and different types of macrophages and point out that the lack of certain iron- acquisition systems, siderophore production in particular, is detrimental for bacterial survival within macrophages.

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P446. *Diaporthe*, an important ally against drought stress

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Agricultural production faces the challenge to produce more food while constrained by a number of abiotic factors such as drought. Tomato (*Solanum lycopersicum* L.) is one of the most nutritionally and economically important crops in the world. This crop is very sensitive to stress and any sudden changes might be unfavorable for production. Development of stress tolerance in plants is a strategy to cope with the negative effects of adverse environmental conditions. Certain endophytes are well recognized for plant growth promotion and production of natural compounds. The capacity of endophytes to induce stress tolerance in plants can be applied to increase crop production. Some *Diaporthe* endophytes that can be found in the roots of plants that inhabit stressful habitats have shown potential functions in plant adaptation to abiotic stress. Therefore, the aim of this work was to evaluate if the symbiosis between tomato plants and a *Diaporthe* strain improves the tolerance to drought stress.
Tomato plants were inoculated with a *Diaporthe* strain and grown for five weeks in a greenhouse. During this time one half of the plants were submitted to drought stress conditions, being watered only two times per week with 10% of the water capacity of the soil. The drought stressed plants showed significantly less height and dry weight (DW), than the non-stressed plants, but drought stressed plants inoculated with *Diaporthe* sp. showed 22% more height and 44% more DW than non-infected plants. Under drought stress the concentration of proline was higher in the shoot system, however, a two-fold proline content increase was observed in *Diaporthe*-inoculated plants respect to uninoculated drought stressed plants. In addition, it was verified that inoculated plants showed enhanced water use efficiency, and lower total phenolic content and antioxidant activity.
Our results suggest that *Diaporthe* sp. seems to limit the drought stress damage and symptoms in tomato during the vegetative stage.

P447. Identification of the determinants of the subcellular localization of CteG, a *Chlamydia trachomatis* Golgi-targeting protein

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Chlamydia trachomatis is an obligate intracellular pathogen that infects cells of the human conjunctiva or urogenital tract, respectively leading to non-congenital blindness, or pelvic inflammatory disease and infertility. During infection of the host cell, *C. trachomatis* resides within a vacuole named inclusion, and expresses a type III secretion (T3S) system. This macromolecular machinery allows the delivery of virulence proteins into the host cell cytoplasm, known as T3S effectors. These proteins manipulate several host cell pathways, favouring bacterial survival and multiplication. In previous work, CteG-2HA was identified as a T3S substrate, and found to be delivered into the host cell cytoplasm. Moreover, the predominant subcellular localizations of CteG-2HA during infection were observed to be the Golgi complex between 16-20 h post-infection (p.i.), and the host plasma membrane between 30-40 h p.i. Further analysis showed that upon ectopic expression, the first 100 amino acid residues of CteG (CteG100) localize to the Golgi, suggesting that this region contains a Golgi-targeting motif. Based on these observations, the goal of this work was to identify the determinants of the subcellular localization of CteG. For this purpose, site directed mutagenesis was applied on CteG and the localization of the generated EGFP-fused mutant proteins was analyzed by immunofluorescence microscopy of transfected HeLa cells. The results showed that the first 20 amino acid residues of CteG are necessary, but not sufficient for its targeting to the Golgi. Substitutions of amino acids within this region of CteG, particularly those that incorporate the hydrophobic face of a putative alpha-helix, affected the localization of CteG100 at the Golgi in transfected HeLa cells. Additionally, deletions performed at the C-terminal region of CteG diminished the frequency of its plasma membrane localization in transfected HeLa cells. This suggests that different regions positioned along the peptide sequence of CteG may be important for this localization.

