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ABSTRACT BOOK

ORAL PRESENTATIONS

SPEAKERS ABSTRACTS

Angélique Gobet, Researcher in microbial ecology in aquaculture

(IFREMER, French Research Institute for the Exploitation of the Sea, and MARBEC, MARine Biodiversity Exploitation and Conservation joint unit)

EXPLORING THE LIMITS OF METABARCODING MARINE MICROBIAL COMMUNITIES

Since almost two decades, environmental DNA (eDNA) metabarcoding is being increasingly used, leading to unprecedented microorganism inventories by identifying abundant, rare, and even unknown species. It includes several steps such as eDNA capture, eDNA extraction, gene amplification and taxonomic identification, each presenting specific biases that should be taken into account depending on the ecological question raised. In this lecture, I will present results from different projects addressing benefits and biases of choices made for some of these steps. The first step, eDNA capture, is essential for a representative description of community diversity. For instance, planktonic protists may be sampled using sequential filtration to discriminate specific cell size-fractions or centrifugation for no size discrimination. Here, I will compare ecological conclusions obtained from the use of the two capture methods. Gene amplification is another crucial step, where the gene and primer choice will have an effect on the resulting diversity. For instance, bacteria are usually targeted using universal primers amplifying 16S rDNA. In the case of bacteria in close vicinity of micro- or macro-algae, the plastidial 16S rDNA gene is also amplified and may represent a major proportion of sequences, reducing the bacterial diversity coverage. Depending on the objective, the following alternatives may be applied: (i) using primers avoiding plastid amplification for a better coverage of bacterial diversity, or (ii) keep using universal 16S rDNA primers and taking advantage of the chloroplastic 16S rDNA to describe ecological patterns of algal communities. Some resulting ecological patterns will be presented for these two examples.

Jasmina Nikodinovic-Runic

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade

WASTE TO VALUE: FROM BUGS TO DRUGS

Our food, cosmetics, clothes, and other products and consumables across our lives need to get leaner and greener and biotechnology solutions can contribute towards responding to this need. Microorganisms are the most abundant group of organisms on Earth. While invisible to the naked eye and thus somewhat intangible, their abundance and diversity underlie their role in maintaining a healthy global ecosystem. Microorganisms have key roles in carbon and nutrient cycling, human, animal and plant health and are also a source of various products with applications across all major industries, including pharmaceutical, chemical, food, environmental, and the agriculture. Microbial diversity provides a massive pool of inimitable chemicals, which nowadays become a treasured source for innovative biotechnology. In this context, metabolic engineering is a key enabling technology for transforming microorganisms into efficient cell factories for these compounds and materials. Microorganisms can provide economic and environmental value via bio-upcycling of variety of waste streams to obtain next generation eco-friendly therapeutics.

The novel eco-sustainable routes towards value-added biologics through biotechnology will be presented on a set of bacterially derived natural products (pyocyanin, prodigiosin, actinomycin and staurosporine) with proven bioactivities (i.e. anticancer, antifungal, antibiofilm, antiviral). Their greener production, processing and, formulation using innovative techniques such as fermentative bioprocess intensification, structural optimization via biocatalysis and formulations using metals, as well as biopolymeric drug carriers will be highlighted. In this way, both environmental and biomedical problems of human are once more addressed by microorganisms.

Keywords: biotechnology, upcycling, waste conversion, sustainability

József Baranyi

University of Debrecen, Hungary

"BIG DATA" METHODS, RESULTS, AND THEIR (MIS-) USE IN FOOD- AND NUTRITION SCIENCES

This talk focuses on the use of "Big Data" in food and nutrition sciences. "Big Data" methods include all computational procedures that make use of the advantages of the "Four V-s" (Volume, Velocity, Variety, Veracity) while modulating the disadvantages these features inevitably generate.

While the first two V-s have been present in scientific data processing for a long time, the latter two (especially the veracity of acquired information) pose more and more problems for food and nutrition science and can mislead potential users. It is difficult to draw a line between innocent misinterpretations of objective results, obtained by statistical and predictive methods, and their manipulative misinterpretation - and here, we do not speak about fake news.

Various real-life-examples will demonstrate these thoughts, mainly from the field of nutrition science. We point out that with the data deluge of the last two decades, our target should be "making sense of data" rather than collecting more data. The correct interpretation of results obtained via statistical methods should have priority versus populist simplifications. Well-defined concepts, standardized vocabulary and quantification is needed in nutrition sciences to catch up with the level of exactness that food biotechnology or predictive food microbiology have reached via using mathematical rigour when establishing their scientific know-how.

Luis M. Rodriguez-R, Ph.D.

Assist. Professor
Department of Microbiology & Digital Science Center (DiSC), University of Innsbruck, Austria

STUDYING GENOMIC ADAPTATIONS UNDER THE LIGHT OF MICROBIAL ECOLOGY

The study of prokaryotes using environmental genomics offers unique opportunities to overcome statistical and technical limitations on the study of communities and populations in situ.

In this lecture, I will showcase recently introduced bioinformatic tools, statistical techniques, and applications of genomics and metagenomics to the study of environmental adaptations to different environments.

I will primarily focus on the microbial response in Pensacola Beach to the 2010 oil spill in the Gulf of Mexico as a model system for the study of succession dynamics and response to disturbance, with additional discussion being presented on freshwater systems and the global distribution of prokaryotic species.

Lisa Y Stein
Professor & Assoc. Dean (Mentorship and Awards)
Climate Change Microbiology Editor in Chief, The ISME Journal, UNIVERSITY OF ALBERTA

INTEGRATING METHANE AND NITROGEN METABOLISM TO MITIGATE GREENHOUSE GASES

Efforts to reduce methane and nitrous oxide emissions from soils must consider microbial communities as they are gatekeepers for the production and consumption of these potent greenhouse gases. While methanogenic archaea are primary producers of methane, methanotrophs consume methane prior to its emission and directly from the atmosphere. Industrialization of methanotrophic bacteria is opening the possibility to employ these microbes to mitigate methane from high emission sources and perhaps eventually remove methane directly from the atmosphere.

Co-benefits of using methanotrophs for this purpose include production of recoverable protein in the form of biomass and value-added bioproducts. In terms of nitrous oxide, emissions from soils from nitrifying and denitrifying microorganisms is accelerating, mainly due to over-application of nitrogenous fertilizers and low nitrogen use efficiency by crops. Biofertilizers, biological nitrification inhibitors and soil-free aquaponics systems are trending technologies that can curtail nitrous oxide emissions while improving crop production. Furthermore, it is important to note that methanotrophic and N-cycling microbes share common enzymes, metabolic pathways, and regulatory features that control GHG efflux.

However, mitigation of one GHG will often exacerbate production of the other, for instance the application of biological nitrification inhibitors can inhibit both ammonia and methane oxidation as they share a common monooxygenase enzyme. The coevolution and co-functionality of microbial processes in the methane and nitrogen cycles emphasizes the need for these cycles to be considered together for reducing GHG emissions at scale.

Sharon E Zytynska
BBSRC David Phillips Research Fellow, Lecturer, Department of Evolution, Ecology and Behaviour
Institute of Infection, Veterinary & Ecological Sciences, Biosciences Building Rm 236, University of Liverpool

UNRAVELLING BENEFICIAL MULTI-SPECIES INTERACTIONS FOR INSECT PEST CONTROL: FROM THE SOIL TO THE LEAF

Plant-associated microbes can prime plant defences that help a plant evade and resist insect pests, resulting in reduced pest outbreaks on crops and minimising yield losses.

Harnessing soil microbiomes for sustainable agriculture is a rapidly developing area, yet strong beneficial effects in controlled studies are not always transferred to the field.

In the first part, I will present an overview of how root-associated plant microbes can reduce aboveground insect pests and highlight the consistency of effects we observe in a barley-bacteria-aphid system across variable environments.

Plant-microbe interactions are rarely studied as complex interacting systems, despite beneficial effects frequently described as being provided by synergistic interactions between microbes.

Thus, I end by discussing how research on microbial communities and interactions will deepen our understanding of microbe-induced plant defence against insects.

TABLE OF CONTENTS

AGRICULTURE

ENVIRONMENT

S2_OP11: Microbial and electrochemincal synergy for sustainable groundwater remediation: combining pyrite-based denitrification and electrochlorination

S2_OP12: The environmental fate and impact on the soil microbial community composition, resistome and mobilome of the veterinary antibiotics sulfomethoxazole, tiamulin and tilmicosin.

S2_OP13: Drivers of the composition of root-associated microbial communities in garrigue ecosystem

S2_OP14: From Micro to Macro - Defining Crete's Macroecology based on its soil Microbial Interactome

S4_OP21: Genomic signatures of symbiotic lifestyle in sponge-associated bacteria

S4_OP22: The Role of Microbes in Mitigating Greenhouse Gas Emissions from a Toxic Cave

S4_OP23: Are microbial communities resilient to heat waves in a shallow intertidal sediment?

S4_OP24: How vulnerable to hydraulic connectivity are phytoplankton assemblages across a salinity gradient?

S4_OP25: The impact of temperature, acidity, and nutrient availability on marine bacteria-bacteriophage interactions

S4_OP26: Microbial diversity and sulfur cycling in a hypersaline marsh microbial mat community

---

BIOTECHNOLOGY

S3_OP15: Exploitation of novel fungal oxidative biocatalysts for the sustainable production of valuable monomers from biobased furans

S3_OP16: Comparative Genomic Analysis of Enterobacter spp. Isolated from Fruit flies : New Insights into bacteria-host interactions

S3_OP17: Hydroxy fatty acid production by chemo-enzymatic conversion of waste cooking oils

S3_OP18: Effect of light wavelength on polysaccharides production by submerged cultivation of Pleurotus ostreatus

S3_OP19: Optimization of a new industrial bioprocess on pilot-scale for 2,3-butanediol by Klebsiella oxytoca ACA-DC 1581 cultivated on biodiesel-derived glycerol

S3_OP20: Valorization of the organic fraction of municipal solid waste: Improving process efficiency via biomass refining and in bio-electrosynthesis of biodegradable plastics

S5_OP27: Fungal copper radical oxidases as new biocatalysts for the green industry.

S5_OP28: Implications of Polysaccharide Oxidative Degradation: Insights into Catalytic Activity and Substrate Specificity of Lytic Polysaccharide Monooxygenases from Thermothelomyces thermophila

S5_OP29: The isolation and characterization of novel pyrethroid hydrolases from biobeds using functional metagenomic approaches

S5_OP30: Discovery of antiaging compounds via the screening of Greek actinobacteria

S5_OP31: Physiology of Yarrowia lipolytica under different fermentation modes: Development of a membrane bioreactor process for production of various biomolecules

S5_OP32: A proof-of-concept study for lactic acid solution purification employing a genetically modified e.coli strain in a membrane bioreactor system

---

ONE HEALTH/OMICS

S7_OP39: Implications of short- and long-term exposure of the microalga Tetraselmis chuii to H2O2-induced oxidative stress, as revealed by multi-omics analysis

S7_OP40: Effector ripe1 of ralstonia solanacearum targets exo70b1 and is recognized by the ptr1 immune receptor

S7_OP41: Phylogenetic and evolutionary analysis of fungal mitogenomes using a novel bioinformatics pipeline

S7_OP42: Multi-omics analysis of a polyurethane-degrading Fusarium oxysporum

S7_OP43: Waterborne parasitic zoonoses: a neglected public health threat?

S7_OP44 (FT): Impact of insect symbionts on parasitoid foraging behaviour

S7_OP45 (FT): The future of 16rRNA gene high throughput sequencing: A comparative analysis of new Illumina and Oxford Nanopore Technologies sequencing methods.

EMERGING APPROACHES

S8_OP46: Isolation and characterization of a Sphingomonas strain able to degrade the pleuromutilin antibiotic tiamulin

S8_OP47: Genome analysis of Lacticaseibacillus paracasei SRX10: An Indigenous Strain for Use as a Multi-Functional Adjunct Culture in Cheese Production.-

S8_OP48: Tracking viral infection temporal dynamics after microbial resuscitation following seasonally y soil rewetting using viromics and stable isotope metagenomics

S8_OP49: Intergenerational memory of single cell division times in a growing microcolon

S8_OP50: Bacteriophage selection alters antibiotic resistance in Vibrio alginolyticus

S8_OP51 (FT): Evaluation of meat spoilage markers for the development of on-package freshness sensors

S8_OP52 (FT): An interactive online stochastic quantitative microbiology tool for risk-based decision support in the Food Industry applied to Bacillus cereus in milk

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FOOD-NUTRITION

S9_OP53: Microbial inactivation: Setting the basis for a risk-based design in food processing

S9_OP54: Combination of spectral and NGS data for the microbiological quality assessment of shellfish

S9_OP55: Vineyard-mediated factors are still operative in spontaneous and commercial fermentations shaping the vinification microbiome and affecting the antioxidant and anticancer properties of wines

S9_OP56: Where Greek cheeses belong within the global cheese microbial map: a comparative integrative analysis of cheeses' microbial signatures

S9_OP57: Monitoring the survival of probiotic bacteria during the shelf life of a novel Greek sheep traditional yogurt and following subsequent in vitro digestion

S9_OP58 (FT): Microbial electrostimulation and electrocution using low intensity electric fields – A novel approach for selective control of microbial growth in vitro and in food matrices

S9_OP59 (FT): Listeria monocytogenes colony growth dynamics after exposure to acidic conditions and disinfectants

S9_OP60 (FT): Short-term effects of fruit juice enriched with probiotics on glycemic responses : A randomized controlled clinical trial in healthy adults

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AGRICULTURE

S1_OP01

POPULATION DYNAMICS OF AERIAL AND EPIPHYTIC ICE-NUCLEATING BACTERIA IN LEMON ORCHARDS IN AEGHION, GREECE

Dimitrios G. Georgakopoulos[1], Dimitrios Kontogiannatos[1], Maria Gini[2], Proomos Fetfatzis[2], Guillaume Charrier[3], Nicolas Dusart[3], **Khalil Geballa-Koukoulas[1]**, Michalis Karvelas[4], Themis Loukopoulos[4], Panagiotis Gkionis[4], Vaios T. Karathanos[5], Georgios Sarigiannidis[6], Ioannis E. Papadakis[7], Konstantinos Eleftheriadis[2]

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Frost is an important weather phenomenon that adversely affects agricultural production. Meteorological, agricultural and microbiological factors almost equally participate in its establishment and appearance within the agro-ecosystem. Epiphytic bacteria of the genus Pseudomonas, seem to be actively involved in enhancing ice formation on plants by virtue of their ice nucleation activity (INA), to catalyze the formation of ice in supercooled water. The accumulation of epiphytic INA bacteria within the agro-ecosystem is influenced by meteorological conditions, aerial immigration/emigration and local microclimatic conditions. In this work we studied the seasonal dynamics and the ice-nucleation activity of epiphytic and aerial microbial populations in two organic lemon orchards in the region of Aeghion, Greece. Through a multidisciplinary approach involving the determination of the diurnal variability of PM1, PM2,5 and PM10 μm particles and their INA, in combination with classical microbiology and molecular biology (qRT-PCR targeting the INA genes), we determined the seasonal fluctuation of fluorescent ice-nucleating bacterial populations as well as the seasonal fluctuation of ice-nucleation activity at the aerial and epiphytic level. The seasonal population dynamics of aerial and epiphytic bacteria seem to follow a similar pattern to the quantitative fluctuation of aerosol particles, allowing us to establish the preliminary basis for constructing computational models to predict the population dynamics of epiphytic INA bacteria and consequently the risk for frost damage in an orchard. Copper sprays were applied to 3-year-old lemon trees in higher altitude orchards to evaluate their effectiveness in reducing epiphytic INA bacterial populations and ice nucleation activity, and to estimate their potential for frost damage mitigation. After 48h, INA bacterial populations and ice nucleation activity were almost completely eliminated from leaf washings.

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S1_OP02

THE EFFECTS OF THE BENEFICIAL ENDOPHYTIC FUNGUS FUSARIUM SOLANI STRAIN K IN TRANSCRIPTOMIC AND METABOLOMIC PROFILE OF TOMATO PLANT UNDER DROUGHT

Feka M[1], Tsiouri O[1], Manresa M[2], Vletsos P[1], Flors V[2], Papadopoulou K[1]
[1]University Of Thessaly, [2]Universitat Jaume I

Plants have evolved defenses against a number of stressors that impede their development and performance. Environmental abiotic factors have an impact on their metabolic and physiological processes (Kavroulakis et al., 2018). Endophytic filamentous fungi help host plants during stress conditions. Fusarium solani strain K (FsK) is a beneficial endophytic fungus that is able to colonize tomato's root system (Solanum lycopersicum) and provide defense against drought and salt stress (Kavroulakis et al., 2007). Endophytes have the ability to alter the metabolic profile of the host, whereas stress triggers the activation of particular transcription factors involved in plant hormone signaling pathways (Jalal et al., 2020)(Orellana et al., 2010). In this work we examined whether FsK colonization on tomato seedlings' root system, grown under normal and drought conditions, had an impact on the transcriptome and on the metabolome. Our data indicated that the inoculation with the endophytic fungus may generally enhance plant growth by activating specific transcription factors and manipulating specific plant metabolites and phytohormones like Abscisic acid, contributing to the inoculated plants' resistance.

Key words: Endophyte, Drought, Transcriptome, Metabolome, Tomato

S1_OP03

INOCULATION-INDUCED MODULATION OF BACTERIAL COMMUNITIES IN THE RHIZOSPHERE OF WINTER RYE REMAINS PRESENT FOLLOWING WINTER DORMANCY

Ioannis Kampouris[1], Behr Jan2[2], . Joerg Geistlinger[3], Michael Baumecker[4], Rita Grosch[2], Kornelia Smalla[1], Doreen Babin[1]

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The inoculation with plant-beneficial microorganisms (B) can increase the performance of important crops. However, it is unknown whether B can survive in a sufficient density over the winter period and retain their activity, despite the harsh conditions that occur during winter dormancy. In the present work, we tested whether the B sufficiently colonise the rhizosphere of winter rye, thereby affecting the composition of the rhizosphere bacterial communities and the plant growth performance. Specifically, winter rye was treated with B in a long-term field trial where fields have been subjected either to organic or integrated farming system.

The B inoculum included two bacterial strains (Bacillus Abi03 and Pseudomonas RU47) and one fungal strain (Trichoderma harzianum OMG16). Samples were taken before and after the winter dormancy (autumn and spring samplings) and rhizosphere bacterial communities were analysed in total community DNA with high-throughput 16S rRNA gene amplicon sequencing. We found that B established in the rhizosphere over the winter period and increased the growth performance of crops, especially when applied under organic farming practice, and that B inoculation increased the crop nutrient acquisition.

The farming practice and B inoculation significantly affected the β-diversity of the bacterial communities in the rhizosphere, with farming practice explaining higher variance of β-diversity in bacterial communities than B inoculation.

In addition, the B significantly altered the composition of bacterial communities in the rhizosphere, in both samplings, but no interaction effect was observed. Meanwhile, several ASVs responded to the farming practices and to a lesser extent to the B inoculation, before and after winter dormancy.

Only a few ASVs were positively or negatively associated with the increased effect of B inoculation on the growth of crops under organic farming practice.

The combination of molecular- and culture-based approaches indicated that the organic farming practice improved the survival of Pseudomonas RU47. In conclusion, the B survive in the rhizosphere of winter rye and alter the bacterial community composition in the rhizosphere, likely contributing to the observed increased growth of winter rye at early plant developmental stage under organic farming practice.

S1_OP04

EXPLORING ENDOPHYTISM OF ENTOMOPATHOGENIC FUNGI THROUGH COMPARATIVE GENOMICS: AN IN-DEPTH STUDY

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Entomopathogenic fungi (EPF) are widely known for their use as biological control agents against a wide range of pests. Alongside this function, they have the ability to affect plant growth and confer plant protection characteristics as both endophytes and rhizosphere colonisers, rendering them promising candidates for the development of a whole range of novel biocontrol products and biofertilizers. This, however, is hindered by limited knowledge related to the mechanisms behind these abilities and the ways that these fungi evolved for this dual mode of living. In this work, an attempt is made to highlight the significance of the deployment of genomics in revealing the underlying mechanisms of endophytism exhibited by entomopathogenic fungi. The whole genome sequencing of Metarhizium brunneum strains ARSEF 4556 and V275, Beauveria bassiana ATHUM 4946, Metarhizium guizhouense ARSEF 819 and Beauveria brongniartii ARSEF 9452 was performed in-house using Oxford Nanopore sequencing, and high-quality whole genomes were produced. Functional annotation results confirmed that entomopathogenic fungal genomes are versatile in respect to their gene content and structure and revealed that all strains have a broad secondary metabolic repertoire that allows them to employ multiple modes of life. Comparative genome analyses revealed that the organisms' genomes share orthologue genes with other endophytic and entomopathogenic strains of the order Hypocreales, as well as several singleton genes. Phylogenetic trees based on matrices of these genes are highly informative regarding the evolutionary relationships among these fungi. Furthermore, synteny analyses showed major syntenic clusters among other endophytic and entomopathogenic strains. Overall, our comparative genome analyses provide insights into the genomic diversity and evolutionary history of these Hypocrealean EPF strains. In addition, our findings shed light on the evolution of the mechanisms and metabolic pathways of endophytism exhibited by EPF and provide answers to questions raised concerning their adoption of this multifaceted mode of life of these species.

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S1_OP06 (FT)

STUDY AND CHARACTERIZATION OF MICROBIAL DIVERSITY IN FISH FAR USING OMICS TECHNOLOGIES

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Sparus aurata is the main cultivable species in Greek aquaculture. Despite strict treatment and disinfection protocols during rearing, symptoms of known or unknown pathogenicity may appear at different age stages, causing epidemics and economic losses. One such condition is the body rotation syndrome, which causes the fish to lose their swimming abilities and die. However, the cause of this symptom is still unknown. As the microbiome plays a key role in animal health, this study examines the association between larval symptoms and their associated bacteria during early developmental stages in an industrial larval production facility. Using amplicon sequencing of the 16S rRNA gene, the bacterial communities present in healthy and symptomatic S. aurata larvae at different developmental stages (3-18 days post-hatch) were compared. The study also examined bacterial communities present in eggs, rearing water, feed, organic matter from the bottom of tanks, and air samples from the surrounding area. Over the development of S. aurata larvae, differences were observed in the bacterial composition between healthy and symptomatic larvae as well as between components of the larviculture system regardless of the disinfection method. A remarkable presence of members of Psychrobacter genus was observed in symptomatic larvae. The healthy larvae harbor different bacterial profiles with a dominance of Vibrio and Bacillus during 3-8dph, various members of Alphaproteobacteria during 11-14dph and Marinifilum at 18dph. The rearing water showed a different bacterial profile compared to the other larviculture components, with a slight effect on healthy larvae at 3-8dph.The feeds used during the rearing of S. aurata larvae contain a diverse array of bacterial genera. These communities play a significant role in shaping the microbiota of both healthy and symptomatic larvae, particularly at 18dph following the introduction of Artemia live feed. Among the larviculture components, the results revealed that the tank disinfection method primarily affects the bacterial community associated with organic matter. This study provides valuable information on the microbial diversity in both healthy and symptomatic larvae, which can be used to estimate the occurrence of the syndrome and determine the optimal disinfection method to prevent future occurrences of the same symptoms.

S1_OP07 (FT)

GILLS AND SKIN BACTERIAL LANDSCAPES OF FIVE FISH SPECIES FROM THE ATLANTIC OCEAN

Sokratis Papaspyrou[1], Jose Luis Varela[1], Antonio Medina[1], Jose Calderón Caro[1], Eleni Nikouli[1], **Konstantinos Kormas[2]**

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Our current knowledge on fish-associated microbiota is highly biased towards farmed compared to wild species. Moreover, most studies focus on intestinal/fecal microbiota. However, the microbiota of wild fish consists a potential pool of novel fish-microbe associations and interactions that could benefit even the farmed species. In this study we present the structural and potentially functional diversity of the gills and skin of five (Auxis sp., Coryphaena hippurus, Euthynnus alletteratus, Sarda sarda, Tetrapturus albidus) fishes caught in the Gulf of Cadiz, Atlantic Ocean. Fishing and sampling took place during sport fishing competitions in summer of 2019. Skin swabs over the right-side lateral line and the second arch of the gills were aseptically removed immediately upon catching and stored at 4 °C and stored immediately at -80°C upon return to the laboratory. Bulk DNA from two or three replicate individuals was extracted with a commercial kit and the V3-V4 region was targeted for high-throughput sequencing on the Illumina MiSeq 2x300 bp platform. The gills microbiota profiles were different, with those from S. sarda and T. albidus being more distinct; these two species had the highest number of unique operational taxonomic units (OTUs). Skin microbiota had a much higher degree of overlap, with the exception of T. albidus, C. hippurus and S. sarda had the highest contribution of unique OTUs. Members of the Moraxellaceae and Vibrionaceae families dominated both the gills and skin of all fishes, while Pseudomonadaceae, Staphylococcaceae members and microalgal chloroplasts were also among the most abundant. The OTUs overlapping in both gills and skin of each fish ranged between 26.8% and 33.2% of all OTUs occurring in each fish. The major inferred microbial functions were dominated by nucleoside and nucleotide metabolism, amino acids biosynthesis, cofactor and vitamins biosynthesis, fatty acids and lipids metabolism. Fermentation and anaerobic pathways were scarce. Functional redundancy was demonstrated by the high overlap between the gills and skin in each fish (94.0-96.5%).

S1_OP08 (FT)

SEABASS GUT MICROBIOME ANALYSIS FED WITH CONVENTIONAL AND INNOVATIVE DIETS

Alexandra Meziti[1], Konstantinos Kormas[2], Elena Mente[3]

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As aquaculture is nowadays the major fish-food production sector, continuous search is taking place for aquafeeds that are alternative to conventional fishmeal in order to enhance its environmental and economic sustainability. The aim of this study was to investigate changes in fish gut microbiota that could be associated with the enhancement of health and productivity of sea bass fed with conventional (C) and innovative diets (I). Midgut (M) and feces (F) samples were collected in replicates from a fish farm in Greece. Apart from different treatments, fishes were also separated based on their growth rates to fast (good; G) and slow (bad; B) growers. After DNA extraction bacterial diversity was assessed by amplification of the V3-V4 region of the bacterial 16S rDNA gene using Illumina MiSeq with universal bacterial primers. Raw sequence reads were processed and analyzed, using the MOTHUR software (v. 1.39.5). Sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold. Our results indicated that Proteobateria prevailed in all samples followed by Firmicutes (MIG/MCB/FIG) or Bacteroidota (FCG/FIB/FCB) while Cyanobacteria were present only in feces. Taxonomic distributions at the OTU level exhibited significant differences between gut and feces samples with representatives of Variovorax and Synechoccus prevailing mostly in gut and feces samples respectively. Regarding different feeds provided, Staphylococcus was more abundant in F diets while Streptococcus prevailed in C. Firmicutes/Bacteroidetes ratio peaked in MFG (5.8) and the respective feces sample (15.3) implying the beneficial effect of innovative diets towards the prevalence of fermentative microbiota.

S6_OP33

INNOVATIVE TOOLS TO COMBAT KEY PESTS OF TOMATO

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Herbivorous arthropod pests and pathogens can be highly destructive to crops causing significant yield losses, often above 30%. As a result, chemical control is often applied to maintain healthy crops and obtain increased yields. Because pesticides do not only target pests but also pose a threat to human health and the environment, there is an increasing demand for alternative pest control solutions. Towards this direction, INTOMED project has studied effective and sustainable tools based on interactions between plants and soil beneficial microbes as well as plant-derived compounds (metabolites and peptides) with the aim to enhance the resistance of economically important Mediterranean crops to major agricultural pests and pathogens. Herein, we present the results of the INTOMED consortium focusing on the tomato crop and its key pests. Transcriptomics, proteomics and metabolomics have been performed to identify promising mechanisms underlying beneficial plant-microbe interactions, and a series of peptides and metabolites including volatile compounds have been identified and assessed for their effects against pests and their natural enemies. Our work highlights the potential of beneficial soil microbes, peptides and metabolites in suppressing key pest populations in sustainable crop production, as well as the need for further research towards this direction.

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S6_OP34

CONTEXT DEPENDENCY ORCHESTRATES THE MECHANISMS BEHIND MYCORRHIZA-INDUCED RESISTANCE

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Mycorrhizal plants show alternative phenotypes resistance when compared to the same plants in the absence of symbiosis. Regardless of the evidence that mycorrhizal plants tolerate better abiotic stresses, there are not many studies of Mycorrhiza-Induced Resistance (MIR) in woody plants against biotic challenges and very few in citrus. The present study ai to investigate how MIR affects a highly susceptible citrus genotype, Citrus reshni to direct resistance in local and systemic tissues as well as antixenosis and attraction to natural enemies. Furthermore, we studied the impact of symbiosis in the hormonal-regulated metabolism of defence, and we include comparative studies with a resistant genotype. The current research of MIR in citrus includes classical symbiosis bioassays, together with an assessment of resistance to T. urticae by analysing leaf damage rates and egg laying. To understand the impact of symbiosis in the defensive metabolism we also have performed a transcriptional analysis of phytohormone-regulated genes, as well as determinations of hormonal levels and non-targeted metabolomics using UPLC coupled to a TQS and qTOF mass spectrometers respectively.

The current study shows that the susceptible genotype Citrus reshni is highly sensitive to MIR displaying local and systemic induced resistance, antixenosis and improved attraction to a specialist carnivorous predator. Transcriptional and hormonal analysis showed enhanced JA-dependent responses in mycorrhizal C. reshni and priming of JA-Ile and the biosynthesis gene LOXD at 24hpi after infestation. Furthermore, symbiosis enhances systemic resistance responses by reducing leaf damage and egg-laying following a secondary infestation. Non-targeted metabolomic analysis evidenced a complex interplay in the secondary metabolites in Rhizophagus irregularis-colonized plants. Mycorrhizal plants exhibited priming of a pool of flavones with protective functions in the presence of mite infestation at 24hpi. Noteworthy, Naringenin shows a strong correlation with overaccumulation of Baicalein as well as OPDA and JA-Ile indicating a likely regulation of flavonoid accumulation by jasmonates in infested mycorrhizal plants. Altogether, symbiosis provides multi-faceted protection of a highly susceptible citrus genotype against the polyphagous T. urticae activating early responses at local and systemic levels.

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S6_OP35

RNAI IN PLANT-MICROBE INTERACTIONS

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RNA interference (RNAi) is a conserved eukaryotic gene regulation mechanism. The great potency of RNAi as a biotechnological crop protection tool in transgene-based (Host-Induced Gene Silencing, HIGS) or transgene-free applications (Spraying-Induced Gene Silencing, SIGS) is well established. What is now emerging is that RNAi is also a key endogenous regulator of plant-microbe interactions. Indeed, a growing body of evidence suggests that bi-directional cross-kingdom RNAi between plants and pathogenic fungi to the advantage of either the host or the pathogen is taking place in several instances. Moreover, our recent data show that trans-kingdom RNAi communication occurs during plant association with beneficial endophytic fungi, suggesting that the latter may establish the mutualism or impose their beneficial impact by translocating RNAi molecules that modulate host gene expression.

S6_OP36

BIOLOGICAL CONTROL OF BOTRYTIS CINEREA USING COMMERCIAL WINE YEASTS

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Botrytis cinerea is a major cause of yield loss in viticulture worldwide. Grapes are particularly susceptible to Botrytis rot at maturity, but the use of chemical products to control the disease is not feasible due to the risk of chemical residues. Additionally, Botrytis easily develops resistance to chemical fungicides. To address these issues, research is focused on biological control methods. This study examined the effectiveness of five commercial wine yeasts (Saccharomyces cerevisae, S. pastorianus, S. bayanus, Lancacea thermotolerans, and Tolulaspora delbrueckii) in controlling the growth of B. cinerea. In vitro tests demonstrated that although the yeast cells, metabolites, or volatiles did not reduce mycelial growth at 3 or 6 days post inoculation, their use significantly reduced the production of Botrytis conidia at the end of the experiment. Ex vivo assays involving detached berries immersed in yeast cultures demonstrated significantly reduced growth of B. cinerea along with reduced conidia production at 15 days post inoculation with the pathogen. Further investigations on the mode of action of the most effective yeast strain, S. bayanus, using Real-time PCR analysis, indicated that it did not induce significant defense responses in PR3, PR4, and PR5 grapevine defense genes, suggesting that its mode of action against Botrytis is due to antagonism for space and nutrients rather than defense induction. The results of this study propose that commercially available wine yeasts can be used for the biological control of Botrytis in vineyards.

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S6_OP37 (FT)

USE OF BOTANICAL EXTRACTS AS POTENTIAL SUPPRESSIVE AGENTS AGAINST CERTAIN PLANT PATHOGENS

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Chemical pesticides are commonly applied to protect plants against various phytopathogens. Even though chemical pesticides are widely applied in managing plant diseases, global efforts are made to reduce their use, due to certain examples of negative impact on the environment and aquatic life. Recently, biocontrol agents with plant protective properties have been gaining scientific attention as part of the efforts to adopt sustainable and efficient strategies for managing plant diseases and preserving plant production. Such agents can be derived from a wide range of aromatic plants, which thrive in the Mediterranean region. For centuries, these plants were recognized for their antimicrobial action. In this work, the potential suppressive properties of certain botanical extracts against major plant pathogens were evaluated. In vitro experiments revealed the phytoprotective properties of selected botanical extracts, e.g. Cistus and Thymus spp., which were further evaluated in planta. It is concluded that botanical pesticides from aromatic plant species that thrive in Mediterranean region can serve as an effective alternative against certain plant pathogens.

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S6_OP38 (FT)

CHARACTERISATION OF BACTERIAL CONTAMINANTS OF AQUATIC PLANT SOURCES

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Aquatic plants, Alaria esculenta, Chlorella vulgaris and Lemna minor, were investigated for the potential as fish feed by identifying the types of microorganisms present in these aquatic plants and determining their contamination levels. Alaria esculenta (type 1: harvested from Dúlra Seaweed, County Mayo, Ireland,), Chlorella vulgaris and Lemna minor (type 1: harvested from an aquaculture site in Mount Lucas Wind Farm, County Offaly, Ireland) were purchased in pre-dried form. Fresh Alaria esculenta (type 2: supplied by Connemara, Organic Seaweed Company, Ireland) and Lemna minor (type 2: purchased from a Greek farmer) were washed, dried and milled. Samples for bacterial DNA extraction were taken from all sources, and bacterial DNA was isolated from plant samples using the DNeasy PowerSoil Pro Kit. A Qubit dsDNA HS assay kit was used to quantify the extracted DNA. The primers 341F (5′ -CCTAYGGGRBGCASCAG- 3′) and 806R (5′ -GGACTACNVGGGTWTCTAAT- 3′) targeting the V3-V4 hypervariable region of bacterial 16S rRNA genes were used to construct amplicon libraries for the sequencing platform. The diverse raw biomass samples were assigned 35 classes representing distinct phyla of fungal kingdoms. Cyanobacteria was found to be the most abundant phylum in Chlorella vulgaris (59.66%), followed by Lemna minor (type 1) (42.08%), Lemna minor (type 2) (35.97%), and Alaria esculenta (type 2) (26.07%). Arthrospira_PCC-7345 (12.39%), Acinetobacter (8.10%), and Sphingobacterium (3.87%) were the most prevalent genera in all raw biomasses. Moreover, a taxonomic-based comparison was carried out to evaluate the differences among the microbiota of all the raw biomass samples. In terms of Unweighted Unifrac b-diversity, type 2 samples were clustered together while substantial differences in prokaryotic communities were identified between raw biomass from type 1. According to the results of the next generation sequencing (NGS) analysis, 13 genera (>1%) dominated the bacterial communities in all of the samples. These findings will be critical in improving the aquaculture plant cultivation, processing, and storage, as well as in developing effective measures to assure their quality and safety for consumption. This research findings will also contribute to develop effective strategies for ensuring the safety of these biomasses as high-protein feeds in aquaculture systems.

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S6_OP38b (FT)

ENDOPHYTES FROM HALOPHYTES: A SOURCE OF BENEFICIAL MICROBES FOR A SUSTAINABLE AGRICULTURE

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The improvement of crop management strategies is needed due to the demands of adaptation to climate change, the emergence of new and more aggressive phytopathogens and the deterioration of agricultural soil quality. Beneficial endophytes are non-pathogenic microbes that live within plant tissues and can provide sufficient protection of their hosts against biotic and abiotic stress.

For potential use in agriculture, we isolated and characterized over 600 endophytic microbes from olive trees and crop wild relative halophytes. We investigated thoroughly 25 beneficial Bacillus isolates using a multi-disciplinary approach and under biotic and abiotic stress conditions.

We sequenced full chromosomes and plasmids of selected 25 Bacillus isolates. Comparative genomics reveal high genetic/genomic dissimilarity, novel secondary metabolism gene clusters and the discovery of new species.

Six isolates grow in-vitro in high salinity (>15%). 15 isolates inhibited the growth of important phytopathogens (eg, Ralstonia, Clavibacter, Fusarium, Botrytis, etc). Several isolates retain these characteristics in-planta. The inhibitions were intensified when testing eluents, obtained from Bacillus cultures using flash column chromatography.

Our studies provide strong evidence that specific beneficial Bacillus endophytes demonstrate high metabolic and genetic diversity and are excellent candidates as Bioinoculants for the enhancement of growth and tolerance of crops under biotic and abiotic stress.

Τα αποτελέσματα της παρούσας μελέτης προέκυψαν στο πλαίσιο του ερευνητικού προγράμματος με τίτλο «Βιώσιμες λύσεις για την Βιολογική Αντιμετώπιση Επιβλαβών Μικροοργανισμών και την Επαγωγή της Αντοχής των Καλλιεργειών στην Αλατότητα» και ακρωνύμιο «BIOCONTROL» (Κωδικός έργου Τ2ΕΔΚ-01859, Κωδικός Πράξης/MIS 5073622. Το Έργο υλοποιείται στο πλαίσιο της Ενιαίας Δράσης Κρατικών Ενισχύσεων Έρευνας, Τεχνολογικής Ανάπτυξης & Καινοτομίας «ΕΡΕΥΝΩ – ΔΗΜΙΟΥΡΓΩ – ΚΑΙΝΟΤΟΜΩ» του Ε.Π. «Ανταγωνιστικότητα, Επιχειρηματικότητα και Καινοτομία» (ΕΠΑνΕΚ) 2014-2020 και συγχρηματοδοτείται από το Ευρωπαϊκό Ταμείο Περιφερειακής Ανάπτυξης (ΕΤΠΑ).

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ENVIROMENT

S2_OP10

COMPARATIVE GENOMICS AND TRANSCRIPTOMIC ANALYSIS REVEALS WHY BACTERIA OF THE GENUS PAENARTHROBACTER ARE SPECICALISTS IN THE DEGRADATION OF THE FUNGICIDE IPRODIONE.

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With the rapid evolution of high throughput sequencing technologies, shotgun metagenomics became a routine analysis for environmental microbial DNA, and, particularly the DNA of enriched naturally occurring microbial consortia during biomining approaches and environmental diagnostics. Current 3 rd generation or combined 3 rd /2 nd generation sequencing strategies along with current assembly and binning algorithms provide unprecedented metagenome reconstruction and metagenome assembled genome (MAG) binning ability. However, differences between DNA structural and phylogenetic markers of mobile genetic elements with the strictly vertically acquired chromosomal elements, greatly humper such efforts, when it comes to assigning these two types of assembled contiguous counterparts into genomes. One powerful culture-independent alternative for retrieving genomes of environmental microbiota is that of single cell genomics, yet, its current cost and complexity renders it inaccessible for the average research facility. We sought to address these issues through the development of a cost effective, efficient, quick and equipment-wise less demanding bacterial single cell sequencing protocol. We attempted to refine our previously generated MAGs of a thiabendazole degrading microbial enrichment. The process followed included the batch performance of the costly reactions in situ (e.g. tagmentation) after the microbial cell fixation. The cells were sorted into individual microplate wells and PCR-tagging was performed for identifying individual cells.

Collectively, we have achieved a post cell-fixation tagmentation of the microbial DNA in situ, as validated by PCR amplification and Sanger sequencing. Cell sorting and DNA indexing was also performed, and high throughput multiplex sequencing of the tagged DNA is on the way. Our results will be presented at the meeting. A successful such protocol is expected to provide accessibility to high quality validated MAGs for members of enriched environmental microbial consortia to a, funding-wise, broad range of research facilities.

S2_OP11

MICROBIAL AND ELECTROCHEMICAL SYNERGY FOR SUSTAINABLE GROUNDWATER REMEDIATION: COMBINING PYRITE-BASED DENITRIFICATION AND ELECTROCHLORINATION

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Groundwater constitutes more than 97% of the planet's freshwaters and serves above 30% of domestic needs. Poor sanitation facilities, release of untreated wastewater, cattle farming and intense fertilising activities are the main drivers of anthropogenic groundwater pollution, designated by increased concentrations of nitrate (NO_3^-), chloride (Cl^-) and coliform bacteria. Human exposure to pathogens and >50 mg NO_3^-/L in potable water has been linked to water-borne diseases and respiratory health issues1. Furthermore, the current water treatment methods suffer from intensive chemical dosing and are therefore unsustainable in resource-limited environments.