P448. Evaluation of olive beneficial rhizobacteria as protectants against drought and salt stresses: examining the potential involvement of bacterial 1-aminocyclopropane-1-carboxylate deaminase activity

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Stress caused by drought and high salinity can affect growth and productivity of olive (*Olea europaea* L.) trees. The phytohormone ethylene plays essential roles in plants but high levels in response to (a)biotic stresses may cause negative effects. Some rhizobacteria have been investigated for its potential to ease these effects due to the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACD). ACD degrades ACC, the immediate ethylene biosynthetic precursor. The aim of this work was to examine whether indigenous olive rhizobacteria showing ACD activity may alleviate salt and drought stress in young olive plants. A collection of 32 previously-isolated olive rhizobacteria was in vitro screened for the presence/absence of ACD activity. The well-characterized beneficial olive root endophyte *Pseudomonas simiae* (fluorescens) PICF7 showed as defective in ACD activity, although genes phylogenetically related to ACD and putatively coding for a D-cysteine desulfhydrase and an unidentified deaminase are present in its genome. *Pseudomonas* sp. PICF6 displayed this activity and sequencing of its genome revealed the presence of a true ACD gene. By confocal laser scanning microscope analysis using fluorescently-labelled derivatives of both strains similar olive root colonization patterns were visualized, including evidence of the endophytic behaviour of strain PICF6. Greenhouse experiments were performed in which olive 'Picual' plants inoculated either with strain PICF6 or PICF7, or with a combination of both strains, were subjected to drought or salt stress. Different physiological and biochemical parameters (chlorophyll and flavonoids contents, stomatal conductance and spectral plant index) were measured along time and compared to the situation in non- stressed and/or non-bacterized plants. Proline content and stem water potential was also scored in plants subjected to salt and drought stress, respectively. Results showed that neither PICF6 (ACD- positive) nor PICF7 (ACD-negative) were able to lessen the negative effects caused by the abiotic stresses tested, although some of the parameters examined (e.g. stomatal conductance or flavonoid content) showed differences in some cases. In summary, inoculation with strain PICF6 does not help olive plants to cope with salt/drought stress, suggesting that ACD activity does not seem to play any protective role under experimental conditions tested.

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III12. Microbial-Host Interactions

P449. Melanin Synthesis dual role in the Protection of *Alternaria* sp. against Antifungals and Immune Response

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Alternaria is a cosmopolitan dematiaceous fungus commonly found in plants, soil, food, and indoor air environment. Alternaria species have been increasingly associated to respiratory allergic diseases and are also etiological agents of serious infections with poor prognosis in immunosuppressed individuals. The present work focuses on *Alternaria infectoria*, which was used as a model organism of the genus, and was designed to unravel melanin production in response to antifungals and to study its role on the immune response to these fungi.

We characterized the DHN-melanin produced by *A. infectoria* by UV-visible spectra, FTIR and 13C NMR and quantified the pigment production when this fungi was exposed to itraconazole, fluconazole, nikkomycin Z, caspofungin and amphotericin B. We demonstrated that *A. infectoria* increased melanin deposition in cell walls in response to the antifungals nikkomycin Z, caspofungin and itraconazole. Moreover, the inhibition of the melanin synthesis by pyroquilon resulted in a lower minimum effective concentration (MEC) of caspofungin, determined according to the M38A protocol (CLSI). We also observed by transmission electron microscopy that the absence of melanin led to A. infectoria cell walls thinner and less organized, indicating that the synthesis of this pigment is involved in the robustness of the cell wall.

To evaluate the immunomodulatory effect of melanin from *A. infectoria* when interacting with RAW 264.7 macrophages, we developed a model of fungal hyphal cell wall nanoparticles (CWNPs) prepared by hyphae fragmentation, when the fungus was grown in the absence and presence of pyroquilon. We observed that these CWNPs were taken up by macrophages by membrane ruffling and then reach membrane compartments inside the cytoplasm. We also demonstrated that the CWNPs containing DHN-melanin were less able to stimulate macrophages while those without melanin (pyrCWNPs) lead to early macrophage activation and, after 6 h of interaction caused a 50% decrease in macrophage viability.

In summary, *A. infectoria* synthesizes melanin as a protective mechanism against antifungal drugs and the presence of the pigment in the cell wall damps the immune system activation.

III12. Microbial-Host Interactions

P450. Antimicrobial activity of olive pomace

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Olive oil consumption and processing is increasing in Portugal and worldwide. Along with its production, several by-products are generated such as olive mill wastewaters and olive pomace (OP). It is known that OP is a rich and low-cost source of natural compounds with high interest for food industry. Therefore, several studies reported OP applications in food-products. Hence, the study of the OP antimicrobial-activity is noteworthy having in view its use in innovative food-products as a natural preservative, attaining the current consumers demand for natural additives.

This work aimed to assess the antimicrobial-activity of an OP functional ingredient (OFI) obtained by a patented process. Extracted physically from OP, OFI is a natural ingredient obtained without any chemical. Four samples from two-phase olive mills (2017/2018 season) were studied: two from the North of Portugal and two from the South. The separation of the liquid phase (water and oil) from solid phase (olive skin, pulp, stone and kernel) was performed by application of a pressing force. The obtained semi-paste was centrifuged and the supernatant (OFI) removed and lyophilized.

OFI samples at 500 mg/mL were screened in different agar methods (incorporation, surface spreading and disk diffusion) for the antimicrobial-activity against *S. aureus*, *E. coli* and *C. albicans*. A growth inhibition diameter greater than the one containing only solvent was considered as a positive result. Positive results were submitted to broth microdilution method in order to determine the minimum inhibitory concentration (MIC).

All the extracts showed antimicrobial-activity against *S. aureus* and *E. coli* strains, in one or more of the three screening methods used, with diameter zone inhibition between 7 and 15 mm for *S. aureus* and 9 to 12 mm for *E. coli*. No antimicrobial-activity against *C. albicans* was verified with this methodology. MIC was determined for the four extracts for *S. aureus* and *E. coli*, with the best results for the extract from Alfândega-da-Fé, followed by the extract from Beja.

According to these results, OFI showed antimicrobial-activity against *S. aureus* and *E. coli*. This result corroborates the importance of OP as a source of value-added biologically active compounds for food and medical purposes.

III12. Microbial-Host Interactions

P451. Metagenomics-assisted monitoring of the fish larvae microbiome: evaluating the efficacy of green technologies to suppress disease incidence in aquaculture settings

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High mortality rates caused by opportunistic bacteria in fish larviculture prevents reared fish production to reach market expectations. Here, we evaluated whether algal-derived, anti-microbial metabolites applied to the live feed (rotifers and artemia) provided to gilthead seabream (*Sparus aurata*) larvae could alter the structure and composition of microbial communities in a fish larviculture system. Next generation 16S rRNA gene sequencing was performed to characterize the microbiomes of rearing water, fish larvae and the live feed themselves when the latter were treated with *Asparagopsis armata* (red alga)-derived secondary metabolites. Bacterial consortia associated with fish larvae were significantly different in structure at 2 and 34 days after hatching (DAH). Under control conditions, the genera *Pseudoalteromonas*, *Marinomonas*, *Acinetobacter*, and *Acidocella* (besides several unclassified Alphaproteobacteria) were dominant at early larval developmental stages (2 DAH), whereas *Actinobacillus*, *Streptococcus*, *Paracoccus*, and *Pseudomonas* were most dominant at later developmental stages (34 DAH). Despite the overall lack of significant differences in the structure of control versus “treated” microbiomes as revealed by Principal Components Analysis coupled to permutational analysis of variance, in fish larvae-associated communities a few individual differences at the “bacterial species” level (i.e. operational taxonomic units – OTUs – defined at 97% 16S rRNA gene similarity) could be identified, involving shifts in relative abundance of potentially probiotic as well as opportunistic bacteria. Indeed, after applying both parametric and non-parametric t-tests to all OTUs (> 60) found across fish larvae microbiomes, nine OTUs showed significantly different relative abundances between control and treatment samples in both tests. Quite strikingly, OTUs found to be enriched in treated fish larvae all belonged to Alphaproteobacteria species with probiotic potential in the genera *Paracoccus*, *Bradyrhizobium*, *Polymorphum* and *Methylobacterium*, whereas the five remaining OTUs found to be depleted in treated fish larvae affiliated with opportunistic, potentially pathogenic bacterial groups such as *Acidocella*, *Klebsiella*, *Enterobacteriales* and *Pseudomonas*. This study reveals that live feed treatment with naturally-occurring antibacterial metabolites can alter the relative abundance of specific fish-associated bacterial populations, encouraging further research on the use of microbiome manipulation approaches to decrease disease incidence in fish larviculture.