This study combined autotrophic denitrification with FeS_2 as electron donor and electrochemical disinfection through Cl^- oxidation to Cl_2 (HOCl + OCl^-) to treat NO_3^- and microbial contamination and simultaneously benefit from the high Cl^- content of the polluted groundwater. Aim was to propose a potentially more sustainable and of reduced chemical input groundwater treatment approach.

A fluidized, FeS_2 (120 g) bed reactor was constructed in a 1 L glass column and operated, first in batch, then in continuous mode (18 h HRT) at 22 (± 2) ^{0}C, to denitrify polluted groundwater from a private well in Co. Galway, Ireland. The denitrified effluent was subsequently disinfected in a membrane divided electrolysis cell, with a Pt/Ti anode (25 cm2), where Cl_2 was produced. The cell was operated in batch at 50 – 150 mA and the production of free chlorine, as well as the removal of total colifor in relation to the electrolysis time and the current input were monitored.

During the 35 days of continuous denitrification, NO_3^- was removed with a consistent 79% NO_3^- efficiency from an initial 178 mg NO_3^-/ L, at an average denitrification rate of 171 mg NO_3^-/L d. The electrochemical unit achieved a 4.5-log decrease in total colifor at a minimum 41.7 Ah/m3 charge density. The potable water limits of NO_3^- < 50 mg/L and 0 CFU/100 mL colifor, were achieved without additional input of chemicals other than the initial FeS_2 addition in the denitrification reactor.

1 Masindi, V. & Foteinis, S. Groundwater contamination in sub-Saharan Africa: Implications for groundwater protection in developing countries. Clean. Eng. Technol. 2, 100038 (2021).

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S2_OP12

THE ENVIRONMENTAL FATE AND IMPACT ON THE SOIL MICROBIAL COMMUNITY COMPOSITION, RESISTOME AND MOBILOME OF THE VETERINARY ANTIBIOTICS SULFOMETHOXAZOLE, TIAMULIN AND TILMICOSIN

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Manure amendments lead to contamination of agricultural soils with veterinary antibiotics (VAs). These might exert toxicity on native microbiota and threaten soil functions, environmental quality, and human health. We assessed the impact of sulfamethoxazole (SMX), tiamulin (TIA) and tilmicosin (TLM) on the soil microbiota, antibiotic resistance gene (ARG) and intl1 abundances, in microcosms repeatedly exposed to VAs either directly or via fortified swine manures. Two soils differing at pH and their VA dissipation capacity were used. Repeated VA applications resulted in accelerated degradation of TIA but not of SMX, and accumulation of TLM, with dissipation patterns differing by soil and application method. We then investigated ecotoxicology-relevant soil N-cycling activities and microorganis. Potential nitrification rates (PNR), and ammonia-oxidizing microorganism (AOM) abundances were reduced by SMX and TIA, but not by TLM. Concerning microbial diversity, VAs had strong structural effects on total prokaryote and AOM communities, whereas fungal and protist communities were affected mostly by manure addition. Furthermore, SMX stimulated the abundance of the associated resistance gene sul1 in both the manure amended and the manure free soils, demonstrating its currently acknowledged broad distribution, while manure was the major factor stimulating the rest tested ARGs and the intl1 horizontal gene transfer surrogate. Zinc supplements received by the animals, putatively induced an ARG co-selection that might have resulted in the increased ARG abundances found in the manure amended soils. Correlation testing identified opportunistic pathogens like Clostridia, Burkholderia-Caballeronia-Paraburkholderia, and Nocardioides as potential ARG reservoirs in soil, with or without manure amendments. These taxa encompass environmentally persistent and potent pathogens, suggesting the urge for putting appropriate associated risk assessment strategies in place. Our results provide unprecedented evidence about the effects of understudied VAs (TIA, TLM) on soil microbiota and showcases environmental, and possibly human-health, risks posed by the dispersal of VA-contaminated manures.

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S2_OP13

DRIVERS OF THE COMPOSITION OF ROOT-ASSOCIATED MICROBIAL COMMUNITIES IN GARRIGUE ECOSYSTE

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Plants dominating garrigue (phryganic) ecosyste of the Mediterranean are exposed to low-soil fertility, seasonal high temperatures and ought-stress. Diversity and structure of root-associated microbial communities of two typical plants were investigated. We aimed to identify ecological drivers that determine community assembly processes of host-associated microbial communities. We hypothesized soil properties, plant taxon and season as major ivers.

The roots, rhizosphere, and adjacent bulk soil of early colonizer Hyparrhenia hirta (Poaceae) and climax shrub Sarcopoterium spinosum (Rosaceae), both naturally occurring in undisturbed sites nearby bentonite and perlite quarrying complexes in Milos island (Greece), were sampled after the y (autumn) and the rain (spring) period. Microbial communities were analysed via MiSeq-Illumina 2x300 bp sequencing of PCR-amplified 16S, ITS2 and 18S rDNA marker genes to capture prokaryotic, fungal and protists communities.

Overall, all habitats (roots, rhizosphere, and adjacent bulk soil) were dominated by oligotrophic slow-growing Actinobacteria (e.g. Thermoleophilia , Rubrobacteria), and spore-forming bacteria (e.g. Bacilli). This pattern is even more conspicuous in the endo-root bacterial communities indicating phylogenetic trait conservatism or simply life cycle commonalities in the bacterial root colonizers of phryganic plants. Fungal microbiome was dominated mostly by Dothideomycetes and Sordariomycetes (Ascomycota), followed by Agaricomycetes (Basidiomycota). Arbuscular mycorrhizal fungi (Glomeromycetes) were present in the bentonite bulk & rhizospheric soil only, and were not present in the endoroot fungal community of both plant species.

NMDS analysis of the Bray-Curtis dissimilarity revealed a partial overlap, in all communities (bacterial, fungal and protist) between the bulk perlite and bentonite soils, suggesting low dispersal limitations. However, a clear divergence leading to separation of all communities investigated in this study, between the perlite and bentonite rhizosphere soils was observed, while it appears that plant taxon possesses a diversifying role in the fungal and protist communities. Biotic (plant host) filtering led to strong convergence in the endo-root microbiomes in samples derived from the two soils. This appears stronger for the climax plant S. spinosum compared to H. hirta, which, is a pioneer plant in garrigue biomes and is exposed to microbial colonization iven by local assemblages and stochastic processes forming more promiscuous associations.

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S2_OP14

FROM MICRO TO MACRO - DEFINING CRETE'S MACROECOLOGY BASED ON ITS SOIL MICROBIAL INTERACTOME

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Ecosystem functioning is an integral part of the sciences of climate change and conservation. Microbes are known for their versatility, abundance and influence on ecosystem functions, yet for a more complete analysis of ecosyste other for of life must be included.

More specifically, the plant-arthropod-soil microbiome interactions are beginning to be studied as a whole to discover important associations. A synthesized knowledge base of biodiversity, in ter of ecological and remote-sensing data remains a major challenge.

Many worldwide studies have been published regarding soil microbiome ecosyste, though there are many gaps to cover the complexity of functioning and biodiversity in the local scale. Islands can be important case studies for this integration, from micro to macro, with Crete being a great example.

Here, we utilize the Island Sampling Day Crete 2016 microbial metabarcoding data and metadata integrated with soil, faunistic and remote sensing data to decipher the drivers of ecosystem function of the island.

Cretan macroecology has been studied for centuries for its diverse and unique geology, fauna and flora. In addition, being a continental island has a distinct natural and evolutionary history with high contrasts in vegetation cover, climatic conditions and geology.

The Island Sampling Day Crete 2016 (co-hosted by the Genomic Standards Consortium (GSC) and the Institute of Marine Biology Biotechnology and Aquaculture), has microbial metabarcoding data 72 distinct topsoil sites all around Crete with ecosystem diversity and FAIR (Findable, Accessible, Interoperable and Reproducible) data by design. With this island wide study the GSC put the standards in action.

Notably, microbiome appears to be influenced pH and by elevation, with higher altitudes having greater biodiversity, a pattern common in other faunistic groups such as arthropods.

These data along with the open access data regarding arthropod fauna, flora and the vast data of remote sensing and biodiversity provide the basis to identify major drivers of biodiversity, evaluate hotspots and contribute to prior knowledge of threatened ecosyste.

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S4_OP21

GENOMIC SIGNATURES OF SYMBIOTIC LIFESTYLE IN SPONGE-ASSOCIATED BACTERIA

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The sponge microbiome has attracted a lot of attention as a potential source of new antibiotics. Indeed, sponge holobionts appear to be treasure troves of secondary products, many of which display bactericidal or bacteriostatic activities. This suggests that sponge symbionts face intense interspecific interactions, the signatures of which may contribute to shaping their genomes. awing evidence from complete genomes of archaeal and bacterial symbionts of Porifera (assembled from metagenome data and/or from laboratory cultures), this presentation will review evidence that sponge-associated bacteria display signatures of a symbiotic lifestyle, such as abundance of mobile genetic elements and insertion sequences compared to their free-living relatives.

S4_OP22

THE ROLE OF MICROBES IN MITIGATING GREENHOUSE GAS EMISSIONS FROM A TOXIC CAVE

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The search for the limits of life has long been a question in the field of microbiology. To understand early life, researchers often study microbial communities from extreme environments. In this work we studied a microbial community from a cave in Romania, highly enriched in greenhouse gasses such as carbon dioxide, as well as methane and hydrogen sulfide[1]. There has been increasing interest in discovering and utilizing microbes that can capture carbon to fight against global warming. From an extremely acidic biofilm (close to pH 1) within the cave we enriched and cultivated a Mycobacterium expanding the known range of methanotrophy into the Actinobacteria clade[1].

Using metagenomics, we investigated the microbial diversity of the cave's biofilm and recovered twenty high-quality metagenome-assembled genomes. In addition to Mycobacterium, we found two dominant archaea taxonomically assigned to the Ferroplasma genus. Pan-genome and phylogenomic analyses indicated that the two Ferroplasma species belong to different phylogenetic clades with distinct genetic backgrounds. Incorporating metaproteomics in the analysis highlighted the potential of both species to fix carbon dioxide via reductive tricarboxylic acid cycle using the cave's available sulfur. In fact, proteins annotated as sulfur oxygenase/reductase and sulfide:quinone oxidoreductase were among the highest in protein intensity. Particularly high protein intensity was found in Ferroplasma's CRISPR proteins, suggesting strong coevolution with viruses. Further virus-specific bioinformatics analysis identified up to 400 potential novel viruses. Dedicated tools for predicting potential virus host range were used and successfully identified the key microbial hosts. From those, a few pointed to Ferroplasma as the host, while the majority, up to 200, pointed to Mycobacterium.

We found a diverse microbial community thriving under highly acidic conditions, consuming greenhouse gases. Previously, we identified the dominant Mycobacterium species feeding on methane. This study revealed two dominant Ferroplasma species likely fixing carbon dioxide, and the potential role of viruses, including Mycobacteria viruses. As Mycobacteria viruses are known for their broad host range, further investigation could advance phage therapy for such renowned pathogens.

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S4_OP23

ARE MICROBIAL COMMUNITIES RESILIENT TO HEAT WAVES IN A SHALLOW INTERTIDAL SEDIMENT?

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The sediment surface in shallow tidal areas and particularly the intertidal zone is a very dynamic environment under strong physical forcing. For example, intertidal sediments are subject to a large variation in temperature during the immersion/emersion cycles. However, the increasing frequency and intensity of heat waves due to climate change can induce changes in the sediment microbial community composition and function.

Here we present the results from short-term temperature change incubations of whole sediment cores from intertidal zones. Sediment cores were exposed from 10 to 42 °C in the laboratory for up to 5 days followed by a one-week recovery period. The microbial community was analysed at each stage together with a series of biogeochemical variables. Temperature stimulated the overall net microbial metabolism in the sediments towards anaerobic processes. Richness and diversity decreased with increasing temperature with a progressive change in the composition of the microbial community observed.

At the Phyla level, little difference in relative abundance of main groups between the lower temperatures but a significant increase in the relative abundance of Firmicutes in and Chloroflexi, which both contain anaerobic high temperature tolerant members, at the highest temperature.

In contrast, significant decreases in Verrucomicrobia and Myxococcota were found, possibly related to the decrease in organic matter. A clear increase in the Methanosarciniales, a group including methanogens using acetate as carbon source amongst other compounds and thermotolerant to thermophilic bacteria, confirm the shift towards anaerobic processes. An increase in the number of sequence reads for denitrification pathway was also detected.

Therefore, heat waves can have a large short term impact on both the microbial community and emissions of greenhouse gases from shallow coastal environments.

Therefore, the most extreme temperature disrupted both community function and taxonomy, whereas the community was more resilient to temp shifts.

S4_OP24

HOW VULNERABLE TO HYDRAULIC CONNECTIVITY ARE PHYTOPLANKTON ASSEMBLAGES ACROSS A SALINITY GRADIENT?

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Salinity gradients, often characterized by a patchwork of different salinity habitats, are widespread in nature, hosting unique phytoplankton assemblages. Such habitats are vulnerable to changes in connectivity levels that occur through altered water levels, due to changes in hydrological regime (e.g. flooding , desiccation) often induced by climate change. Although some knowledge exists on assemblage composition and stability in these habitats to seasonal variations in the water cycle, very little is known about effects of connectivity. Herein, we investigate the effect of increasing connectivity, arising from water exchanges between habitats that differ in salinity and assemblage composition, on the resistance and recovery of species and functional traits, as an indication of the mechanisms behind stability of ecosystem functions. We deployed mesocosms within an ecologically important, manmade saltpan system and hydraulically connected enclosures of three salinity levels (40, 46, and 61 psu) at three connectivity levels (0.002d-1,0.02d-1,0.2d-1). In this system, even though species originate from the coastal environment, they present different preferences on salinity, with large diatoms thriving at low salinity and small flagellates and coccoid green algae thriving at high salinity. We hypothesized that high salinity assemblages would be more resistant than low salinity assemblages, as extreme environments are generally more stable with fewer species that can withstand high salinity levels. We further hypothesized that high connectivity levels would decrease both resistance and recovery, promoting homogenization. Indeed, high salinity assemblages proved to be more resistant at all connectivity levels than low salinity assemblages. Moreover, high salinity assemblages of high connectivity were more vulnerable, as they recovered less than assemblages of low connectivity. On the other hand, low salinity assemblages of high connectivity recovered more compared to assemblages of low connectivity, an unexpected finding that needs further investigation. Species size emerged as an important functional trait driving resistance and recovery, with small species replacing the larger ones during the connectivity phase at higher salinity, hindering the recovery at high connectivity. Overall, our study showed that phytoplankton assemblages in an environment comprised of patchwork habitats driven by salinity gradients could be vulnerable to changes of hydrological regimes, with implications on ecosystem functions.

S4_OP25

THE IMPACT OF TEMPERATURE, ACIDITY, AND NUTRIENT AVAILABILITY ON MARINE BACTERIA-BACTERIOPHAGE INTERACTIONS

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Climate change adaptations of microbes are going to be pivotal for the future understanding of marine ecology.

The increasing temperature could affect biological processes as well as circulation and stratification, influencing how organisms are dispersed and nutrients are transported.

Microbial community composition and function are also affected by nutrient inputs from sources such as air, rivers, and estuaries, all of which are subjected to climate change. In addition, increased levels of carbon dioxide in the oceans can lead to acidification of water.

Climate change could influence bacterial interactions with their natural predators. Bacteriophage-bacteria interactions are among the most frequent engagements in marine habitats, contributing to marine biochemistry circles.

In this study, we used a Mediterranean marine-abundant bacterial species, Vibrio alginolyticus, and two Caudoviricetes lytic bacteriophages, both owning a myovirus morphotype but with distinctly different genome sizes.

The lytic cycles of bacteriophages were monitored upon different temperatures, pH levels, and nutrient availability and it was shown that bacteriophages with a larger genomic size had the least impact.

An rt-qPCR platform was developed to monitor the differentially expressed patterns of well-known bacteriophagic genes as well as potentially induced auxiliary metabolic genes. Our findings showcase that bacteriophages with larger genomes may endure efficient host cell hijacking in different microenvironments and continue normal host lysis.

This advantage could favor their abundance in marine habitats under future climate shifts.

S4_OP26

MICROBIAL DIVERSITY AND SULFUR CYCLING IN A HYPERSALINE MARSH MICROBIAL MAT COMMUNITY

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Microbial mats are vertically stratified communities of microorganisms characterized by pronounced physiochemical gradients allowing for high species diversity and a wide range of metabolic capabilities. The present study combines 16S rRNA amplicon sequencing and shotgun metagenomics on a hypersaline marsh in Tristomo bay (Karpathos, Greece).

Samples were collected in July 2018 and November 2019 from microbial mats, deeper sediment, aggregates observed in the water overlying the sediment, as well as sediment samples with no apparent layering.

Metagenomic samples' co-assembly and binning revealed 250 bacterial and 39 archaeal metagenome-assembled genomes (MAGs), with completeness estimates higher than 70% and contamination less than 5%. 46 phyla had at least 1 KEGG Orthology term belonging to the sulfur cycle, with 9 being from the Archaeal domain (Halobacteriota,Thermoproteota, Asgardarchaeota, Thermoplasmatota, Nanoarchaeota, Altiarchaeota, Aenigmatarchaeota,Nanohaloarchaeota, Micrarchaeota).

In addition, several bacterial candidate phyla were found potentially involved in sulfur cycling (KSB1, OLB16, QNDG01, AABM5-125-24, UBP15, RBG-13-61-14, FCPU426, UBP14, VGIX01).

All samples had the capacity for sulfate reduction, dissimilatory arsenic reduction and conversion of pyruvate to oxaloacetate. Overall, both sequencing methodologies resulted in similar taxonomic compositions and revealed that the formation of the microbial mat in this marsh exhibits seasonal variation.

BIOTECHONOLOGY

S3_OP15

EXPLOITATION OF NOVEL FUNGAL OXIDATIVE BIOCATALYSTS FOR THE SUSTAINABLE PRODUCTION OF VALUABLE MONOMERS FROM BIOBASED FURANS

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The dependence on fossil fuels for the production of chemical building blocks with significant interest to the polymer industry has drawn attention not only in the utilization of renewable resources such as lignocellulosic biomass, but also in the application of biocatalysis to replace chemical reagents toward the development of greener and sustainable processes. Furans, such as 5-hydroxymethylfurfural (HMF) and furfural (FA) obtained from the lignocellulose-derived polysaccharides, have emerged as crucial precursors in chemical synthesis reactions, since they can be transformed to a wide range of derivatives (2,5-furandicarboxylic acid, 2-furancarboxylic acid etc.) with exceptional applications. Biocatalytic oxidation of furans with redox enzymes offers a facile and regioselective route of reaction under mild conditions [1]. The current study targeted at the enzymatic biotransformation of HMF and FA using novel fungal biocatalysts from the Auxiliary Activity AA3 and AA5 families of CAZy database [2]. Through intelligent exploration of Ganoderma lucidum genome, it was possible to retrieve one sequence with putative glyoxal oxidase activity (GlGlyOx1) and one with aryl-alcohol oxidase (GlAAOx1) activity based on their homology with known furan-transforming fungal catalytic activities. The genes were heterologously expressed in yeast Pichia pastoris, the respective enzymes were purified to their homogeneity and biochemically characterized. GlGlyOx1 and GlAAOx1 were evaluated, both individually and synergistically (along with the presence of in-house produced galactose oxidase FoGalOx from Fusarium oxysporum [3] and a commercially available horseradish peroxidase HRP), for their ability to act on furans and produce value-added oxidized derivatives. Our results demonstrate the potential of G. lucidum enzymes for obtaining furan-based monomers from lignocellulosic biomass residues, which can be used as building blocks for the production of biobased polymers.

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S3_OP16

COMPARATIVE GENOMIC ANALYSIS OF ENTEROBACTER SPP. ISOLATED FROM FRUIT FLIES: NEW INSIGHTS INTO BACTERIA-HOST INTERACTIONS

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Enterobacter species are commonly found in the gut microbiota of fruit flies, and they are known to play an important role in the physiology of their host. Some Enterobacter spp. have been shown to benefit fruit flies by promoting their growth, and overall health. The knowledge regarding the genomic features of Enterobacter spp. and the mechanisms of interaction with their host remains limited. Here, we sequenced 12 Enterobacter spp. isolated from different fruit flies including Zeugodacus cucurbitae (n=3), Ceratitis capitata (n=3), Bactrocera zonata (n=4) and Anastrepha fraterculus (n=2). Genomic sequencing revealed that each species harbors two Enterobacter spp., one unique to each insect host while the other was shared between them, except for A. fraterculus which is found to harbor a single Enterobacter sp. The unique isolates were identified as Enterobacter mori (n=2) in Z. cucurbitae, E. asburia (n=1) in C. capitata, and E. roggenkampii (n=1) in B. zonata. While the shared isolates were identified as E. hormaechei (n=8). The Enterobacter spp. aft genomes ranging in size from 4.5-to-5.1Mb and a CG content of around 55%. These genomes were composed of 27-to-85 contigs encoding between 3856 and 4780 genes, with completeness levels above 98%.

The genomic analysis revealed the presence of three distinct secretion systems mechanisms, that enable Gram-negative bacteria including Enterobacter spp. to interact with their environments by transporting proteins or other molecules across their cell membranes. The identified mechanisms include T1SS and T2SS which are conserved within most Gram-negative bacteria where they transport proteins into the extracellular environment. Interestingly, the analysis revealed that all studied Enterobacter spp. had at least one cluster of the 13 core genes encoding for T6SS. In addition to transporting proteins into the extracellular environment, T6SS had the ability to deliver a diverse array of effector proteins directly into target cells, including other bacteria, and eukaryotic cells. The T6SS in Enterobacter spp. are supposed to promote the health and growth of their host by eliminating competing pathogenic bacteria. This is achieved by giving a competitive advantage to Enterobacter, which can then provide essential nutrients to the host and help it resist pathogen infection

S3_OP17

HYDROXY FATTY ACID PRODUCTION BY CHEMO-ENZYMATIC CONVERSION OF WASTE COOKING OILS

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The global production of Waste Cooking Oils (WCOs) are estimated to be 11-20 million metric tons per year (Teixeira et al., 2018). WCOs are mainly produced by households, hospitality sectors, and the food industry and due to incorrect disposal, is considered a hazard to the environment and human health. A proper WCOs management, in a circular economy approach, can help to minimize environmental and socio-economic problems. WCOs represents a good feedstock for several biorefinery processes.

The present study regards the conversion of WCOs, through a chemo-enzymatic approach, into Hydroxy Fatty Acids. These molecules are of highest interest for industrial applications since they represent building blocks for the production of polymers, plasticizers, surfactants, lubricants, and detergents (Biundo et al., 2023). For the biocatalytic hydration of crude WCO-derived unsaturated free fatty acids, Escherichia coli whole cell biocatalyst containing the recombinantly produced Elizabethkingia meningioseptica Oleate hydratases (Em_OhyA) was used. Furthermore, in order to improve the economic feasibility of the process through enzyme recovery, biocatalysis tests were carried out with the catalyst immobilized in sodium-alginate beads. The exploitation of other feedstocks and the production of different products are the future goals of this study which aims to implement green processes for the valorization of waste feedstocks and the production of bio-based industrial products.

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S3_OP18

EFFECT OF LIGHT WAVELENGTH ON POLYSACCHARIDES PRODUCTION BY SUBMERGED CULTIVATION OF PLEUROTUS OSTREATUS

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Light is an important abiotic factor that regulates fundamental processes in organisms including fungi1. Different fungal species use light as a signal to regulate their developmental and metabolic processes. Most fungi respond to blue, red, and green light2. The activation of photoreceptors starts the signal transduction pathway that leads to adaptations in their metabolic pathways, especially with alterations in the carbohydrate and polysaccharide metabolism3. Polysaccharides production by submerged cultivation of basidiomycetes in bioreactors is promising due to the advantages of controlling culture conditions. These polysaccharides are separated into two categories, intra-cellular polysaccharides (IPS) and extra-cellular polysaccharides (EPS) which play a special role in food, biotechnological and pharmaceutical industries with high economic significance4. In this work, the effect of different light wavelengths on the production of polysaccharides in submerged culture of Pleurotus ostreatus was studied. Samples were withdrawn from the culture for biomass, EPS and IPS determination. To do a more systematic study of how light affects Pleurotus ostreatus a proteomic analysis was performed. Submerged culture in red and green light enhanced polysaccharide production by Pleurotus ostreatus, producing 4.2 g/L EPS and an IPS content of up to 45%. Similar productions were achieved in a 3.5L bioreactor. Antioxidant activities of the precipitated EPS were tested using ABTS radical cation decolorization assay. EPS showed antioxidant activities so could be considered potential novel antioxidant for functional food.

Keywords: *mushrooms, submerged cultivation, light, polysaccharides, bioreactors, nutraceuticals*

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S3_OP19

OPTIMIZATION OF A NEW INDUSTRIAL BIOPROCESS ON PILOT-SCALE FOR 2,3-BUTANEDIOL BY KLEBSIELLA OXYTOCA ACA-DC 1581 CULTIVATED ON BIODIESEL-DERIVED GLYCEROL

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Biodiesel production, having a constantly growing industrial application and a continuously increasing production the last many years, generates biodiesel derived-glycerol as the main side product of the process, (for 10 kg of biodiesel produced, 1 kg of glycerol is generated). As a result, valorization of glycerol through microbial and/or chemical conversions presents enormous interest.

The aim of the present study was to optimize the bioprocess, concerning the fermentation of biodiesel-derived glycerol to 2,3-butanediol (BDO), by the most promising bacterial strain (viz., Klebsiella oxytoca ACA-DC 1581), as previous screening of several bacterial strains has shown.

A new culture medium was developed in order to use other by-products/wastes and more economical sources of nitrogen (e.g., corn steep liquor, biogas digestate and other inorganic nitrogen compounds). A new pre-culture medium was studied, as well as a new method of aeration (e.g., two-stage aeration) and pH adjustment. After optimizing these parameters, four new fed-batch fermentations were performed in a bioreactor system. In all cases K. oxytoca produced almost 70 g/L, but after optimization, the volumetric productivity (Pr) increased 2.5 times (to c. 0.60 g/L/h) while the yield of BDO production to glycerol consumption (YBDO/Glv) remained ≈0.40 g/g. At this point it should be mentioned that, in the first 48h of the most successful fed-batch fermentation, Pr=1.10, YBDO/Glv=0.46, while the ratio of BDO to the sum of metabolic products was 96.3%, which indicates the significant high level of bioprocess selectivity in BDO. In the next step, the upscaling of the optimized bioprocess was successfully carried out in a pilot-scale bioreactor (250L) at Verd S.A. plant. Finally, a downstream process was developed since salting-out extraction (SOE) was applied to separate BDO from fermentation broth. A solution of ethanol and K2HPO4 gave a recovery of BDO c. 90%. The partition coefficient of glycerol needs further investigation, as the remaining glycerol was equally distributed in both phases.

These results indicate the feasibility of industrial-scale fermentation, while the BDO titer, productivity and YBDO/Gly obtained are among the highest in the international literature.

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S3_OP20

VALORIZATION OF THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE: IMPROVING PROCESS EFFICIENCY VIA BIOMASS REFINING AND IN BIO-ELECTROSYNTHESIS OF BIODEGRADABLE PLASTICS

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The development of sustainable biorefineries using crude renewable resources for the production of various bio-based products is essential in order to achieve a smooth transition to the circular economy era. The organic fraction of municipal solid waste (OFW) contains proteins, lipids, pectins and polysaccharides that could be used in various applications. The fractionation and conversion of OFW should be combined with the development of innovative technologies, such as the integrated bioconversion and electrochemical separation process presented in this study. OFW has been used as the sole substrate for the production of crude enzyme consortia via solid state fermentation of Aspergillus awamori. Extraction of lipids, proteins and pectins was evaluated using untreated or enzymatically treated OFW and material balances were estimated in order to identify the optimal refining scheme. The OFW carbohydrates were enzymatically hydrolyzed and the sugar-rich hydrolysate was used for polyhyoxybutyrate (PHB) production via fermentation with the bacterial strain Paraburkholderia sacchari. An electrochemical bioreactor was applied for the bio-electrochemical synthesis of PHB. The electrochemical system led to higher yield and productivity of PHB than the control fermentation.

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S5_OP27

FUNGAL COPPER RADICAL OXIDASES AS NEW BIOCATALYSTS FOR THE GREEN INDUSTRY.

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The development of new biotech processes has boomed and offers today viable solutions for expanding a renewable carbon-based bio-economy. Bio-catalysis is being used to an increasing extent by industries for the synthesis of complex molecules, and plant cell wall - a mixture of polysaccharides and poly-aromatic polymers - represents an essential resource that can be promoted into high value-added products for further commercial purposes [1,2]. Fungal saprotrophs and phytopathogens naturally target plant cell wall components and produce a large panel of hydrolytic and oxidative enzymes. Hence, fungi are currently the most important source of chemo-, regio- and stereoselective enzymes for biomass conversion. Recently, we selected, expressed, and deeply characterized almost 40 new members of the copper-radical oxidase (CRO) family from the Auxiliary Activity 5_subfamily 2 (AA5_2) of the Carbohyate-Active Enzymes Classification (CAZy, www.cazy.org) able to oxidize aliphatic and aromatic alcohols to their corresponding aldehydes, revealing a new reservoir of biocatalysts with high potential for green industry within the fungal kingdom [3-5]. In this context, we describe here (i) the survey of the biocatalytic diversity of AA5_2s, (ii) the knowledge gained about their catalytic mechanism, the structure/activity relationships affecting substrate specificity within the AA5_2 subfamily, (iii) their engineering, and (iv) their biocatalytic potential under applied conditions for the flavor and fragrance industries [6-10].

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S5_OP28

IMPLICATIONS OF POLYSACCHARIDE OXIDATIVE DEGRADATION: INSIGHTS INTO CATALYTIC ACTIVITY AND SUBSTRATE SPECIFICITY OF LYTIC POLYSACCHARIDE MONOOXYGENASES FROM THERMOTHELOMYCES THERMOPHILA

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Lytic Polysaccharide Monooxygenases (LPMOs) play a pivotal role in the enzymatic degradation of polysaccharides including cellulose and hemicellulose, which are abundant in plant biomass. This study investigated the catalytic activity and substrate specificity of LPMOs from the thermophilic fungus Thermothelomyces thermophila, specifically focusing on AA9 and AA16 families. The findings revealed that the AA9 LPMO, TthLPMO9G, exhibited a remarkable C1-regioselectivity and a dual cellulolytic/xylanolytic activity by effectively cleaving the β-glycosidic bonds of various hemicelluloses, thus it was successfully used in a multi-enzymatic process to produce nanocellulose from beechwood. A novel AA16 LPMO, TthLPMO16A, was also characterized, showing oxidative cleavage of xylan, providing insights into the decomposition of this complex polysaccharide for the first time. Notably, TthLPMO16A demonstrated activity even in the absence of cellulose, suggesting a unique mode of action. Furthermore, point mutations in TthLPMO9G, specifically H140A and S28A, were generated and characterized. The H140A mutant showed weakened catalytic activity towards cellulose, highlighting the crucial role of histidine in substrate recognition and catalysis. The S28A mutant exhibited altered product patterns on cellulose, suggesting a modified mode of action compared to the wild-type enzyme. In conclusion, this study provides valuable insights into the catalytic activity, substrate specificity and electron donor specificity of LPMOs from T. thermophila, contributing to the understanding of enzymatic oxidative degradation of polysaccharides and potential applications in bio-material processes.

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Acknowledgements
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Keywords: lytic polysaccharide monooxygenases, cellulolytic/xylanolytic oxidation, nanocellulose, enzyme-mediated processes, point mutations, electron donors

S5_OP29

THE ISOLATION AND CHARACTERIZATION OF NOVEL PYRETHROID HYOLASES FROM BIOBEDS USING FUNCTIONAL METAGENOMIC APPROACHES

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Insecticides are mainly used to control crop pest but also insects that are vectors of diseases threatening public health. However, their widespread use poses a major risk for the environment, due to accumulation of their residues in natural resources, and human health, due to presence of their residues in agricultural products. Our study focused on the isolation and characterization of novel pyrethroid hydrolases from on-farm biobed systems with prior exposure to pyrethroid compounds using functional metagenomics. A fosmid library of 10000 clones was tested through fast-track phenotypic tests for the presence of esterase activity, and twelve positive clones showed the desired phenotype in butyrate and magenta caprylate hydrolysis assays. Testing all 12 clones for their capacity to degrade α-cypermethrin, as a model pyrethroid, revealed active degradation for only one clone which was further studied. Through a transposon mutagenesis approach, 100 mutated clones were derived and tested for their degradation ability towards α-cypermethrin. Amongst them, 12 showed no degradation capacity against cypermethrin and the region of transposon insertion was sequenced to reveal the interrupted gene and its potential function. Our results pointed to an interruption of the bioH gene, which encodes for an esterase, as a possible pyrethroid degrading enzyme. Heterologous expression of bioH in E. coli followed by in vitro functional assays of the purified enzyme confirmed its capacity to hydrolyze cypermethrin. On going efforts focus on the full biochemical characterization of BioH and investigation of its activity against other pyrethroids. Functional metagenomics led to the identification of novel pyrethroid hydrolases precluding the need of microbial cultivation which can be restrictive for biotechnological exploitation. Such novel enzymes could be used in the development of novel cleaning products for the removal of pesticide residues from treated fruits and vegetables.

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S5_OP30

DISCOVERY OF ANTIAGING COMPOUNDS VIA THE SCREENING OF GREEK ACTINOBACTERIA

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The Athens University Bacterial & Archaea Culture Collection (ATHUBA) contains a large number of actinobacterial isolates from Greek environments with potential industrial utility. 1000 strains of the ATHUBA culture collection were screened for antiaging activity, more specifically for bacteria producing secondary metabolites capable of inhibiting elastase (which breaks down skin collagen and can cause wrinkles) and tyrosinase (which produces melanin and can cause liver spots). The strains were grown in liquid culture and their secondary metabolites were extracted twice, first with ethyl acetate and then with methanol. The ethyl acetate and the methanol extracts were then tested for inhibition of elastase and tyrosinase using in vitro enzymatic assays. The screening demonstrated that 1.4% of strains produced elastase inhibitors and 26.4% produced tyrosinase inhibitors and that ethyl acetate is a more efficient extraction solvent than methanol. The 70 most active of these extracts were then tested for cytotoxicity on two human cell lines. 30 extracts were found to be non-cytotoxic, and they were then tested for tyrosinase inhibition and for elastase inhibition on human cell lines. 3 extracts were found to inhibit tyrosinase and 1 extract was found to inhibit elastase in human cells. These 4 extracts are undergoing fractionation and mass spectrometry in order to identify the active molecules and the most suitable compounds will be used in the manufacture of antiaging skin creams by our industrial partner.

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S5_OP31

PHYSIOLOGY OF YARROWIA LIPOLYTICA UNDER DIFFERENT FERMENTATION MODES: DEVELOPMENT OF A MEMBRANE BIOREACTOR PROCESS FOR PRODUCTION OF VARIOUS BIOMOLECULES

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Oleaginous yeasts are suitable microbial platforms for single-cell-oil production. For this purpose, Yarrowia lipolytica has been widely studied, as it can efficiently utilize low-cost carbon sources, such as crude glycerol [1]. Furthermore, Y. lipolytica has shown great potential for producing various high-value products, e.g. terpenoids, lipases, citric acid, succinic acid, etc. Process enhancement is pursued through development of new strains, using metabolic engineering techniques, and fermentation optimization to improve process efficiency.

This study investigates how different cultivation modes alter physiology-related parameters (growth, uptake, secretion rates) of Y. lipolytica fermentations. Initially, batch fermentation was conducted in a 3L bioreactor to examine the effect of critical fermentation parameters (i.e. pH, crude glycerol and nitrogen concentration) on growth, lipogenesis and citric acid secretion. Based on these results, a semi-continuous fermentation process was designed, applying two harvesting/ feeding steps at 24h and 48h, when 50% v/v of culture was harvested and the same volume of fresh substrate was added, while the cultivation lasted 72 h in total. Next, a custom-made submerged UF membrane module was employed, resulting in a Membrane Bioreactor (MBR) system. The latter allowed the retention of active biomass inside the bioreactor during these steps, while removing 11% v/v of the consumed substrate. MBR resulted in high concentration cultures, up to 50 g/L of y biomass. A continuous fermentation mode, which was initiated after 24 h of batch cultivation, was also implemented in the MBR, leading to a 3.5- and 6- fold increase in y biomass and lipid yields, respectively, after 10h operation. Further, a simulation model of the different fermentation modes was developed, using MATLAB and Simulink, which is currently under calibration and validation. The performance of the novel MBR system is currently being evaluated using various in-house constructed recombinant Y. lipolytica strains, including a strain obtained after cell surface display of lipase LIP2 and used as a whole-cell biocatalyst.

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S5_OP32

A PROOF-OF-CONCEPT STUDY FOR LACTIC ACID SOLUTION PURIFICATION EMPLOYING A GENETICALLY MODIFIED E. COLI STRAIN IN A MEMBRANE BIOREACTOR SYSTEM

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Lactic Acid (LA) is a widely used biomolecule in multiple fields, such as polymer (PLA), food and pharmaceutical industry[1]. LA production is achieved primarily through sugar fermentation; however, LA recovery from grass silage juice (a by-product of green grass bio-refinery) is a potential alternative[2]. LA separation and purification is a key issue during LA industrial production; it is reported that LA purification from the fermentation broth may comprise between 30%[3] and 80%[4] of its production cost, depending of the required purity level. A biologically-based purification process may comprise a potential alternative to the conventional purification processes, due to its high selectivity. A hybrid biotechnological process combining bioreactor fermentation and ultrafiltration membrane separation in a single process step (Membrane Bioreactor – MBR) can be used to overcome many challenges of conventional biotechnological processes. In this study, a genetically engineered E.coli strain (GMM), that cannot catabolize LA, has been used to selectively remove impurities in a synthetic medium comprising LA, glucose, acetate, and salt (NaCl), as typical components of green grass silage. Synthetic leachate composition was optimized in shake-flasks experiments, followed by process scale-up in bench-scale bioreactor experiments for assessing optimum fermentation conditions. Subsequently, a submerged ultrafiltration membrane was employed in the bioreactor to separate the purified medium from the active cells. The hybrid biotechnological/membrane separation process (MBR) was assessed under different semi-continuous operating conditions, resulting in a bacteria-free effluent and 100% glucose and acetate depletion, thus achieving higher purity of the LA effluent solution. This study demonstrates the technical feasibility of a novel MBR process for LA purification in a synthetic leachate broth containing glucose and acetate as typical impurities, paving the way for the development of a biological-based purification method of real LA fermentation solutions.

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S7_OP39

IMPLICATIONS OF SHORT- AND LONG-TERM EXPOSURE OF THE MICROALGA TETRASELMIS CHUII TO H2O2-INDUCED OXIDATIVE STRESS, AS REVEALED BY MULTI-OMICS ANALYSIS

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Microalgae species encounter oxidative stress conditions in their natural habitats and microenvironments, and as a result, have developed species-specific adaptation mechanisms (Barone et al., 2021). Clarifying these mechanisms in depth could enhance biotechnological exploitation of microalgae, through metabolic manipulation (Qiao et al., 2021). In that context, in the present study the response of Tetraselmis chuii, which is extensively used in industry as feedstock, to hydrogen peroxide (H2O2)-induced oxidative stress, was studied. Exposure to 0.5-mM H2O2 resulted in reduced cell viability, while exposure to higher concentrations caused a dramatic decline. Furthermore, 1 h exposure to 0.5-mM negatively affected photosynthetic capacity (Qy) and the reduction became even more pronounced when the stress was continued for 6 h. Our global multi-omics analysis revealed that T. chuii response to H2O2-induced oxidative stress occurred within the first hour, leading to profound changes to both transcriptomic and metabolomic profiles. Carbon and energy flow were among the cellular functions that were negatively impacted. In summary, our results suggest that T. chuii has developed a quick sense and response to oxidative stress, but long exposure has detrimental effects on it.

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S7_OP40

EFFECTOR RIPE1 OF RALSTONIA SOLANACEARUM TARGETS EXO70B1 AND IS RECOGNIZED BY THE PTR1 IMMUNE RECEPTOR

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Ralstonia solanacearum depends on numerous virulence factors, also known as effectors, to promote disease in a wide range of economically important host plants. Although some of these effectors have been characterized, none have yet been shown to target the host's secretion machinery. Here, we used an extended library of plant Nucleotide-binding Leucine-rich repeats Receptor (NLR) integrated domains (IDs), to identify new effector targets. The screen uncovered that the core effector RipE1, of the R. solanacearum species complex, among other targets, associates with Arabidopsis exocyst component Exo70B1. RipE1, in accordance with its predicted cysteine protease activity, cleaves Exo70B1 in vitro and also results in the activation of TN2-dependent ectopic cell death in planta. TN2 is an atypical NLR that has been proposed to guard Exo70B1. Despite the fact that RipE1 has been previously reported to activate defense responses in model plant species, here we present a Nicotiana species, in which RipE1 expression does not activate cell death. In addition, we discovered that RipE1 associates with RIN4, a known interactor of Exo70B1 and other exocyst components and guardee of Ptr1. Here we show that RipE1 is recognized by Ptr1, a Nicotiana benthamiana Coiled-coil (CC)-NLR, via its cysteine protease activity. Overall, this study uncovers a new RipE1 host target and a new RipE1-activated NLR, while providing evidence and novel tools to advance in-depth studies of RipE1 and homologous effectors.

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S7_OP41

PHYLOGENETIC AND EVOLUTIONARY ANALYSIS OF FUNGAL MITOGENOMES USING A NOVEL BIOINFORMATICS PIPELINE

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Mitochondria contain multiple copies of a small genome, the mitochondrial (mt) genome, that shows great variability. This renders them very useful tools in evolutionary and phylogenetic studies, as the evolutionary relationship of organisms is reflected in their whole genome. Moreover, mtDNAs possess additional useful traits, such as a variety of introns and intergenic regions, which do not succumb to larger evolutionary pressure and thus, they may diverge at a different rate than their nuclear counterparts. As a result, mtDNA provides molecular markers which can be utilized along with their nuclear counterparts for phylogenetic studies.

In this study, a pipeline was constructed in order to retrieve all the fungal mitogenomes available at the NCBI Nucleotide database and download them locally in GenBank format. The ter "Fungi AND Mitochondrion" were used for the initial search, however, additional filters were employed in the algorithms as more than half of the results represented fungal chromosomes or contigs. An algorithm was written to separate annotated mitogenomes, to collect all coding sequences and to print their synteny. Gene order is a source of valuable phylogenetic information, since conclusions can be made about the evolutionary processes that took place in each genome, and for that reason it was imperative to be included in this pipeline.

More than 2300 whole mitochondrial genomes of fungi were collected from NCBI and were annotated, if needed, to be analysed further in this work. By employing this pipeline as a fast tool to be used for determining the phylogeny and evolution of fungi, representatives from all fungal phyla were selected and the synteny of their mtDNAs was studied. A significant degree of conservation was observed in the order of protein-coding genes, especially between members of the same genus and in cases of the same order. Nevertheless, tRNAs formed syntenic groups which showed high mobility within the genome, suggesting that they could be responsible for genetic shuffling events and rearrangements observed in fungal mt genomes.

Keywords: fungi; mitogenomes; phylogeny; bioinformatics; synteny/gene order

S7_OP42

MULTI-OMICS ANALYSIS OF A POLYURETHANE-DEGRADING FUSARIUM OXYSPORUM

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Plastics, the most prevalent artificial polymers in modern societies, present a growing environmental challenge because of their resistance and durability. To address this issue, the exploration of microbial species involved in plastic degradation has gained significant attention. Although genomic databases offer valuable insights, the discovery of novel enzymes with low sequence similarity to known counterparts requires a multi-omics approach. By screening a fungal library, we identified Fusarium oxysporum BPOP18 for its potential for plastic degradation. This strain was capable of utilizing Impranil® DLN-SD, a commercial polyester-polyurethane dispersion, as its sole carbon source. To gain a comprehensive understanding of its enzymatic repertoire, we performed RNA-seq and secretomic / analysis at two time points selected based on assays of enzyme activities. The analysis of differential expression at the transcript and protein level will provide valuable insights into the enzymatic machinery of Fusarium oxysporum BPOP18 and facilitate the targeted selection of novel enzymes for further plastic degradation evaluation. This study demonstrates the importance of a multi-omics approach for identifying enzymes with significant bioremediation potential.

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Keywords: Fusarium oxysporum, plastic degradation, proteomics, RNAseq, multiomics

S7_OP43

WATERBORNE PARASITIC ZOONOSES: A NEGLECTED PUBLIC HEALTH THREAT?

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Cryptosporidium and Giardia are important parasitic protozoa, causing gastrointestinal disease in both humans and animals, and both are considered to be zoonotic. The most common route of transmission is through contaminated water and/or food, thus being responsible for many waterborne (sometimes also foodborne) outbreaks of disease worldwide, having important impact on public health. Contamination of water (and consequently of foodstuff) with both protozoa may be due to runoffs from agricultural areas, drainage from manure storage, wastewaters overflows or improper sewage systems. During a 2-year period, using a longitudinal, repeated sampling approach, 12 locations in 4 rivers of Northern Greece, irrigation canals and the water production company of Thessaloniki, were monitored for Cryptosporidium and Giardia, using standard methods. In an effort to identify potential sources of water contamination, 254 faecal samples from farm animals (15 cattle and 12 sheep far) located near the water sampling points were screened for both protozoa. Moreover, for this reason, treated wastewaters from 3 wastewater treatment plants were monitored for both parasites for a 6-month period. River-water samples were frequently contaminated with Cryptosporidium (47.1%) and Giardia (66.2%), while oocysts of both parasites were also detected in inking water (<1/litre). A seasonal pattern of contamination has been revealed, with higher contamination rates during winter rainy months. All animals tested were infected with both protozoa, and 22% of the treated wastewaters were contaminated with Cryptosporidium and 72% with Giardia. The same potentially zoonotic species of both parasites and more importantly C. parvum and G. duodenalis assemblage AII, both responsible for documented waterborne disease outbreaks, were identified in both water and animal samples, with animals being the main source of water contamination through run-off from the surrounding far. A machine-learning model that can predict contamination intensity with Cryptosporidium (75% accuracy) and Giardia (69% accuracy), combining biological, physicochemical, and meteorological factors, was also developed. Although the prediction accuracies of the machine learning model may be insufficient for public health purposes, it could be useful for augmenting and informing risk-based sampling plans.

S7_OP44 (FT)

IMPACT OF INSECT SYMBIONTS ON PARASITOID FORAGING BEHAVIOUR

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Parasitoids are small insects that before laying eggs have the remarkable task of locating, and successfully attacking a suitable host. Once the egg is laid, many herbivorous insects carry defensive symbionts that prevent parasitoid development. Some symbioses can act ahead of these defenses by reducing parasitoid foraging efficiency, while others may betray their hosts by producing chemical cues that attract parasitoids. I will provide examples of symbionts altering the different steps that adult parasitoids need to fulfil to achieve egg laying. I will also discuss how habitat complexity, plants and herbivores modulate the way symbionts affect parasitoid foraging, and parasitoid evaluation of patch quality. Such quality may depend on risk cues derived from parasitoid antagonists like competing parasitoids and predator.

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S7_OP45 (FT)

THE FUTURE OF 16RRNA GENE HIGH THROUGHPUT SEQUENCING: A COMPARATIVE ANALYSIS OF NEW ILLUMINA AND OXFORD NANOPORE TECHNOLOGIES SEQUENCING METHODS

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In the last decades, the sequencing of the bacterial 16S rRNA gene has been extensively utilized for the identification, classification and compositional determination of bacterial communities. Based on current usage and market share, Illumina owns the dominating sequencing technology with the capacity to process multiple samples in a single run with a relatively low cost, high accuracy and low error rate. Because in amplicon-based microbiome research size matters, Illumina's MiSeq sequencer has been especially popular as the only model capable of 600 (2x300) cycles. In December 2022, Illumina developed a new 600-cycle flow cell for the NextSeq 2000 system that has a significantly increased throughput of up to 120Gb of data per run compared to 15 Gb of data per run of the MiSeq 600-cycle one. In contrast, Oxford Nanopore Technologies produce long reads that can easily cover the full length of the 16S rRNA, but it has been historically associated with high error rates, reducing the resolution of the technology to genus-level classifications. However, their latest R10.4 chemistry has a proclaimed accuracy of 95% -99% which can support the taxonomic resolution at species and even strain level.

Despite their potential to greatly accelerate microbiome research, only a few studies yet exist that are reporting on the practical evaluation of those advancements. To fill this information gap, an extensive comparative study was performed at the Microbiome Facility at the University of Crete which operates both NextSeq2000 and MinIon, to evaluate the new flow cells in terms of accuracy, throughput, cost-effectiveness and turnaround time. Our results, show that sequencing with the aforementioned flowcells outperforms, in terms of cost and accuracy, their previous versions. Overall, although Illumina's NextSeq 2000 offer a cheaper solution compared with MiSeq, Nanopore's enhancements in accuracy combined with the long reads and affordable instrument cost makes it a reliable solution for small and medium amplicon studies.

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EMERGING APPROACHES

S8_OP46

ISOLATION AND CHARACTERIZATION OF A SPHINGOMONAS STRAIN ABLE TO DEGRADE THE PLEUROMUTILIN ANTIBIOTIC TIAMULIN.

Sotirios Vasileiadis, **Chiara Perruchon**, Niki Tagkalidou, Eleni Katsivelou, Panagiotis Karas, Natasa Kalogiouri, Ourania Menkisoglou, Dimitrios Karpouzas

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Tiamulin (TIA) is a veterinary antibiotic commonly used in livestock farming which is persistent in the animal digestive system and downstream receiving environments, it is ecotoxicologically relevant, and its environmental dispersal entails high-risk for antibiotic resistance development according to our previous work 1,2. Little is known regarding its biodegradation potential in soil where it can end up upon manuring. Antibiophagy is a desirable microbial trait which could be exploited in bioaugmentation strategies for the reduction of antibiotics pressure in environmental settings. Aiming to develop such a strategy, a Sphingomonas strain able to degrade TIA was isolated from soil exhibiting accelerated biodegradation through liquid enrichment in minimal media where the antibiotic was the sole carbon/nitrogen source. The capacity of the strain to degrade TIA was characterized at a range of antibiotic concentrations, pHs, and temperatures, while genomic, transcriptomic and metabolomic analysis were performed in an attempt to further characterize the genetic background of the strain relative to its biodegrdation capacity and elucidate the TIA metabolic pathway. The isolate completely degraded TIA at concentrations up to 100 mg l-1 within 3 days, with pH and temperature optima of 6.5-7.5 and 25 °C respectively. Phylogenomics analysis indicated a novel species (83.87 % average nucleotide identity with Sphingomonas laterariae, ≤ 95 %), which we coined candidatus Sphingomonas perruchonii. Transcriptomics showed the enhanced (log2 fold-change value of ≥ 6) upregulation of several oxidoreductases, transporters, and hydrolases, a lyase, a transcription regulator, and secondary metabolites biosynthetic genes under the TIA treatment compared with succinate as carbon source, while a Bcr/CflA family resistance gene (efflux pump coding, homologous to bicyclomycin, chloramphenicol, florphenicol, and tetracycline resistance genes) was also significantly upregulated. Most of the highly upregulated genes under TIA lacked homologues in other sphingomonads according to pangenome analysis. Metabolomics identified five metabolites, although only one of them, a dioxygenated derivative of TIA was formed in significantly higher amounts in the presence of the bacterium and its formation pattern was conducive with TIA degradation. The current study is the first to report a TIA-degrading isolate with the potential for use in the bioaugmentation of contaminated manures.

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S8_OP47

GENOME ANALYSIS OF LACTICASEIBACILLUS PARACASEI SRX10: AN INDIGENOUS STRAIN FOR USE AS A MULTI-FUNCTIONAL ADJUNCT CULTURE IN CHEESE PRODUCTION

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Molecular and genomic techniques have become increasingly important for the characterization and selection of bacterial strains for use as adjunct cultures in cheese production. In this study, we present the annotated whole genome sequence of Lacticaseibacillus paracasei SRX10, a multi-functional indigenous strain, which was isolated from fermented goat cheese. The genome of Lc. paracasei SRX10 is comprised by a circular chromosome with a total length of 2.81 Mb and a GC content of 46.40%. Average nucleotide identity (ANI) analysis using the python module Pyani, showed that the strain possessed unique genomic sequences, and further confirmed its classification in the Lc. paracasei species.

The presence of genes and proteins potentially involved in the technological characteristics and health-promoting phenotype of Lc. paracasei SRX10 was investigated using EggNOG, BlastKOALA and BLAST+ (Basic Local Alignment Search Tool). Genes coding for proteins related to technological characteristics, including acid tolerance response (e.g., atpA, atpB, atpC, atpD), proteolytic enzymes (e.g., pepN, pepX, and pepC), proteins related to fatty acid biosynthesis (e.g., fabD, fabH, fabZ) and exopolysaccharide (EPS) biosynthesis (e.g., epsA, epsB, and epsC) were annotated in the genome of the strain, which are known to contribute to the texture, flavor and aroma of cheese.

Furthermore, proteins involved in gastrointestinal tract survival (e.g., adhesins, moonlighting proteins) and vitamin B1 production were identified, highlighting the health-promoting potential of the novel strain. Importantly, Lc. paracasei SRX10 does not harbor any acquired antimicrobial resistance nor virulence genes, indicating its safety for use in the dairy industry. Overall, the genetic and functional characterization of the Lc. paracasei SRX10 supports its use as an adjunct culture in the production of various types of cheese, contributing to the improvement of the overall quality and nutritional attributes of the final product.

S8_OP48

TRACKING VIRAL INFECTION TEMPORAL DYNAMICS AFTER MICROBIAL RESUSCITATION FOLLOWING SEASONALLY Y SOIL REWETTING USING VIROMICS AND STABLE ISOTOPE METAGENOMICS

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Viruses are highly active during ecosystem perturbations in aquatic systems, but these dynamics in soil ecosystems are unknown. Previous studies have shown that soil rewetting propels a succession of the microbial community with specific lineages growing and dying at different times. This varied taxonomic mortality of bacteria could be driven by host specific viral lysis. Here, we investigated lineage-specific virus-host dynamics in grassland soil following soil rewetting, when resident microbes are both resuscitated and lysed after a prolonged y period.

To characterize actively infecting viruses and host succession, we used a replicated time-series including a combination of viromes and 18O-water stable isotope probing (SIP) targeted metagenomics.

We found that y soil held a diverse but low biomass reservoir of virions, of which only a subset thrived following wet-up. Viral richness decreased by 50% within 24 h post wet-up, while viral biomass increased four-fold within one week.

Counter to recent hypotheses suggesting temperate viruses predominate in soil, our evidence indicates that wet-up is dominated by viruses in lytic cycles. We used isotope incorporation into viral and microbial DNA to characterize virus-host temporal dynamics and found taxon-specific trends in viral-host dynamics wherein viruses may follow their microbial hosts or, perhaps, control host populations.

We estimate that viruses drive a measurable and continuous rate of cell lysis, with up to 46% of bacterial death driven by viral lysis one week following wet-up, resembling rates in marine systems that yield 20% of the dissolved organic carbon pool. Thus, viruses contribute to turnover of soil microbial biomass and the widely reported CO2 efflux following wet-up of seasonally y soils, with a potentially significant viral shunt that likely contributes to soil carbon sequestration.

S8_OP49

INTERGENERATIONAL MEMORY OF SINGLE CELL DIVISION TIMES IN A GROWING MICROCOLONY

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Individual-cell heterogeneity is a major source of variability in biological systems affecting importantly, among others, microbial behavior. In a previous study on the stochasticity in colonial growth dynamics of individual bacterial cells our research team showed that when the number of cells in a microcolony originated from a single cell exceeded 20-25, growth rate reached a constant value which varied significantly among microcolonies. The question arising from this observation is whether intergenerational memory of single cells has an impact on the latter variability. Thus, the objective of the present study was to monitor the colonial growth of individual cells using time-lapse microscopy and analyze the results in order to reveal the relation between single-cell division time and growth dynamics of bacterial colonies and evaluate the role of intergenerational memory in division times. For this, the colonial growth of probiotic Lactiplantibacillus plantarum and Escherichia coli cells on solid media was studied using (phase-contrast) time-lapse microscopy. Z-stacked images were acquired every 5 min for 24h. Individual images were compiled giving a sequence of frames showing the behavior of each cell over time. Division times up to 6 generations and growth kinetic parameters for each microcolony originating from a single cell were assessed and used for the development of stochastic models for colonial growth. Based on the division times of single cells, a birth model was utilized to simulate the growth of microcolonies. Using Monte Carlo simulation, birth-based growth kinetics were estimated for 100 microcolonies which were further compared with the growth kinetics obtained by fitting the observed growth curves for microcolonies originating from a single cell with different intergenerational memory simulation scenarios. The results of the comparison revealed the relation between the division times of single cells and the growth kinetics of microcolonies. The results showed that the variability in the growth kinetics among microcolonies cannot be only explained under certain intergenerational memory simulation scenarios. The findings of the present study can be proved useful in elucidating the underlying biological mechanisms regarding growth dynamics of microcolonies and ultimately result in the development of more accurate stochastic growth models.

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S8_OP50

BACTERIOPHAGE SELECTION ALTERS ANTIBIOTIC RESISTANCE IN VIBRIO ALGINOLYTICUS

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Vibrio alginolyticus is a Mediterranean-abundant species and a major opportunistic fish pathogen in aquacultures. Investigating its antibiotic resistance can prove pivotal for establishing novel treatments against it. The prevalence of multi-resistance strains of antibiotics is a primary concern for environmental bacterial species. Resistance to some antibiotics has been shown as a result of overexpression patterns of membrane efflux pump proteins, an acquired metabolic strategy, rather than antibiotic-resistant genes. Bacteriophages, which are viruses that specifically kill bacteria, have been considered alternative agents for reducing bacterial load in fish hatcheries. In the past, bacterial metabolic adaptation has been shown to instigate bacteriophage resistance. This metabolic adaptation strategy included the diminishing of expression patterns of well-characterized membrane and transmembrane proteins, including antibiotic resistance-related proteins. By generating in vitro antibiotic-resistant strains and multiphage-resistant strains we were able to investigate if acquired phage resistance could induce antibiotic susceptibility in Vibrio alginolyticus. Additionally, by using an rt-QPCR platform we monitored the expression patterns of membrane proteins and genes related to major metabolic pathways for elucidating their fate during the development of antibiotic resistance and/or susceptibility. Microbiological assays revealed the interplay between antibiotic and phage resistance susceptibility, as well as, phenotypic trade-offs. Gene expression results showed differential expression of genes related to antibiotic resistance. Future results will evaluate the possible synergy and mechanism of action of specific antibiotics and lytic phages against multi-drug marine resistance strains.

S8_OP51 (FT)

EVALUATION OF MEAT SPOILAGE MARKERS FOR THE DEVELOPMENT OF ON-PACKAGE FRESHNESS SENSORS

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The aim of the current study was to associate meat spoilage with analytes/ metabolic markers to develop packaging sensors. Fresh beef cuts were stored under modified atmosphere packaging (80% O2/ 20% CO2) at chill (0, 5°C) and abuse (10, 15°C) temperature conditions that may occur during the chill chain. During meat storage, microbiological analyses, pH measurements, quantification of total volatile basic nitrogen (TVB-N), organic acids by HPLC and volatile compounds by HS-SPME/GC- and organoleptic assessment took place. The microbiological analyses showed that the initial meat microbiota consisted of Pseudomonas spp., H2S reducing bacteria, Brochothrix thermosphacta, yeasts, lactic acid bacteria and Enterobacteriaceae, while their dynamics and their contribution to the final microbial associated was affected by the storage temperature. The main organic acids that were detected and quantified were lactic, succinic, propionic, malic and citric acid, while the changes in their concentration was also effected by the storage temperature. The TVB-N values were found to increase at all temperatures during storage. Regarding the volatile compounds assessment, a large number of organic compounds were detected (ca. 110-160, depending on the storage temperature), many of which have been previously associated with the metabolic activity of meat microbiota. Approximately 20 compounds that included alcohols, aldehydes, ketones, esters, organic acids, hydrocarbons and furans were found to increase during meat storage. In conclusion, the results of the study were promising in terms of developing easy-to-read packaging sensors, by exploiting the data base that was created.

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S8_OP52 (FT)

AN INTERACTIVE ONLINE STOCHASTIC QUANTITATIVE MICROBIOLOGY TOOL FOR RISK-BASED DECISION SUPPORT IN THE FOOD INDUSTRY APPLIED TO BACILLUS CEREUS IN MILK

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Quantitative microbiology is a reliable tool for predicting the effect of various endogenous and exogenous factors and industrial processes on the microbial ecology of food products. The multitude of mathematical models developed by researchers make it possible to estimate the growth and/or inactivation of pathogenic and spoilage microorganisms in foods. However, models described in the scientific literature are often not available in a format that would make understanding and applying them to a larger extent in practical industrial applications possible, without the necessary scientific background. The integration of these models into tertiary easy-to-use software could bridge this gap. The current trend in quantitative microbiological growth prediction and risk assessment tools is the development of such easy-to-use tertiary models that will meet the needs of potential users with diverse backgrounds. Most existing quantitative microbiology tools, such as ComBase, follow the deterministic approach and do not take into account the variability and uncertainty that characterises all entered parameters, as well as the model's final prediction. The R programming language through the Shiny package makes it possible to design web applications with a graphical user interface. Such web applications can be designed with the end user in mind, aiming to improve user experience. Furthermore, the accuracy and visualisation of the final prediction of microbial behaviour can be improved for more realistic risk assessments. The proposed tool is a web application that introduces stochasticity into ComBase's growth models making it possible to quantify the inherent variability of growth parameters in food. Bacillus cereus, a spore-forming bacterium, can grow in milk even after pasteurisation and depending on the strain can either present a health hazard via the production of toxins, or result in spoilage. The dairy industry is in a precarious position daily, seeking to reduce the risk to consumers and minimise food waste due to microbiological spoilage. Determining processing conditions and establishing shelf life for products are examples of crucial risk management choices that can be aided by easy-to-use stochastic quantitative microbiology tools. In this way, these choices can be made based on the calculated risk and not empirically.

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FOOD-NUTRITION

S9_OP53

MICROBIAL INACTIVATION: SETTING THE BASIS FOR A RISK-BASED DESIGN IN FOOD PROCESSING

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Phenotypic heterogeneity seems to be an important component leading to biological individuality and is of great importance in the case of microbial inactivation. Bacterial cells are characterized by their own resistance to stresses. In a previous study we showed that this inherent stochasticity is reflected in microbial survival curve which, in this context, can be considered as cumulative probability distribution of single cell inactivation times. The objective of the present study was to present an overview on the assessment and quantification of variability and uncertainty in microbial inactivation and set the basis for a risk-based design in the processing of foods.

We show that the time of inactivation leading to zero survivors follows a Gumbel distribution with location and scale parameters depending on the D-value and the initial contamination level of the food. The biological meaning of the Euler–Mascheroni constant appearing in the Gumbel distribution in relation to single bacterial cell death is discussed.

The cumulative density function of the Gumbel distribution can be used to estimate a treatment time which provides a target probability of zero survivors at the end of the processing. In the case of "real" thermal processing scenarios with dynamic temperature treatment, the target probability of zero survivors can be translated to an F-value by integrating the function with respect to time based on the Dref and the z-value of the organism of concern. We also show that other variability sources including prevalence and concentration of the target organism before the treatment as well as strain-to-strain differences in the inactivation behavior can be integrated in the Gumbel distribution to provide realistic probability estimates for the time to zero survivors. Application examples of the above are provided for both probiotic and pathogenic bacteria.

The applicability of the above stochastic approach as a practical decision support tool for the food industry is presented through the demonstration of a user-friendly software tool for risk-based design and real time monitoring of food processing.

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S9_OP54

COMBINATION OF SPECTRAL AND NGS DATA FOR THE MICROBIOLOGICAL QUALITY ASSESSMENT OF SHELLFISH

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The last decade omics technologies considered to be as promising approach for monitoring the safety and quality of food. To implement this approach the massive amount (i.e., big data) of heterogenous data (either structured or unstructured) derived across the food value chain should be integrated into management system.

In this study, various heterogeneous -omics data (i.e., FTIR spectroscopy, Multispectral Imaging -I, NGS) were obtained analyzing mussels of Greek and Spanish origin, in an attempt to acquire a comprehensive view about the quality of these products. More specifically, spectral data were collected using FT-IR and I analysis (n=300), while the overall profile of microorganisms present in these samples, affecting quality and safety of mussels throughout storage, was determined through Next Generation Sequencing (NGS) applying 16S rRNA metabarcoding analysis (n=30). In parallel, conventional microbiological analysis for the estimation of culturable spoilage microorganisms (total aerobes, Pseudomonas spp., B. thermosphacta, Enterobacteriaceae) was applied. Different machine learning techniques (MLT) (Partial Least Square (PLS), Support Vector and Extra Trees regression) were applied assessing the potential of FTIR, and I to provide useful information which can be related to mussels' microbiological quality. These models were also validated using independent, external datasets.

Microbial counts ranged from 3.5 to 9.0 log CFU/g, while NGS revealed several bacterial genera e.g., Leuconostoc, Acinetobacter and Corynebacterium on fresh samples, while Psychrobacter and Pseudoalteromonas were dominant at the end of shelf-life. Extra Trees Regression algorithm was more efficient using FTIR (slope a; 0,58, R2;0.89, RE; 0,74) while PLS-R algorithm was more suitable for predicting the microbial counts in mussels using I (R2; 0,74, RE; 0.78). The obtained spectra were further analyzed to find out any association between genomics data and specific spectral regions.

The application of "multi-omics" in seafood supply chain can provide higher quality information regarding food quality and safety compared to the conventional microbiology and as such can be considered as a holistic approach.

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S9_OP55

VINEYARD-MEDIATED FACTORS ARE STILL OPERATIVE IN SPONTANEOUS AND COMMERCIAL FERMENTATIONS SHAPING THE VINIFICATION MICROBIOME AND AFFECTING THE ANTIOXIDANT AND ANTICANCER PROPERTIES OF WINES

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The grapevine and vinification microbiome strongly influence the characteristics of the produced wines. Currently we have a good understanding of the role and the influence of vineyard-derived factors like cultivar, vintage and terroir in shaping the grapevine microbiome. However, the endurance of these factors along the vinification process remains unknown. We determined the influence of these factors on (a) the vinification microbiome succession (bacterial and fungi) and (b) the antioxidant, antimutagenic and anticancer potential of the produced wines, under three different vinification strategies used in winemaking (spontaneous V1, spontaneous with preservatives V2, commercial V3) performed separately for two cultivars (Roditis and Sideritis), two terroir and two vintages. Cultivar and vintage were key determinants of the vinification microbiome, unlike terroir whose effect became weaker from vineyard and early fermentation stages, where non-Saccharomyces yeasts and filamentous fungi (Aureobasidium, Cladosporium, Lachancea, Alternaria, Aspergillus, Torulaspora), and acetic acid bacteria (Gluconobacter, Acetobacter, Komagataeibacter) dominated, to late fermentation stages where Saccharomyces and Oenococcus become prevalent. The vinification process employed was the strongest determinant of the fungal microbiome compared to bacteria, were effects varied per cultivar. Vintage and vinification type were the main factors affecting the antioxidant, antimutagenic and anticancer potential of the produced wines. Further analysis identified significant positive correlations between vinification microbiome members like the yeasts Torulaspora debrueckii and Lachancea quebecensis and the anticancer and antioxidant properties of wines. These findings could be exploited towards a microbiome-mediated vinification process for the production of wines with particular and desirable traits and enhanced geographical character.

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S9_OP56

WHERE GREEK CHEESES BELONG WITHIN THE GLOBAL CHEESE MICROBIAL MAP: A COMPARATIVE INTEGRATIVE ANALYSIS OF CHEESES' MICROBIAL SIGNATURES

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The quality and safety of cheese are largely given by the resident bacteria, but comparative analyses of the cheese microbiota across cheese types are scarce. The present research was conducted to test the hypothesis that the biotic and abiotic factors (terroir, cheese character and milk origin) can differentiate the microbial diversity across cheese types, demonstrating different microbial consortia, and shed light on universal patterns of ecological assembly in cheese microbiomes.

To further delve into the relationship among all cheese types and their resident microbiome, we obtained publicly available sequences of the 16S rRNA gene and performed for the first time an integrative analysis of cheese types across the globe with different Greek cheeses also been incorporated. This dataset included sequences from 323 cheese samples, spanning across 27 cheese types and 7 countries. In total, we detected 850 distinct OTUs. To investigate similarities among cheeses, we performed a cluster analysis across all cheese types which led to the identification of four main cheese clusters, which exhibited distinct dominant communities. Furthermore, 9 subclusters were observed within these four clusters. All clusters were compositionally diverse from each other (permanova $p=0.001$) with Cluster 1 additionally having the highest effective richness among them ($p<0.05$ for all Wilcoxon pairwise tests). Of the 27 cheese types included in the cluster analysis, Greek cheese samples were classified into multiple cluster groups indicating that they are characterized by a more diverse microbiome, not restricted by the geographical location. Additionally, we examined the relationship between the relative abundance of members of the phylum Bacillota and richness in the whole community observing that the proportion of lactic acid bacteria (LAB) in the community affected community richness in cheeses. Interestingly, the majority of these LAB OTUs have not been classified until now.

In conclusion, four main global cheese clusters were identified and therefore a new classification system of the cheese microbiome could be proposed including novel OTUs that could characterize the unique microbial signature of each cheese type.

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S9_OP57

MONITORING THE SURVIVAL OF PROBIOTIC BACTERIA DURING THE SHELF LIFE OF A NOVEL GREEK SHEEP TRADITIONAL YOGURT AND FOLLOWING SUBSEQUENT IN VITRO DIGESTION

Ioanna Gkitsaki[1], Panagiota Potsaki[2], Ioanna Dimou[2], Zoi Laskari[3], Antonios Koutelidakis[2], Efstathios Giaouris[1]

[1]Laboratory of Food Microbiology and Hygiene, Department of Food Science and Nutrition, School of the Environment, University of the Aegeanthe shelf life of a novel Greek sheep traditional yogurt and following subsequent in vitro digestion, Myrina, Lemnos, Greece, [2]Laboratory of Nutrition and Public Health, Department of Food Science and Nutrition, School of the Environment, University of the Aegean, Myrina, Lemnos, Greece, [3]Mystakelli Traditional Dairy Products, Mantamados, Lesvos, Greece

Greek yogurt, a fermented dairy product of high nutritional value, is a suitable matrix for the delivery of probiotics. Probiotic viability in yogurt during shelf life and subsequent survival after passage through the gastrointestinal tract, is crucial in determining the health benefits of the product. Besides nutritional value, the enhancement of yogurt's sensory characteristics contributes to the consumer's acceptance. The aim of this study was to: a) obtain a new probiotic sheep yogurt with upgraded quality characteristics, b) monitor the population of lactic acid bacteria (LAB) species throughout the product's shelf life, and c) evaluate the survival of probiotic bacteria in a static in vitro digestion model (SIVDM). The populations of total viable counts (TVC), Enterobacteriaceae, yeasts and molds grown in yogurt were also determined. To achieve our ai, yogurt was manufactured the traditional way by fermenting pasteurized milk with either only the commercial starter culture (Lactobacillus delbrueckii subsp. bulgaricus (LB), Streptococcus thermophilus (ST); YF-L812 Chr Hansen) (CY), or together with the potential probiotic strain 708 of Lacticaseibacillus rhamnosus (LR), previously isolated in our lab from raw sheep milk (PY). The survival of all three LAB species was monitored throughout the product shelf life (incubation at 4 oC for 20 d), and also following exposure to SIVDM. At each sampling day (5, 11, 16 and 20 d after production), both yogurt samples (CY, PY) were also evaluated for their main sensory characteristics (appearance, odor, taste, texture, and overall acceptance) by 15 panelists. The population of probiotic LR remained stable during the shelf life (and above 108 CFU/g). Similar good was also the survival of ST (with counts always above 109 CFU/g). On the contrary, the initial population of LB (106 CFU/g) was not detected from the 11th d and afterwards (<102 CFU/g). Yogurt starters were not detected following SIVDM, whereas LR (in PY) presented a reduction of about only one log (95.7%). Sensory analysis did not reveal differences between the two yogurt types during the shelf life. To sum, the novel yogurt had good sensory attributes and was able to deliver to consumer a high amount of potentially probiotic cells.

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S9_OP58 (FT)

MICROBIAL ELECTROSTIMULATION AND ELECTROCUTION USING LOW INTENSITY ELECTRIC FIELDS – A NOVEL APPROACH FOR SELECTIVE CONTROL OF MICROBIAL GROWTH IN VITRO AND IN FOOD MATRICES

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Low intensity electric current (LIEC) can affect microbial cell membrane permeability and cell apoptosis, as well as stimulate metabolism. This technology was tested in liquid synthetic media and beverages (cider and beer) aiming to either inactivate pathogenic bacteria and enteroviruses, or stimulate beneficial microbes involved in fermentation.

LIEC was used either as a single treatment, or in tandem with low temperature pasteurization (65ºC). Different combinations of electric current frequency, intensity and time were applied.

A LIEF of 800 Hz, with either 1 or 10 mA, was effective against E. coli, S. typhimurium, C. jejuni, S. aureus, L. monocytogenes, C. perfringens and high titers of Enteroviruses, when applied for 10 or 30min in vitro. When applied prior to pasteurization, LIEC significantly improved the destructive effects of thermal treatment, when LIEC was applied simultaneously with pasteurization, a much greater lethal effect was achieved (up to 3 log cfu/ml higher lethality compared to single pasteurization). However, low intensity (2Hz) and short time of application of LIEC (1 or 10 min) could stimulate microbial growth of some bacteria, which was further studied in fermented foods.

In fermented cider and beer, a LIEC of 800 Hz reduced spoilage bacteria (Lactic acid bacteria, Acetobacter, Zymomonas) in a similar manner to pasteurization. The microbiocidal effect of pasteurization was significantly improved by 2-3 log cfu/ml when combined with tandem LIEC. Yeasts were more resistant to the lethal effects of LIEC, but a short application (2-15 min) of low frequency LIEC (2 or 90 Hz) could stimulate the growth and metabolism of Saccharomyces cerevisiae. An improved lactic acid or ethanol fermentation was noticeable under specific stimulatory conditions of low intensity electric current.

This new approach offers a low cost, effective method for selective cell electrocution of electrostimulation of microbes, using low intensity conductive electric current, in contrast to the expensive and purely lethal high intensity inductive Pulsed Electric Fields (PEF) that have been previously used only for cell destruction. In a broader perspective, it introduces the concept of Electroculturomics, for a selective treatment, stimulation and isolation of beneficial or undesirable microbiota in medical, food or environmental applications.

S9_OP59 (FT)

LISTERIA MONOCYTOGENES COLONY GROWTH DYNAMICS AFTER EXPOSURE TO ACIDIC CONDITIONS AND DISINFECTANTS

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Listeria monocytogenes exposure to sublethal stresses may induce different physiological states i.e., sublethal injury or dormancy, that present variable resuscitation capacity. The objectives of the present study were: (i) to monitor real-time resuscitation of L. monocytogenes Exposure to acetic acid-AA and hydrochloric acid-HCl (pH 3.0-2.5; for 5h), peracetic acid-PAA (10, 20 and 30ppm; for 3h) and sodium hypochlorite-SH (200 and 250ppm; for 3h) at 20oC were used to induce different physiological states in L. monocytogenes. After stress exposure, colonial growth of single-cells was monitored, on Tryptic Soy Agar supplemented with 0.6% Yeast Extract by time-lapse microscopy, at 37°C. Images were acquired every 5min and were analyzed using BaSCA pipeline. The obtained colonial growth curves were fitted to the model of Baranyi and Roberts and the Trilinear model for the estimation of lag time-λ and maximum specific growth rate-μmax. Data analysis and modelling were done in R.

Growth of 113 untreated single-cells was monitored. Eleven (9.73%) were non-dividing. After treatment with Assessing the heterogeneity of the colonial growth dynamics after stress exposure offers quantitative stressed single-cells; (ii) to examine the variability in the division time of single-cells; (ii) to estimate the colonial outgrowth kinetics; and (iii) to detect non-dividing fractions.

AA pH 3.0, 12 (24%) out of 50 single-cells found as non-dividing. The average λ value and μmax of growing cells was 1.68h (SD=0.53h) and 14.61h-1 (SD=49.93h), respectively, compared to 1.57h (SD=0.57h) and 3.46h-1 (SD=24.74h) of the control. Increasing stress intensity to pH 2.5 resulted in an average λ value of 11.45h and μmax 2.41 h-1. The behavior of 61, 59, 58 single-cells treated with HCl at pH 3.0, 2.7 and 2.5, respectively, was investigated. The average colonial λ value of HCl-treated cells ranged from 1.11h (pH 2.7) to 1.57h (pH 3.0). Exposure to PAA 10ppm resulted in 34 non-dividing cells out of 81 (41.98%). The mean colonial λ value ranged from 0.47h to 2.84h. After treatment with SH 200ppm the average colonial λ value and μmax of 104 growing cells was 13.68h (SD=1.36h) and 0.83h-1 (SD=1.49h), respectively.

insights on the impact of stress on residual risk associated with survivors.

The research work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: HFRI-FM17-3268).

S9_OP60 (FT)

SHORT-TERM EFFECTS OF FRUIT JUICE ENRICHED WITH PROBIOTICS ON GLYCEMIC RESPONSES: A RANDOMIZED CONTROLLED CLINICAL TRIAL IN HEALTHY ADULTS

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Introduction: Health benefits from eating fruits are well established. Fruit juice is the product from the extraction or pressing of the natural liquid contained in fruits. Probiotics are considered as health-promoting live microorganisms that can fit on the people's daily diet. Probiotic foods have positive effects on intestinal microbiota composition and overall health. Probiotics are defined as microorganisms that provide benefits to the host when administered in appropriate amounts (10^8 cfu/mL). The glycemic index (GI) is a tool developed to systematically classify carbohydrate containing foods according to time integrated effects on postprandial glucose response. This study aimed to determine effects of consuming a mixed fruit juice (apple, orange, grape, pomegranate; control fruit juice) and the same fruit juice fortified with two probiotics strains (Lacticaseibacillus casei Shirota and Lacticaseibacillus rhamnosus GG) and with the combination of vitamin D3, n-3 PUFA, and the two probiotic strains, on glycemic and salivary insulin responses, and subjective appetite.

Methods: Clinically and metabolically healthy men and women participated in this randomized, double-blind, cross-over, clinical trial. Inclusion criteria for participation were a body mass index (BMI) between 18 and 25 kg/m^2 and age between 18 and 55 years old. In a randomized controlled, double-blind, crossover study, 11 healthy participants (25±2 years; five women; BMI=23±1 kg/m^2) were randomly *assigned* to receive 3 types of fruit juices and glucose as reference ink, all containing 50 g available carbohydrate.

Results: All fruit juices provided low glycemic index values (control: 54; probiotics: 50; vitD-n-3-probiotics combination: 52, on glucose scale). Conclusion: All fruit juice types provided lower peak glucose values, lower mean glycemic and insulinemic responses, were more pleasurable, and affected satiety scores compared to glucose. All fruit juice types, regardless of the added probiotics and biofunctional ingredients, attenuated postprandial glycemic responses, and improved appetite scores, which may offer advantages to glycemic and body weight control.

Acknowledgments: This research has been co-financed by the EU and Greek national funds through the Operational Program under the call RESEARCH-CREATE-IN_NOVATE (T2EDK-01922, FunJuice).

POSTER PRESENTATIONS

TABLE OF CONTENTS

AGRICULTURE

PP015: Cross-talk of Abscisic acid and Ethylene -mediated pathways in the interaction of tomato plant with the beneficial fungus Fusarium solani strain K

PP016: Three newly acquired mycophilic genomes elucidate the fungicolous secondary metabolism: a comparative study

PP017: Exploring the biochemical mode of action and spectrum of activity of synthetic and biological nitrification inhibitors

PP018: Studies on the role of Bacillus subtilis cyclophilin in bacterial growth and development

PP019: Oxytetracycline treatment led to persistent gut microbiome dysbiosis, the rise of parasites and growth impairment of European Sea Bass in open sea aquaculture

PP020: Forecasting and protecting fruit crops from frost damage: the EU/LIFE FrostDefend project

PP021: Withdrawn

PP022: Characterization and use of plant growth promoting bacteria from vineyard soils

PP023: Withdrawn

PP024: Development of a fast-track/ high throughput screening assay on soil ammonia-oxidizing bacteria for the discovery of novel biological nitrification inhibitors

PP025: Evaluation of biological and synthetic plant protection products against the pathogen Taphrina deformans causing peach leaf curl

PP026: Preliminary results on the relative abundance of certain functional groups of microorganisms present in the rhizosphere of herbicide-resistant and non-resistant weeds

PP027: Seasonal shifts in soil microbiome structure are associated with the cultivation of the local runner bean variety around the lake mikri prespa

PP028: The suppressive effect of bacterial strains isolated from halophytes against important plant pathogens

PP029: Evaluation of the impact of synthetic and biological nitrification inhibitors on the composition and activity of the soil microbial community and the emissions of greenhouse gases

PP030: Bacterial community structure and functioning in Cyprus vineyard ecosystem

PP031: Enhancement of production of pathogen suppressing volatiles by precursor molecules: towards sustainable control of soilborne diseases

PP032: Combining Rhizoglomus irregulare QS69 with systemin results in better control of tomato pests.

PP033: Using knowledge from wild relatives of plants to combat soilborne phytopathogens

PP034: Do ammonia oxidizers adapt to nitrification inhibitors upon repeated exposure? First evidence from in vitro assessment

PP035: Exploring the potential of plant-derived terpenoids as biological nitrification inhibitors

PP036: Withdrawn

PP037: **Withdrawn**

PP038: Genomic analysis of Nontuberculous Mycobacteria (NTM) highlights the potential of reverse vaccinology in aquaculture

PP039: Assessment of population viability in compost enriched with nitrogen-fixing bacteria stored at ambient and low temperature

PP040: Combined application of compost and plant growth promoting bacteria improves growth and nutrient uptake by lettuce plants grown under three different phosphorus sources

PP041: Tripartite association between legumes, nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi: deciphering the microbial interactions

PP042: Title: Comparison of secondary metabolite biosynthetic clusters between pathogenic and non-pathogenic fungi: The case of the endophytic fungus Fusarium solani strain K.