III12. Microbial-Host Interactions

P452. Epiphytic and endophytic bacteria on olive tree phyllosphere: cultivar- and tissue preferences

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The olive tree (*Olea europaea*) is a worldwide important culture with a great impact for the Mediterranean region. Although plant phyllosphere harbours a panoply of microorganisms known to play diverse and important roles for the plant health, the number of studies focused on the phyllospheric olive tree bacterial communities and their shaping factors are currently scarce. In the present work, surface and internal bacterial microbiota of two olive cultivars (Cobrançosa and Verdeal Transmontana) were evaluated in twigs and leaves. Although three phyla (Proteobacteria, Actinobacteria and Firmicutes) were consistently found in epiphytic and endophytic communities, a higher abundance of Actinobacteria and the presence of Bacteroidetes phylum was characteristic of epiphytic communities. Comparing both cultivars, cv. Verdeal Transmontana presented bacterial communities with higher richness and diversity than cv. Cobrançosa, while twigs presented higher bacterial abundance than leaves. Bacterial populations showed to be highly influenced by the cultivar, followed by the plant organ, with a number of taxa being specific to those different plant micro- environments. Altogether, the results presented here increase the knowledge on the structure of bacterial microbiome in the olive tree, which could play a significant role for olive tree be able to cope with typical Mediterranean climates (warm and dry summers and wet winters), as well as the impact that host-associated variables exert on this microorganisms.

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III12. Microbial-Host Interactions

P453. Crop rotation and *Azospirillum brasilense* on grain yield and root colonization of upland rice by endophytic fungi

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The antecessor plant residues resulting from crop rotation in the no-tillage (NT) system remain in the area, ensuring production and maintenance of straw and the crop yield in succession. This practice raises the levels of organic matter, benefiting the microbiota, important by its interactions with plants and for acting in several process in the soil. Atmospheric nitrogen-fixing bacteria, such as those of the genus *Azospirillum*, recommended for the ability that promotes the plant growth, can also produce phytohormones that can enable root system elongation, allowing the plants to explore a larger area of soil, searching for water and nutrients. Beneficial microorganisms include arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSF). Both colonize the root system of plants, favoring the acquisition of water and minerals through their hyphae that colonize the substrate, and supplying the plants with phosphorus. The aim of this study was to evaluate the effects of seed inoculation of *Azospirillum brasilense* on antecessor (maize and *Urochloa ruziziensis*) and successor (upland rice) plants on grain yield and rice root colonization (COL) by FMA and DSF. This study was conducted at the experimental area of UNESP-Sao Paulo State University, Ilha Solteira Campus, which is located in Selvíria-MS, Brazil. The experimental design was randomized blocks arranged in a strip scheme with 4 replications and 8 inoculation treatments in the predecessors were: 1) maize; 2) maize-I; 3) *Urochloa ruziziensis*; 4) Urochloa-I; 5) maize +Urochoa; 6) maize-I+Urochoa; 7) maize+Urochoa-I; 8) maize-I+Urochoa-I. The seed inoculation of the predecessors and successor with *A. brasilense* promoted an increase (19%) in rice grain yield. Inoculation also provided increases in COL-FMA (7%), but there was a decrease of 7.6% in COL-DSF. For the interactions between inoculation and root colonization, Urochloa-I was the treatment that allowed the highest percentages of COL-FMA, regardless of rice inoculation treatment, followed by maize+Urochoa, without rice inoculation. COL-DSF was higher in the treatment with maize (treat. 1) as a antecessor plant without rice inoculation, while the lowest were with maize-I (treat. 2) and maize-I+Urochoa (treat. 6), also without rice inoculation.

P454. *Diaporthe*, "endophyghting" for life: *Fusarium* wilt challenge

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The establishment of stress-tolerant plants is a key challenge for more sustainable, healthy and efficient crop production. In the last years, endophytic microorganisms have attracted a great interest because some are able to improve the performance of plants under stress situations due to a mutualistic relationship. *Diaporthe* sp. is an endophytic fungus abundant in some plants that grow in unhospitable environments, such as *Festuca rubra* subsp. *pruinosa*. In previous studies, this endophyte was shown to be beneficial to plant performance under stress conditions. Tomato (*Solanum lycopersicum* L.) is one of the most important commercial crops and is very sensitive to attacks of pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici* (Fol), a fungus that causes vascular wilt disease. Thus, the objective of this work was to determine if tomato plants are able to host *Diaporthe* sp. and if this interaction induces resistance against Fol.
Tomato plants were inoculated with a *Diaporthe* strain isolated from *F. rubra* plants and were grown for six weeks in a greenhouse. One half of the plants was inoculated with Fol and the other was Fol free. All the control plants infected with Fol showed severe disease symptoms and some eventually died. In contrast, only 40% of the plants inoculated with *Diaporthe* showed Fol disease symptoms. A disease index was scored on a scale from 0 to 5 based on the presence of Fol in the vasculature at the cotyledon and crown levels. All control plants infected with Fol showed infection in both levels and only 50% of the *Diaporthe*-inoculated plants showed some infection at the cotyledon level. The height of the Fol infected control plants decreased by 81% but only 42% in plants co-inoculated with *Diaporthe*, in comparison with non-infected control plants. Regarding dry weight, a decreased of 95% and 61% in Fol infected plants was detected in *Diaporthe* free and *Diaporthe*-inoculated plants respectively.
In conclusion, a positive effect on tomato plants by *Diaporthe* strain was observed against Fol. The severity of Fusarium wilt was lower or absent in *Diaporthe*-inoculated plants, suggesting that this endophyte seems to restrict the colonization of tomato plants by Fol.

P455. Unraveling the interaction between *Vitis vinifera* and the fungal pathogen *Lasiodiplodia theobromae* through Dual RNA-Sequencing

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Grapevine is an extremely important fruit crop worldwide. The fungus *Lasiodiplodia theobromae* has been increasingly recognized as an important pathogen of grapevine (*Vitis vinifera*) and one of the most aggressive agents of the grapevine trunk disease Botryosphaeria dieback. *Lasiodiplodia theobromae* colonizes grapevine wood structures, causing an internal necrotic canker that affects fruit production. The interaction between these two organisms at the molecular level is still poorly understood. Through a dual RNA-sequencing approach this study aimed to give a broader perspective on the infection strategy deployed by *L. theobromae*, while understanding grapevine response. In this study, 1-year-old seedlings of *V. vinifera* were inoculated with *L. theobromae* and four sampling points were defined: 1, 3, 7, and 10 days post inoculation (dpi). Approximately 0.05% and 90% of the reads were mapped to the genomes of *L. theobromae* and *V. vinifera*, respectively. Over 2500 genes were significantly differentially expressed in infected grapevine plants in comparison to mock inoculated plants, many of which are involved in the inducible defense mechanisms of grapevine. Gene expression analysis showed changes in fungal metabolism of phenolic compounds, carbohydrate metabolism, transmembrane transport and toxin synthesis. These functions are related to pathogenicity mechanisms involved in plant cell wall degradation and fungal defense against antimicrobial substances produced by the host. Genes encoding for degradation of plant phenylpropanoid precursors were up-regulated suggesting that the fungus could evade the host defense response using the phenylpropanoid pathway. The up-regulation of many distinct components of the phenylpropanoid pathway in planta supports this hypothesis. Moreover, genes related to phytoalexin biosynthesis, hormone metabolism, cell wall modification enzymes and pathogenesis-related proteins seem to be involved in the host responses observed. This study provides additional insights into the molecular mechanisms of *L. theobromae* and *V. vinifera* interactions.