PP043: Characterization of Colletotrichum spp. from olive for growth under different temperatures and virulence on flowers

PP044: Towards the development of a novel biostimulant for use in sustainable cultivation of horticultural crops

PP045: Development of multiplex ssr approaches and hrm markers for the accurate genotyping of taphrina deformans populations in Greek peach orchards

PP045a: Plant growth promoting rhizobacteria produce volatiles with potential activity against Verticillium dahliae

PP045b: Mycorrhiza-assisted phytoremediation potential of hemp: Preliminary results of a field experiment

PP045c: Plant-mediated effects of beneficial soil microbes on herbivore populations in the greenhouse

---

ENVIROMENT

PP046: Combining culture-dependent and -independent approaches for identifying plastic-degrading bacteria and enzymes in soil samples

PP047: Composition of prokaryotic and eukaryotic microbial communities across the water column of the Eastern Mediterranean Sea

PP048: Preliminary data on the diversity of the genus agaricus section minores in Greece

PP049: Optimizing Bacterial Cellulose Production using Post-Consumed TPA as a Non-Nutritional Adjunct in Twin-Bioreactor System

PP050: MicroGLO: Effect based bioassays for antibiotic detection

PP051: Exploring the enzymatic ability of strains isolated from plastic-polluted environments for enhancing synthetic and natural biopolymers' biodegradation

PP052: Municipal wastewater treatment: assessment of ecotoxicity and dispersion of bacterial antibiotic resistance

PP053: Removal of antibiotic-resistant Escherichia coli and antibiotic resistance genes in wastewater through wastewater chlorination

PP054: SynComs in Microbial Ecotoxicology: Development and standardization of in vitro tests to assess the toxicity of pesticides on N microbial networks

PP055: Effect of synthetic and biodegradable microplastics. either alone or in the presence of pesticides or veterinary drugs on the soil microbiota

PP056: Biocompatible Eco-plastic Blends with Enhanced Antimicrobial Activity by Addition of Essential Oils

PP057: **Withdrawn**

PP058: Biogas production using raw glycerol on a sunflower-seed-hulls-residues packed digester

PP059: Utilization of Mortierella ramanniana lipid fermentation wastewater as maceration water for Pleurotus and Ganoderma cultivation

PP060: Impact of different soil salinity levels on the concentration of the polysaccharide acemannan and Plant Associated Microbial Communities in Aloe vera (Aloe Barbadensis Miller) plants

PP061: Exploring the diversity and antimicrobial potential of Streptomyces strains in Greek environments

PP062: **Withdrawn**

PP063: Bacterial and biochar-assisted phytoremediation of multi-metal contaminated soil using crotalaria retusa

PP064: Establishment of synthetic microbial communities across trophic levels to assess pesticide soil microbial toxicity in an ecosystem relevant in vitro system

PP065: Evaluation of the toxicity of three fungicides on Arbuscular Mycorrhizal Fungi (DAOM, Rhizophagus irregularis) using an in-situ monoxenic sandwich system

PP066: In vitro assessment of the toxicity of anthelmintics on soil ammonia-oxidizing archaea and bacteria: Benchmarking the soil microbial toxicity of anthelminthic veterinary drugs

PP067: Biodiversity study of rice commercial samples with RAPDS

PP068: Antimicrobial and antibiofilm activities of three Pseudoalteromonas strains against fish pathogen Tenacibaculum discolor strain B487

PP069: DIMITRA: an upcoming database on effects of pesticides on the soil microbiome and meta-analytic analysis

PP070: Microbial populations on the surface of a marble monument in Athens, Greece

PP071: Protocatechuate 4,5-dioxygenase directed evolution: sometimes small changes are all that it takes

PP072: **Withdrawn**

PP073: Characterizing the symbiotic profile of natural and laboratory populations of Aedes albopictus

Greek Ae. albopictus populations

PP074: The linkage between boreal peatlands and greenhouse gases: A review of recent efforts to characterize the diversity and functionality of the Sphagnum microbiome

PP075: Municipal wastewater treatment by means of pilot scale constructed wetlands: elimination of microorganisms and antibiotic resistance genes

PP076: Study of the ecotoxicity and biodegradation of bioplastics

PP076a: The use of microbial cultures with microalgal species for the degradation of bioplastics (PHB and TPS)

BIOTECHONOLOGY

PP077: Bioconversion of unrecyclable PET waste into biodegradable polyhyoxyalkanoates by a newly isolated Delftia sp. strain

PP078: Antimicrobial and antioxidant activity of natural extracts of byproducts derived from the olive oil and winemaking processing

PP079: Unchain the metabolic potential of plastics as carbon source for biotechnology

PP080: Recycling mushrooms' cultivation by-products to produce Pleurotus ostreatus new crops

PP081: Exploring the fungal potential for natural and synthetic polymer degradation

PP082: Accumulation of high-nutritional-value omega-3 fatty acids by the heterotrophic microalgae crypthecodinium cohnii on organosolv fractionated agricultural residues from the flour milling industry

PP083: **Withdrawn**

PP084: Exposure to salinity stress conditions of an isolate from Kalloni solar saltworks on Lesvos island leads to biomass production with promising traits

PP085: Molecular classification of five microalgae strains isolated from Kalloni solar saltworks on Lesvos island

PP086: Changes on nodule sulfate metabolism triggered upon carbon starvation induced by prolonged darkness

PP087: Analysis of the bacterial and fungal microbiome of stone surfaces of monuments: towards the development of mitigation strategies for protection of cultural heritage

PP088: Exploitation of novel fungal oxidative biocatalysts for the sustainable production of valuable monomers from biobased furans

PP089: Assessing growth kinetics and accumulation of high-value metabolites in freshwater and marine microalgae using crude glycerol as a carbon source

PP090: Biotechnological production of bacterial pigment prodigiosin and bioactive properties of its metal complexes with cu(ii) and zn(ii)

PP091 : Enzyme-assisted extraction of bioactive compounds from the rosehips of rosa canina l. applying a box-behnken experimental design

PP092: Assessment of the biofilm-forming ability of marine microalgae and cyanobacteria

PP093: Characterization of isolated microalgae stains for hydroponic wastewater bioremediation

PP094: Effects of culture and bioprocess conditions on the mycelium growth and production of bioactive substances of ganoderma lucidum and monascus purpureus

PP095: Symbiotic interactions in the model legume Lotus japonicus: Mutation analysis of the SHAGGY-like LjLSK1 kinase gene and phenotypic analysis of nodulation

PP096: Spent from Pleurotus ostreatus and Pleurotus eryngii: a potential source of exopolysaccharides and enzymes

PP097: Investigating the role of triterpene biosynthetic genes in I. japonicus during colonization by an endophytic fungus

PP098: A novel CE16 exo-deacetylase from Thermothelomyces thermophilus with biotechnological potential

PP099: Conversion of mixed plastic waste containing PET into biopolymer bacterial nanocellulose

PP100: Characterization of a novel cyclofructan transferase from Janthinobacterium BJB-304

PP101: Parameters optimization of glycerol's bioconversion into gamma-linolenic acid by fungus Cunninghamella elegans cultivated in shake-flask and batch-bioreactor experiments

PP102: Isolation and characterization of the multi-component carbazole dioxygenase driving the transformation of thiabendazole by a soil bacterial consortium

PP103: Secretome analysis of Pleurotus citrinopileatus grown on different lignocellulosic substrates

PP104: Enzymatic treatment of extracts derived from natural sources for the enhancement of their biological activities

PP105: The enzymatic machinery of the white-rot basidiomycete Abortiporus biennis can cause significant modifications to polystyrene structure

PP106: Sustainable Exploitation of Bio-Based Compounds Revealed and Engineered from Natural Sources

PP107: Novel Yarrowia lipolytica strains obtained through adaptive laboratory evolution and targeted genetic modifications for single-cell-oil production

PP108: Enhancing Heterologous Protein Secretion in Yeasts: Combining Secretory Peptides and Genetic Modifications

PP109: Lignocellulosic biomass to biogas: A biotechnological approach for Spent Mushroom Substrate valorization

PP110: Extraction, characterization and potential applications of microalgal bioactive polysaccharides of strains isolated from Greek coastal lagoons

PP111: A fungal transformation validation workflow for crispr/cas9 mediated rnai mutants

PP112: Evaluation of in vitro protective effect against oxidative stress on human colon cells of microalgae extracts derived from hyoponic effluents

PP113: Enhancement of Desulfurization via Targeted Genetic Engineering in the Model Biocatalyst Rhodococcus qingshengii IGTS8

PP113a: Advancing Sustainable Bioplastics through Microbial Upcycling for Novel Biopolymer Production

PP113b: Insights into a bifunctional catalase-phenol oxidase from a marine-derived Cladosporium species

PP113c: Exploiting the Greek microbial diversity for the discovery and development of novel antiaging molecules

PP113d: Catabolic Sphe3 genes: gears in an E.coli cis, cis-muconic acid producing apparatus

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ONE HEALTH/OMICS

PP114: Utilization of real-time monitoring technologies in a food Risk Assessment framework

PP115: **Withdrawn**

PP116: The co-evolution of mitochondrial and nuclear genes encoding proteins implicated in the cellular respiration of fungi

PP117: Food waste management for the production of animal feed

PP118: Incorporation of pomegranate juice and its concentrate in sodium alginate for the production of "Smart" edible packaging for chicken minced meat

PP119: The complex GH32 orchestra from Priestia spp. holds the key to better discriminate sucrose and fructan metabolism in bacteria

PP120: Less is more: design of a minimal zymomonas mobilis genome by generating targeted deletions in strain atcc 29191

PP121: A beneficial fungal endophyte triggers RNA silencing to an intronless but not to an intron-containing host reporter gene

PP122: Laurus nobilis and rosa canina plant extracts as potent functional regulators of food microbiome

PP123: Medium chain length polyhydroxyalkanoates (mcl-PHA) model compounds for the discovery of novel PHA depolymerases

PP124: Elucidating the evolution of early diverging fungi: the role of the mitochondrial genome

PP125: **Withdrawn**

PP126: **Withdrawn**

PP127: Speed up Mosquito Control: Innovative Approaches to Identify Invasive Species and Detect Vector-Borne Viruses

PP128: Evaluating the effects of novel probiotic strains in healthy mice

PP129: Novel Greek Streptomyces strain with antimicrobial activity against multi-drug resistant pathogens

PP130: Understanding the formation and inactivation of biofilms of the pathogenic microorganism Legionella pneumophila

PP131: **Withdrawn**

PP131a: A panoramic view of the genomic landscape of the genus Streptomyces

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EMERGING APPROACHES

PP132: Evaluation of biofilm formation on oenological surfaces by new isolated Brettanomyces bruxellensis strains

PP133: Endometrial microbiota and in vitro fertilization

PP135: Genomic analysis, probiotic properties and antagonistic activity of lactic acid bacteria isolated from raw sheep milk against mastitis-causing agents

PP137: Investigation of the biological actions of selected probiotics on breast cancer cells in vitro

PP138: Modification of tomato hormonal pathways during a tripartite plant-microbe-arthropod interaction

PP140: Development and validation of a stochastic model for the effect of temperature on the growth kinetics of Bacillus cereus strains in pasteurized milk

PP140a: Developing a new yeast selection method for wine fermentation starters

PP140b : Progressing Ultragreen, Energy-Efficient Biobased Depolymerization of Poly(ethylene terephthalate) via Microwave-Assisted Green Deep Eutectic Solvent and Enzymatic Treatment

---

FOOD-NUTRITION

PP141: Effect of storage temperature on the production of volatile metabolites in chicken meat of similar microbiological quality, based on the microbial counts

PP142: Inactivation behavior of salmonella under acidic food preservation stress: individual cell heterogeneity and population dynamics (hetero - inactivation)

PP143: **Withdrawn**

PP144: Microbial stability of home-made dried fruits and vegetables

PP145: Determination, characterization and bioprotective properties of the microflora of naturally fermented traditional Greek yogurt from sheep and cow milk of different artisanal dairy producers

PP146: Effect of 12-week probiotic consumption immobilized on oat flakes on blood and urine biomarkers and human microbiome

PP147: Multispectral imaging (msi) for the discrimination of different microorganisms forming biofilms on stainless steel surfaces

PP148: Physicochemical and microbiological characteristics of yogurts fortified with apple pulp

PP149: Application of ATR-FTIR spectroscopy as a PAT tool for discriminating among Tuber species, their origin and maturity status

PP150: Directed fermentation of black olives cv. Konservolia by functional cultures in lab and industrial scale

PP151: Effect of organic herbs on the quality and safety of chicken broth

PP152: Microbiological quality and safety assessment of various chicken meat products as influenced by manual processing operations

PP153: Survey: Microbiological quality of fish from retail

PP154: Occurrence and contamination levels of ochratoxin A and aflatoxins in corn-based products from markets in Greece

PP155: Evaluation of the Greek pdo anevato cheese with metagenomics and volatolomics

PP156: Assessment of the microbiota of industrial dry sourdoughs through MALDI-TOF and metagenomics

PP157: Development of novel biomarkers as time-temperature indicators of freshness or spoilage of fresh, vacuum-packed prawns and squid

PP158: Cytotoxic effect of selected cell-free probiotic supernatants on colon cancer cell lines

PP159: Evaluation of different encapsulation methods of lactobacillus plantarum 2035 to enhance its viability in beer environment

PP160: "Application of alternative preservation methods for fruits and production of novel highly preservable products"

PP161: Production of wine with enhanced varietal aroma from cv. "Moschofilero"

PP162: Fungal mycobiota isolated from Kefalonian cheeses

PP163: **Withdrawn**

PP164: Bacterial communities of fresh mussels stored at 2 and 4oC

PP165: Developing stable functional ingredients for the food industry containing immobilized cells of a wild-type plant-based presumptive probiotic strain on high-fiber content supports

PP166: Effectiveness of probiotic immobilized on lyophilized banana powder on patients with Irritable Bowel Syndrome

PP167: Discrimination of biofilm cells from planktonic and of different microbial groups using ft-ir

PP168: Tracking of Salmonella in sesame edible seeds imported in Greece

PP169: Volatile metabolites analysis coupled with machine learning for the rapid quality assessment of chicken meat

PP170: Bioprotective effect of probiotic lactic acid bacteria against Salmonella enterica in orange juice

PP171: **Withdrawn**

PP172: Potential of Fourier transform infrared spectroscopy for the rapid monitoring of ochratoxin A and aflatoxin B1 in foods

PP173: Predicting the survival of probiotic Lactiplantibacillus plantarum in fruit juice as a function of pH and storage temperature

PP174: **Withdrawn**

PP175: Comparative genomics of Bacillus cereus isolates responsible for sweet curdling of milk

PP176: **Withdrawn**

PP177: Modeling the survival of two probiotic strains encapsulated in a whey protein isolate - gum arabic coacervate matrix under harsh conditions

PP178: Survival of Lactic acid bacteria and pathogens in a fermented product made from soy beverage during cold storage

PP178a: Design and development of functional strained yoghurt with probiotic cultures encapsulated in prebiotic matrices

PP178b: Fate of Salmonella enterica in orange juice and in consequent simulated human gastrointestinal system in the presence of free or encapsulated probiotic lactic acid bacteria

PP178c: Natural Fruit Juices Enriched With Probiotic Bacteria And Other Biofunctional Constituents In Encapsulated Form

PP178d: Effect of microencapsulation on the survival of probiotic bacteria in model food and in orange juice during heat treatment

PP178e: Application of novel olive fruit processing methods and technologies for the high-efficiency 0production of olive oil and olive paste with improved quality and nutritional characteristics

AGRICULTURE

PP001

Mitochondrial Ef-Tu: a Trump Card in pathogenesis of Verticillium dahliae

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The translation elongation factor (Ef-tu) protein is crucial (but not only) for fungi as it is necessary for protein synthesis in mitochondria. The ortholog gene in bacteria besides the main role in protein synthesis, it also triggers the immune system of plants as a PAMP (Pathogen-Associated-Molecular-Pattern), via a 18 amino-acid N-terminal residue (elf18) recognized by the specific receptor EFR which is found in species within Brassicaceae family. Interestingly this peptide is well remained also in V. dahliae. In this study we investigated the subcellular localization and role in virulence of the orthologous protein Vtu in V. dahliae. The vtu gene was fused with the enhanced green fluorescent protein (egfp) gene under the control of a strong fungal promoter. This construct was used to transform a race 1 strain of V. dahliae via Agrobacterium tumefaciens-mediated transformation. Localization of Vtu-Egfp was detected using confocal and live Airyscan super resolution microscopy, which showed that the Vtu protein was localized in mitochondria and in membrane vehicles located in the vicinity of cell wall. Presence of the chimera in these vehicles indicates the potential of Vtu for secretion. V. dahliae transformants expressing more copies of the endogenous gene were evaluated for virulence compared with the wild type by infecting Arabidopsis strains Col-0 and EFR1 as well as tomatoes. The parameters as amount of disease (AUDPC) and relative biomass of pathogen into the infected tissues were evaluated with qPCR. Our results showed significant reduction in pathogenicity of both mutant and wild type V. dahliae in Arabidopsis EFR1 and increase of Vtu mutant in Col-0. Consequently, we support the hypothesis that V. dahliae manipulates the induced response, probably via ethylene pathway, by the interaction of Vtu with EFR for enhanced virulence.

PP003

Mycoflora and mycotoxin characterisation of berries cultivated in Mediterranean farms

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Berries are perishable fruits and very susceptible to fungi contamination due to their high acidity and water activity. The most predominant fungal contaminants reported in literature are Botrytis cinerea, Alternaria spp., Cladosporium spp., Penicillium spp. and Rhizopus spp. The purpose of this study was to characterise the mycoflora found on the surface of fresh berries that are cultivated in the Mediterranean region and determine the potential presence of mycotoxins (e.g., ochratoxin A, patulin and alternariol). Fresh blueberries (2), blackberries (2), strawberries (2), and raspberries (5) were collected from different farmers in Greece and Cyprus and were stored in freezing conditions (-20o C). Each sample was incubated at 25o C for 5 days in DG18, PDA and MEA media. A direct plating method was used with DG18 in order to isolate and identify the fungi. PDA medium was used for the enumeration of fungi and yeasts, while MEA for growing the isolates. Isolates were characterized by macro- and microscopic observation to the genus level, and by Sanger sequencing at the ITS locus for species identification. Mycotoxins were determined by using HPLC – FD and HPLC – DAD methods. A total of 181 isolates were collected from fresh berries and nearly half of the microscopically characterized fungi belonged to Penicillium (29.3 %), Cladosporium (18.8 %) and Aspergillus section Nigri (14.4 %) genus. Other identified isolates included Alternaria (11 %), Botrytis (8.8 %) and Rhizopus (5.5 %) species, while the rest of the isolates, (12.2 %), assigned to other fungal genera such as, Ulocladium, Seiridium and Arthrinium. Quantification of fungi and yeasts varied between 2.18 – 2.98 log cfu/g and 1.67 – 3.00 log cfu/g, respectively.

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PP004

Impact of nitrification inhibitors on the soil microbial community after repeated exposure: a soil microcosm story

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Nitrification inhibitors (NIs) such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) are routinely incorporated into fertilizers to stabilize the supply of N available in soil by delaying the microbial conversion of ammonium-N to nitrate-N (nitrification). Considering the massive global annual N fertilizer input, this practice could lead to a systematic exposure of agricultural soils to NIs with potential undesirable effects on the environment and public health. Little is still known about the specific activity of these NIs on the different groups of ammonia-oxidizing microorganisms (AOM) and mostly their effects on diversity and function of other soil microbiota. We aimed to explore the outcome of the complex interactions of NIs with the soil microbial community. We employ microcosm tests in soils with or without previous exposure to the studied NIs which were repeatedly fortified with NIs. Our hypothesis is that repetitive exposure will lead to enhanced biodegradation of NIs in soils with prior exposure and to accumulation of NIs residues and eventual toxicity to off-target microbial groups in soils without prior exposure. To evaluate these hypotheses we monitor (a) the soil dissipation of NIs as a measure of the development of enhanced biodegradation of NIs (b) the activity of NIs on the functional diversity of target microorganisms (ammonia oxidizers) using q-PCR, amplicon sequencing and relevant functional measurements (potential nitrification, NO3--N, NH4+-N concentrations), (c) the impact of NIs on NOB and denitrifying bacteria modulating downstream processes in N cycling using q-PCR, (d) the potential ecotoxicological effects of NIs on the functional and phylogenetic diversity of non-target microorganisms (total prokaryotes, fungi) via amplicon sequencing. Current data, derived after two successive applications of NIs in soil, suggest no relevant NIs dissipation regardless of previous or not soil exposure to NIs, while a significant and persistent reduction in nitrification was observed in all tested soils. We envision that our findings will contribute to a better understanding of the potential risks associated with the prolonged use of NIs in agriculture and may provide insights into the development of more sustainable agricultural practices.

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PP005

Elimination of Olive Leaf Spot disease by Mg(OH)2 Porous Microparticle treatment

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Olive peacock spot, or Olive Leaf Spot (Cycloconium oleaginum) is considered by many as the most serious disease that affects the Mediterranean olive groves. It is caused by Venturia oleaginea, a fungal pathogen and can lead to severe economic losses in olive production. Severe and recurrent attacks by the fungus cause intense defoliation, poor growth and dieback of the defoliated branches. The most common practice of chemical control of the OLS disease is the application of copper fungicides, which has raised serious concerns for the high levels of accumulation in soil and groundwater, and the toxicity to plants and humans.

Recently, we showed that Porous Micron-scale Mg(OH)2 Particles (PMPs) sprayed on tomato leaves cause major changes in the phylloplane microbiota and a drastic fungal load reduction1. To assess their effects on plant disease, we applied treatment on an olive tree with severe manifestations of OLS, destined for destruction. The effect of spraying PMPs on disease symptoms on leaves and branches was drastic 20 days later, effectively eliminating visual signs of the disease. Scanning Electron Microscopy showed that infected leaves were saturated with conidia, prior to spraying, whereas at day 20 fewer conidia are present and leaves are covered with PMP crust. DNA from the phyllosphere of the infected tree and two healthy neighboring trees submitted to amplicon metabarcoding analysis using the ITS1, 18SrRNA as taxonomic identification markers. The results show that prior to spraying 20% of the fungal phyllosphere consisted of the Venturia pathogen confirming visual observations of OLS. Subsequent to PMP treatment, the abundance decreased to <1% and the fungal profile was similar to the neighboring healthy trees. Mg(OH)2 is deemed as safe by FDA and the European Chemical Agency (ECHA) has concluded that it is not persistent, bioaccumulative or toxic. Moreover, magnesium is an essential nutrient for living organisms. The potent activity of Mg(OH)2 PMPs against OLS offers a powerful attractive alternative in disease control.

References: 1. Andreadelli, A. et al. Effects of magnesium oxide and magnesium hyoxide microparticle foliar treatment on tomato pr gene expression and leaf microbiome. Microorganis 9, 1–4 (2021).

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PP006

Species-specific skin microbiome in gilthead seabream and European seabass is strongly influenced by the rearing location

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Fish is a highly perishable product, and its shelf-life is determined by intrinsic and external factors. Fish skin microbiome at harvest is expected to contribute to the dynamics of the shelf-life and building knowledge on the factors that determine its variations can be important for designing informed protocols for harvest and preservation. Mediterranean marine fish farming takes place in floating cages where the fish is in continuous interaction with the sea microbiota. This study explores how fish skin microbiome differs between fish species that are farmed in the same location and how it differs between locations. The skin microbiome of the gilthead sea bream (Sparus aurata) was compared to that of the European seabass (Dicentrarchus labrax), both farmed either in the Ionian or the Aegean Sea.

Samples were collected from two commercial fish far, located in the Ionian Sea and Aegean Sea. Fish at commercial size were harvested in ice water at 21°C water temperature, and skin microbiome samples were obtained using a sterile swab on the right upper lateral part of the fish. DNA was extracted and sent to BGI Genomics for 16S rDNA sequencing of the V3-V4 region using the HiSeq 2500 Illumina platform. Data analysis was performed using the DADA2 pipeline in R studio, aligning sequences to the SILVA reference database.

Skin microbiome differentiated with species and geography. Only 8 ASVs were shared amongst all comparisons, and were of two genera, namely Shewanella and Psychrobacter, which were overrepresented in both species and far. Pseudoalteromonas, Chryseobacterium, Pseudomonas and Flavobacterium were the other genera with high relative abundance among comparisons. Gilthead seabream had a much richer, in ASV number and relative abundance, microbiome compared with European sea bass. Location had a clear influence on skin microbiome composition with 45%- 65% unique ASVs per location in each species. Our results corroborate previous reports of bacterial diversity differences between the Ionian and Aegean Sea, and they indicate that fish species actually has the capacity to moderate its skin microbiome as the two species shared just 24 and 45 ASVs in the Aegean and the Ionian Sea far, respectively.

PP007

SYNERGISTIC EFFECTS OF COMBINED USE OF MICROBIAL INOCULA AND METABOLITES ON THE STRESS RESPONSE OF TOMATO PLANTS UNDER FIELD CONDITIONS

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Innovative and alternative ways to combat plant stresses are currently mandatory in order to secure our future food in a sustainable manner. Beneficial microorganisms and external applications of plant metabolites are known to promote plant growth and enhance their resilience to biotic and abiotic stresses. In this study, we evaluated the roles of the root endophytic fungi Fusarium solani strain K (FsK) and Trichoderma spp., as well as the effect of plant metabolites on tomato plants (Solanum lycopersicum) infected with either the pathogenic fungus Verticillium dahliae or with natural infestations from the insect pest Tuta absoluta. Agrochemical inputs were kept to a minimum and plant performance was assessed across developmental stages based on photosynthetic parameters, nutrient composition of leaves and fruits, harvest quality of the fruits and overall infestation and disease severity.

PP010

PHYLOTYPING OF VIBRIO HARVEYI STRAINS ISOLATED FROM DISEASED FISH FROM GREEK AQUACULTURE OUTBREAKS

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Vibrio harveyi is a well-recognized and serious bacterial pathogen of marine fish and invertebrates, including penaeid shrimp, in aquaculture. Fish vibriosis can cause vasculitis, gastro-enteritis, and eye lesions. Vibrio outbreaks in aquaculture far may result in high mortality of farmed organisms or considerable deterioration in the quality of the produce, affecting the marketability of the product and thus contributing to major economic losses. The aim of this study was to isolate and characterize the diversity of Vibrio sp. in marine systems by a cultivation-dependent approach. A total of 18 Vibrio sp. strains from different aquaculture outbreaks all over Greece were isolated. Fish specimens were dissected, and the strains were recovered in general and selective media. Molecular identification of the Vibrio isolates was based on the amplification and sequencing of different genes. One common approach to Vibrio phylotyping is to analyze the sequence of the 16S rRNA gene, which is present in all bacteria and is highly conserved across different species. However, this method may not provide enough resolution to differentiate between closely related Vibrio strains. Based on the 16S rRNA gene sequence phylogeny the isolates were assigned to different phylotypes by high probability to the species level. Genomic fingerprinting indicated an even higher genetic diversity of Vibrio sp. at the strain (genotype) level. Isolates of this phylotype showed highest 16S rRNA gene sequence similarity to type strains of Vibrio harveyi. Alternatively, whole-genome sequencing can provide a more detailed overview of Vibrio diversity and evolution. Overall, Vibrio phylotyping can be a valuable tool for understanding the biology and ecology of these bacteria, as well as for developing strategies to prevent and control Vibrio-associated diseases. Development in genomics and bioinformatics will continue to lead to new discoveries in this field, with potential applications in environmental monitoring, biotechnology, and vaccine design, making it an important tool in the aquaculture industry for the prevention and control of disease spread in fish farming populations of Greek aquaculture far.

PP011

New bio-based tools to control Bactrocera oleae (Diptera, Tephritidae) and elucidation of their mode of action

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The olive fruit fly, Bactrocera oleae (Rossi) is one of the most important olive pest, causing economic losses worldwide. Currently, control strategies of this pest relies mostly on the application of chemical insecticides with harmful effect on the environment and man. Thus, induced host plant resistance via the application of resistance inducers could be an additional sustainable valuable tool to manage olive fly. In this work, both laboratory and field assays were undertaken to explore the potential of the endophyte Alcaligenes faecalis and of the peptide systemin to control olive fruit fly via induction of olive tree resistance. The involvement of volatile organic compounds (VOCs) in the induction of host resistance triggered by the endophyte and peptide was also assessed by HS-SPME and GC-MS. Accordingly, olive trees were inoculated with the endophyte, systemin or buffer (control), and the fruits were used to performed two-choice and olfactometer assays. Both the endophyte and systemin reduced significantly the number of ovipositions (up to 31% and 67%, respectively) and repelled B. oleae females when compared to control. The deterrent activity of the primed olives was ascribed to the emission of specific VOCs. In particular, the alkenes o-cymene, D-limonene, gamma-terpinene, beta-myrcene and 1-dodecanol emitted by olives inoculated with A. faecalis were found to be negatively correlated with oviposition. The deterrent effect induced by systemine was ascribed to the emission of the alcohol 1-hexanol, 2-ethyl, the aromatic hydrocarbon benzene, 1,2,3-trimethyl- and the ester 4-tert-butylcyclohexyl. Systemine-treated olives also showed the ability to protect neighbouring non-treated olives against olive fruit fly infestation and might trigger priming responses through the emission of these volatiles. Overall, these findings suggest that both A. faecalis and systemin could be used as resistance inducers in olive tree to control olive fruit fly.

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PP012

Identifying the key determinants, cultivar or terroir units, of the carpospheric grapevine microbiome in the viticultural zone of Drama

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Grapevine cultivation along with wine production constitute two globally and commercially well-established agricultural sectors. Grapevine microbiome see to have a major contribution on wine's regional characteristics, underlining, this way, the special identity of regionally established cultivars. Recent scientific evidence have documented the key role of abiotic and biotic factors on the composition of the fungal and bacterial grapevine microbiome, and suggested the establishment of a microbial terroir for a specific viticultural region. In this context, we aimed to fully characterize the factors shaping the grapevine microbiome in the main red wine grape varieties cultivated in the ama viticultural zone (Merlot, Agiorgitiko, Cabernet Sauvignon). In the frame of the project, the Terroir Units (TU) of the specific viticultural zones were initially determined based on climate, soil, and topography using GIS mapping approaches. Vineyards located in the different TU of the viticultural zone were selected per cultivar and the composition of the carpospheric fungal and bacterial microbiome were determined via amplicon sequencing (Illumina Hiseq, 2x250bp). Our results based on the harvest of the year 2022 showed that the fungal and bacterial microbiome composition is driven primarily by the TU indexes followed by cultivar which showed a limited but significant effect. The same sampling strategy will be followed in 2023 and the results from the two vintages will be compared. Differential abundance analysis for both fungi and bacteria highlighted ASVs that seem to be cultivar-depended and contribute to the local character of the wines produced. Strains of the genus Saccharomyces sp. which are the most important contributors to winemaking were detected in only a few samples and always at low relative abundance <0.01%.

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PP013

GUT BACTERIAL DIVERSITY OF INSECTICIDE-SUSCEPTIBLE AND -RESISTANT STRAINS OF THE MEDITERRANEAN FRUIT FLY CERATITIS CAPITATA AND A POSSIBLE RELATIONSHIP WITH INSECTICIDE RESISTANCE

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Insecticides are applied worldwide for the control of agricultural insect pests. The evolution of insecticide resistance has led to the failure of pest control in the field. The enhanced detoxifying enzyme activity and target mutations play important roles in the resistance of insects to insecticides. Emerging evidence indicates a central role of the gut symbionts in insect pest resistance by degrading pesticides, but there are few reports on the relationship between midgut microbiota and insecticide resistance in Tephritidae. Using metagenomic sequencing, we can analyze and study the population diversity, biological activity, and functional roles of midgut microbiota. In this study, a susceptible strain (SS), a Malathion-resistant strain (MLR), a dimethoate-resistant strain (D) and Spinosad-resistant strain (SPR) of the Mediterranean fruit fly Ceratitis capitata were selected for metagenomic sequencing. The microbial diversity and metabolic functions of different strains were analyzed in order to clarify the relationship between the symbiotic gut microbiota of C. capitata and its resistance to insecticide and to provide a new target for the biological control of medfly. The results revealed differences in intestinal microbiota structures among the insecticide resistance strains of C. capitata compared to the susceptible strain. At the species level, Enterococcus sulfureus and Klebsiella oxytoca were more abundant in the Malathion- and Dimethoate-resistant strains. The Spinosad-resistant strain was dominated by Enterococcus sulfureus and Cronobacter sakazakii. The insecticide resistant strains had also some microorganisms with lower abundance compared to SS strain, including Serratia marcescens, Morganella morganii and Lactococcus lactis. Comparison of predicted KEGG function of the gut bacteria of the SS strain had significantly different metagenomic functions than the three resistant strains (MLR, D and SPR). Our findings will provide a basis for future studies elucidating of the roles of the gut bacteria in the insecticide resistance associated symbiotic relationship and on the design of novel strategies for the management of medfly.

PP014

SUPPRESSION OF VERTICILLIUM DAHLIAE AND PROMOTION OF PLANT GROWTH IN EGGPLANTS THROUGH COMBINED APPLICATION OF COMPOST TEA AND ERWINIA RHAPONTICI

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In intensive cropping systems, soil-borne plant diseases have become a significant issue affecting plant health and yield. To counter this, there has been increasing interest in utilizing beneficial microbes and organic amendments, such as compost and aerated compost tea, as alternatives to synthetic pesticides for soil-borne disease suppression. One of the most challenging soil-borne plant pathogens is Verticillium dahliae, which has a wide range of hosts and can survive for extended periods in the soil as microsclerotia. In this study, we evaluated the efficacy of using compost produced from olive pruning and olive mill waste, its aerated compost tea, and Erwinia rhapontici (a plant growth promoting bacterium with in vitro antagonistic effect on V. dahliae) to protect eggplants from V. dahliae and to assess their potential effects on plant growth and nutrient uptake.

The results of pathogenicity experiments performed under greenhouse conditions showed that singly applied compost tea and E. rhapontici reduced the disease severity (wilt symptoms) by 28% in both cases, compared to the control. However, the combined application of compost tea + E. rhapontici and compost + E. rhapontici was more effective in reducing the disease severity by 35% and 40% respectively, compared to the control. In terms of plant growth and nutrient status, plants treated with compost tea or E. rhapontici produced more biomass than non-amended control plants, while the combined application of compost tea + E. rhapontici and compost + E. rhapontici did not differ from the control. However, plants that received the combined application of compost tea + E. rhapontici and compost + E. rhapontici had higher nutrient concentrations than the control.

In conclusion, our study highlights the potential of using organic amendments and microbial inoculants, specifically compost tea and E. rhapontici, for the suppression of soil-borne pathogens and promotion of plant growth. Further research is needed to investigate the mechanisms of action underlying these effects.

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PP015

Cross-talk of Abscisic acid and Ethylene -mediated pathways in the interaction of tomato plant with the beneficial fungus Fusarium solani strain K

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The physiology of plants can be influenced by a variety of endogenous signaling and regulatory molecules such as hormones. They frequently move from places of synthesis to far-off parts of action, and they function at incredibly low concentrations (Smith et al., 2017). Endophytic filamentous fungi help host plants during stress conditions. Fusarium solani strain K (FsK) is a beneficial endophytic fungus that is able to colonize tomato's root system (Solanum lycopersicum) and provide defense against ought and salt stress (Kavroulakis et al., 2007). Endophytes have the ability to alter the phenotype and the hormone profile of the host. In this work we examined whether FsK colonization of tomato wild type, ABA mutant (flacca) and Ethylene mutant (never ripe) seedlings' root system, grown under normal, ought and in vitro conditions, had an impact on the root architecture and on the ABA/Ethylene biosynthetic pathways. Our data indicated that the inoculation with the endophytic fungus may generally affect plant tolerance to abiotic stress by rewiring the cross talking between those phytohormones, contributing to the perseverance of the inoculated plants.

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PP016

Three newly acquired mycophilic genomes elucidate the fungicolous secondary metabolism: a comparative study

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Hypocrealean fungi present a plethora of biological interactions with various hosts. Fungicolous (or mycophilic) species are associated with other fungi, mainly belonging to family Hypocreaceae. The biotechnological importance of these fungi is constantly becoming larger since they colonize both wild and cultivated mushrooms causing great damage in the production of the edible ones. Currently, there is limited knowledge regarding the genetic mechanisms of fungicolous interactions, which is crucial for enhancing the cultivation efficiency using environmentally friendly solutions. From a different angle, some fungi have been examined for their potential use as biological control agents against fungal pathogens.

Mycophilic species are characterized by a very rich secondary metabolism, which is strongly associated with their mode of life. Studies has shown that the production of inhibitory metabolites, wall-degrading hydrolytic enzymes and the fungicolous intense pigmentation is highly important for these fungi. All these factors indicate that the elucidation of fungicolous interactions is a necessity. In this study, the complete genomes of the mycophilic species Cladobotryum mycophilum ATHUM6906, Cladobotryum sp. ATHUM6904 and Trichoderma aggressivum MBE03 are presented and annotated. High-quality DNA sequencing was performed in-house by MinION sequencer (Oxford Nanopore Technologies). Emphasis was given to the secondary metabolism which provides extra advantages to mycophilic fungi during the colonization of their hosts.

Therefore, the pathogenicity related genes and Biosynthetic Gene Clusters (BGC) for the biosynthesis of plethora of secondary metabolites were determined and implemented in a comparative genomic analysis including all other mycophilic species whose genomes are publicly available. Interestingly, the genes related to the secondary metabolic pathways presented a notable diversity among the species examined. This work provides the genomic data for studying the mycophilic mode of life.

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PP017

EXPLORING THE BIOCHEMICAL MODE OF ACTION AND SPECTRUM OF ACTIVITY OF SYNTHETIC AND BIOLOGICAL NITRIFICATION INHIBITORS

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The direct inhibition of soil ammonia-oxidizing microorganisms (AOM) using synthetic nitrification inhibitors (SNIs) along with N fertilizers, is a well-established strategy for improving nitrogen (N) use efficiency in agricultural ecosystems and restricting groundwater and atmospheric pollution. In addition, functionally similar plant derived compounds that inhibit nitrification, called biological nitrification inhibitors (BNIs), have recently received increasing attention as safer and potentially more effective alternatives to SNIs. However, the efficacy of NIs in regulating soil N transformations is highly variable across soils and often suboptimal due to the variable sensitivity of soil AOM to different NIs, and the dependency of NIs performance on the composition of the metabolically active AOM community in soil. Importantly, while the inhibition mechanisms of commercially available SNIs, and characterized BNIs, have been presumed to be associated with the inactivation of ammonia monooxygenase, their actual biochemical mode of action remains unknown, impeding any prediction about their biological spectrum of inhibition against the different groups of AOM and posing an important drawback for the improvement of N fertilizer use efficiency. Within the framework of the European Union's Horizon 2021-2027 research and innovation programme ACTIONr, we aim (a) to define the mode of action of selected synthetic and biological NIs against soil representative strains of ammonia-oxidizing bacteria (AOB) (Nitrosospira multiformis and Nitrosomonas communis) and ammonia-oxidizing archaea (AOA) (Nitrososphaera viennensis, Ca. Nitrosotalea sinensis, Ca. Nitrosocosmicus franklandus) using proteomic and transcriptomic approaches, and (b) to develop, optimize and use synthetic microbial communities of nitrifiers as an ecologically relevant tool for NI activity screening by co-culturing two or more selected strains with distinct functions in nitrification (e.g., AOB/NOB, AOA/NOB and AOB/AOA/NOB). Overall, our work is expected (i) to contribute to the development of novel NI N-fertilisers, with improved performance, combining NIs with a complementary spectrum of activity against the main groups of nitrifying microorganisms, and (ii) to provide innovative methodological tools for the in vitro study of the impact of NIs and other established agrochemicals on soil ecosystem functioning.

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PP018

Studies on the role of Bacillus subtilis cyclophilin in bacterial growth and development

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Cyclophilin PpiB belongs to a group of proteins showing peptidyl-prolyl cis/trans isomerase activity which enables them to accelerate the isomerization of peptidyl-prolyl bonds, a rate limiting step in protein folding, and in some cases to control the duration and the amplitude of different cellular processes. Bacillus subtilis, a gram-positive spore-forming soil bacterium with plant growth promoting activity, possesses one gene, ppiB, coding for a cyclophilin. In order to understand the function of PpiB, we have replaced the ppiB gene of an undomesticated strain of B. subtilis with a kanamycin resistance cassette and have used the engineered strain to examine the possible involvement of PpiB in bacterial growth and development in vitro and in association with plants. We have observed various bacterial developmental and plant growth promoting phenotypes that seem to change when the bacteria have remained in the rhizosphere of the model plant Arabidopsis thaliana for extended periods of time possibly due to adaptation to this particular environment. As the initial phenotypes of the ΔppiB strain are probably due to the incorrect peptidyl-prolyl cis/trans isomerisation of certain PpiB target proteins, we have computationally analyzed the publicly available experimentally determined structures of B. subtilis proteins for prolines adopting the cis conformation. We have identified several proteins with cis prolines in their structure which are involved in various bacterial developmental and metabolic pathways. Future functional and structural studies will show whether these proteins are functionally related to PpiB. The deeper understanding of the association of B. subtilis with plants will lead to greater consistency and efficiency in the performance of plant beneficial microorganisms in agricultural practices.