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P456. Study of the antimicrobial potential of red tulip flowers (*Tulipa gesneriana*) against strains of *Escherichia coli, Staphylococcus aureus* and *Candida albicans*

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Introduction: In the petals of *Tulipa gesneriana*, there can be found several types of flavonoids, compound that plays an important role in the plant's antimicrobial activity. It is posited that those compounds would reach, inside the cell, different loci of action from currently known antibiotics, which would be active against resistant pathogens. Aims: To evaluate the antimicrobial effect of red petals of *Tulipa gesneriana* against strains of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* isolated from hospital infections. Methods: Fifteen strains of *E. coli*, 15 of *S. aureus* and 15 of *C. albicans* were isolated from hospital infections. The petals were dried in an incubator, ground up and mixed with alcohol. After 26 days at rest, they were filtered to form the extract. For the analysis of the antimicrobial activity of Tulipa flowers, the broth microdilution methodology was used. Results: At the concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12,5mg/ml, 6.25mg/ml and 3,125mg/ml there was total antimicrobial effect of the extract against strains of *E. coli*, *S. aureus* and *C. albicans*. Conclusion: A antimicrobial activity was found in the petals of Tulip against strains of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* at the concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml,12.5 mg/ml, 6,25 mg/ml and 3,125 mg/ml.

III12. Microbial-Host Interactions

P457. Statins as new candidates for the treatment of Buruli Ulcer

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Mycobacterium ulcerans is the pathogenic agent of Buruli ulcer (BU), a neglected emerging infectious disease that has been notified in over 30 countries, particularly in West Africa. Younger patients are the most affected, especially children within 5 and 15 years of age. Clinical stages evolve from early nodular lesions to characteristic undermined edges lesions, which can even spread to deep tissues causing devastating disfigurations and disability.

Treatment varies from antibiotherapy for early presentations, to a combination of antibiotics and extensive grafting surgeries for severe ulcers. Current antibiotic regimen includes a combination of rifampicin and streptomycin. However, adherence to a long-term therapy of intramuscular streptomycin injections associated to the considerable side effects, such as ototoxicity or lower effectivity in older ages, are some of the reasons to continue the research of new therapeutic candidates. Statins are lipid- lowering drugs broadly used for dyslipidaemia treatment. Recent evidence has shown pleiotropic antimicrobial activity of these drugs against several bacteria, including *M. tuberculosis* and *M. leprae*. Therefore, it is feasible to think that these drugs could have an activity against *M. ulcerans*. In this sense, we have tested IC_{50} of several statins (atorvastatin, simvastatin, fluvastatin, lovastatin) using highly virulent strains of *M. ulcerans* to determine their antimicrobial efficacy *in vitro* as well as in an *in vivo* model of murine infection. The validation of antibiotic activity of statins against *M. ulcerans* could become these drugs on potential candidates of easy administration and affordable cost for the treatment of Buruli Ulcer.

P458. Characterization of small non coding RNAs differentially expressed by *Burkholderia cenocepacia* when infecting *Caenorhabditis elegan*

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Small non-coding RNAs (sRNAs) are key regulators of post-transcriptional gene expression in bacteria. These molecules can interact with mRNAs or proteins, affecting a variety of bacterial functions. Regulation of gene expression can be achieved by blocking the mRNAs access to ribosomes and/or directing them to degradation, or by enhancing the translation of key stress-regulated transcriptional factors. Despite the identification of hundreds of bacterial sRNAs mainly due to high throughput techniques, such as RNA sequencing (RNASeq), their roles on bacteria physiology and virulence remain largely unknown. This is the case of bacteria of the *Burkholderia cepacia* complex (Bcc), a group of opportunistic pathogens capable of causing lethal lung infections among cystic fibrosis (CF) patients. Although recent upgrade on the identification of sRNAs on Bcc bacteria, an approach using a host infection model is still missing. The aim of the present work is to unveil sRNAs expressed by Bcc bacteria when infecting a host. The nematode *Caenorhabditis elegans* was used as an infection model, being infected with the epidemic CF strain *B. cenocepacia* J2315. The RNA extracted from infecting bacteria was used for RNASeq. Bioinformatics analyses were performed to identify the non- coding RNAs expressed and the level of expression. *In silico* web tools were used for sRNA targets prediction and conservation analysis. 97 sRNAs with a predicted Rho independent terminator were identified, most of them located on chromosome 1. Some of those sRNAs were previously identified [3], validating the methodology and the results obtained. The sRNAs expression levels, their conservation among Bcc bacteria, the predicted interaction with both Hfq and Hfq2 proteins and the relevance of the predicted targets were used as criteria to select 4 sRNAs for further validation and functional characterization. Northern blotting analysis and Rapid amplification of cDNA ends (RACE) are in progress to assess the expression and the exact length of the selected sRNAs. Phenotypic analysis by overexpression or silencing the sRNAs will be performed, to gain new clues about the role of these sRNAs on Bcc bacteria. Electrophoretic mobility shift assays (EMSA) will be used to check sRNAs interactions with predicted targets and the Hfq chaperone.

III12. Microbial-Host Interactions

P459. Vaginal Microbiome Responsible for Female Health

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Microbiota plays a fundamental role in the overall development and defences of human beings. The majority of indigenous microbiota exists in a mutually beneficial relationship with their hosts, while few of these are opportunistic pathogens that can lead to life-threatening diseases and chronic infections. These microbial communities constitute the primary defence against infections induced by non-indigenous invasive organisms. Female vaginal ecosystem thought to have been shaped over the years by co-evolutionary processes occurring between the particular microbial partners and human host. Vaginal secretions contain numerous microorganisms and the host provides them nutrients for their growth and development. Disruptions in vaginal association with the microbiomes lead to the change in vaginal environment, which enhanced the risk of acquiring diseases including sexually transmitted infections, bacterial vaginosis, fungal infections, preterm birth etc. The focus of this study is on the detailed analysis of vaginal microbiome interplay and its overall impact on female health. The mutualistic relationship between vagina and residing microbial species has been well described.

III12. Microbial-Host Interactions

P460. The role of serratomolides produced by bacteria of genus *Serratia* in nematicidal activity

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The pinewood nematode (PWN), *Bursaphelencus xylophilus*, is known as the pathogenic agent of Pine Wilt Disease (PWD) that affects pine trees around the world. Infection is spreading globally through wood international commerce and locally by vector beetles, threatening wood world economy. As climate changes, more countries are becoming susceptible to PWD and, to prevent disease spread and limit economic and ecological losses, better knowledge about this pathogenic agent is needed. *Serratia* strains, present in endophytic community of pine trees and carried by PWN, may play an important role in PWD. This work aims to better understand interaction between eighteen *Serratia* strains and *B. xylophilus*, to assess nematicidal potential of serratomolides (serrawettin W1 and W2) produced by *Serratia* strains and to identify genes involved in serratomolides biosynthesis process. Serrawettin genes presence was evaluated in selected *Serratia* strains. Using AntiSMASH software, twenty eight *Serratia* strains with serratomolides genes were identified and serrawettins clusters were analysed. Phylogenetic and comparative genomic approaches were performed both in serrawettin W1 and W2 clusters and cluster proteins were identified through NCBI Blast. Mortality tests were performed with bacteria, supernatants and extracted amino lipids against *B. xylophilus* and *C. elegans* (model organism) to determine their nematicidal potential. Moreover, attraction tests were performed with *C. elegans*. Cluster analysis was performed between *Serratia* strains with serratomolides genes. All concentrated supernatants were able to kill more than 57% and concentrated supernatants of *Serratia* strains with serrawettins were able to kill more than 77% of *B. xylophilus* after 72 h. Five *Serratia* strains were able to attract more *C. elegans* than *E. coli* OP50 and four supernatants from *Serratia* strains were able to attract more *C. elegans* than *E. coli* OP50. Eight specific amino lipids showed a high nematicidal activity against *B. xylophilus*, killing more than 46% of *B. xylophilus* in 48 h. Cluster analysis of serrawettin W1 and W2 biosynthesis genes revealed several new proteins involved in serratomolides biosynthesis process, eight common to all serrawettin W1 and W2 clusters. We conclude that some *Serratia* strains, their supernatants and specific amino lipids show nematicidal activity against *B. xylophilus*.