PP019

Oxytetracycline treatment led to persistent gut microbiome dysbiosis, the rise of parasites and growth impairment of European Sea Bass in open sea aquaculture

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The use of antibiotics in open-water aquacultures is often unavoidable when faced with pathogens with high mortality rates. Those seasonal pathogen surges are becoming more common and more intensive over the years. Besides the apparent cost of antibiotic treatment, it has been observed that in aquaculture practice the surviving fish often display measurable growth impairment. This growth delay can be in the range of 2-12% of the commercial-sized fish weight. In order to understand better the role of gut microbiota on the observed growth impairment we follow an incidence of Photobacterium damselae subsp. piscicida in a Sea Bass commercial open-water aquaculture setting in Galaxidi (Greece).

Fish around 10 months of age were fed with food containing oxytetracycline (120 mg/kg/day) for twelve days, followed by a twelve-day withdrawal period, followed by another eighteen days of treatment. Fishes were sampled 19 days before the start of the treatment and a month after the end of the second treatment cycle. Sequencing of the 16S rRNA gene was used to measure the changes in the gut microbiome. Overall, the gut microbiota community even a month after treatment was highly dysbiotic and characterized by very low alpha diversity. High abundances of alkalophilic bacteria in the post-antibiotic-treated fishes indicate a rise in pH that was coupled with a significant rise of gut parasites. This study indicates that OTC treatment causes persistent dysbiosis even a month after withdrawal, providing a more suitable environment for the raise of parasites and highlighting the need for interventions to restore a healthy and beneficial gut microbiome.

Keywords: Sea Bass; oxytetracycline; aquaculture; fish microbiome; dysbiosis, fish parasites

PP020

Forecasting and protecting fruit crops from frost damage: the EU/LIFE FrostDefend project

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Climate change (CC) induced an increase in climate variability, and various harmful weather events occur at the global and regional scale with increased frequency and intensity. CC is among the most serious challenges to society, and the need for adaptation is particularly acute, considering its impact on many communities and sectors that depend on natural resources. Crops are sensitive to weather. A frost event can wipe out an entire crop. Frost is responsible for serious crop losses in Greece. During the cold periods of 2004 and 2007, severe frosts in Aeghion, Greece resulted in the complete loss of lemon production. In France, in 2016, 2017 and 2019 a combination of premature vegetation and late frost caused signifi¬cant damage to fruit-tree and grape crops. In Europe, frost-related crop losses are estimated to reach 3,3 b € per year.

We develop a smart Internet of Things (IoT)-based system to monitor relevant atmospheric, meteorological and plant indicators in an orchard, to predict the risk for frost damage. This tool will make use of parameters monitored in real-time with cost-effective sensors to predict growth of populations of epiphytic ice nucleation-active bacteria, key factors in frost damage of crops. Furthermore, this tool will provide reliable warnings and guidelines to farmers for simple, low cost and sustainable actions to mitigate potential frost damage to tree crops by reducing these populations ahead of an anticipated frost event. FrostDefend is based on cross-disciplinary research that covers agronomical, biological, meteorological and atmospheric sciences. The system will take into account:

. the mechanism of plant frost damage and plant frost adaptation strategies.

. the biology of epiphytic ice nucleation active bacteria which facilitate the development of frost on plants.

. the atmospheric processes and properties of atmospheric aerosols leading to ice nucleation, freezing and precipitation events.

. the importance of long-range transport and mixing of aerosol sources over the Mediterranean basin.

The EU/LIFE-FROSTDEFEND project ai to design, develop and demonstrate the benefi¬ts of a novel monitoring and frost forecasting tool to mitigate frost injury in tree crops. The project started in September 2021 and will last 4 years.

LIFE FROSTDEFEND

PP022

Characterization and use of plant growth promoting bacteria from vineyard soils

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Climate change demands the re-innovation of agriculture and the development of accurate and smart tools that can confer increased adaptation and protection to main crops in each country. Several studies showed plant growth promoting bacteria (PGPB) could be an alternative tool for the reduction of N fertilization and the adaptation of several crops in increased salinity and ought. In the current study, we isolated and characterized environmental isolates from the main wine making areas of the country namely Panagia, Kyperounda and Koilani. We totally isolated 149 colonies and tested for plant 43 exhibited plant growth promoting traits like N fixation, IAA and siderophore production and solubilization of P and K. From those, 4 isolates characterized as Pseudomonas sp (1), Bacillus aryabhappai (2), Staphylococcus epidermidis (3) and Acinetobacter viviannii (4) applied individually or in combination in vine cuttings in peat:vermiculite to assess their plant growth effect in the above and below ground biomass in a complete randomized design. Mock inoculum was used as control while cuttings grown without any addition of chemical fertilizet. The results showed that Acinetobacter viviannii caused a substantial increase of vine cuttings biomass. A competition between the different isolates applied was observed regarding their effect on vine cuttings biomass production. In detail, the cuttings inoculated with all isolates exhibited similar biomass production with those received no bacteria. Our findings suggest that the isolation, characterization and development of autochthonous bacterial inocula could be a promising tool to produce sturdy and vine seedlings.

PP024

Development of a fast-track/ high throughput screening assay on soil ammonia-oxidizing bacteria for the discovery of novel biological nitrification inhibitors

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Nitrification, the microbially-mediated oxidation of ammonia (NH3/NH4+) to nitrate (NO3-), is associated with significant losses of fertilizer-derived NH4+ from agroecosystems through NO3- leaching and N oxides emissions, raising agricultural production costs and contributing to environmental pollution and climate change. The use of nitrification inhibitors (NIs), a diverse group of both synthetic (SNIs) and plant derived, biological (BNIs) compounds that inhibit the activity of soil ammonia-oxidizing microbes (AOM), holds a great potential to effectively reduce such losses. Recent benchmarking research of our group pointed to the use of inhibition assays with pure cultures of a diverse range of soil derived AOM as a necessary step to evaluate the inhibition potential and define the spectrum of activity of NIs destined for use in agriculture. In vitro assays give a good, conservative estimate of NIs impact on AOM, and have been previously used for determining the activity of both synthetic and biological NIs, but due to the low specific growth rates of AOM, could be a time-consuming and labor-intensive approach. To enable the systematic screening of the NI activity of multiple in-house produced root exudate fractions, we aim to develop, optimize, and use a fast-track, simple, and sensitive assay which will be able to implement on multiple nitrifier species. Here, we focus on optimizing the culturing of Nitrosospira multiformis, representing a highly abundant lineage of ammonia-oxidizing bacteria (AOB) found in soil. To this end, we developed a method for concentrating and efficiently harvesting N. multiformis cells to obtain a high cell density culture (1.5x concentrated cells), reducing by half the AOB growth response (from 156 h to 60-70h). Further optimization, targeting to a higher level of concentrated growth (2x concentrated cells) and subsequent standardization and validation of the new assay using known NIs will follow. Overall, our work is expected to pave the way for the development of novel, rapid, and accurate tools for the massive screening of the activity of multiple NI promising chemical candidates on nitrifiers, which in the long term could be harnessed for the discovery of novel NI compounds.

Acknowledgements: This work is part of the project “Pipeline for Development and Commercialization of Biological Nitrification Inhibitors to mitigate GHG Emissions from Cultivated Soils” which is funded by the Grantham Foundation.

PP025

Evaluation of biological and synthetic plant protection products against the pathogen Taphrina deformans causing peach leaf curl

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Peach leaf curl is a fungal disease that affects peach trees, caused by the pathogen Taphrina deformans. This important fungal disease has been particularly pronounced in recent years in Greece, causing extensive infections characterized by severe distortion and hyperplasia of host leaves, premature defoliation and weakness of the tree's overall health. The pathogen persists over the winter and infects emerging buds in cool, wet spring weather. The disease is managed by application of several sprays of synthetic plant protection products (PPPs). The goal of this study was to evaluate the efficacy of selected synthetic and biological PPPs in in vitro studies. Four synthetic PPPs (Flint Max®, Syllit®, Heliocuivre®, and Signum®) were evaluated with five different concentrations each in liquid media inoculated with the pathogen yeasts cells. Inhibition rates and EC50 values were calculated for each synthetic PPP. Additionally, the efficiency of active ingredients and metabolites (obtained from the supernatant of their liquid cultures) of several bioPPPs (Serenade®, Serifel® and Botector®) and a non-commercial biological control agent (Bacillus velezensis) was also evaluated. All tested synthetic and biological treatments were efficient in inhibiting pathogen's growth. The treatments of the most effective biocontrol agents' metabolites are currently investigated at transcriptomics level to unravel time-course differential gene expression patterns of T. deformans.

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PP026

Preliminary results on the relative abundance of certain functional groups of microorganisms present in the rhizosphere of herbicide-resistant and non-resistant weeds

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Herbicide-resistant weeds evolved mainly due to the widespread and continuous selection pressure triggered by the long-term application of herbicides. In this study, total microbial counts and the heterogeneity of microbial communities in the rhizosphere of herbicide-resistant and non-resistant weeds have been investigated. Bacterial and fungal populations associated with two biotypes (herbicide-resistant and non-resistant) of Avena sterilis subsp. ludoviciana, Lolium rigidum, and Bromus sterilis. cultivated on three different sterilized and non-sterilized soils were assessed. The rhizosphere of the two L. rigidum biotypes was further examined for the presence of Pseudomonas fluorescens, colifor, ligninolytic fungi and bacteria, chitinolytic and cellulolytic microorganisms, phosphate solubilizers, spore-forming bacteria, nitrogen-fixing microorganisms, and nitrifiers. Fungal populations in the herbicide-resistant biotype were considerably higher compared to the non-resistant biotype. In addition, the origin of soil samples had an impact on both the fungal and bacterial populations, while the microbial populations in the rhizosphere were noticeably richer than those of the bulk soil. Moreover, the two L. rigidum's biotypes had a varying impact on the diversity and abundance of particular microbial communities in the rhizosphere, which in turn may have affected the herbicide-resistant biotype's competitiveness and rapid growth in comparison to the non-resistant biotype. The rhizosphere in the herbicide non-resistant biotype was dominated by potential weed (and other plant) pathogens and plant growth-promoting microorganisms, whereas plant growth-promoting microorganisms, biocontrol agents and microorganisms with herbicide degradation potential were detected in the rhizosphere of the herbicide-resistant biotype. The root exudates of each weed biotype have a unique composition that can be determined by plant gene expression; those exudates could affect the prevalence of the particular biotype in either a beneficial or detrimental manner.

PP027

SEASONAL SHIFTS IN SOIL MICROBIOME STRUCTURE ARE ASSOCIATED WITH THE CULTIVATION OF THE LOCAL RUNNER BEAN VARIETY AROUND THE LAKE MIKRI PRESPA

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Leguminous crops play a key role in food production and agroecosystem sustainability. However, climate change and agricultural intensification have a significant impact on the available arable land, soil microbiome functions, and ultimately, crop productivity. The "Prespa bean" (Phaseolous coccineous L.) is an important leguminous crop for the agricultural economy of the rural areas surrounding the lake, Mikri Prespa, which is of significant ecological importance. The seasonal effects on soil microbiome structure, diversity and functions associated with the runner bean cultivation were investigated using 16S rRNA amplicon sequencing. The results indicated that the presence of the runner bean differentially shaped the soil microbial community structure. The runner bean was implicated in the recruitment of specific bacteria, by favouring or excluding specific classes or even phyla. Soil functions involved in nutrient availability and carbon metabolism, among other pathways, were associated with microbiome–plant interactions. The temporal relative abundance shifts could be explained by the impact of soil organic matter, the fertilization regime, and the equilibrium in carbon metabolic processes. This research has shown the effect of runner bean cultivation on the soil microbiome which, in future, may potentially contribute to research into sustainable agricultural productivity and the protection of soil ecosystem services.

PP028

The suppressive effect of bacterial strains isolated from halophytes against important plant pathogens

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In the present study, the suppressive effect of 173 bacterial strains isolated from halophytes against significant phytopathogens (Verticillium dahliae-V.d., Botrytis cinerea-B.c., Fusarium oxysporum f.sp. radicis-cucumerinum-F.o.r.c. and Phytophthora infestans-P.i.) was tested. 173 bacterial strains were initially screened in-vitro and the 20 most efficient strains per pathogen were evaluated thoroughly by conducting dual culture (confrontation) and dual plate (volatile) assays. Amongst them, 9 strains inhibited significantly the growth of B.c., whereas 15, 10, and 18 strains inhibited the growth of V.d., F.o.r.c. and P.i., respectively. The most effective strains were evaluated furtherly in planta for their capacity to provide sufficient plant protection against the pathogens. Results showed that 9 strains reduced significantly the symptoms of grey mould in tomato, 1 strain suppressed verticillium wilt symptoms on eggplant and 2 strains on tomato, 2 strains reduced disease severity of root and stem rot (caused by F.o.r.c.) in cucumber plants, and 4 strains suppressed symptoms of late blight (caused by P.i.) in tomato leaves. Finally, the 2 most effective bacterial strains that suppressed grey mould in-planta under controlled conditions were evaluated furtherly in a commercially used greenhouse. It was revealed that one strain (code SAR 128) was able to provide significant protection of tomato plants against B. cinerea. Data of the present study suggest the potential of certain bacterial strains isolated from halophytes to protect vegetable crops against significant plant pathogens, and thus, to be used as biocontrol agents.

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PP029

EVALUATION OF THE IMPACT OF SYNTHETIC AND BIOLOGICAL NITRIFICATION INHIBITORS ON THE COMPOSITION AND ACTIVITY OF THE SOIL MICROBIAL COMMUNITY AND THE EMISSIONS OF GREENHOUSE GASES

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Nitrification inhibitors (NIs) delay the microbial conversion of ammonium to nitrate (nitrification), reducing the risk of N loss through leaching (in the form of NO_3-/N) or denitrification (emissions of nitrous oxides like N_2O), and thereby increasing the N use efficiency (NUE) of fertilizers and reducing the detrimental environmental effects of the reactive nitrogen (Nr) cascade. To date, little is known regarding the effect of nitrification inhibitors (NIs) on the metabolic activity of the different groups of soil ammonia-oxidizing microorganisms (AOM). Within the framework of the European Union's Horizon 2021-2027 research and innovation programme ACTIONr we aim to investigate the complex interactions of NIs with N cycling microbial players both at functional and community structure level with ultimate goal to establish new tools and pathways to optimise NUE, decelerate the N cycle, and decrease the environmental footprint of Nr. To this end, the effect of selected synthetic and biological NIs, applied individually or in mixtures targeting either different parts of the ammonia oxidation pathway or different groups of AOM, is investigated in soil microcosm and pot experiments, under a range of conditions known to affect the activity of soil AOM and the performance of NIs (e.g., soil pH and N fertilization type). Our assessment will expand to other microbial groups directly (e.g., denitrifiers fuelled by substrates produced during nitrification) or not directly associated with nitrification (e.g., bacteria, fungi, viruses, protists) gaining insights into the effects of NIs on the community composition and activity of off-target soil communities and highlight possible interrelated effects of NIs on broader cross-kingdom networks. Gas emission rates of CO_2, CH_4 and N_2O will be measured in parallel to gain insight of the impact of NIs on nitrifier and total microbial contributions to GHG emissions. Overall, our work is expected to enable a deep understanding on the complex interactions between NIs and soil nitrifying microorganisms, which in the short term could be harnessed for the development of innovative fertilization products, while in the long-term it will contribute to more efficient N management in agricultural settings.

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PP030

Bacterial community structure and functioning in Cyprus vineyard ecosystem

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Understanding the intricate relationships between bacterial communities and their environment is crucial for sustainable viticulture. This study explores the structure and functioning of bacterial communities in Cyprus vineyard ecosystems, a setting characterized by unique climatic conditions and traditional viticultural practices. High-throughput 16S rRNA gene sequencing was used to identify bacterial diversity, while metagenomic sequencing and qPCR were employed to explore the presence and functionality of nitrogen-cycling genes. Results revealed a rich bacterial diversity, with distinct community structures between vineyards location. Soil pH was the decisive factor shaping the bacterial structure in Cyprus soils while the dominant taxa found were assigned in the phylum of Proteobacteria, Actinobacteria, and Plantomycetota. Shotgun metagenomic analysis revealed an array of bacterial metabolic pathways related to nutrient cycling and plant growth promotion. Notably, bacterial populations related with nitrification and denitrification were detected and significant differences between vineyards location were observed. The differences were associated with soil physicochemical properties and environmental conditions. These findings underscore the potential of microbial management as a strategy for improving vineyard health and productivity. Further research is needed to unravel the complex interactions between these bacterial communities, vine plants, and the broader vineyard ecosystem to enhance the sustainability and resilience of Cyprus viticulture.

PP031

ENHANCEMENT OF PRODUCTION OF PATHOGEN SUPRESSING VOLATILES BY PRECURSOR MOLECULES: TOWARDS SUSTAINBLE CONTROL OF SOILBORNE DISEASES

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Bacterial volatile organic compounds (VOCs) can play a significant role in antagonistic interactions between microorganisms. Enhancing the production of bacterial VOCs suppressing the growth of soil-borne plant pathogenic fungi, has perspective as a sustainable disease control strategy. In the present study, we explored the potential of stimulating production of pathogen suppressing VOCs by provision of precursor molecules, such as amino acids. Burkholderia AD24 was supplied with different amino acids, in mixture and individually, and the composition and suppressive effect of the volatile blend against Fusarium culmorum and Rhizoctonia solani was evaluated. Coincident presence of different amino acids in the bacterial growth medium, resulted in higher suppression of both pathogens via produced volatiles. Subsequent analysis of the composition of the volatile blend produced by Burkholderia AD24 in the presence and absence of amino acids, showed higher abundance of antifungal compounds in the former, including sulfur compounds (DMDS) and heterocyclic oxygen compounds (dioxane, dioxolane). Follow-up treatment with single amino acids revealed a pathogen specific effect, where F. culmorum was significantly suppressed by the volatile blend produced when Burkholderia AD24 was cultivated in the presence of glutamine, asparagine and arginine, whereas R. solani was suppressed in the presence of glycine. Analysis of the volatile blend composition also showed differences between the different amino acid treatments. Our results show that precursor molecules, like amino acids, can enhance the production of pathogen suppressing bacterial VOCs. Next step, will be to test this under conditions used in practice.

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PP032

Combining Rhizoglomus irregulare QS69 with systemin results in better control of tomato pests

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The tomato russet mite Aculops lycopersici and the whitefly Bemisia tabaci are key tomato pests causing serious yield losses if left uncontrolled. Methods other than chemicals are required to control the populations of both pests. In this study, we assessed the effects of the peptide systemin and the mycorrhizal fungus Rhizoglomus irregulare QS69 when applied as soil drench, against A. lycopersici and B. tabaci in tomato. Plants were infested with a standard number of tomato russet mites or whitefly females after being treated with the peptide, the fungus, or their combination. After two weeks or five days, we assessed the number of live A. lycopersici individuals or B. tabaci females, respectively. In the latter case, we also counted the number of eggs on each plant. The same experiment was also performed under field conditions in screen cages to assess the effects of systemin, R. irregulare QS69 and their combination on the pest population dynamics. Our results highlight the potential of peptides and beneficial soil microbes and their combination in pest control, as well as the need for further research in this field.

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PP033

USING KNOWLEDGE FROM WILD RELATIVES OF PLANTS TO COMBAT SOILBORNE PHYTOPATHOGENS

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Modern agriculture relies on the extensive use of chemicals to deal with plant pathogens and at the same time cope with the relentless increase of global food demand. Nevertheless, the use of chemical methods as a preventive strategy against plant diseases, can negatively affect plant productivity, human health, as well as environmental stability. Therefore, it is crucial to find new methods in order to deal with such diseases effectively and in a more sustainable way. A promising approach is to exploit the microorganisms present in the rhizosphere of plants which influence plant growth and disease resistance. These microorganisms and their functional repertoire are collectively known as the root-associated microbiome. In addition, crop wild relatives possess a range of stress-resistant traits that need to be unearthed and used for the improvement of cultivated plants. The aim of this study is to clarify how selected wild tomato relatives demonstrate increased resistance to the phytopathogenic soilborne fungus Verticillium dahliae, which heavily infects cultivated tomato plants. The contribution of specific defense responses (suberin, lignin, callose) to the increased resistance of the wild relatives will be determined, while the expression of genes with a role in the plant's defense will also be studied. At the same time, the contribution of the metabolism and the microbiome to the increased levels of disease resistance will be characterized and then these responses will be compared with those of susceptible cultivated plants. Ultimately, our findings can form the basis for the development of approaches towards the improvement of plant disease management leading to a more sustainable and stress-resistant agriculture.

PP034

Do ammonia oxidizers adapt to nitrification inhibitors upon repeated exposure? First evidence from in vitro assessment

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Dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) are synthetic nitrification inhibitors (NIs) routinely used in agriculture along with N fertilizers to delay the microbial conversion of ammonium-N to nitrate-N (nitrification) and increase N use efficiency in intensive farming systems. Despite the systematic exposure of agricultural soils to these compounds, the response of target nitrifying microorganisms to repeated NIs applications has not been explored yet. In vitro assays with representative terrestrial strains of ammonia oxidizing bacteria (Nitrosospira multiformis) and archaea (Ca. Nitrisocosmicus franklandianus) and nitrite oxidizing bacteria (Nitrobacter sp. NHBI) were employed to explore their response under continuous exposure to sub-inhibitory levels of NIs for at least five cycles. The hypothesis tested is that repeated exposure to sublethal levels of NIs will either trigger the evolution of subtypes which will tolerate or actively degrade NIs or will lead to increasingly stressed subtypes of nitrifiers. For each exposed generation of nitrifying isolates, inhibition threshold levels (EC50) and NIs degradation half-life (DT50) are calculated, to check the potential evolution of tolerance, increasing sensitivity or enhanced catabolic capacities towards NIs. In cases where a differential response of the target organisms to NIs will be detected (increasing EC50, decreasing DT50), the mechanism driving this differential response will be explored through transcriptomic analysis. Current data appear to contradict the hypothesis of tolerance or enhanced biodegradation given that the repeated exposure to NIs has so far induced a general stress condition on the tested microorganisms which is perpetuated from cycle to cycle and lead to a decrease in the EC50 values indicating a reduced tolerance to NIs. Final results and conclusions will be presented in the conference.

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PP035

Exploring the potential of plant-derived terpenoids as biological nitrification inhibitors

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Nitrogen fertilizers are widely used in agriculture to increase crop yields, but they often lead to environmental issues related to reactive nitrogen (Nr) losses. Such losses are directly linked to the microbially mediated process of nitrification which generates the soil-mobile anion NO_3– from relatively immobile ammonium (NH_4+) pools, making inorganic N susceptible to losses through NO_3– leaching, and N_2O emissions. The direct inhibition of soil nitrifying prokaryotes using synthetic nitrification inhibitors (SNIs) along with N fertilizers, is a well-established strategy for reducing the negative ecological impact of nitrification and improving N use efficiency in agriculture. In addition to SNIs, functionally similar plant-derived compounds that inhibit nitrification, called biological nitrification inhibitors (BNIs), have recently received increased attention as safer alternatives to SNIs. Until now, several BNI compounds have been identified from plant roots including sorgoleone, sakuranetin, and methyl 3-(4-hyoxyphenyl)propionate from sorghum, 1,9-decanediol from rice, and zeanone from maize. Exploiting the intrinsic plant potential to produce BNIs is a promising path toward more sustainable agriculture. Terpenoids are a diverse group of plant secondary metabolites with an acknowledged role as plant defensive agents against pathogenic microbes and herbivores. Still, their potential to inhibit nitrification in soil has not been explored. We aimed to assess the inhibition capacity of terpenoids on nitrification and specifically on ammonia-oxidation and its microbial mediators. To this end, we are currently assaying in vitro the inhibitory activity of multiple triterpenoid compounds -purified from different plant species that had previously been identified to possess highly antimicrobial and antifeedant activity- against two soil ammonia-oxidizing microbes (Nitrosospira multiformis, ammonia-oxidizing bacterium, and Ca. Nitrosocosmicus franklandianus, ammonia-oxidizing archaeon) both representing widely distributed lineages of ammonia-oxidizing microbes in the soil. Triterpenes able to reduce ammonia-oxidation rates by up to 50% compared to the control treatment will be tested against a wider range of ammonia- and nitrite-oxidizing isolates to further characterize the inhibition potential as well as to investigate the mechanisms underlying the inhibitory activity. Overall, our work is expected to provide benchmarking knowledge on the impact of terpenoids on soil Nitrification nitrifiers elucidating the potential for their exploitation as BNIs.

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PP038

Genomic analysis of Nontuberculous Mycobacteria (NTM) highlights the potential of reverse vaccinology in aquaculture

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Non-tuberculous mycobacteria (NTM) are a group of bacteria that are widely distributed in aquatic environments, including freshwater, seawater, and soil. Mycobacteriosis caused by NTM is a chronic and progressive disease that affects various fish species and can lead to significant economic losses in aquaculture operations. Several basic aspects of mycobacterial pathobiology in aquatic animals remain poorly understood, although several important recent developments have been made, especially with respect to identification of novel Mycobacterium spp. infecting fish. Currently there are no widely accepted treatments for fish mycobacteriosis. Therefore, prevention and control measures, such as good biosecurity practices are crucial for the management of NTM in fish populations. The development of vaccines for NTM has been challenging due to the diversity of NTM species, the lack of understanding of their pathogenic mechanisms, their extremely slow growth, and the complexity of their cell wall, which contains a unique lipid structure that is not found in other bacteria. In this study, we report the isolation and characterization of two NTM, from meagre and European sea bass, originating from aquaculture farms in Western Greece. Reverse vaccinology was deployed to explore the antigenic diversity of the newly isolated strains. In this way, selection of candidate genes was based on a series of different in silico predictions, using publicly available or newly sequenced genomic and proteomic data. These genes were assembled by cloning in the pcDNA3.1 eukaryotic expression vector and their immunogenicity effects were estimated in zebrafish upon vaccination. Genes associated with MHC antigen processing were analyzed by qPCR and the results revealed activated MHC-I and MHC-II pathways even during the early stage of vaccine immunization. The strongest gene target candidates will be prioritized in future efforts to prevent Mycobacteriosis outbreaks.

PP039

Assessment of population viability in compost enriched with nitrogen-fixing bacteria stored at ambient and low temperature

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Enrichment of organic compost with beneficial microorganisms, such as nitrogen-fixing bacteria, leads to improvement of its agricultural and environmental effects. However, prolonged periods of storage may impact the viability of the microbial inoculants and limit their effectiveness in the field, especially in the case of non-sporing bacteria. In the current study, the establishment and viability of a nitrogen-fixing bacterial consortium, consisting of Azotobacter vinelandii, Pseudomonas stutzeri, and two strains of Azospirillum brasilense were evaluated in a compost produced from spent mushroom substrates and cotton gin residues, stored at two different temperatures. After ensuring the non-competitive suppression among the selected strains, the compost was enriched by spraying 100 ml of the bacterial consortium per kg of the compost and was following stored at either ambient (25 ^{0}C) or low temperature (4 ^{0}C) for 184 days. The nitrogen-fixing bacterial population in the enriched and the control (non-enriched) series was enumerated periodically using the Most Probable Number method. At the initiation of storage, the nitrogen-fixing population in the enriched compost was equal to 3.08×10^5 cfu/g and was significantly higher compared to that in the control series (equal to 33 cfu/g), indicating the successful establishment of the bacterial consortium after the enrichment procedure. During the next 10 days of storage, the number of viable nitrogen-fixing cells increased to 4.49×10^5 cfu/g at 25^{0}C, while at 4^{0}C declined to 3.5×10^4 cfu/g. Between day 10 and day 35 the population at 25 ^{0}C gradually decreased to 1×10^2 cfu/g and then remained almost steady. By day 35, the nitrogen-fixing population of the enriched compost at 4 ^{0}C had reached a plateau of $1.1\text{x}10^4$ cfu/g which remained stable during the next two months. After day 101 the bacterial population suffered a further decrease to all the series tested. It could be therefore concluded that an enriched compost could be kept under ambient conditions for a short storage, but in the case of a long-term future use, storage under refrigerated conditions could serve to maintaining the nitrogen-fixing population up to satisfactory levels for at least a three-months storage period.

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PP040

COMBINED APPLICATION OF COMPOST AND PLANT GROWTH PROMOTING BACTERIA IMPROVES GROWTH AND NUTRIENT UPTAKE BY LETTUCE PLANTS GROWN UNDER THREE DIFFERENT PHOSPHORUS SOURCES

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Phosphorus (P), is a major plant nutrient, often shown to be limiting agricultural production, and soil ecosystem functioning more than nitrogen. In alkaline, calcareous soils, typical in Greece, phosphorus is bound to calcium, forming insoluble Ca-phosphates, therefore substantial P inputs are required to secure crop growth and high yields. However, sustainable management of agroecosystems, demands reductions in the use of fertilizers without compromising productivity. In this context, modern farming systems aim at the rational use of natural resources and the application of soil improvement products such as composts, as well as of beneficial microorganisms (microbial biostimulants) that promote plant growth and facilitate nutrition. In this work, the effects of the application of a compost product and of beneficial microorganisms on plant growth, nutrient uptake, and P availability in the rhizosphere were investigated in lettuce plants, grown in pots under greenhouse conditions and supplied with different P sources. Pots were filled either with a sandy slightly alkaline soil alone, or with a soil-compost mixture in a 10:1 ratio. P was applied at a rate of 200 mg in three different for: triple superphosphate fertilizer-TSP (inorganic-P, highly soluble and plant available), rock phosphate-RP (inorganic-P, highly insoluble and unavailable) and phytate (organic-P, storage form of P in many plant tissues). After transplantation, plants were inoculated with two different bacterial consortia of in vitro tested Plant Growth Promoting P-solubilizers (BAC-I and BAC-II). Our results demonstrated that compost addition improved plant growth, nutrient uptake, plant physiology and P-availability (Olsen-P) in the rhizosphere under all three different P sources. More importantly, although P availability was low in the soil with RP, leading to growth arrest, this was offset by the compost addition. The BAC-I inoculum further increased the above-ground lettuce biomass in soil with TSP and phytate, while BAC-II showed a similar increase in soil+compost. This differentiation was probably due to the different needs of the two consortia for carbon sources, highlighting the importance of microbial inoculum compatibility with soil organic amendments, however the relevant mechanisms of action need to be specifically investigated.

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PP041

Tripartite association between legumes, nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi: deciphering the microbial interactions

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Plants establish symbiotic relationships with soil bacteria or fungi, which colonize the plant root and provide the plant with inorganic nutrients, in exchange for photosynthetic products. In nature, simultaneous microbial interactions usually take place on the same host. Legume plants establish symbioses with both nitrogen-fixing rhizobia and arbuscular mycorrhizal fungi (AMF), forming a tripartite association. We examine how AMF and rhizobia interact during the tripartite symbiosis by performing co-inoculations. Using the well-studied model legume Lotus japonicus and performing inoculations with its microsymbiont Mesorhizobium loti and AMF, we observed that rhizobia and AMF do affect one another during the colonization of the same host root. We identified that nodulation is positively affected by AMF, whereas the impact of rhizobia on mycorrhizal root colonization is AMF strain dependent [1]. To determine the symbiotic stages that rhizobium and AMF interact, we performed single and double inoculations at different time points, testing different co-inoculation strategies, and monitored the symbiotic phenotypes. Moreover, we examined the expression of specific genes in single and double inoculated plants to identify molecular components that influence the symbiotic outcome. The present study ai to enhance our understanding on how host plants support symbiosis with multiple microbial partners in order to optimize the application of microbial biofertilizers to crops.

[1] Tsikou D, Nikolaou CN, Tsiknia M, Papadopoulou KK, Ehaliotis C. Interplay between rhizobial nodulation and arbuscular mycorrhizal fungal colonization in Lotus japonicus roots. J Appl Microbiol. 2023. 134(1):lxac010

PP042

Comparison of secondary metabolite biosynthetic clusters between pathogenic and non-pathogenic fungi: The case of the endophytic fungus Fusarium solani strain K.

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Secondary metabolite biosynthetic clusters play a key role in plant-fungal interactions as they can synthesize molecules with a variety of functions, such as increasing virulence and toxicity of the fungus on the plant or by producing compounds that can act as modulators of growth and by altering the plant's response to biotic and abiotic stresses. A fungus whose predicted secondary metabolite biosynthetic clusters are worth exploring is Fusarium solani strain K (FsK), which acts protectively against root and foliar pathogens, spider mites and zoophytophagous predators in tomato as well as alleviating the response of the plant to ought. FsK is the only member of the Fusarium solani species complex that is being described as a non-pathogen. To uncover the biosynthetic clusters that may be putatively responsible for the protective role of the fungus, a comparison with symbiotic Basidiomycete and Glomeromycetes Serendipita indica and Rhizophagus irregularis as well as with the closely-related pathogenic Fusarium vanettenii 77-13-4 was conducted by comparative genomics approaches. Using hybrid next generation sequencing methods as well as bioinformatic tools, we genome annotated FsK and we uncovered FsK-specific clusters with putative roles in virulence and plant immunity, suggesting for a novel plant colonization mechanism.

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H.F.R.I. Hellenic Foundation for Research & Innovation

PP043

Characterization of Colletotrichum spp. from olive for growth under different temperatures and virulence on flowers

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Anthracnose disease on olive is endemic and has been reported from the 1960's in the region of Preveza, situated in the north west part of Greece. After a se-rious outbreak in 2009, the disease causes every year serious losses with the olivegrowers struggling to maintain their olive production. Nevertheless, there is limited information on epidemiological aspects and virulence of Colletotrichum species in the region in order to establish effective disease management. In the present study, five Colletotrichum spp. strains from different regions of Greece were evaluated for their ability to grow under different temperatures and to infect olive flowers of the Kalamon and the Koroneiki variety. The three strains were isolated from the north-west region of Greece (Mirsini, Preveza), one of them belonging to C. godetiae based on preliminary sequence analysis of the ITS (internal transcribed spacer region). These strains were compared to a C. nymphae isolate from the southwest part of Greece (Messinia, Peloponese) and the C. acutatum reference strain (island Zakynthos, west central). Results show that strains from the northwest part of Greece where more capable to grow on lower temperature (15°C), compared to the intermediately growing C. nymphae isolate and the C. acutatum reference strain, which showed the slowest growth. The same strains from the northern part of Greece did not grow on high temperatures (30°C). Interestingly, the C. nymphaea isolate exhibited restricted growth, whereas C. acutatum was able to grow on high temperature (30°C). These results indicate inferior aggressiveness of Colletotrichum strains from the northwest part of Greece on flower infection; moreover these strains show adaptation to lower and sensitivity to higher temperatures compared to the C. acutatum reference strain. Correlation of these results with the population structure of Colletotrichum spp. in the region, will give an insight to population dynamics and epidemiology in order to develop effective disease management protocols.

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PP044

Towards the development of a novel biostimulant for use in sustainable cultivation of horticultural crops

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Fusarium solani strain K (FsK) is a beneficial endophytic fungus isolated from tomato plants, capable of conferring plant resistance against pathogenic fungi of both roots and leaves as well as enhancing nutrient acquisition and plant tolerance to reduced water availability. Therefore, FsK could be utilized as a microbial inoculum (bio-stimulant) in agriculture. The main challenge in its use for agricultural purposes, lies with the long-term preservation and transportation of the fungal structures, which we aimed to overcome by encapsulating the FsK mycelium within stable structures, in the form of biopolymer beads. The encapsulation successfully protected FsK during ying, while still enabling mycelial growth in different soils and cultivation media in vitro.

Furthermore, we comparatively studied the capability of the encapsulated FsK and the conventional conidial suspension inoculum in promoting endophytism in tomato plants. The endophytism of FsK was verified though a qPCR analysis , while the plant tolerance to ought stress was confirmed by measuring the relative water content of the plants. At the same time, the capability of the encapsulated FsK in promoting plant performance was also tested in the field.

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PP045

DEVELOPMENT OF MULTIPLEX SSR APPROACHES AND HRM MARKERS FOR THE ACCURATE GENOTYPING OF TAPHRINA DEFORMANS POPULATIONS IN GREEK PEACH ORCHARDS

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The pathogen Taphrina deformans is the causative agent of leaf curl disease of peach. This important fungal disease has been particularly pronounced in recent years in Greece, causing extensive infections characterized by severe distortion and hyperplasia of host leaves. In order to investigate the genetic differentiation and diversity of pathogen populations in Greek peach orchards, accurate SSR (microsatellite) genotyping approaches coupled with high-resolution melting (HRM) analyses were employed. Leaf samples were collected from four main peach producing areas and fungal strains were isolated using the "spore-fall" method. Sequencing with species specific primers led to the molecular identification of the strains as T. deformans. Genomic DNA was extracted from isolated fungal strains and underwent a large scale SSR genotyping to assess the genetic polymorphism among the isolates. Six SSR primers pairs revealed a higher polymorphism in multiplex PCR assays. In parallel, two specific polymorphic markers with distinct HRM melting profiles were identified allowing the efficient genotypic differentiation among the isolates. The results showed a higher genetic polymorphism between strains from different Greek peach producing areas than within each area. This study concluded in the importance of powerful SSR and HRM markers towards the robust population genotyping of T. deformans in Greece.

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PP045a

Plant growth promoting rhizobacteria produce volatiles with potential activity against Verticillium dahliae

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Plant pathogens causing vascular wilts are responsible for highly destructive diseases of crop plants and significant yield losses. Among them, the soil-borne fungal pathogen Verticillium dahliae which infects a wide range of hosts worldwide, cannot be effectively controlled with current agricultural practices because of its ability to form microsclerotia and its resistance to fungicides. In the absence of effective chemical means, biocontrol of the pathogen by antagonistic microorganisms is a promising and sustainable alternative. Under this scope, the present study aimed to evaluate plant growth rhizobacteria (PGPR) as potential biological agents against V. dahliae, through their ability to synthesize antifungal volatile organic compounds (VOCs). As a first step, antifungal activity of PGP strains due to their VOCs was evaluated in laboratory bioassays. Among twenty-five tested bacterial strains, eighteen were found antagonistic against V. dahliae and this antagonism was attributed to their VOCs. As a second step, Gas chromatography-mass spectrometry analysis of bacterial headspace bouquets was conducted for three bacterial strains that were proven able to produce astic VOCs, when co-inoculated with the fungal pathogen. The bacterial volatiles with antifungal activity were identified as dimethyl trisulfide, 3-methylbutanoic acid, 2-nonanone, and 2-undecanone. PGP strains were further evaluated as antifungal agents against V. dahliae in a pot experiment, while their impact on agronomic characteristics of the host plant Capsicum annuum was also assessed. The in vivo trials showed that pepper plants treated with the Chryseobacterium strain Rs24 significantly reduced disease symptoms caused by V. dahliae compared to the infected control plants. Moreover, plant growth and photosynthesis of host plants were enhanced in response to bacterial treatment in the absence of the pathogen. Further experimentation will elucidate the contribution of bacterial VOCs activity in the pathosystem under study.

PP045b

Mycorrhiza-assisted phytoremediation potential of hemp: Preliminary results of a field experiment

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The last decade contaminated lands have received wide scientific and policy attention as they could become a soil resource that can actually create value. This can be achieved by applying the technology of phytoremediation, which is the use of plants and associated microorganisms to remove, contain, inactivate, or degrade harmful environmental contaminants. Arbuscular mycorrhizal fungi (AMF) form a symbiotic partnership with plant roots, facilitating nutrient absorption, fortifying plant resilience, and expediting the remediation process. Phytoremediation becomes more efficient and profitable when the plants used are high yielding non-food cash crops, and a promising candidate is industrial hemp (Cannabis sativa L.).

The aim of this work was to evaluate -under real field conditions- the impact of mycorrhizal fungi on the growth and phytoremediation ability of industrial hemp (var. Futura 75). The experiment was conducted in a mining and metallurgical site in Attica, Greece, in which the soil is contaminated with multiple heavy metals and metalloids (HM&M), including Cd at 25.0 mg/kg, Pb at 10797.7 mg/kg, Zn at 4958.5 mg/kg, Ni at 172.1 mg/kg, Cu at 138.0 mg/kg and Sb at 92.0 mg/kg. The research involved measuring AMF root colonization after staining with trypan blue. At harvest plant height, shoot diameter, dry biomass, and the HM&M concentrations (ICP-OES) in the aerial biomass were measured.

Preliminary results indicate that mycorrhiza symbiosis benefits the plant growth; the treated with AMF plots gave significantly taller plants and larger shoot diameters when compared with the control plots (no addition of AMF). The mean values for height and shoot diameter in treated plots were 120.18 cm and 7.04 mm, respectively, compared to 108.38 cm and 5.75 mm in untreated plots. Additionally, there was a significant difference in dry biomass yields, with treated plants yielding significantly higher results at 1.35 tn/ha compared to the 0.97 tn/ha measured in untreated plants. ICP analysis demonstrated that treated plants showed an increase tendency to absorb Zn, Pb, Cu, and Sb, except for Ni and Cd. Moreover, they exhibited improved ability to accumulate these elements in their above-ground biomass, except for Ni.