P461. Plasmid diversity of bacterial communities associated with the marine sponge *Cinachyrella* sp

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Multiply-primed rolling circle amplification (RCA) is a technique for isothermal amplification of circular templates using phi29 DNA polymerase. In this study we aimed to develop a sequence-specific multiply-primed RCA for selective plasmid amplification from sponge samples and further characterize the sponge plasmidome via Illumina next-generation sequencing. Plasmid DNA obtained from sponge (*Cinachyrella* sp.) microbial communities and a bacterial symbiont (*Vibrio* sp. CyArs1) isolated from the same sponge species (carrying unknown plasmid) were used to develop and validate our methodology for selective RCA. The selective RCA was performed during (16h) with 141 plasmid-specific primers covering all known circular plasmid groups. The amplified products were purified and subjected to a re- amplification with random hexamer primers (2h). The resulting amplification products were sequenced using Illumina MiSeq (2 x 300 bp) and analysed with bioinformatics pipelines. The developed method resulted in the successful amplification and characterization of the sponge plasmidome, including plasmids larger than 20kbp, and allowed us to detect plasmids present in the bacterial symbiont *Vibrio* sp. CyArs1 in the sponge host. Besides this a higher number of small (less than 2kb) and cryptic plasmids were also amplified in sponge samples. Functional analysis identified proteins involved in the control of plasmid partitioning, maintenance and replication. In conclusion, our approach allowed us to selectively enrich and characterize the plasmid DNA from sponge-associated bacterial communities.

III12. Microbial-Host Interactions

P462. Metagenomic analysis of the sponge *Cinachyrella* sp., seawater and sediment of a Taiwanese coral reef ecosystem

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Marine sponges are sessile filter-feeders and have been shown to harbour exceptionally high microbial densities. These metazoans are known to play important functional roles in water column, benthos and the coupling between these two zones. Other functions that can be added to this repertoire are those putatively associated with sponge microbial community (e.g., denitrification; nitrification, carbon fixation; biosynthesis of secondary metabolites, antibiotics, cofactors and vitamins). Although an increasing number of studies have generated sponge metagenomic information, only a minority have recovered endosymbionts genomes through binning approaches. Here, using a hybrid bin extraction algorithm and a consolidation approach, the whole community metagenome of the coral reef sponge species *Cinachyrella* sp., seawater and sediment (Aimen, Penghu archipelago, Taiwan), was investigated in order to maximize the recovery of symbiont genomes (bins).

50 seawater, 14 sponge and 3 sediment prokaryotic reassembled bin genomes (≥ 50% completeness; ≤ 10% contamination) were recovered. The bins were assigned to Proteobacteria (21 Alphaproteobacteria and 24 Gammaproteobacteria), Actinobacteriota (3), Crenarchaeota (3), Cyanobacteria (2), Bacteroidota (2) and Dadabacteria (1). Of these, 14 seawater and 5 sponge recover bins had ≥ 90% completeness and ≤ 3% contamination. For sediment all the 3 bins recovered had a completeness ranging from 50% to 58% with contaminations ≤ 5%. The only recovered bin with 100% completeness and 0 % contamination was found in sponge prokaryotic community and was assigned to the archaeal genus *Cenarchaeum*.

The sponge-pbin.1 assigned to the alphaproteobacterial order Rhodobacterales, was by far the most abundant sponge bin, with coverages 4 times higher than that of the second most abundant sponge bin. Interestingly this was also the order to which the most abundant seawater and sediment bins were assigned to.

The metagenome analysis still ongoing and current efforts are focused on the functional analysis and its integration with taxonomy to understand the microbial specificity of the sponge specie *Cinachyrella* sp. comparing with the surrounding environment.

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