In summary, this research shows that mycorrhizal fungi can improve the phytoremediation performance of industrial hemp grown on HM&M polluted soils.

Keywords: In situ phytoremediation; arbuscular mycorrhizal fungi; phytomanagement, Cannabis sativa, biomass

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PP045c

Plant-mediated effects of beneficial soil microbes on herbivore populations in the greenhouse

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Plants have evolved sophisticated mechanisms to defend themselves against their enemies. Besides the latter, they also interact with beneficial organisms such as soil microbes and zoophytophagous predators which are known to prime plants against future attacks via plant defense elicitation. In this work, we assessed the plant-mediated effects of two beneficial soil microbes, namely *Trichoderma harzianum* T22 and *Bacillus amyloliquefaciens* QST713, shown previously to negatively affect herbivore performance in the lab, on the population dynamics of the two spotted spider mite *Tetranychus urticae* and the whitefly *Trialeurodes vaporariorum* with greenhouse experiments. Our results show that inoculating tomato plants with microbes can result in decreased herbivore performance in the greenhouse. Furthermore, biological control with the release of mirid predators was not affected by microbial inoculation of the plants. Overall, our study highlights the added value of beneficial soil microbes in pest control as well as their compatibility with natural enemies.

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PP046

Combining culture-dependent and -independent approaches for identifying plastic-degrading bacteria and enzymes in soil samples

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Plastic waste accumulation is a global issue that negatively impacts natural habitats, living organis, as well as the economy. Biotechnologies offer promising solutions to address plastic pollution, including the discovery of plastic-degrading degrading bacteria and enzymes. Different approaches exist to identify plastic-degrading bacteria and related enzymes, including culture-dependent (e.g. enrichment of microbial communities) and -independent (e.g. microbial community analysis) methods. In this study, we used both approaches with the aim to expand the toolbox needed to address plastic pollution. We have applied and combined both culture-dependent and -independent methods to identify plastic-degrading bacteria and enzymes. A plastic-polluted soil sample originating from a plastic-polluted area in Serbia was enriched using virgin plastic (LLDPE) or a mix of plastics, representative of the composition of consumer waste, as a sole carbon source. We analysed the composition of the enriched microbial community using 16S metagenomic sequencing to identify key genera potentially involved in plastic degradation. During the enrichment experiment, we have also isolated 54 strains with the aim of matching genera identified via our community analysis. Then, three relevant strains were selected to fully sequence their genome using nanopore sequencing. The annotated genomes were analysed, with a focus on enzymes with a potential for plastic degradation. Some of the relevant proteins identified through this analysis were then expressed in E. coli.

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PP047

Composition of prokaryotic and eukaryotic microbial communities across the water column of the Eastern Mediterranean Sea.

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The waters of the Eastern Mediterranean Sea (E) present numerous, complex oceanic processes and are stratified into three distinct layers based on thermohaline circulation. The upper surface (0-150m) consists of Atlantic Water (AW) entering from the Straits of Gibraltar, the Levantine Intermediate Water (LIW) accumulates in water depths from 150-400m moving westwards and the Eastern Mediterranean Deep Water (EMDW) occupies depths beyond 400m. The oceanographic profile of this semi-enclosed basin in combination with its unique properties (high salinity, elevated bottom-sea temperatures, phosphorus limitation) drives the selection of distinct microbial taxa and determines the structure of microbial communities. To our knowledge, microbial community studies across the E water column have been performed near the coasts of Israel and the Nile Delta River while in areas south of Crete (Northeastern Mediterranean), molecular studies focused only on eukaryotic communities or on particular strains. For this study, two sampling cruises were performed off South Crete, near Koufonisi (August 2019) and Gavdos (February 2020) during which seawater was retrieved from several depths across the three-water layer system. In total, fourteen (14) samples were dispatched for DNA metabarcoding analysis of prokaryotic taxa and nine (9) for eukaryotic. In addition, CTD data were recorded during the sampling cruises and nutrient analysis was also performed to address which environmental parameters impact the microbial community composition. Finally, correlation network analysis indicated positive and negative interactions between the two life domains of the surface (AW) and deep (EMDW) water layers. Overall, this work ai to enrich our insight regarding the composition and interactions of background prokaryotic and eukaryotic communities in the E water column

PP048

PRELIMINARY DATA ON THE DIVERSITY OF THE GENUS AGARICUS SECTION MINORES IN GREECE

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The genus Agaricus is a large and cosmopolitan genus of basidiomycetes comprising approximately 500 saprotrophic species; 111 taxa assigned in five subgenera and 14 sections are reported to occur in Europe (Cappelli & Parra, 2022). In particular as regards A. sect. Minores (i.e., one of the largest sections in Agaricus), it includes more than 80 species (Chen et al., 2017), the majority of which are found in the tropics while 23 have been recorded in Europe. To date, ca. 40 Agaricus species are reported in Greece; despite their significant economic importance (commercial cultivation, nutritional and pharmaceutical value), no systematic investigation of the genus diversity has been performed so far. Seventy-one specimens of A. sect.

Minores deriving from dried samples (ACAM; Laboratory of General and Agricultural Microbiology, Agricultural University of Athens) and from field collections were studied by examining both the macro- and micro-morphoanatomical characters of basidiomes, and by performing phylogenetic analysis of sequences corresponding to the internal transcribed spacer (ITS) region of nuclear rDNA. Basidiomes of A. sect. Minores are generally delicate, with a characteristic bitter almond or anise-like smell and a more or less yellow discoloration of their pileal surface and stipe base after bruising. Results revealed the existence of 11 species, i.e., A. aridicola, A. brunneolus, A. comtulus, A. dulcidulus, A. iesu-et-marthae, and A. pseudolutosus, as well as A. gemlii, A. heinamannianus, A. jacobi, A. kerriganii and A. marisae; the last five are recorded for the first time in Greece. In addition, some specimens are likely to represent new species for science, but further investigation with additional molecular markers is needed for a definitive conclusion.

PP049

Optimizing Bacterial Cellulose Production using Post-Consumed TPA as a Non-Nutritional Adjunct in Twin-Bioreactor System

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Bacterial cellulose (BC) is the purest form of cellulose available and the most area-efficient one since it is lignin, pectin and hemicellulose are free and require less area to grow[1]. These and many other mechanical and biocompatible properties make it a promising material in the quest for a petroleum-based and non-degradable plastics-free circular economy substitutes. However, its drawback related to the high production cost and mechanical efficiency is a key factor which direct the effortces for improvements of the BC proprities that could alowing its viablility as a sustainable alternative. In recent years, studies have focused on capturing a higher diversity of cellulose-producing bacterial strains, optimizing production media by using inexpensive nutrient sources, or improving the production and mechanical properties of BC by adding variety of supplements[1,2].

In this study, we aimed to optimize the production of BC by utilizing post-consumed terephthalic acid (pcTPA), resulting from polyethylene terephthalate (PET) depolymerization, as a non-nutritional adjunct in a twin-bioreactor system under different agitation conditions. BC was synthesised by Komagataeibacter medellinensis ID13488 in modified Hestrin–Schramm medium (HS) (glucose 2 g/L, peptone 0.5 g/L, yeast extract 0.5 g/L, disodium phosphate 0.27 g/L and citric acid 0.15 g/L) modified with up to 50% substitution of glucose with pcTPA. Four batches (and their respective positive controls) were tested in both dynamic and static conditions. Our results showed a significant positive impact of pcTPA in BC production in all batches, compared with the control. Additionally, derived materials' mechanical strength and morphology were assessed.

Substitution of glucose with pcTPA up to 50% has led to the yields comparable to the positive control indicating that pcTPA has the potential to be a cost-effective and sustainable adjunct for BC production. Materials' mechanical properties were not affected by this substitution. TPA left in medium could possibly be further reused as a substrate for other bioproducts synthesis which will be examined in our future studies.

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PP050

MicroGLO: Effect based bioassays for antibiotic detection

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Ever since the discovery of penicillin in 1928, antibiotics have played an important role in modern medicine and have contributed to the eradication of several bacterial diseases in the western world. Nowadays there are a whole range of different antibiotics that exert different modes of action when.

Most antibiotics have one feature in common, however, which is that, over time, bacteria develop resistance against their effectiveness. Resistance to antibiotics often occurs when bacteria are faced with lower than optimal antibiotic concentrations such as during unfinished treatments but it can also develop in the environment if antibiotics are present.

Because of rising resistance against antibiotics, it is of vital importance that new antibiotics are being developed as well as that the influx of current antibiotics into the environment is closely monitored and minimized. We have developed a panel of effect-based bioassays, named MicroGLO, which use a combination of gene promoters that respond specifically to certain types of antibiotics coupled to a modified bacterial luciferase for high and sensitive antibiotic measurement.

This panel can be used for both screening for new antimicrobial compounds as well as determining antibiotics in environmental and food/feedstock samples. Five classes of antibiotics are covered by the panel: tetracyclines, macrolides, sulfonamides, beta-lacta and quinolones. The reporters are based on an improved bacterial luciferase reporter operon for fast and sensitive measurement.

Additionally, there is a general cytoxicity assay that can capture effects not measured by the five specific bioassays. For light measurement we use a modified bacterial luciferase operon.

Using bacterial luciferase operons as reporter genes gives two advantages over using firefly luciferase.

First, the bacterial luciferase operon produces its own luciferase substrate so no luciferin is needed for light measurements and second, measurements are non-destructive so studying time series of induction is easy and straightforward.

The different specific reporters respond with clear dose effect curves to their model compounds (used as positive control) with minimum crosstalk between the reporter bioassays

PP051

Exploring the enzymatic ability of strains isolated from plastic-polluted environments for enhancing synthetic and natural biopolymers' biodegradation

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Background: Renowned oil-based polymers represent an environmental nuisance, due to their well-known permanency in nature, known to disrupt ecosystems. In response, bio-based polymers have emerged as an alternative, providing a sustainable blueprint for plastic circularity1. However, despite being classified as biodegradable, these polymers can still show resistance to biodegradation when not presented with optimal environmental conditions such as specific temperature and pH, amongst other key factors in their breakdown. Due to this, microbial degradation results in an interesting path to promote biodegradation of such polymers.2

Objective: This study aims to evaluate the ability of selected strains isolated from polluted environments to improve the rate of biopolymers' biodegradation such as Polyhydroxy butyrate (PHB), Polylactic acid (PLA), and Bacterial cellulose (BC) when exposed to strains with specific enzymatic tools to aid in its degradation.

Methods: Selected strains were previously isolated from plastic-polluted soil samples. Strains were transferred to Mineral Salt media (MSM) biopolymer-supplemented plates (7d, 30℃) (MSM (15 g/l Agar, 9 g/l Na2HPO4 x 12H2O, 1.5 g/l KH2PO4, 1 g/l NH4Cl, 0.2 g/l MgSO4 x 7H2O, 0.2 g/l CaCl2 x 2H2O, Fe(III)NH4-citrate 0.0012 g/l), (1-3%) biopolymers (PHB, BC, and PLA) growth and clear-zone method were used to determine strains' possible enzymatic activity. Selected strains were transferred to flasks containing PHB, PLA, and BC films, respectively, in MSM and incubated for 20 days at 30℃, 120 rpm. Samples were washed and dried and their level of degradation was assessed by FTIR, weight loss, and scanning electron microscopy (SEM). The ability of the best performer strain to degrade PHB was additionally assessed using Respirometer (Echo Instruments) to determine the biodegradability of said films under a controlled temperature (25°C) with a flow rate of 500ml/min.

Results: Significant weight loss was observed in samples exposed to strains, meaning biodegradability was achieved in an important percentage, proving their capability to degrade the proposed biopolymers, compared to samples lacking microbial presence.

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PP052

Municipal wastewater treatment: assessment of ecotoxicity and dispersion of bacterial antibiotic resistance

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Antimicrobial resistance is a long-term threat to public health. In the era of multi-drug resistance, humanity faces the risk of being unable to treat infections caused by common bacteria. Municipal wastewater treatment plants (WWTPs), as recipients of human waste, are ideal environments for the development and spread of antibiotic resistance. In this context, the present study examines the microbiological quality of urban wastewater (influent and effluent) derived from the WWTP of Platanias (Chania-GR).

Sampling took place from October 2021 to May 2022 and samples were taken from: a)influent, b)secondary treatment and c)effluent (after chlorination). The aim was to test the efficiency of WWTP, regarding the removal of important bacterial species, namely, Escherichia coli, Enterococcus sp., Klebsiella pneumoniae and Staphylococcus aureus and then to test the resistance of the residual strains to specific antibiotics (Amoxicillin, Ciprofloxacin and Sulfamethoxazole). The method applied was the estimation of the minimum inhibitory concentration (MIC60), which represents the concentration of the antibiotic that inhibits the growth of 60% of the bacterial population. Most of the bacterial strains, isolated from samples before and after wastewater treatment were resistant to Amoxicillin, while the least resistant strains were related to Ciprofloxacin. In the case of Sulfamethoxazole, strains after treatment were more susceptible. Between inlet and effluent, no accurate conclusion can be drawn, as there were cases where the treated strains showed greater resistance after wastewater decontamination.

Further tests included the detection and quantification of the genes sulII and ampC in wastewater (influent and effluent). The results showed a reduction of both genes in the effluents, namely, 2.2 and 2Logs for sulII and ampC, respectively.

The ecotoxicity of the samples was tested using Artemia nauplii and Vibrio fischeri as biomarkers. The mortality ranged from 90 to 100% when influent samples were tested at concentrations of 37-80%v/v. Effluent samples showed lower toxicity levels, as the mortality of bioindicators reached approximately 20% of their initial population.

In conclusion, WWTPs are efficient reducing bacterial load and removing organic and other pollutants. However, they do not appear to successfully eliminate resistance genes, increasing the concerns about the spread of bacterial resistance during wastewater treatment.

PP053

Removal of antibiotic-resistant Escherichia coli and antibiotic resistance genes in wastewater through wastewater chlorination

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Natural water resources are threatened by climate change, population growth, urbanization, and industrialization. To alleviate the demand on freshwater sources, treated wastewater can be used for a variety of purposes such as irrigation and industrial use. However, wastewater treatment plants (WWTPs) are unable to remove antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) released from anthropogenic sources, leading to environmental and public health concerns.

There is the potential for ARGs to be transferred to soil microorganisms through horizontal gene transfer, potentially contributing to the spread of antibiotic resistance. Disinfection methods are an important barrier to the spread of ARB and ARGs in the environment. In this study we determined the removal of ampicillin, erythromycin, ciloxacin, and tetracycline-resistant Escherichia coli, as well as ARGs (blaTEM, qnrS, ermB) and class 1 integron (intI1) from secondary treated wastewater by chlorination with different chlorine doses (30, 35 and 50 $mgCl_2min/L$).

After 30 minutes of chlorination, a sodium thiosulfate solution was added to remove residual chlorine before enumeration of bacteria and ARGs. The membrane filtration method was used to count Escherichia coli.

For the enumeration of ARB, antibiotics were added to the selective media. Gene abundance was quantified by quantitative polymerase chain reaction (qPCR) using SYBR-Green chemistry.

At a dose of 50 $mgCl_2min/L$, ARB was completely removed, and the total amount of Escherichia coli was less than 10 CFU/100 mL.

However, the chlorine doses were not effective in removing ARGs, possibly due to the presence of residual DNA in the effluent after cell lysis of ARB. Consequently, higher chlorine doses are required for inactivation of ARGs.

PP054

SynComs in Microbial Ecotoxicology: Development and standardization of in vitro tests to assess the toxicity of pesticides on N microbial networks

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To date single-species tests are routinely used as a highly conservative step to determine pesticides toxicity on aquatic and terrestrial organisms. This has not been explored for soil microorganisms due to the lack of identified microbial indicators, and the lack of standardization of in vitro assays. Recent benchmarking research of our group pointed to ammonia-oxidizing microbes (AOM) as ideal microbial indicators of the effects of pesticides on the soil microbial community, and subsequently developed and used robust in vitro tests for the systematic assessment of pesticides toxicity on phylogenetically and ecophysiologically distinct soil AOM, as a proxy to the single-species tests used in other environmental compartments and organis. Here, we expand the evaluation of pesticides toxicity beyond assays with single nitrifiers strains towards more ecologically relevant in vitro tests. In this frame we aimed to develop, optimize, and use synthetic microbial communities (SynCo) of nitrifiers by co-culturing selected strains with distinct functions in nitrification [e.g., ammonia-oxidizing archaea (AOA) and/or bacteria (AOB), and nitrite-oxidizing bacteria (NOB)], as a more realistic assessment step of the toxicity of pesticides on soil ecosystem functioning. We focused on stabilizing two co-cultures: (i) Ca. Nitrosotalea sinensis (AOA) and Nitrobacter sp. NHB1 (NOB), and (ii) Nitrosospira multiformis (AOB) and Nitrobacter winogradskyi (NOB), occupying contrasting ecological niches (acidophilic vs. neutrophilic), respectively, in communal liquid nutrient media capable of supporting nitrifiers growth and function under stable and defined conditions. To identify optimal growth conditions for these co-cultures we monitored nitrite and nitrate production and/or consumption and the abundance of amoA and nxrB genes, under a range of conditions (NH_4+-N concentration, growth media composition, temperature) known to affect nitrification kinetics, both in axenic and mixed cultures. Based on our previous results at the single species level, the obtained SynCo were subsequently used to assess the toxicity of selected pesticides aiming to identify potential synergism or antagonism that minimize or maximize pesticide effects at the nitrifying microbial networks. Our findings are expected to provide benchmarking knowledge enabling the in vitro assessment of the toxicity of pesticides on natural assemblages of soil nitrifiers.

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PP055

EFFECT OF SYNTHETIC AND BIODEGRADABLE MICROPLASTICS. EITHER ALONE OR IN THE PRESENCE OF PESTICIDES OR VETERINARY DRUGS ON THE SOIL MICROBIOTA

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Plastic use in agriculture has tremendously increased in the past decades resulting in soil pollution with plastic residues forming microplastics. Still, the impact of microplastics on the soil microbiota, which constitute the growth engines of terrestrial ecosystems, remains elusive. In addition to microplastics, organic pollutants like pesticides and veterinary ugs, which are also widespread in agricultural soils, are expected to interact with plastics. This interaction can further affect the soil physical and chemical properties, and eventually soil microbiota. To determine the effects of microplastics either individually or in combination with other stressors on the soil microbial community, a microcosm experiment was performed. Three different types of microplastic including a synthetic plastic like LDPE (low-density polyethylene) and biodegradable plastics like PBAT-based (Polybutylene adipate terephthalate), and starch-based at dimension of < 250 μm were applied in the soil at two dose rates 0.01 and 0.1%. Two types of organic pollutants consisting of pyraclostrobin (fungicide) and albendazole (anthelmintic) were used as representatives of different organic pollutant sources in agricultural fields (direct or through manuring). In treatments without pesticide application, microplastic addition significantly increased the potential nitrification rate and soil nitrate concentration on day 30, while no effects were evident on day 90. In addition, the different doses of microplastic application (0.01% w/w and 0.1% w/w) had a minor effect on the potential nitrification rate and soil nitrate concentration. Compared with the treatment with microplastic addition but without organic pollutant application, the interaction between microplastic with either albendazole individually or a combination of both pyraclostrobin and albendazole significantly inhibited potential nitrification rate, and decreased soil nitrate concentration on day 30. However recovery from these effects were evident at day 90. Our study suggested that the presence of microplastic can boost soil microbial nitrification process, although this effect is temporary. Co-existence of microplastic and some organic pollutant (albendazole in our case) can pose a threat on the soil microbial nitrification process. On going measurements will further address this looking at potential effects on the abundance of nitrifiers and the overall soil microbial diversity using q-PCR and amplicon sequencing.

PP056

BIOCOMPATIBLE ECO-PLASTIC BLENDS WITH ENHANCED ANTIMICROBIAL ACTIVITY BY ADDITION OF ESSENTIAL OILS

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In recent years, biopolymers have emerged as a promising sustainable alternative to petroleum-based polymers. Among them, bacterial cellulose (BC), polylactic acid (PLA), and polyhydroxybutyrate (PHB) have gained significant attention due to their renewable, biodegradable, and unique properties. However, these materials still face some challenges, including mechanical properties and production costs [1]. In this study, we aimed to improve the mechanical properties of dry BC films by blending them with biopolymers such as PLA or PHB and adding essential oils as antimicrobial agents [2].

BC films were produced by Komagataeibacter medellinensis ID13488 in Hestrin-Schramm medium (HS) (glucose 2 g/L, peptone 0.5 g/L, yeast extract 0.5 g/L, disodium phosphate 0.27 g/L and citric acid 0.15 g/L), at 30°C under static conditions, for 14 days. The produced BC films were autoclaved and dried overnight at room temperature. The blends were prepared by dissolving 10 g or PHB or PLA in 150 ml of acetic acid, assisted by heating until complete dissolution was observed. The BC films were immersed in the solutions of PHB or PLA, and incubated overnight at 60°C. The films were then left to dry for 2 hours at room temperature and washed with double-distilled water. Finally, the blends were immersed in an ethanol solution containing 0.15 g of essential oil per mL of ethanol. The antimicrobial activity of the films was evaluated against Escherichia coli (ATCC 9001), Staphylococcus aureus (ATCC 25923) and Candida albicans (ATCC 10231). Furthermore, the biocompatibility of the blends was evaluated in vitro and in vivo, against MC3T3 osteoblasts and against Caenorhabditis elegans, respectively.

Our results show that the BC blends with the addition of essential oils exhibit excellent antimicrobial activity and biocompatibility, making them potential candidates for food packaging and biomedical applications. Further research can optimize these blends' properties and explore their potential in a circular economy.

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PP058

Biogas production using raw glycerol on a sunflower-seed-hulls-residues packed digester

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Raw glycerol has been extensively investigated for AD, where most of the studies focus on co-digesting raw glycerol with other substrates in order to boost the biogas production. Still, the generation of digestate in these bioprocesses cannot be avoided. Moreover, the local availability of co-substrates for a stable AD bioprocess is not always guaranteed and therefore it would be of interest to develop a process where glycerol would be used as the sole feedstock for in-situs biogas production without generating any digestates. Given that glycerol is an organic compound that it is completely degradable, it was hypothesized that glycerol will be completely bioconverted to biogas without generating any liquid effluents and that probable the withdrawal part of the digesting liquor would be necessary only when electrical conductivity would be increased in inhibitory levels. Moreover, the addition of a matrix where microbes could be attached, it was hypothesized that would result in a better overall bio-conversion performance. To this end, sunflower seed hulls (SSH) residues, which are an additional by-product from the whole plant-based biodiesel production-chain, were considered as a potential source of the packed material that could be used for attached growth AD. Thus, aim of this study was to test these hypotheses by conducting lab-scale experiments for optimizing the bioprocess and then to test the best conditions in a small-scale pilot unit.

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PP059

Utilization of Mortierella ramanniana lipid fermentation wastewater as maceration water for Pleurotus and Ganoderma cultivation

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The growing number of wastes (e.g., lignocellulosic residues, food-processing residues, biodiesel-derived glycerol) produced by modern agriculture and agricultural-based industries has a significant negative environmental impact. Their proper degradation or recycling by microbial valorization may be an alternate option to limit their impact by transforming them into high nutritional/ medicinal value products, such as mushrooms. The bioconversion effectiveness of Ganoderma resinaceum and Pleurotus ostreatus species during the colonization phase was investigated utilizing four agro-industrial residues (wheat straw-WS, beech wood shavings-BWS, coffee residues-CR, and olive crop residues-OCR). Additionally, lipid fermentation wastewater from Mortierella ramanniana (FW) containing 1.5 g/L wastewater of glycerol and salts was utilized as maceration water instead of tap water, as an additional mean of waste decrease. Solid-state fermentations were conducted using substrates of CR and OCR mixed with BWS for G. resinaceum and CR and OCR mixed with WS for P. ostreatus, wetted with FW and tap water (control substrates). Due to the significance of C/N ratio of the substrate in mushroom cultivation, two C/N ratios were examined, 15-20 and 35-40. After substrate addition in glass Petri dishes, sterilization and inoculation with mycelium agar-plug, incubation took place at 26 ± 0.5 °C. Preliminary evaluation included determination of mycelium growth rate (Kr, mm/d) and biomass production (estimated as glucosamine content). Results showed that the addition of FW had no significant effect on Kr, whereas C/N 15-20 ratio favored the quick growth of G. resinaceum and C/N 35-40 ratio favored that of P. ostreatus. In particular, G. resinaceum was the fastest colonizer with Kr values ranging from 17.52 to 22.21 mm/d. On the other hand, C/N ratio and FW seemed to positively affect the biomass production of G. resinaceum that presented higher values at C/N 15-20 ratio (323.75 mg/g d.w. - control substrate, 340.97 mg/g d.w. FW - substrate) compared to the C/N 35-40 ratio (206.29 mg/g d.w. - control substrate, 185.07 mg/g d.w. FW - substrate). Therefore, it appears that new substrates from various agro-industrial waste supported efficiently mycelial growth, with lipid fermentation wastewater being successfully used as maceration water in a zero-waste approach.

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PP060

Impact of different soil salinity levels on the concentration of the polysaccharide acemannan and Plant Associated Microbial Communities in Aloe vera (Aloe Barbadensis Miller) plants

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Aloe vera (Aloe Barbadensis Miller) is a xerophytic succulent plant which constitutes a major source of bioactive compounds. Beta-(1→4) acetylated glucomannan, also, known as acemannan, is one of the most important polysaccharides held inside the pulp of Aloe vera leaves, since it shows a wide range of pharmaceutical properties. Acemannan serves primarily for storage of water inside the leaf pulp, supporting the plant's tolerance to abiotic stresses related to water deficit. Soil salinization, apart from negatively impacting soil fertility, it also induces plant water stress. Furthermore, an increase in soil salinity not only influences the composition of plant-associated microbial communities, established in the rhizosphere and the endoroot habitat, but also affects soil microbial processes linked to nutrient biochemical cycles. In the present work, we examined the effects of increased levels of soil salinity (electrical conductivity 9.10 and 6.38 dS/m) on the concentration of acemannan, plant nutrient acquisition and plant-associated microbial communities in 3 years old Aloe vera plants grown in field in. The concentration of acemannan was significantly increased in the plants grown at higher salinity levels. In addition, rhizospheric and adjacent bulk soil samples were collected for analysis of soil properties and extracellular enzymes activities (EEAs). Microbial community profiles were determined with amplicon sequencing via Illumina MiSeq platform (2x300bp) in bulk and rhizospheric soil and also in Aloe vera roots, targeting 16S rRNA gene (for the prokaryotic community), ITS2 region (for the fungal community) and 18S rRNA gene (for the AMF community). We hypothesized that the degree of soil salinity influences the accumulation of acemannan in Aloe vera leaves and we aimed to examine links to changes in the examined microbial communities and processes. The results of the analysis of amplicon sequencing and of EEAs will be discussed at the conference.

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PP061

Exploring the diversity and antimicrobial potential of Streptomyces strains in Greek environments

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Streptomyces is a highly widespread bacterial genus found in diverse environments, known for its remarkable capacity to produce a wide range of valuable natural products with significant biological activities, impacting fields such as medicine, environmental science, food industries, and agronomy. According to recent studies, the varied geomorphological and climatic conditions in Greece have resulted in soil reservoirs that are abundant in multi-producer Streptomyces strains, which remain mostly unexplored. In this study, we aimed to investigate the abundance and diversity of streptomycetes in various habitats throughout Greece and assess whether there is a correlation between their antimicrobial activity and the characteristics of the sampling site.

We collected a total of 3102 Streptomyces strains from 55 different sites and have currently tested 1704 strains for their antimicrobial activity using a diffusion method against six indicator strains, including two Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), two Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), and two yeasts (Candida albicans, Saccharomyces cerevisiae). 16S rRNA gene sequences of the isolated strains were used to determine their phylogenetic relationships.

Our results show that the evergreen sclerophyllous plants rhizosphere is a habitant that harbors strains with interesting antimicrobial profiles. In particular, strains isolated from olive and carob trees, as well as Ebenus and Cistus shrubs exhibited high antimicrobial capacity, with over 50% of them inhibiting the growth of three or more indicator strains, regardless of the sampling site.

The isolates from sites contaminated with fertilizers and pesticides had a higher percentage of streptomycete populations with antimicrobial properties, with most of them effectively inhibiting the growth of three or more indicator microorganis.

The rhizospheres of both herbaceous and aromatic plants displayed consistent antimicrobial properties across all sampling sites.

The phylogenetic analysis revealed that there are clusters of Streptomyces isolates widely distributed across the examined habitats, while other clusters were present in fewer than three. We therefore observed that the antimicrobial capacity of our Streptomyces isolates was influenced by their microniches, plant-derived polyphenols, and by oil contamination. Overall, our study demonstrates the rich diversity of Streptomyces strains in Greek environments and their potential as a source of novel pharmaceuticals.

PP063

BACTERIAL AND BIOCHAR-ASSISTED PHYTOREMEDIATION OF MULTI-METAL CONTAMINATED SOIL USING CROTALARIA RETUSA

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In this study, different bioremediation strategies for multi-metal (Pb and Cd)-contaminated soils, including (a) phytoremediation (rattleweed (Crotalaria retusa)), and b) assisted phytoremediation (using a bacterial consortium, biochar or any of their combinations) were evaluated. The biochar was prepared via pyrolysis of rice husk – a locally available waste biomass from rice milling industries. The multi-metal tolerant bacterial consortium comprising Alcaligenes faecalis, Pseudomonas paralactis and Alcaligenes sp., was assembled using pure culture bacterial isolates growing in high metal concentrations (700 mg/L, ratio of 1:1, Cd: Pb). Bacterial counts, throughout the remediation process, varied in response to heavy metal stress. Contaminated soils amended with consortium supported the highest bacterial counts (5.85 x 106 CFU g-1), in contrast with their untreated counterparts which, at 3.15 x 106 CFU g-1, supported the least bacterial growth. C. retusa tolerated and grew in the presence of high concentrations of Pb (500 mg/kg) and Cd (600 mg/kg), in all treatments, which promoted plant growth and appeared to alleviate plant stress. Significantly ($p < 0.05$) more metal accumulation occurred in shoots than roots for both Pb and Cd. The highest Pb uptake for both shoot and root (76.25 and 34.43 µg plant-1, respectively) occurred when the treatment combining C. retusa and the bacterial consortium was used. Both Pb and Cd were effectively translocated by the plant (TF > 1). There was a statistically significant impact ($p < 0.05$) of the combination of biochar and consortium in the translocation of Cd (TF = 2.65). These findings demonstrate the biochar and bacterial consortium enhanced phytoextraction potentials of C. retusa for the removal Pb and Cd from multi-metal polluted environment

PP064

ESTABLISHMENT OF SYNTHETIC MICROBIAL COMMUNITIES ACROSS TROPHIC LEVELS TO ASSESS PESTICIDE SOIL MICROBIAL TOXICITY IN AN ECOSYSTEM RELEVANT IN VITRO SYSTEM

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Nitrification is a primary process within the global nitrogen (N) cycle that leads to significant fertilizer loss, atmospheric and groundwater pollution, and is principally driven by soil microorganisms. Ammonia oxidation, the first and rate-limiting step of nitrification, is mediated by ammonia-oxidising archaea and bacteria (AOA and AOB) that perform the initial oxidation of ammonia (NH3) to nitrite (NO2-), followed by subsequent oxidation to nitrate (NO3-) by nitrite-oxidising bacteria (NOB). In addition to the prokaryotic nitrifiers, there is evidence of NH3 excretion by unicellular eukaryotic microorganisms, protists, that prey upon bacteria and archaea. The current study aimed at developing synthetic microbial networks of different players in N cycling, including nitrifiers and protists, with the long-term prospect to use such synthetic communities (SynComs) for assessing the toxicity of pesticides towards an ecologically relevant in vitro testing. SynComs of nitrifiers were initiated and the growth of the different members was optimized under different growth conditions. Predator-prey system testing was introduced using the Tetrahymena ciliate as model predator grazing on different individual nitrifier microbes and their SynComs. Two SynComs were established and stabilized following several generations: (i) SynCom 1 composed of an acidophile AOA (Ca. Nitrosotalea sinensis) and a NOB (Nitrobacter sp. NHB1), and (ii) SynCom 2 composed of a neutrophile AOB (Nitrosospira multiformis) and a NOB (Nitrobacter winogradkyi). During our first experiments, the ciliate-nitrifier network demonstrated release of nitrogen as a result of the predatory action, potentially in concert with the higher C:N ratio of protists compared to their prey. It has long been evident that predation controls nutrient cycling and microbial population dynamics, therefore it is of critical importance to shed light into the dynamics of nitrifier-protist interplay as a mean to determine soil ecosystem functioning while considering complex interactions across trophic levels. Overall, the establishment of synthetic microbial communities constitutes an innovative and useful tool for the assessment of stressors' effects at N microbial networks. The insights provided into predator-prey interactions are anticipated to support an ecosystem-level assessment of induced stressors on microorganisms from different trophic levels within the soil food-web, and extend understanding of the consequences on soil ecosystem processes.

Acknowledgements
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PP065

Evaluation of the toxicity of three fungicides on Arbuscular Mycorrhizal Fungi (DAOM, Rhizophagus irregularis) using an in-situ monoxenic sandwich system

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Pesticides are major environmental pollutants. To protect human health and the environment, European Commission (EC) has prescribed a stringent regulatory scheme for pesticides (Regulation 1107/2009). The assessment of pesticide toxicity on soil microorganisms is based on assays that do not accurately determine their potency against microbes. Arbuscular Mycorrhizal Fungi (AMF), based on their key role in soil fertility and structure but also their sensitivity to pesticides, have been identified as key functional group that could be used as surrogates for assessing the toxicity of pesticides on the soil microbiota. However apart from the spore emergence test which determines effects on AMF in the absence of plant, no other test is available. Thus, novel experimental approaches for more realistic and robust pesticide toxicity assessment on AMF are necessary.

In the current work the AMF in situ sandwich system was used to evaluate the effect of three fungicides, hymexazol (DNA/RNA synthesis inhibitor), pyraclostrobin (Respiratory complex III inhibitor) and fludioxonil (Inhibitor of transport-associated phosphorylation of glucose) on AMF colonization and functionality. In this assay three plantlets of Lotus japonicus (gifu) and 300 spores of AMF DAOM (R. irregularis) inoculum were placed into a pair of nitrocellulose round filter paper. Plants were grown for five weeks in magenta boxes containing baked y sand and they were watered with Long-Ashton solution (SLA) amended or not amended with pesticides at five concentration levels To estimate if the pesticides have phytotoxic effect on plants at the different concentration levels, the y mass of belowground plant parts were determined and compared with the control. As a first screening we determined effects on percentage root colonization by R. irregularis.

None of the fungicides showed significant phytotoxicity at the concentrations tested. Hymexazol had no effect on AMF colonization. In contrast, pyraclostrobin and fludioxonil showed clear dose-response effect on colonization levels. Calculated EC50 values for hymexazol, pyraclostrobin and fludioxonil were >50ppm, 11,362ppm and 0,804ppm respectively. Follow up measurements will monitor the expression of genes LjSbtM1, LjPT4, LjAMT2.2 as indicators of the arbuscules functionality.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 03255).

H.F.R.I.
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PP066

In vitro assessment of the toxicity of anthelmintics on soil ammonia-oxidizing archaea and bacteria: Benchmarking the soil microbial toxicity of anthelminthic veterinary drugs

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Anthelmintics (AHs) are used to control infestations of ruminants by gastrointestinal nematodes. They are only partially metabolized in animals, ending in animal excreta whose use as manures leads to AHs dispersal in agricultural soils. Once in soil, AHs interact with the soil microbiota, with recent benchmarking research suggesting their strong inhibitory effect on the activity and dynamics of soil ammonia-oxidizing bacteria (AOB) and archaea (AOA). These findings build on previous evidence on the sensitivity of ammonia-oxidizing microorganisms (AOM) to other pollutants (e.g., pesticides), reinforcing their potential utilization as indicators of the effects of abiotic stressors on the soil microbial community in environmental risk assessment schemes. To further explore the ecotoxicological response of AOM to AHs, we are currently assaying the toxicity of some of the most widely used in veterinary medicine AHs, namely the bendimidazoles albendazole, ricobendazole, and fenbendazole, the macrocyclic lactones-avermectins ivermectin and eprinomectin, and the imidazothiazole levamisole on a range of phenotypically and ecologically distinct strains of AOB (e.g., Nitrosospira multiformis, Nitrosospira briensis, and Nitrosomonas ureae), and AOA (e.g., Ca. Nitrosotalea sinensis, Ca. Nitrosocosmicus franklandianus, and Nitrososphaera viennensis) representing globally distributed lineages found in soil. Toxicity is assessed at the functional level by measuring nitrite production in liquid cultures of AOM, amended with a broad range of AHs concentrations, and relevant toxicity endpoints (EC50s) are calculated. The stability of AHs during laboratory incubation is determined in parallel. Ongoing data suggest a higher sensitivity of the AOA Ca. N. sinensis compared to the AOB N. multiformis to selected AHs (ricobendazole, ivermectin and eprinomectin), with the activity of the AOB isolate being affected only by eprinomectin. Our findings are expected to benchmark the ecotoxicological response of the different microbial moderators of the ammonia oxidation process to

PP067

Biodiversity study of rice commercial samples with RAPDS

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Rice biodiversity is important in rice germplasm maintenance with high environmental impact as large semi aquatic areas are used for rice cultivation. Four commercial rice samples were analyzed for genetic similarity based on genotyping techniques using RAPD markers with Real time PCR. From the tested RAPDs, the ones with the highest discriminant power were selected after electrophoresis of their PCR products and data were evaluated algorithmically using bioinformatics analysis. Dendrograms were constructed using the UPGMA and WPGMA algorithms. Samples formed two distinct groups, with two samples per group having high level of similarity. It is also observed that, all samples except one did not show internal variation between replications, indicative of homogenous populations. The proposed methodology can be applicable in biodiversity monitoring.

PP068

Antimicrobial and antibiofilm activities of three Pseudoalteromonas strains against fish pathogen Tenacibaculum discolor strain B487

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Tenacibaculosis is one of the major bacterial diseases occurring in aquaculture with Tenacibaculum discolor often being characterized as the causative agent. Instead of the use of antibiotics, biological alternatives could be investigated. Marine bacteria of the Pseudoalteromonas species are known to exhibit anti-bacterial activities by producing antimicrobial metabolites. In this study, the antimicrobial and antibiofilm activities of three Pseudoalteromonas strains were assessed against T. discolor.

T. discolor strain B487 was isolated in a marine recirculated aquaculture system (RAS) and identified by whole genome sequencing. Three Pseudoalteromonas strains were selected: Pseudoalteromonas sp. strain GY795 from the collection of IFREMER Institute, Pseudoalteromonas spongiae isolated from a marine RAS and Pseudoalteromonas tetraodonis isolated from brown algae. The dynamics of dual species biofilm were evaluated. Empty petri dishes were inoculated with combinations of each Pseudoalteromonas strain with T. discolor and allowed to attach for 3 hours. Planktic cells were removed and adhered cells were left to form a biofilm for 48h. Sessile cells were sampled and enumerated immediately after 3 hours, at 24 and 48 hours. Single species controls were also performed. Experiments were executed thrice with 3 technical replicates. The supernatants (SNs) of the Pseudoalteromonas strains cultures were obtained and their antimicrobial and antibiofilm activity against T. discolor was evaluated in microplates, where the strain had previously formed a biofilm (OD600 and Crystal Violet assay). All assays were performed thrice with 16 technical replicates to validate results.

In the dual species biofil, all strains were attached at equal levels, but all of Pseudoalteromonas strains sessile population exceeded those of T. discolor by 2 log cfu/cm2 after 24 hours. OD 600 of T. discolor was significantly decreased when the SN of P. spongiae was added, but remained at similar levels to the control in all other treatments. However, CV assays showed that adding the SNs of all three Pseudoalteromonas strains enhanced the biofilm of T. discolor, implying that biofilm extracellular matrix was increased.

This study was carried out under the frame of IMPAQT project, funded by the EU H2020 research and innovation programme under Grant Agreement No 774109.

PP069

DIMITRA: an upcoming database on effects of pesticides on the soil microbiome and meta-analytic analysis

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Pesticides are a cornerstone of modern agriculture. Their effects on soil microorganisms, a group responsible for maintaining soil health through regulation of nutrient cycling, structure and diversity of flora and fauna, has attracted considerable attention, reflected through increasing numbers of publications on this topic. However, this wealth of knowledge remains fragmented and should be assessed in a systematic way, allowing the development of tools used for defining new target organisms in the soil microbiome. Through the project DIMITRA funded by SYNGENTA, the foundation of a novel database has been set using data gathered and consolidated from global studies to date, studying the effects of pesticides on key functional microbial groups, such as arbuscular mycorrhizal fungi, distinct soil microbial functional endpoints relative to plant nutrient cycling (urease, acid & alkaline phosphatase and arylsulfatase), organic matter decomposition (dehyogenase and β-glucosidase) and key soil functional gene markers associated with N cycling. A meta-analysis, based on observations (n) made from relevant studies (K), identified significant reductions in total enzyme response in relation to total pesticide applications ($K = 35$, $n = 565$, $p = 0.03$), weighted towards fungicide application ($K = 8$, $n = 63$, $p = 0.034$) and urease and arylsulfatase activities ($p = 0.03$ & $p = 0.01$, respectively). Ammonia oxidizing bacteria (AOB) and archaea (AOA) gene abundances decreased significantly for pesticides application ($K = 10$, $n = 222$, $p = 0.002$), and pesticide groups (fungicide, herbicide and insecticide) ($K = 3$, $n = 48$, $p = < 0.001$; $K = 5$, $n = 94$, $p = 0.03$; $K = 5$, $n = 80$, $p = 0.02$, respectively). Individual assessment revealed significant inhibition of AOA by fungicides and insecticides ($p = 0.01$ & $p = 0.04$, respectively) and AOB by herbicides ($p = 0.004$). DIMITRA identified key microbial indicators, which display higher sensitivity to pesticides. Such indicators can be used in our quest for a robust pesticide risk assessment for soil microorganisms. In future, this database will provide access to curated amplicon sequencing datasets for bacteria, fungi and functional microbial groups (ammonia-oxidizing microorganisms, arbuscular mycorrhizal fungi and protists) used for multi-purpose meta-analysis.

PP070

MICROBIAL POPULATIONS ON THE SURFACE OF A MARBLE MONUMENT IN ATHENS, GREECE

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Microorganisms can inhabit the surfaces of stone and marble resulting in aesthetic and structural damage on monuments, contributing thus to its decay. The diversity of the microbial communities, including fungi and bacteria, on the surface of a marble monumental sculpture in the First Cemetery of Athens was examined to identify taxa which could have an effect on it. The aim was to detect the microorganisms of the different types of biodeterioration on the monument and to identify the native taxa that may cause biodeterioration of the surface or may act beneficially through facilitation of calcium carbonate precipitation. Samples were collected from different types of decay on the sculpture's surface using sterile cotton swabs. The active microbiota were grown to four different agar media. Following counts of morphologically similar colonies in the same medium, clones with relative abundance of CFUs > 1 % of the total number were selected for further molecular analysis. Each selected clone was subjected to PCR using primers targeting either the bacterial 16S ribosomal RNA gene, or the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA, and the PCR products were sequenced with Sanger technology. Overall, eleven fungi genera were identified belonging to Cladosporium, Knufia, Radulidium, Coniosporum, Talaromyces, Neodidymelliopsis, Pleosporales, Aerobasidium, Fusarium, Aspergillus and Penicillium. These genera include potentially hazardous members (Cladosporium, Knufia, Coniosporium, Aerobasidium, Fusarium, Aspergillus) but also some known airborne opportunistic species or related to plants (Radulidium, Talaromyces, Neodidymelliopsis, Pleosporales), which could be incidentally retrieved, while some genera may include both hazardous and beneficial members (e.g. Penicillium). Furthermore, seven genera of bacteria were identified belonging to Arthrobacter, Micrococcus, Actinotalea, Bacillus, Rhodococcus, Rathayibacter and Kocuria. These genera include potentially beneficial members (Bacillus, Micrococcus, Arthrobacter) through microbial calcium carbonate precipitation, but also potentially hazardous members (Rhodococcus) or genera that colonize both healthy and damaged stone surfaces (Actinotalea, Rathayibacter, Kocuria). Further examination is in progress to connect the types of decay with the presence and prevalence of specific taxa towards the development of bioremediation strategies.

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PP071

Protocatechuate 4,5-dioxygenase directed evolution: sometimes small changes are all that it takes

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Polycyclic Aromatic Hyocarbons (PAHs) belong to a class of organic priority pollutants with detrimental impact on environmental and public health. Removing PAHs from the contaminated areas is critical for repairing the ecological damage and one of the most effective ways to do so, is the process of biodegradation, which is the use of microbial cells with the ability to catabolize PAHs to simpler compounds. Despite their heterogenicity, the aerobic degradation of PAHs proceeds through a handful of diol intermediates such as protocatechuic (PCA) and gentisic acid and catechol that are further funneled in TCA cycle. The first step of this conversion is the cleavage of the aromatic ring cleavage by enzymes called dioxygenases (Shahsavari et al., 2019).

In the present study 4,5-dioxygenase of PCA (PCD45), an enzyme with narrow substrate specificity (Tsagogiannis et al., 2021), underwent point mutations of selected amino acid residues that react with the substrate. The point of these mutations was to broaden the substrate specificity of PCD45. Point-mutation experiments lead to the acquisition of two mutant enzymes with the ability of cleaving compounds that PCD45 couldn't: PCD45/F93A and PCD45/VPA.

In PCD45/F93A, Phe-93 has been substituted by alanine. This substitution resulted in the PCD45/F93A mutant's utilization of caffeate, 3- and 4-hyoxybenzoates, 3,5-dinitrosalicylate and 2,4,6-trichlorophenol as substrates. In PCD45/VPA, Val-13 and Pro-14 residues have been substituted by alanine. This mutation led to an enzyme with the ability to react with 3,4-dihyoxyphenylacetate as well as 3-and4-hyoxybenzoates.

Both mutants retain the ability to cleave PCA and gallate which are the only substrates recognized by PCD45.

The advantage of directed mutagenesis approach is the emergence of a handful of enzymes that could be used for the catabolism of a broader range of aromatic compounds, facilitating the more sustainable bioremediation of PAH-polluted areas.

Shahsavari, E. et al (2019). Biological Degradation of Polycyclic Aromatic Compounds (PAHs) in Soil: a Current Perspective. Current Pollution Reports, 5(3), 84–92. https://doi.org/10.1007/S40726-019-00113-8/FIGURES/3

Tsagogiannis, E. et al (2021). Characterization of Protocatechuate 4,5-Dioxygenase from Pseudarthrobacter phenanthrenivorans Sphe3 and In Situ Reaction Monitoring in the N Tube. International Journal of Molecular Sciences. https://doi.org/10.3390/ij

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PP073

Characterizing the symbiotic profile of natural and laboratory populations of Aedes albopictus

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Mosquitoes (Diptera, Culicidae) are one of the most diverse arthropod groups in the world and their feeding behavior as hematophagous gives them the ability to transmit an enormous number of pathogens from one host to another. Symbiotic bacteria contribute to a multitude of important biological functions such as nutrition, reproduction, protection against pathogens and communication, affecting multiple physiological factors of their insect hosts, including fitness, longevity and survival. The composition of the mosquito microbiome varies across mosquito populations; however, the factors that contribute to this variation are poorly understood. Understanding the factors that shape the mosquito microbiome will inform how mosquito-borne disease transmission varies among environments and help to develop effective disease-mitigating strategies. In this study, we assessed the diversity and variation of the bacteriome of a medically important mosquito, Aedes albopictus from natural and laboratory populations. PCR amplification of the V4 region of the 16S rRNA gene, and sequencing with the Illumina Platform will enable us to provide useful information on the bacterial profile of the Greek Ae. albopictus populations.

PP074

The linkage between boreal peatlands and greenhouse gases: A review of recent efforts to characterize the diversity and functionality of the Sphagnum microbiome

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Boreal peatlands have successfully cooled the world temperature by acting as a long-term carbon dioxide sink, at the same time they absorb and emit greenhouse gases. A major species inhabiting the Canadian and North American boreal peatlands is Sphagnum, which is considered an inherently acidic organism and ecosystem engineer for the peatland habitat. This bryophyte is inhabited by various bacteria, cyanobacteria, archaea and fungi, having varied characteristics, such as methanogens, methanotrophs, diazotrophs, while there is a connection between this moss and the trees and plants, especially with Ericaceae family. Sphagnum moss is home to an extremely diverse range of microbial communities, but little is known about their interactions. It can be suggested that, there are some functional guilds of microorganisms tightly connected with Sphagnum and that these bacteria can contribute to the productivity and health of the plant host (such as N2 fixation and cyanobacterial diazotrophs for sphagnum biomass growth), along with the regulation of the cycles of the greenhouse gases. In this bryophyte, Alphaproteobacteria and Acidobacteria appear to dominate, during the investigation of the applied metagenomic approaches. In this work, we present that the North American and Canadian sphagnum-dominated peatlands would most probably be more methanogenic with future climate change. Additionally, it's expected that the decomposition of Sphagnum will be accelerated by future climate warming, thus the carbon sequestration will decrease.

PP075

Municipal wastewater treatment by means of pilot scale constructed wetlands: elimination of microorganisms and antibiotic resistance genes

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The performance efficiency of three pilot scale constructed wetlands (CWs) was investigated, regarding the treatment of municipal wastewater and the removal of pathogenic microorganisms and antibiotic resistance genes (ARGs). Specifically, three vertical sub-surface flow CWs were placed in the campus of TUC and operated for 11 months. The two units were planted with Phragmites australis and had gravel and recycled plastic HDPE, respectively, as filling materials, while the third unit was the control (without vegetation but with HDPE filler). The three CWs are labeled as CWG, CWP and CWC. Samples were taken monthly and were examined for the removal of bacterial indicators, adenoviruses (AdVs) and ARGs. Also, the possible differentiation of antibiotic resistance profile of the bacterial strains to various antibiotics was investigated.

Three bacterial indicators were examined, namely, Escherichia coli, Staphylococcus sp. and Enterococcus sp. Generally, the average bacterial removal in CWC, CWP and CWG for E.coli was 84.6%, 97.3% and 90.3%, respectively and for Enterococcus sp. was 90.1%, 99.4% and 94.9% respectively. On the contrary, no substantial removal was recorded for S.aureus in any unit. Enterococcus sp. showed better removal rates due to the high sedimentation speeds compared to E.coli.

The combination of HDPE and vegetation in CWP makes it the most efficient wetland. AdVs were sufficiently eliminated, reaching removal rates up to 4.5 Logs. The best performance was recorded in CWC and CWP.

The potential differentiation of the resistance ile of the bacteria was examined for Ciloxacin (CIP), Amoxicillin (AMX) and Sulfamethoxazole (SMX). The results showed that the strains from CWC effluent were highly resistant to almost all antibiotics. In CWG and CWP, the results showed that perhaps there is an indirect contribution of plants to the reduction of resistance. However, there is no clear pattern on how the resistance profile of bacteria changes. The ARGs under study were qnrA, ampC and sul ll, whose reduction reached up to approximately 2Logs in some cases.

Concluding, CWs can be used as alternative, environmentally friendly technologies for wastewater treatment, as they can achieve satisfactory elimination of pathogens and ARGs, which are threats to the environment and human health.

PP076

Study of the ecotoxicity and biodegradation of bioplastics

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Plastic waste produced worldwide ends up in ecosystems, resulting in chemical pollution and raising concerns for public health. The excessive use of plastics has made them major pollutants. The production of bioplastics has emerged as an alternative, as they are considered environmentally friendly materials. However, microplastics and nanoplastics produced during their aging process are a global concern, due to their wide distribution and large quantities.

In this context, the aim of the present study was to evaluate the ecotoxicity and biodegradation potential of PLA and PBAT bioplastics, which are two of the most widely used materials. The ecotoxicity was studied on biomarkers, namely, the bacterium Vibrio fischeri and the fungus Penicillium rubrum in aqueous matrix and in two different types of soil. Biodegradability was tested in the same matrices, measuring the weight reduction of bioplastics during a period of 135d. Finally, the biofilm generation on bioplastics was studied in the same period.

Regarding the results from the toxicity tests of the bioplastics against V. fischeri and P. rubrum in aqueous matrix, after 30 and 14d, respectively, no reduction of their population was observed, as both of them remained almost constant or showed small differences throughout incubation. A small increase in the fungal population was observed, considering that P. rubrum may use bioplastics as a carbon source thus contributing to its faster growth. Furthermore, tests in soils samples, during a period of 60d, revealed that none of the bioplastic samples was toxic against soil bacteria and fungi. The concentration of bacteria remained constant, while the concentration of the fungal population increased in both soil types. Bioplastics are inherently complex materials in their composition but non-toxic in specific amounts in the soil. Biodegradation tests showed substantial reduction of bioplastics weight in aqueous and soil matrix, reaching values of 15.7 and 51.6%, respectively. Biofilm formation was obviousa on all tested materials and bacterial density was up to almost 107 CFUs/cm2.

Bioplastics, upon proper design and production, seem to be environmentally friendly materials with a good level of biodegradability.

Keywords: bioplastics; ecotoxicity; biodegradation; soil; bacteria.

PP076a

The use of microbial cultures with microalgal species for the degradation of bioplastics (PHB and TPS)

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Bioplastics that are biobased polymers, are currently produced at a scale of ~1.5 million t per year. They are considered as integral part of future circular economies to help achieve some of the United Nations' (UN) Sustainable Development Goals, while their application is fostered by the European Union. Bioplastics have a lower carbon footprint and exhibit advantageous material properties in comparison with fossil-based plastics; moreover, some offer biodegradation as an end-of-life scenario if performed under controlled or predictable environments.

However, bioplastics should overcome certain limitations such as reduction in manufacturing costs, and improved biodegradability in order to completely replace traditional fossil-based plastics. In this context, the present study investigated the potential of microbial consortia alone and in mixed cultures with microalgal species aiming to degrade the bioplastics poly-β-hyoxybutyrate (PHB) and thermoplastic starch (TPS).

Several soil communities were found able to decrease the weight of the pellets after 1 month cultivation, while higher weight decrease was observed for TPS in comparison with PHB. For example, the TPS2 community reduced the weight by 24.5% and the highest weight decrease observed for PHB was 18%. A decrease in the intention of the peaks was observed after exposure of both pellets to the microbial communities in accordance with weight reduction. New peaks can also be detected on the surface of TPS pellets. When the algal strains Chlorella vulgaris and Scenedesmus obliquus were added in the cultures with the biofilm covered pellets, no growth inhibition of the strains was observed. Instead the mixed cultures displayed increase in the concentration of the cells and two separate layers were observed on the surface of pellets. To conclude, mixed cultures of microbial communities and microalgal species can be considered as a sustainable and efficient approach for the degradation of bioplastics.

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BIOTECHNOLOGY

PP077

Bioconversion of unrecyclable PET waste into biodegradable polyhyoxyalkanoates by a newly isolated Delftia sp. strain

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Polyethylene terephthalate (PET), a thermoplastic composed of repeating terephthalic acid (TPA) and ethylene glycol (EG) units [1], accounts for a significant portion of the plastic market, with applications in construction, electronics, packaging, household ite, textile, medicine and many other products [1,2]. Because of its large scale worldwide production and great resistance to biological decomposition, it has become a persistent pollutant worldwide[2], with approximately 72% of plastic waste not being recovered[3]. Current recycling rates for PET waste, especially when mixed with other plastics (i.e., multilayer packaging or fibers), are low, prompting research into finding new sustainable technologies for PET recycling or valorization [3,4].

This work reports a new biotechnological process for the conversion of unrecyclable post use PET into biodegradable, biocompatible, thermoplastic polymers (i.e., polyhyoxyalkanoates, PHA) [5]. The bacterium Delftia sp. has been reported to use TPA, the main monomer in PET composition, as the sole carbon source for energy and cell growth[6]. In this work, a Delftia sp. strain isolated from an aerobic activated sludge plant was investigated for PHA production using TPA of chemically degraded mixed PET plastic waste as feedstock. Envisaging the optimization of the bioprocess, cell growth was evaluated under different pH (6–8) and temperature (20–40 ^{0}C) values, as well as different ammonium sources (NH4Cl, (NH4)2HPO4, NH4NO3, (NH4)2SO3). In shake flask assays, cell growth was maximized by using (NH4)2SO3, and incubating at 30 ^{0}C with initial pH 7, during 3 days. The bioprocess was implemented in a 2 L fermenter, where the unrecyclable PET waste was successfully converted into biomass and PHA. These results demonstrate the potential of Delftia sp. in the upcycling of unrecyclable petrochemical-derived plastic waste into value-added biopolymers, providing a seamless route to resolving pervasive PET plastic pollution.

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PP078

Antimicrobial and antioxidant activity of natural extracts of byproducts derived from the olive oil and winemaking processing

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Olive oil and wine industries produce high amounts of by-products annually. Olive Pomace (OP) and Wine Lees (WL) are among the main wastes derived from these industries, with increasing research interest. Olive pomace consists a solid residue recovered after the centrifugation of olive oil extraction, and wine lees are defined as the residue precipitating during storage or after centrifugation or filtration [1,2]. These bio-wastes are rich sources of phenolic compounds. (Poly)phenolic compounds, such as gallic acid, caffeic acid, hydroxytyrosol etc., are well known for their antioxidant and antimicrobial activities [3]. Several studies demonstrate the potential of extraction of bioactive phenolic compounds from agro-industrial by products, such as OP and WL, promoting the principles of circular economy [4].

In this study, OP and WL were employed as starting materials for the preparation of natural extracts. The y extracts were characterized for their Total Phenolic Content (TPC) and were tested for their antimicrobial and antioxidant activity. The results showed that both extracts demonstrated high TPC values, as well as high antioxidant and antimicrobial properties.

Acknowledgements

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PP079

Unchain the metabolic potential of plastics as carbon source for biotechnology

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The worldwide plastic crisis is real and requires drastic measures, especially for the plastics' end-of-life. The overwhelming challenges are the 4.8 billion tons of plastics in poorly managed landfills and the annually close to 400 Mt of new plastic produced. Without measures to reduce plastics usage, this amount is likely to increase significantly in addition to the already 8 billion tons in Earth's system, and the less than 10% of new plastic recycled even once (1, 2). Social costs from plastic pollution (incl., e.g., clean-up, ecosystem destruction) exceed already 100 billion $ per year (3). Mixed plastic fractions are currently difficult to recycle, but microbial metabolism might open new pathways. With new technologies for degrading plastics to oligo- and monomers, these carbon sources can be used in biotechnology to upcycle plastic waste to valuable products, such as bioplastics and biosurfactants.

The EU Horizon 2020 project MIX-UP (MIXed plastics biodegradation and UPcycling using microbial communities) focuses on changing plastics' traditional linear value chain to a sustainable, biodegradable one (4). The main process steps are the consecutively controlled enzymatic and microbial degradation of mechanically pre-treated plastic wastes combined with subsequent microbial conversion to polymers and value-added chemicals by mixed cultures. Known plastic-degrading enzymes have been optimised by integrated protein engineering to achieve high specific binding capacities, stability, and catalytic efficacy towards a broad spectrum of plastic polymers under high salt and temperature conditions. Other focuses lie in searching for and isolating novel enzymes active on the more recalcitrant polymers, designing enzyme cocktails tailored to specific waste streams and striving to enhance enzyme production significantly. In vivo and in vitro application of these cocktails enables stable, self-sustaining microbiomes to convert the released plastic monomers selectively into value-added products, key building blocks, and biomass. Any remaining material recalcitrant to the enzymatic activities will be recirculated into the process by physicochemical treatment.

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PP080

Recycling mushroom's cultivation by-products to produce Pleurotus ostreatus new crops

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Mushrooms' cultivation by-products (spent mushroom substrate-S, stipes and malformed mushrooms-MW) are wastes that confront many disposal issues, but they also constitute potential sources of essential compounds that can be used in new mushroom production, resulting in economic growth and environmental protection. In this study, several combinations of fresh agro-industrial residues,(produced in previous experiments on Laboratory of Edible Fungi/ITAP/ELGO-Dimitra) and MW were used to form new substrates for a 2nd cycle mushroom cultivation. Experiments with P. ostreatus AL 150 took place in glass-tubes, where 20% of MW was added in 80% of fresh or spent substrates of wheat straw (WS), barley and oats straw (BOS) and coffee residues (CR), in respect to fresh substrates (control). Evaluation included mushrooms' biological efficiency (BE%; weight of fresh mushrooms/weight of y substrate x 100), mean fresh weight, pileus diameter and stipe length and thickness. According to the results, the by-products did not seem to have significantly affected the morphological characteristics of mushrooms. Regarding pileus diameter, values ranged from 35.16 mm (20% MW + 80% S-WS) to 45.38 mm (20% MW + 80% S-BOS). The stipe length varied from 20.92 mm (20% MW + 80% BOS) to 27.56 mm (100% CR), while stipe thickness values were in the range of 9.09 mm (100% BOS) and 10.87 mm (20% MW + 80% BOS). Contrariwise, the by-products enhanced BE% and the average fresh weight. BE% performance was the highest on 20% MW + 80% S-WS and 20% MW + 80% S-BOS substrates, whereas the average fresh weight values were the highest on 20% MW+ 80% WS (4.16 g) and 20% MW + 80% BOS (3.92 g). In the case of the 20% MW + 80% S-WS substrate, a two-fold BE value (124.68%) was recorded, compared to the with fresh residues (20% MW + 80% WS; 53.76%) and to control substrates (100% WS; 69.76%). Hence, mushroom by-products had beneficial effect on production parameters of P. ostreatus mushroom, making feasible their re-use through combination of mushroom waste new cultivations. However, further research into the nutritional profile of mushrooms would provide more information on the impact of these by-products.

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PP081

Exploring the fungal potential for natural and synthetic polymer degradation

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Fungi are versatile microorganisms with a crucial role in organic carbon mineralization and dead matter degradation in nature, due to their ability to break down complex natural polymeric structures. Many fungal strains are also very efficient in degradation of not only recalcitrant organic compounds, such as lignin, cutin, and waxes, but also xenobiotics with similar structure, including synthetic polymers [1,2]. In this work, four fungal strains (one ascomycete and three basidiomycetes) were selected based on preliminary screening results on agar plates and evaluated for their ability to grow with complex polymers as sole carbon sources in submerged fermentations. Anionic aliphatic polyester-polyurethane and long-chain alkanes dispersions were used to probe the fungal degradation potential towards polyurethane and polyolefins, respectively, while corn bran was used to check for the delignification capacity of the selected strains. During fermentation, culture supernatants were regularly screened for their protein content, and selected enzymatic oxidative and hydrolytic activities were quantified via spectrophotometric assays. Upon termination of fermentation, substrate degradation was evaluated through compositional analysis of corn bran and gel permeation chromatography of synthetic compounds. Culture supernatants were sent for shotgun proteomics analyses, targeting at sequences homologous to known Carbohyate Active Enzymes (CAZymes) [3] and activities related to synthetic polymers degradation [4]. This study highlights the potential of fungi and their enzymatic machinery as powerful tools for the degradation of these recalcitrant compounds.

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PP082

ACCUMULATION OF HIGH-NUTRITIONAL-VALUE OMEGA-3 FATTY ACIDS BY THE HETEROTROPHIC MICROALGAE CRYPTHECODINIUM COHNII ON ORGANOSOLV FRACTIONATED AGRICULTURAL RESIDUES FROM THE FLOUR MILLING INDUSTRY

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The main scope of this study was to assess the utilization of low-value agricultural residues from the Greek flour milling industry for the production of polyunsaturated omega-3 fatty acids, which are recognized as important nutraceuticals affecting human/animal physiology and promoting brain and heart health. Both straw and bran from wheat and barley were pretreated using a novel OxiOrganosolv fractionation process [1], assisted by solid acid catalysts. The impact of various catalysts and of different catalyst-to-biomass ratios on the production of sugar-rich streams from the pretreatment was investigated. Moreover, the susceptibility of the sugar-rich streams to enzymatic degradation towards fermentable sugars was examined. The enzymatic hydrolysates were subsequently used as a carbon source for the growth of the heterotrophic marine microalgae Crypthecodinium cohnii. This microalgal species is able to grow on variable carbon sources and has the ability to accumulate polyunsaturated omega-3 fatty acids and more specifically docosahexaenoic acid (DHA) [2,3]. The growth and lipid accumulation of C. cohnii cells was evaluated after cultivation of the microalgae, initially in flasks and then in bioreactors, under optimal growth conditions. The results demonstrate the possibility of valorizing abundant, currently underutilized lignocellulosic residues for the production of high-value nutraceuticals through physicochemical pretreatment, enzymatic saccharification and microbial fermentation.

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PP084

Exposure to salinity stress conditions of an isolate from Kalloni solar saltworks on Lesvos island leads to biomass production with promising traits

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Microalgae inhabit a variety of ecosystems, utilizing the unique adaptations they have developed (Park et al., 2022). Some microalgae can grow successfully in high salinity conditions, such as those of the genus Dunaliella, whose properties are still largely unknown (Chen & Jiang, 2009). In this context, sampling was carried from two concentration ponds of Kalloni solar saltworks on Lesvos island and the isolated strains were classified using ITS, rbcL and tufA molecular markers. In the present study, one of the isolates was exposed to salinity stress conditions, specifically 0 M, 1 M and 2 M NaCl. Under these conditions, culture growth was determined for ten days. In addition, the chlorophyll content and the total protein content of the cells were determined using photometric methods. The antioxidant activity of the biomass was evaluated by Ferric Reducing Antioxidant Power Assay (FRAP). The results highlighted the ability of this strain to grow successfully under high salinity conditions by modulating its metabolism and producing biomass with promising traits in regard to antioxidant potential and protein content.

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PP085

Molecular classification of five microalgae strains isolated from Kalloni solar saltworks on Lesvos island

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Microalgae inhabit all aquatic ecosystems on Earth, even under harsh conditions, utilizing their metabolic plasticity including secondary metabolites (Park et al., 2022). This attribute can lead to their potential application as feedstocks, for producing nutraceuticals, pharmaceuticals as well as biofuels (Bhalamurugan et al., 2018). As the classification of microalgae using traditional approaches is often hindered by the complexity of their structure and morphology, molecular markers have been established with the usage of proper genes, such as tufA (Vieira et al., 2016). In the present study, five microalgae strains were isolated from water samples collected in October 2021 from two concentration ponds of a solar saltwork located in Kalloni, on Lesvos island. The ponds had a density of 23 and 40 Baume, a unit used to express the concentration of salt in brines. Sodium chloride crystallizes at around 26 Baume, so the two samples are characteristic of salty water just before and after the crystallization of NaCl. Microalgae strains were studied using nuclear ITS region and plastid rbcL and tufA molecular markers. In-depth analysis was accomplished with the generation of a phylogenetic tree based on concatenated sequences of all three molecular markers. Therefore, herein we present the molecular classification of five microalgal strains based on three single DNA-barcoding markers alongside with a new suggestion for the combined usage of ITS region with the tufA and rbcL genes.

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PP086

Changes on nodule sulfate metabolism triggered upon carbon starvation induced by prolonged darkness

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Symbiotic N-fixation takes place in legume root nodules, where symbiotic for of rhizobia reduce atmospheric nitrogen to ammonia and in return the rhizobia receive organic carbon and other nutrients from the plant (Oloyd et al., 2005). Our previous work revealed that N-fixing nodules, except from being the main source of assimilated nitrogen for the legumes, represent a strong source of assimilated sulfur for the whole plant (Kalloniati et al, 2015). To investigate the impact of photosynthetic carbon deficiency on S-metabolism in nodules of the model legume L. japonicus inoculated with the rhizobium Mesorhizobium loti, photosynthesis was limited by exposure of the plants to prolonged darkness and then transcript accumulation of plant and rhizobial genes involved in S-uptake and metabolism was studied using real-time qPCR. In addition, APR activity, as well as the S-containing metabolites levels in nodules were determined. Most of the plant and rhizobial genes were downregulated during the extended dark period, and APR activity was dramatically reduced. Thiols content was increased except for glutathione which was reduced under prolonged darkness. Integrated biochemical and -omics analysis, in nodules clearly indicate that the strong S-assimilation in nodules is tightly connected to the supply of photosynthetic carbon, while protein recycling could represent a major thiol source under C-limiting conditions.

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PP087

Analysis of the bacterial and fungal microbiome of stone surfaces of monuments: towards the development of mitigation strategies for protection of cultural heritage

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The induced deterioration and degradation of historical stone monuments contributes mainly to the loss of important cultural heritage worldwide, especially when exposed to environmental conditions and climate change. Microorganisms (bacteria and fungi) are in the most cases the cause of the biodeterioration processes, witch typically involves biochemical and microbial processes. The main aim of the current study was to define the composition of the bacterial and fungal communities developing on deteriorated surfaces of historical stone monuments in Greece using amplicon sequencing and bioinformatics. These data along with shotgun metagenomic analysis, will be used for the identification of microorganisms which contribute to the deterioration or to the protection of the monuments.

The studied monuments were the Eupalinian Aqueduct, in Pythagoreion of Samos (UNESCO underground monumental structure), the sculpted monuments of Afentaki and Diligianni in the First Cemetery of Athens (marble surfaces exposed to atmospheric pollution) and the Catholicon of Varnakova Monastery, in Naupaktos. Samples for DNA extraction were collected from several locations per monument that were showing biodeterioration and they were subjected to amplicon sequencing analysis for bacteria and fungi. The analysis of the bacterial community identified, at medium to high abundance, bacterial belonging to Pseudomonas, Acinetobacter, Flavobacterium and Streptococcus, which are known to have beneficial effects on monuments preservation. On the other hand, the fungal community was dominated by fungal genera (e.g. Cladosporium, Penicillium, Aspergillus and Alternaria) with known biodeteriorating capacities. On-going metagenomic analysis will further verify the identity of microorganisms that carry attributes contributing to the preservation or deterioration of monuments. This will dictate the next microbiome modulating strategies for monument recovery and preservation.

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PP088

Exploitation of novel fungal oxidative biocatalysts for the sustainable production of valuable monomers from biobased furans

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The dependence on fossil fuels for the production of chemical building blocks with significant interest to the polymer industry has awn attention not only in the utilization of renewable resources such as lignocellulosic biomass, but also in the application of biocatalysis to replace chemical reagents toward the development of greener and sustainable processes. Furans, such as 5-hyoxymethylfurfural (HMF) and furfural (FA) obtained from the lignocellulose-derived polysaccharides, have emerged as crucial precursors in chemical synthesis reactions, since they can be transformed to a wide range of derivatives (2,5-furandicarboxylic acid, 2-furancarboxylic acid etc.) with exceptional applications. Biocatalytic oxidation of furans with redox enzymes offers a facile and regioselective route of reaction under mild conditions [1]. The current study targeted at the enzymatic biotransformation of HMF and FA using novel fungal biocatalysts from the Auxiliary Activity AA3 and AA5 families of CAZy database [2]. Through intelligent exploration of Ganoderma lucidum genome, it was possible to retrieve one sequence with putative glyoxal oxidase activity (GlGlyOx1) and one with aryl-alcohol oxidase (GlAAOx1) activity based on their homology with known furan-transforming fungal catalytic activities. The genes were heterologously expressed in yeast Pichia pastoris, the respective enzymes were purified to their homogeneity and biochemically characterized. GlGlyOx1 and GlAAOx1 were evaluated, both individually and synergistically (along with the presence of in-house produced galactose oxidase FoGalOx from Fusarium oxysporum [3] and a commercially available horseradish peroxidase HRP), for their ability to act on furans and produce value-added oxidized derivatives. Our results demonstrate the potential of G. lucidum enzymes for obtaining furan-based monomers from lignocellulosic biomass residues, which can be used as building blocks for the production of biobased polymers.

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PP089

Assessing growth kinetics and accumulation of high-value metabolites in freshwater and marine microalgae using crude glycerol as a carbon source

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Microalgae have been gaining attention in the last decade as potential sources of high-value metabolites with a great importance as nutraceuticals and cosmeceuticals [1,2]. Compared to autotrophic, when growing under mixotrophic or heterotrophic conditions, several species often show higher biomass concentrations and lipid productivities [3]. In this study, the growth and production of metabolites in two freshwater species, namely Scenedesmus quaicauda and Chlorella vulgaris, were evaluated in crude glycerol obtained as a byproduct from the biodiesel industry as a carbon source, under heterotrophic and mixotrophic conditions with monochromatic illumination using LEDs. The results indicated that mixotrophy resulted in higher biomass yields than heterotrophy in both species, while different light sources significantly affected the growth and the cell biochemical composition. S. quaicauda displayed more promising results reaching 1.86 g of y biomass/L when cultivated on 10 g/L glycerol, under yellow light, while lipid accumulation was higher under red light, with a value of 0.36 g/L (20 wt% of y cell weight). Carotenoids and protein content were higher in C. vulgaris under heterotrophic conditions. The marine heterotrophic Crypthecodinium cohnii was also assessed due to its ability to produce oil rich in docosahexaenoic acid (DHA). When growing on 9 g/L crude glycerol, cells accumulated about 30% of total lipids, verifying the ability of the strain to metabolize this carbon source. Overall, this study demonstrated the possibility of crude glycerol valorization towards the production of value-added compounds through microalgal fermentation.

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PP090

BIOTECHNOLOGICAL PRODUCTION OF BACTERIAL PIGMENT PRODIGIOSIN AND BIOACTIVE PROPERTIES OF ITS METAL COMPLEXES WITH Cu(II) AND Zn(II)

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Prodigiosin (PG, Fig. 1a) is a biologically active pyrrolylpyrromethene alkaloid whose structure was first confirmed in 1962 [1]. PG is commonly produced by Gram-negative bacteria, such as Serratia spp. and has an eco-physiological role [2]. Its biological activities were extensively researched, and numerous pharmacological properties were established, including anticancer and immunosuppressive [3].

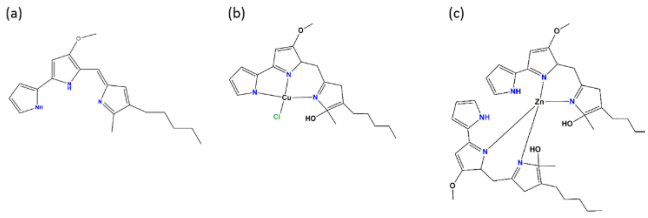

Fig. 1. (a) Structure of prodigiosin (PG) and its metal complexes (b) [Cu(PG)Cl] and (c) [$Zn(PG)_2$].

However, high cost of extraction and purification still represent the bottleneck in the microbial production of PG. Meat and fish processing wastes have high potential as raw materials for conversion into useful products of higher value. In this study, meat offcuts were assessed as the sole nutrient for the fermentative production of PG from S. marcescens. Using this substrate lowered the cultivation medium cost and shortened the fermentation time to 12 h, while allowing a satisfying PG yield of 83.1 mg/L. The isolated PG was used in one-step reactions with $CuCl_2$ or $ZnCl_2$ in terc-BuOH at 25 °C. The obtained [Cu(PG)Cl] (Fig. 1b) and [$Zn(PG)_2$] (Fig. 1c) complexes were characterized by UV-Vis and IR spectroscopy and their bioactivity potential was assessed.

Antimicrobial activity was assessed in a disc assay against 4 human pathogens: Escherichia coli NCTC 9001, Pseudomonas aeruginosa ATCC 10332, Staphylococcus aureus NCTC 6571, Candida albicans ATCC 10231, but no effect was observed for the tested concentrations of 200 µg per disc and lower. However, the anticancer potential of the new derivatives is promising and the bovine serum albumin (BSA) binding study revealed that complexes bind to BSA tightly and reversibly [4].

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PP091

ENZYME-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM THE ROSEHIPS OF ROSA CANINA L. APPLYING A BOX-BEHNKEN EXPERIMENTAL DESIGN

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The pseudo-fruit of Rosa canina L. (rosehip) is a rich source of biologically active compounds with antioxidant, anti-inflammatory, anti-cancer, immunomodulatory, cardioprotective, gastroprotective, and antimicrobial effects. Plant cell walls consist of a series of complex structural polysaccharides such as cellulose, hemicellulose, pectin as well as lignin and protein. Enzymes with specific hydrolytic properties can be used to rupture this matrix in order to facilitate the release of the bioactive components from within the cytosolic spaces and even those bound to the cellular walls. Enzyme assisted extraction (EAE) method is a green extraction technology.

In the present study, EAE was applied in the extraction of bioactive compounds from the pseudo-fruit of Rosa canina L. of Greek origin. Initially, four different commercial enzyme preparations namely, Cellic® CTec3 (cellulolytic enzymes), Pectinex® Ultra Color (pectinolytic enzymes), Neutrase® (proteolytic enzymes), and Viscoferm® (hemicellulolytic enzymes) were screened. The selection was based on the compositional analysis of the rosehips. Application of Cellic® CTec3 in EAE resulted in higher extraction yields compared to other enzyme preparations. A Box-Behnken experimental design was applied to determine the optimal conditions of EAE using CellicCtec3. The impact of three factors namely, enzyme load (0.5, 1.0, 1.5% v/v), solid-to-liquid ratio (SLR) (4, 6, 8% w/v), and extraction time (2, 4, 6 h) on three responses i.e., total phenolic content (TPC), total flavonoids content (TFC) and the antioxidant activity (IC50 based on DPPH radical scavenging method) was studied. Response surface methodology (RSM) was used to analyze the relationship between the measured responses and the individual and combined effects. Simultaneous optimization of the three responses was performed by Derringer's desirability function method. Applying this methodology, the optimal levels of the parameters were found LSR=6.1% w/v, enzyme load=0.53% v/v, and extraction time = 2.84 h with the corresponding desirability (D) value of 0.708. The antimicrobial activity of the extract was assessed against the bacterium E. coli.

KEYWORDS: Rosa Canina L. fruits, Enzyme Assisted Extraction (EAE), Box-Behnken experimental design; Total flavonoids content (TFC), Total phenolic content (TPC), Antioxidant activity

This work was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (grant number: T2ΕΔΚ-0233).

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PP092

Assessment of the biofilm-forming ability of marine microalgae and cyanobacteria

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Autotrophic microorganisms present great research interest, mainly due to their promising utilization in various biotechnological applications. Given the increasing preference of attached over conventional cultivation systems in such applications, biofilm formation by photosynthetic microorganisms is, among others, an important asset when selecting microbial strains. The objective of this study was the in vitro evaluation of the biofilm formation of different strains of marine microalgae and cyanobacteria. Specifically, 20 strains of microalgae and four strains of cyanobacteria were studied regarding their biofilm-forming ability in artificial sea water of different pH values (6.0-9.0), at 25°C, under continuous lighting conditions, and after incubation for distinct time periods (4, 7 and 10 days). Biofilm quantification was performed in polystyrene microtiter plates using crystal violet staining, while for four selected microalgal strains the y biofilm biomass formed in plastic Petri dishes also was determined. Beyond the extensive strain variability that was recorded, both the substrate's pH and the incubation duration significantly affected the biofilm formation behavior of microalgae, with most of them forming more biofilm at pH 7.0 and after 7 days. Overall, the highest biofilm-forming capacity was noted for microalgal strains belonging to the genera Nannochloropsis and Tetraselmis, while Chlorella sp. and Picochlorum costavermella strains exhibited poor biofilm growth. With reference to the studied cyanobacteria, prominent biofilm-forming ability was recorded for two strains of cyanobacteria (Oscillatoria sp. and Geitlerinema sp.), whereas the substrate's pH did not appear to affect the biofilm formation phenotype of the studied species. The collected data should be useful in strain selection for utilization in attached-cultivation techniques applied in (i) wastewater treatment, and (ii) the joined (with wastewater treatment) or independent recovery of high added-value metabolites as a cost-efficient means of biomass harvesting in large-scale applications.

PP093

CHARACTERIZATION OF ISOLATED MICROALGAE STAINS FOR HYOPONIC WASTEWATER BIOREMEDIATION

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In the frame of environmental and energetic crisis, agriculture development needs to develop a more sustainable hydroponic waste management. Microalgae can utilize nutrient elements of hydroponic wastewater for their growth, offering an economically viable solution for bioremediation of hydroponic eluents. In the present study, effluent water from hydroponic cultures was used for isolating naturally present microalgal species that may possibly be of biochemical interest and play a crucial role in wastewater treatment. Four different microalgae strains (PR1, PR2, PR3, and PR4) were isolated and characterized with morphological, biokinetic and molecular taxonomy methods. Moreover, the biomass of the isolated stains cultured in common growth media and hydroponic effluent was characterized for their content including both primary and secondary metabolites. Protein content was determined by Kjeldahl method, while total lipids and polysaccharides were evaluated spectophotometrically by phospho-vanillin and Dubois assays, respectively. The phenolic content were determined by Folin-Ciocalteu method, while flavonoids by aluminum chloride assay. Comprehensive microalgal metabolite profiling was assessed by GC- analysis. Furthermore, antioxidant potential of microalgae strains was evaluated via FRAP Assay and ABTS-radical scavenging activity. All isolated microalgae were tested for macro- and micro- element removal from hdryoponic effluents. More specific, nitrate and phosphate were determined photometrically, potassium and sodium by flame photometry, while minerals (Fe, Cu, Mn, Ca, Mg, and Zn) were estimated by atomic absorption spectroscopy. Among isolated microalgae strains, PR4 demonstrated great performance on nutrient removal from hydroponic effluent, rendering it as a promising candidate microalgae strain for wastewater treatment.

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PP094

EFFECTS OF CULTURE AND BIOPROCESS CONDITIONS ON THE MYCELIUM GROWTH AND PRODUCTION OF BIOACTIVE SUBSTANCES OF GANODERMA LUCIDUM AND MONASCUS PURPUREUS

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Ganoderma lucidum (Reishi mushroom) and Monascus purpureus (the fungus or "Red rice") are two fungi that have been used for centuries in traditional Chinese medicine. Both fungi are known for their health benefits due to the production of numerous bioactive compounds. Usually, in industrial scale the two fungi grow in solid substrates such as straw or wood trunks (for Ganoderma lucidum) or rice (for Monascus purpureus) in greenhouses, but this a lengthy process, only partly controlled and vulnerable to contamination. The submerged culture in liquid synthetic media is an alternative method which can lead to high productivity of fungal mycelium under fully controlled conditions.

After initial bioprocess optimization in shake flasks (regarding the pH, medium composition, agitation rate), the two fungi were cultured in bioreactors in order to optimize other bioprocess parameters (e.g. aeration rate and dissolved oxygen) and finally the yield of mycelium and bioactive substances. The filtered mycelia, as well as the fermentation broth with all extracellular bioactive metabolites were isolated and dried and studied for their antioxidant and antimicrobial activity, in relation to polysaccharide and pigment production. These bioactive properties were compared with (a) commercially available Ganoderma lucidum powder from fruiting bodies of the mushroom and (b) red rice with Monascus purpureus produced in a solid state fermentation in the lab.

The results showed different degrees of antimicrobial and antioxidant effect between fruiting bodies and mycelium of the two fungi. The phenol content and antioxidant activity were comparative higher in red rice compared to mushroom powder Ganoderma lucidum. In general, the ethanol extracts of dried mycelium of the submerged cultivation were significantly effective against E. coli, L. monocytogenes, S. aureus and P. expansum compared to water extracts which were not effective against the tested microorganisms.

The functional properties of these edible fungi can be utilized in novel food additives and supplements.

PP095

Symbiotic interactions in the model legume Lotus japonicus: Mutation analysis of the SHAGGY-like LjLSK1 kinase gene and phenotypic analysis of nodulation

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Lotus japonicus belongs to the family of legumes (Fabaceae), plants of great agricultural and biological interest. It is considered a model plant because of its small diploid genome, short generation time and its ability to be easily genetically transformed. A property that distinguishes legumes from other plant families is their ability to form symbiotic relationships with Rhizobia during which atmospheric nitrogen is converted to ammonia making it easier for the plant to absorb it. The LjLSK1 gene encoding for a SHAGGY-like kinase has been shown to affect the number of nodules formed in the roots. In this study, we aim to analyze mutations of the LjLSK1 gene caused by the CRISPR/Cas9 system, using the T7 endonuclease assay and compare the nodulation phenotype in Agrobacterium rhizogenes-produced transgenic and non-transgenic hairy roots

PP096

Spent mushroom substrates from Pleurotus ostreatus and Pleurotus eryngii: a potential source of exopolysaccharides and enzymes

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The increasing development of the mushroom industry leads to the creation of million tons of wastes. The most abundant waste produced at the end of solid-state fermentation of mushrooms is spent mushroom substrate (S). This lignocellulosic by-product is composed of mushroom mycelia, cellulose, hemicellulose and lignin that remained unused after cultivation, enzymes secreted during substrate colonization, such as the oxidative enzyme laccase and the hydrolytic enzyme endoglucanase and other bioactive compounds including exopolysaccharides (EPS) with remarkable medicinal properties. Therefore, the biosynthesis of laccase, endoglucanase and EPS was estimated in Ss consisted of five different agro-residues (wheat straw, barley and oats straw, beech wood shavings, rice bark, coffee residue) derived from Greek far and industries after P. ostreatus (strains AL 144, 150) and P. eryngii (strains AL 166, 173-6) cultivation. Results showed that all Ss contained significant amount of EPS and laccase. Specifically, all Ss derived from P. ostreatus cultivation presented higher quantity of EPS than those from P. eryngii (12.43-24.01 mg/g and 4.29-17.13 mg/g, respectively). Similarly, the greatest laccase production was detected in Ss from P. ostreatus (1086-1830 U/g substrate d.w., 1008-1699 U/g substrate d.w.; Ss from P. eryngii). On the contrary, endoglucanase, produced in low concentration, was found to be higher in Ss from P. eryngii cultivation (0.18-0.42 U/g substrate d.w., 0.12-0.37 U/g substrate d.w.). Furthermore, it see that EPS production and enzymes' activity were not affected by the type of lignocellulosic substrate, but it was strain dependent. However, the lowest values of EPS and laccase activity were detected inconsisted of beech wood shavings for all Pleurotus strains. Overall, the potential for recovery of high value-added products that could be extracted from S, such as enzymes and polysaccharides, see particularly promising in financial and environmental ter, due to their use in various applications in food and beverage industry.

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-05027), scientifically coordinated by the Hellenic Agricultural Organization–DEMETER (Institute of Technology of Agricultural Products/ Laboratory of Edible Fungi).

PP097

INVESTIGATING THE ROLE OF TRITERPENE BIOSYNTHETIC GENES IN L. JAPONICUS DURING COLONIZATION BY AN ENDOPHYTIC FUNGUS

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Lotus japonicus is a model legume plant better known for its ability to participate in symbiotic interactions with various microbes but, also, studied for a very rich production of specialized metabolites. Triterpenes, a major subgroup of the terpene superfamily of plant specialized metabolites, arise from oxidosqualene with the help of oxidosqualene cyclases and get catalyzed into different active for by P450 cytochromes. They have attracted the scientific community for their medicinal values and, recently, for their ability to affect symbiotic relationships. Evidently, a non-pathogenic strain of Fusarium solani (strain K – FsK) that colonizes the legume is not an exception, as its endophytic ability see to be interlinked with major triterpene biosynthetic genes. We decided to investigate the effect of amy2, an oxidosqualene cyclase, and cyp71d353, a P450 cytochrome, on FsK symbiosis by generating a collection of mutant plants, deprived of said genes. The knock-out was achieved utilizing an optimized CRISPR/Cas9 system for L. japonicus with different gRNAs targeting the two genes. The genetic system was introduced to the plants via A. rhizogenes-mediated hairy root transformation. After infecting the transformed hairy roots with the endophytic FsK, we aimed to evaluate the mutation efficacy of each gene-targeting gRNA and assess colonization effects in the mutated and non-mutated plants.

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PP098

A novel CE16 exo-deacetylase from Thermothelomyces thermophilus with biotechnological potential

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Biotechnological utilization of hemicellulose is an enticing concept, hindered by the recalcitrance of this biomaterial against biodegradation. Acetyl-substitutions of the main-chain carbohyates pose a severe inhibitory effect on hemicellulose- and especially xylan-targeting enzymes, as the xylopyranosyl (Xylp) residues of the backbone are mono- or di-acetylated at positions O-2 and O-3, or even O-4 of the non-reducing-end Xylp. The carbohyate esterase family CE16 is a growing group of enzymes, involving acetyl esterases, that target acetyl-esters and exhibit an exceptional diversity regarding substrate specificity, regioselectivity and preference on oligomeric or polymeric substrates. However, further insight into the CE16 family is required for their efficient exploitation in the biodegradation of lignocellulosic biomass.

In this study, the acetyl esterase TtCE16B from Thermothelomyces thermophilus was heterologously expressed in Pichia pastoris and its mode of action was determined using monoacetates of 4-nitrophenyl β-D-xylopyranosides and multiply acetylated methyl β-D-xylopyranosides. The first crystal structure of a CE16 representative was determined, in apo- and product (acetate) bound form to 1.9 and 1.42 Å resolution, respectively. TtCE16B structure was solved by molecular replacement, using an AlphaFold prediction as starting model. Finally, the synergistic effect of TtCE16B with a number of hemicellulose-targeting enzymes, during hyolysis of pretreated biomass samples, was examined.

TtCE16B is an exo-acting deacetylase that perfor optimally on xylooligosaccharides. The esterase targets the non-reducing-end Xylp, removing acetyl groups from positions O-3 and O-4, given that the other vicinal hyoxyl group is free, regardless acetylation at position O-2. Catalyzing the O-4-deacetylation TtCE16B exhibits complementary regiospecificity to CE6 esterases, which are unable to deesterify this position. Regarding deconstruction of pretreated biomass, TtCE16B exhibited minor synergistic effects with main-chain degrading enzymes and more prominent synergies with oligomer-acting enzymes. Overall, the discovery of biocatalysts with distinctive specificities could become an asset for the design of efficient hemicellulolytic cocktails and assist in the upcycling of residual biomass.

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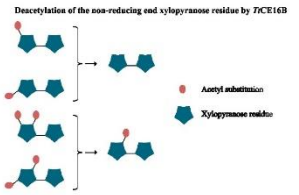

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PP099

Conversion of mixed plastic waste containing PET into biopolymer bacterial nanocellulose

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The rapid increase in global plastics production is also causing an accelerated environmental pollution. Recently, biotechnological solutions and enzymatic recycling of poly(ethylene terephthalate) (PET) waste stream have been put forward and commercialized1. Increasing recycling and upcycling rates is the most effective model approach to plastic circularity. However, mixed plastic waste is still quite a challenge for both recycling and upcycling technologies. This study is focused on the eco-conversion of plastic waste containing poly(ethylene terephthalate), PET, into biopolymer, bacterial nanocellulose. Polymer mix contained selection of commercial biodegradable plastics (poly(lactic acid), PLA, poly(ε-caprolactone), PCL, poly(hyoxyl butyrate), PHB) and PET. This mixture was hydrolysed under aqueous conditions and hydrolysate was used as carbon source for Komagataeibacter medellinensis ID13488 and bacterial nanocellulose (BNC) production. HPLC analysis confirmed the presence of monomers and dimers of polymer mix components indicating existence of potential substrates for BNC production. BNC production by K. medellinensis was investigated and optimized in ter of the amount of carbon source and growth conditions. Under the most efficient rate in terms of yield, BNC production was scaled up and the obtained biopolymer was characterized. The structure of produced BNC was confirmed by FTIR analysis, thermal properties by DSC/TG analysis, and the morphology of material by optical microscopy and SEM analysis. This research demonstrates how to put the mixed plastic waste stream into a circular loop through the biotechnological conversion into valuable biopolymer.

Keywords: plastic waste, PET, bacterial nanocellulose, upcycling, plastic sustainability

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PP100

Characterization of a novel cyclofructan transferase from Janthinobacterium BJB-304

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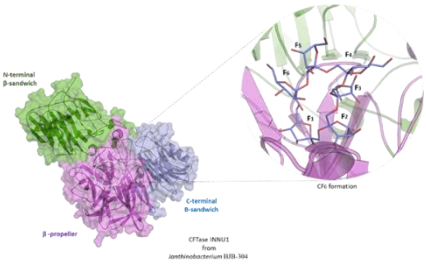
Janthinobacterium sp. BJB-304 is a purple-colored, violacein producing bacterium isolated from the Norrie Pond, Hudson Valley in NY, USA (Bettina et al. 2018), belonging to the Oxalobacteriaceae, Proteobacteria. Janthinobacteria are Gram-negative, motile, rod-shaped and obligate aerobic bacteria that preferentially occur as microbiota in soil and aquatic environments. Janthinobacteria has anticancer and antimicrobial activities, e.g. the inhibition of multi-resistant Staphylococcus aureus (Choi et al., 2021) and plant pathogenic fungi such as Fusarium graminearum (Haack et al., 2016).

Here we characterized a novel cyclofructan transferase (CFTase) from Janthinobacterium BJB-304, producing cyclic small inulin molecules from long linear chain-inulin. This particular enzyme belongs to a new subgroup of the glycoside hydrolase family 32 (GH32) with three structural domains. The extra N-terminal β-sandwich domain shows identity to a carbohydrate-binding module of family GH43, harboring arabinanases different from classic 2 domain e GH32 structures consisting of an N-terminal β-propeller and a C-terminal β-sandwich.

This enzyme showed a pH optimum of 6.5 and a temperature optimum of 24°C. Michaelis- Menten kinetics were observed with inulin (HP; Orafti© chicory) as substrate. Kinetic constants were as follows: Vmax 1.106 mM/min, Km 2.55 (µmol [S]/µmol[E]), and Kcat 1.52 (µmol [S]/µmol[E]*s).

Bacterial culturing and in vitro enzymatic assays showed that CFTase activities reside in the extracellular environment. With HPAEC-IPAD, two main products were detected from inulin as substrate, with differential sensitivity to treatment with a fructan 1-exohyolase (1-FEH) and cellular uptake by Janthinobacterium The 1-FEH resistant product remains in the extracellular environment and likely represents a cyclic inulohexose CF6, awaiting further confirmation by N Similar to cyclodextrins, cyclofructans may also act as signaling molecules involved in quorum sensing, biofilm formation, and plant-microbe interaction (Almagro et al. 2020), awaiting further investigation.



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PP101

Parameters optimization of glycerol's bioconversion into gamma-linolenic acid by fungus Cunninghamella elegans cultivated in shake-flask and batch-bioreactor experiments

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The valorization of metabolites derived from the microbial cultivation in organic industrial residues, as an antidote to the depletion of planet's natural resources in terms of sustainable development and circular economy is of high interest. Polyunsaturated fatty acids (PUFAs) and especially gamma-linolenic acid (GLA) have important nutritional and therapeutic properties (as anti-cancer factors or against rheumatoid arthritis, atherosclerosis, etc.) and are therefore of high pharmaceutical interest. A remarkable content of GLA has been observed in lipids of Zygomycetes strains. In the present study, the fungus Cunninghamella elegans NRRL 1392 was evaluated for its ability to catabolize glycerol, the main by-product of biodiesel production plants, and produce GLA-rich lipids. First, different carbon-to-nitrogen ratios (C/N =11, 110, 220 mol/mol) were examined in batch shake flask experiments with ≈60000 spores/mL of broth, at an initial glycerol concentration (S0 ≈30 g/L), and the highest GLA production =0.23 g/L (productivity PGLA =0.050 g/L/d) occurring under nitrogen excess conditions. Residual nitrogen in the culture medium resulted in new cultivations with higher ratio =20 mol/mol either by increasing the S0 =55 g/L or by decreasing the available nitrogen sources, while keeping the S0 =30 g/L. The first case resulted in GLA =0.36 g/L (PGLA =0.045 g/L/d), while in the second one the highest productivity was observed =0.057 g/L/d (GLA =0.23 g/L). Then, the effect of the inoculum size (fungal spores per mL of broth) on the substrate's uptake rate was studied, using 2 different inoculations (≈6000 and 600000 spores/mL), resulting in a high assimilation rate when the inoculation was higher. The effect of agitation on morphology and glycerol's uptake rate was evaluated in batch bioreactor experiments at 200 and 800 rpm, resulting in pellet formation when in low agitation, while mycelia were formed in high agitation, decelerating the glycerol consumption. The above results indicate that glycerol is a competitive substrate for biomass and PUFAs/GLA production by C. elegans, the biosynthesis of PUFAs is strongly dependent on mycelial growth (favored in low C/N ratios), as their main function is related to their presence into the mycelial membranes, while pellet formation combined with high inoculum size enhance high productivity.

The current study was funded by the project entitled "Biotransformation of glycerol into high pharmaceutical-value poly-unsaturated fatty acids (PUFAs)" (Acronym: Glycerol2PUFAs, project code HFRI-FM17-1839), financed by the Hellenic Foundation for Research & Innovation (H.F.R.I.), Nea Smyrni - Greece (project action: "1st Call for H.F.R.I. Research Projects to Support Faculty Members & Researchers and Procure High-Value Research Equipment").

PP102

Isolation and characterization of the multi-component carbazole dioxygenase driving the transformation of thiabendazole by a soil bacterial consortium

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Thiabendazole (TBZ) is a benzimidazole fungicide that is utilized for preventing postharvest fungal infestations in fruits. In Europe, monitoring programs have shown that TBZ is frequently detected in fruits at levels that pose a risk to consumers. At the same time, it is also one of the most prevalent pollutants found in surface water systems in the Mediterranean region. In view of this, it is essential to take immediate action to limit its dispersion. Previous research conducted in our laboratory has demonstrated that a bacterial consortium was efficient in degrading the fungicide TBZ, and has highlighted the vital role of Sphingomonas in its degradation. Meta-transcriptomic and meta-proteomic analyses have suggested that Sphingomonas activates a carbazole dioxygenase operon during the initial cleavage of TBZ. Carbazole is an N-heterocyclic aromatic hydrocarbon that is structurally similar to TBZ. The aim of this study is to verify the role of carbazole dioxygenase (CarAaAcF), the meta-cleavage enzyme (CarBaBb) and the meta-cleavage compound hydrolase (CarC) in the transformation of TBZ. Each protein was overexpressed in Escherichia coli strain BL21 (DE3) with a histidine tag and was subsequently purified. The functionality of the purified proteins in the degradation of their substrates has already been successfully tested in in vitro assays. Currently, the ability of the purified proteins to degrade TBZ is being tested and the result will be presented at the conference. These enzymes will be the first ones reported to be capable of detoxifying TBZ. Their application in appropriate formulations would be an innovative approach in removing TBZ from agro-food produce and the environment.

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PP103

Secretome analysis of Pleurotus citrinopileatus grown on different lignocellulosic substrates

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White-rot basidiomycetes possess a unique ability to produce both hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) enzymes, making them highly desirable for the discovery of novel enzymes with exceptional properties for lignocellulosic biomass degradation. In this study, we focused on Pleurotus citrinopileatus LGAM 28684, a wild strain of Basidiomycetes fungus collected from Greek habitats. Our aim was to investigate the enzymatic response of P. citrinopileatus when grown on different lignocellulosic substrates. Proteomic analysis was performed on the secretomes induced by corn stover, xylose and beachwood. The secretome analysis of P. citrinopileatus enabled us to elucidate the enzyme arsenal employed by the fungus for efficient degradation of lignocellulosic substrates. Furthermore, the study revealed the expression patterns of individual carbohyate-active enzymes (CAZymes) in response to the specific substrates, and network analysis of the protein intensities identified clusters of co-expressed enzymes. This comprehensive characterization of its enzymatic repertoire and substrate-specific expression patterns will facilitate the targeted selection of novel enzymes with diverse functionalities for a broad range of biocatalytic applications requiring the saccharification of lignocellulose as a first step.

Acknowledgements

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H.F.R.I. Hellenic Foundation for Research & Innovation

Keywords: *Pleurotus citrinopileatus*, lignocellulose degradation, proteomics, secretome, CAZymes

PP104

Enzymatic treatment of extracts derived from natural sources for the enhancement of their biological activities

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Origanum dictamnus (dittany) is a native herb to the island of Crete which has been employed since antiquity as a remedy for various diseases1. Recent evidence has supported the biological activities of extracts derived from dittany, such as antioxidant and antimicrobial, which are mainly attributed to their unique polyphenolic ile2. On the other hand, biocatalysis is often used for the targeted modification of extracts of natural origin in a try to enhance their biological activities. Among the enzymes that are often used are oxidoreductases3. Alongside, hydrogels employing phenolic compounds are gaining more and more interest for their application in pharmaceutical industry, for example in wound healing, due to their antioxidant and antibacterial activities4,5. In the present study, aqueous and methanolic extracts from Origanum dictamnus, rich in phenolic compounds, were prepared and studied for their polyphenolic profile, antioxidant and antimicrobial activity against Gram positive and Gram negative bacteria, before and after their enzymatic treatment with oxidoreductases. The main polyphenolic compounds contained in the extracts were further successfully used for the preparation of enzymatically crosslinked biopolymer-based hydrogels in the presence of a deep eutectic solvent (DES), which were studied for their antimicrobial activity.

Keywords: dittany; extract; oxidoreductase; antimicrobial; hyogel; DES

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PP105

The enzymatic machinery of the white-rot basidiomycete Abortiporus biennis can cause significant modifications to polystyrene structure

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Abortiporous biennis LGAM 436 strain is a saprophytic white-rot fungus, known for its ability to express oxidoreductive enzymes such as laccases and other lignocellulose-acting enzymes (Zerva et al., 2023). This basidiomycete possesses the enzymatic arsenal to oxidize phenol-containing media, such as olive oil mill effluents and waste (Aggelis et al., 2002), so the potential of this strain was also tested in polystyrene (PS); a recalcitrant plastic with a backbone of phenyl groups. Interestingly, A. biennis could sufficiently grow on different PS materials such as amorphous PS and commercial expanded polystyrene (EPS) foam, expressing high titers of laccase activity. Considering plastic properties after treatment, the basidiomycete caused modifications to PS molecular fingerprint, oxidizing carbon ato located in benzene rings while microscopy analyses detected alterations in the polymer microstructure. The molecular weight reduction was one more piece of evidence that verified PS biodegradation demonstrating the biotechnological potential of the unexplored enzymatic machinery of this white-rot basidiomycete. Proteomics analysis in the presence of PS and expression of the most promising enzymes are the future goals of this study which ai to decode and analyze the mechanism of PS functionalization by a wood-decomposing microorganism.

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PP106

Sustainable Exploitation of Bio-Based Compounds Revealed and Engineered from Natural Sources

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The SECRETed project aim to tap into the potential of marine and extremophilic bacteria to produce tailor-made amphiphilic compounds for the agrochemical, pharmaceutical, cosmetic, and chemistry sector. Novel hybrid compounds will be developed by leveraging the potential of aquatic biotechnology and by employing systems and synthetic biology tools. Biosurfactants, which possess surface-active properties and tend to adsorb at interfaces, and siderophores, which can chelate and transport Fe3+ ions, play a crucial role in this endeavor since the amphiphilic nature of these biosurfactants and marine siderophores presents an exciting opportunity to develop biosynthesis methods that facilitate the interchange of their hydrophobic and hydrophilic chemical components. By combining the unique properties of these compounds, new applications can be explored. The key objectives of the project include; constructing a comprehensive database that encompasses genomic and chemical information related to siderophore and biosurfactant production pathways, gene clusters, chemical structures, and physiochemical properties; optimizing the production and purification processes of these compounds; and throughout machine learning algorithms, based on new experimental and computational data, to build a unique microbial amphiphilic compound chemical space to identify the desired genetic mechanism. Detected genes will be reverse engineered to standardize and modularize associated metabolic elements for the production of non-natural biosurfactants and siderophores with a purpose to expand their benefits for industrial-driven formulations on suitable microbial hosts.

SECRETed project was funded by the European Union's Horizon2020 Research and Innovation Programme (Grant Agreement No. 101000794).

PP107

Novel Yarrowia lipolytica strains obtained through adaptive laboratory evolution and targeted genetic modifications for single-cell-oil production

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The increase in energy demand, oil prices, global political instability and climate change have intensified the need for development of alternative 2nd generation biofuels. Therefore, researchers have focused on microbial oils from oleaginous yeasts, which have similar fatty acid composition with vegetable oils, to attain a sustainable biodiesel production[1]. Among the oleaginous yeasts, Yarrowia lipolytica is a popular candidate for single-cell-oil production. Y. lipolytica has the ability to accumulate and store intracellular lipids, growing on crude glycerol (a by-product of the biodiesel industry), as a low-cost carbon source.

To increase the lipid content of the yeast, two successive approaches for metabolic enhancement were followed. At first, Y. lipolytica MUCL 28849 underwent Adaptive Laboratory Evolution, resulting in 450 strains with improved crude glycerol metabolic characteristics[2]. Then, the superior evolved strain, YLE155, was modified through genetic engineering to alter the overexpression or to delete genes important for lipid bioaccumulation. In this strain, an additional copy of the endogenous diacylglycerol acyltransferase type 2 gene (DGA2) and the phospholipid:diacylglycerol acyltransferase gene (LRO1), were integrated into the genome of Y. lipolytica under the control of native strong promoters. In the engineered strain with the additional DGA2 and LRO1 genes, carnitine O-acetyltransferase gene (CAT2) of S. cerevisiae was integrated. The novel engineered strains were grown in 7.5% v/v crude glycerol-synthetic medium and examined for their physiology, i.e., biomass accumulation, glycerol consumption and lipid accumulation. Lipid characterization, using flow cytometry and a colorimetric method based on sulfo-phospho-vanillin reaction (SPV), showed an increase in fluorescence for the engineered strains, reaching a lipid content 1.6-times higher than the parental strain. These results indicate the beneficial synergistic action of the three genes in lipogenesis and the formation of lipid bodies, and provide strong evidence that the novel engineered strains can achieve higher lipid yields, operating as efficient “lipid production factories”.

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PP108

Enhancing Heterologous Protein Secretion in Yeasts: Combining Secretory Peptides and Genetic Modifications

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Fungi have become increasingly popular as tools for heterologous protein production and secretion due to their ability to provide a sufficient yield of secreted proteins and carry out post-translational modifications required for producing Eukaryotic proteins (1). In this study, we aimed to explore new ways and combine secretory peptides with simple genetic modifications in Saccharomyces cerevisiae to enhance protein secretion. Two secretion vectors were constructed containing secretory peptides SP1 (2) and OST1-SP (3), along with a fluorescent reporter gene. These vectors were transformed into yeast strains EGY48 and BY4741, as well as ten mutant strains, carrying single gene deletions, found to increase overall protein secretion. We also tested the overexpression of COPII protein ERV29, which is involved in Golgi transportation (3). The effect of these modifications was measured through fluorescence intensity detected in the cultures' supernatant.

Analysis showed that the signal peptide OST1-SP was more effective in secretion than SP1. Three of the tested mutant strains, Δgos1, Δvps5, and Δykr078w, were also found to be compatible with OST1-SP, yielding higher mCherry secretion than the parental strain. Overexpression of ERV29 was shown to be the most effective modification on BY4741 cells but did not synergize with the tested mutant strains.

Overall, this study has identified new ways to improve protein secretion in S. cerevisiae, by combining specific signal peptides and genetic modifications, paving the way for improved methods on biotechnological research and engineering. Current research addresses the synergy of double mutation backgrounds and the transferability of the system into other yeasts.

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PP109

Lignocellulosic biomass to biogas: A biotechnological approach for Spent Mushroom Substrate valorization

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The proper valorization of lignocellulosic biomass, which is commonly produced in agro-industrial activities (global production ~180 billion metric tons, annually), can effectively decrease waste deposition, as an antidote to the environmental burden and provide useful raw materials if processed correctly, in terms of circular economy. Spent Mushroom Substrate (SMS) is an example of such a biomass; it is the main byproduct of mushroom cultivation and has become increasingly abundant due to the growing global production of edible and medicinal mushrooms (e.g. shiitake, oyster mushrooms, etc.), which are products of high nutraceutical and cosmeceutical value. The rising demand for energy has led to the exploration of alternative sustainable sources combined with biowaste utilization, such as the anaerobic digestion of agro-industrial residues. The current study focuses on the pretreatment of SMS and the biotechnological utilization of SMS hydrolysates for biogas production. Various pretreatment processes were examined, with a focus on chemical hydrolysis combined with thermal treatment of the waste streams. The hydrolysate obtained from the acidic chemical hydrolysis, which presented the highest concentration of free sugars (approximately 36 grams per 100 grams of dry SMS with a hydrolysis yield of approximately 75% w/w of holocellulose), was mixed with cattle manure and evaluated as a potential feedstock for biomethane production in a laboratory bench-scale digester. During a 15-day trial of anaerobic co-digestion, 52 liters of biogas per kilogram of volatile solids (VS) were produced, containing 65% methane. Conversely, the alkaline hydrolysate resulted in a pulp-like substance due to the disruption of the lignocellulosic matrix, without releasing additional sugars, and the biogas production was delayed for several days. The biogas yield value for this process was 37 liters of biogas per kilogram of VS, containing 62% methane. Based on these findings, it can be concluded that SMS can be valorized as an alternative medium for anaerobic digestion when pretreated with both chemical and hydrothermal hydrolysis.

This current investigation has been co-financed by the European Regional Development Fund of the European Union and Greek national funds (European Social Fund – ESF) through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE -INNOVATE (project code: T1ΕΔΚ-05027), scientifically coordinated by the Hellenic Agricultural Organization – DEMETER (Institute of Technology of Agricultural Products/ Laboratory of Edible Fungi).

PP110

Extraction, characterization and potential applications of microalgal bioactive polysaccharides of strains isolated from Greek coastal lagoons

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Microalgae have been of great scientific and industrial interest in recent years as alternative and sustainable sources of high-value metabolites, such as polysaccharides, lipids, proteins, vitamins or biopigments, due to their nutritional and pharmaceutical properties. The intracellular, structural, cell-bound and released extracellular bioactive polysaccharides (PS), particularly rich in sulfated groups, beta-glucans, galactans, etc., are of high interest in food, biomedical and cosmetic applications due to their antimicrobial, anti-inflammatory, antioxidant, hypolipidemic and hypoglycemic properties and are also biocompatible, biodegradable, and non-toxic. In the present study, 14 newly isolated strains of microalgae from Greek coastal lagoons, mainly belonging to the genera Tetraselmis sp. and Dunaliela sp. were evaluated to produce bioactive PS. First, the content of total sugars was determined; the 2 strains, namely Tetraselmis verrucosa f. rubens PLA1-2 and Tetraselmis sp. T3-1 presented the highest contents (40.9 and 32.6 g/100 g total y biomass - TDB, respectively), and the content of beta-glucans was enzymatically determined in the 4 strains with PS content higher than 20 g/100 g TDB. The two aforementioned strains also had the highest contents, i.e. 27 and 25 g β-glucans per 100 g total sugars, respectively. For these two strains, PS extraction was performed, and crude PS extracts were isolated after cell disruption and removal of proteins, lipophilic and residual substances. The crude extracts were rich in glucans (about 66-74%), followed by galactans (about 21%) according to monosaccharides analysis. Then, fractionation was performed by ion exchange (DEAE cellulose and NaCl solutions 0.2, 1, 2 M for elution) and three different eluents were obtained. Subsequently, they were characterized for the concentration of total sugars, sulfated PS, β-glucans, and their antioxidant capacity. Among the eluents, the two fractions (1 M and 2 M NaCl elution) of the strain T. verrucosa f. rubens PLA1-2 presented high contents of sulfated polysaccharides (74% and 85% of total sugars, respectively), antioxidant activity (ca. 3 μM Trolox), while only in the latter fraction β-glucans were detected (20%), considering it an eluent rich in bioactive molecules, with anti-inflammatory, antimicrobial and antioxidant activity and potential application in the healing of chronic or acute wounds.

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PP111

A FUNGAL TRANSFORMATION VALIDATION WORKFLOW FOR CRISPR/CAS9 MEDIATED RNAi MUTANTS

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Endophytic filamentous fungi have been previously shown to have a great potential in agriculture, due to their unique properties that enhance the performance of the host plants. Our main focus is the non-model organism Fusarium solani strain K (FsK), a strain that provides biotic and abiotic stress tolerance to host plants. Aiming to develop an efficient and fast platform to obtain mutant strains of the endophyte to study its mode of action, we opted for, a CRISPR/Cas9- based tool. Filamentous fungi pose a high level of difficulty in integrating the CRISPR/Cas9 technology due to their complex DNA repair mechanisms. To achieve reliable FsK mutant strains we propose a workflow, for the evaluation of both the fungal transformation, as well as the desired mutagenesis efficacy. The first step of evaluation relies on the presence of selective markers and reporter genes, thus providing macroscopic phenotypic evidence of a successful transformation of FsK. Then the workflow focuses solely on the extraction of the genomic fungal DNA and the further treatment that must undergo, to examine the efficiency of the CRISPR/Cas9 system on the desired genetic loci, through sequencing and in silico analysis. In the current study, progress in targeting the genetic loci for core RNAi genes of the endophyte will be described.

The project is funded by the General Secretariat for Research and Technology of the Ministry of Development and Investments under the PRIMA Programme. PRIMA is an Art.185 initiative supported and co-funded under Horizon 2020, the European Union's Programme for Research and Innovation (PRIMA2018-05).

PP112

EVALUATION OF IN VITRO PROTECTIVE EFFECT AGAINST OXIDATIVE STRESS ON HUMAN COLON CELLS OF MICROALGAE EXTRACTS DERIVED FROM HYOPONIC EFFLUENTS

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Microalgae can be a promising and sustainable candidate in wastewater treatment, as they have the capacity of efficiently recovering nutrients from agricultural wastewaters, while simultaneously producing high value added bioproducts with various biotechnological applications1. In the present study, effluent water from tomato hydroponic cultures was used for isolating naturally present microalgal species that could possibly be of biotechnological interest. The microalgal strains were subsequently cultured under steady conditions in both commonly used media and in hydroponic effluent to estimate the bioactivity of the biomass obtained. Thus, extracts were produced by freeze-dried biomass of the microalgae strains cultured in both regimes with effective cell disruption protocols. Characterization of the produced extracts was carried out for evaluating their antioxidant capacity as well as total phenolic and flavonoid content. In vitro cytotoxicity of the microalgae extracts was also assessed on human colon cell line (Caco-2). Finally, the protective role and potential applications of the extracts against H2O2 induced cells, was evaluated by MTT method. In order to have a deeper insight into the molecular mechanisms of the microalgae strains biological activity, we studied transcript accumulation of oxidative stress related genes. The study showed that extracts produced by species cultured in wastewater provide new insights into their beneficial role as sustainable feedstock of value-added extracts for many applications.

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The project is funded by the General Secretariat for Research and Technology of the Ministry of Development and Investments under the PRIMA Programme. PRIMA is an Art.185 initiative supported and co-funded under Horizon 2020, the European Union's Programme for Research and Innovation.

PP113

Enhancement of Desulfurization via Targeted Genetic Engineering in the Model Biocatalyst Rhodococcus qingshengii IGTS8

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Biodesulfurization poses as an ideal replacement to the high cost hydrodesulfurization of the recalcitrant heterocyclic sulfur compounds, such as dibenzothiophene (DBT) and its derivatives. The increasingly stringent limits on fuel sulfur content intensify the need for improved desulfurization biocatalysts, without sacrificing the calorific value of the fuel. Selective sulfur removal in a wide range of biodesulfurization strains, as well as in the model biocatalyst Rhodococcus qingshengii IGTS8, occurs via the 4S metabolic pathway that involves the dszABC operon, which encodes enzymes that catalyze the generation of 2-hydroxybiphenyl and sulfite from DBT. Here, using a homologous recombination process, we generate two recombinant IGTS8 biocatalysts, harboring native or rearranged, nonrepressible desulfurization operons, within the native dsz locus. The alleviation of sulfate-, methionine-, and cysteine-mediated dsz repression is achieved through the exchange of the native promoter Pdsz, with the nonrepressible Pkap1 promoter. The Dsz-mediated desulfurization from DBT was monitored at three growth phases, through HPLC analysis of end-product levels. Notably, an 86-fold enhancement of desulfurization activity was documented in the presence of selected repressive sulfur sources for the recombinant biocatalyst harboring a combination of three targeted genetic modifications, namely, a dsz operon rearrangement, a native promoter exchange, and a dszA-dszB overlap removal. In addition, transcript level comparison highlighted the diverse effects of our genetic engineering approaches on dsz mRNA ratios and revealed a gene-specific differential increase in mRNA levels.

PP113a

Advancing Sustainable Bioplastics through Microbial Upcycling for Novel Biopolymer Production

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This study presents an upcycling approach that involves converting commercially available bioplastics, such as polylactide (PLA), polycaprolactone (PCL), and polyhydroxyalkanoates (PHA), into carbon-rich feedstocks. These feedstocks can be used for the biotechnological production of raw biopolymers like PHA and bacterial cellulose (BC). The project's biotechnological platform aims to accelerate the green bioeconomy and make significant contributions to sustainability targets and goals, aligning with nine of the United Nations' Sustainable Development Goals (SDGs). These goals encompass improving health and well-being, waste and resource management, clean water and sanitation, and sustainable industrialization.

The methodology involved the development of fermentation strategies for carbon-rich feedstocks, with a focus on microbial conversion to PHAs. In the initial stage, flask experiments were conducted to test the ability of several bacterial strains to grow and produce PHA biopolymers. Hydroxyalated fatty acids were identified as suitable substrates, albeit exhibiting slight toxicity. Pulse feeding strategies were developed for each tested compound. Batch cultivations were carried out to obtain fundamental fermentation parameters. Based on these results, feeding strategies were optimized to maximize cell density (CDW) and PHA content. PHA polymers were extracted using solvent processing at room or elevated temperatures.

Preliminary results indicate promising potential for the production of PHA polymers using various bacterial strains tested in shake flasks. Hydroxyalated fatty acids have shown to be suitable substrates, despite their slight toxicity. Pulse feeding strategies have been developed for these compounds. Additionally, initial batch fermentations have provided fundamental fermentation parameters. These findings highlight the potential of these substrates for producing different types of PHA polymers.

This research contributes to the advancement of sustainable biomaterials, biopackaging innovation, affordable and clean energy, and land conservation, while addressing important global sustainability goals.

Keywords:

Bio-upcycling, Bioplastics , Biodegradable materials, PHA polymers, Fermentation strategies, Sustainable biomaterials

PP113b

Insights into a bifunctional catalase-phenol oxidase from a marine-derived Cladosporium species

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The biodiversity of fungi and their extensive enzymatic wealth have placed these microorganisms at the forefront of contemporary environmental and industrial research. The last decade, marine-associated Cladosporium species have gained substantial scientific attention, owning to the production of a wide array of metabolites and the expression of diverse enzymes, pertinent to biotechnological applications [1]. A recent function-based study on the bioremediation potential of marine fungi against aromatic pollutants evinced Cladosporium sp. TM138-S3 as a strong candidate for polychlorinated biphenyls removal, and a typical monofunctional catalase was found upregulated under relevant culture conditions [2]. In this study, this catalase -hereafter termed CPO (Catalase Phenol Oxidase)- was recombinantly expressed in the yeast host *Pichia pastoris* and its biochemical and structural properties together with its catalytic potential are being assessed. CPO is a 250-kDa homotetramer of heme *b* containing subunits, capable of hydrogen peroxide decomposition following non-Michaelis Menten kinetics. It exhibits mildly alkaline pH optimum, mesophilic temperature stability, and halotolerant catalytic behavior. Its side phenol oxidase activity was assessed spectrophotometrically towards phenol, catechol, biphenyls, and select chlorinated compounds. CPO demonstrates clear catechol oxidase activity but absence of cresolase activity, indicative of substrate specificity towards benzene rings with two hydroxy substituents. In vitro inhibition studies appear to support the notion that heme *b* is the active site of the oxidative activity observed [3, 4]. Structural studies on ligand-bound CPO together with more extensive investigation of its catalytic potential are under way. This work provides a fresh view on a long-known class of enzymes and their unexplored capabilities for bioremediation and oxidation of unconventional substrates, as opposed to their traditional uses to the present day.

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Keywords
marine fungi; catalase; phenol oxidase; bioremediation; biocatalysis

PP113c

Exploiting the Greek microbial diversity for the discovery and development of novel antiaging molecules.

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Actinobacteria from Greek ecosystems are renowned for producing bioactive compounds. To this purpose, the "AntiAging" project sought to uncover new, promising natural compounds with anti-aging properties, which could be incorporated into cosmeceutical/nutraceutical formulations. Herein, 1000 isolates originating from the Greek natural environment and belonging to the ATHUBA collection (Athens University Bacterial & Archaea Culture Collection), were investigated. A unique library of ~2000 extracts (EtOAc and MeOH/H2O) was generated, and the tyrosinase and elastase inhibition activity was evaluated via enzymatic assays. Out of the top 100 extracts with the highest inhibitory activity, 28 were found to be non-cytotoxic when tested on cell-based assays. Those extracts were then analyzed by HPLC-DAD-ELSD and UPLC-HRMS. The top 3 microbial strains, belonging to *Amycolatopsis* sp. and *Streptomyces* sp. families, were subsequently grown using four different cultivation media. The most promising cultures were subjected to a scale-up process (2L) and fractionation, and their bioactivity was further assessed. A customized bio-guided workflow was then applied, combining a high-throughput dereplication and molecular networking method (UPLC-HRMS), along with the bioactivity of each fraction. To meet the requirements of the isolation procedure, the microbial strain M43B was subjected to an 8L cultivation, extracted with EtOAc and fractionated using Sephadex® LH-20. The purified molecules were characterized by an extensive analysis of NMR and HRMS spectra data and will be further evaluated for their antioxidant activity, the proteasome activation and their impact on longevity of *in vivo* models (*Drosophilla* sp.). Our ultimate goal is the targeted identification and isolation of bioactive compounds, which can be integrated into cosmeceutical formulations. The plethora of identified molecules highlight the significant potential for exploiting Greek microbial diversity in the realm of anti-aging research.

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PP113d

Catabolic Sphe3 genes: gears in an E.coli cis, cis-muconic acid producing apparatus

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Pseudarthrobacter phenanthrenivorans Sphe3 is capable of catabolizing various aromatic compounds and was recently found to efficiently utilize phenol as the sole source of carbon and energy, at concentrations up to 1500 mg/L, mainly via the catechol ortho-cleavage route. HPLC analysis suggested, apart from catechol, the presence of cis, cis-muconic acid (ccMA), an intermediate of the ortho-pathway (1).

In the present study, the function of Sphe3 genes possibly coding for phenol hydroxylase and catechol 1,2-dioxygenase (C12O) was validated by heterologous expression in Escherichia coli and quantitative real-time PCR followed by biochemical characterization of the above enzymes.

On the other hand, ccMA is known for its industrial importance as a precursor molecule employed for the synthesis of a broad range of economically valuable compounds of polymeric in nature and can be converted into adipic acid and terephthalic which serve as the major platform chemicals for the production of bioplastics and polyesters including polyethylene, terephthalate and nylon-6,6 (2).

Considering ccMA is produced in a single step by the ring cleavage of catechol by C12O through the β-ketoadipate pathway, recombinant E. coli cells expressing C12O gene from Sphe3 were used as microbial factories for the biotechnological production of cis, cis-muconic acid as a high value-added bio-product. Recombinant E. coli cells were able to produce over 400 mg/L of ccMA an hour after being supplemented with 20 mM catechol, a yield much higher compared to other studies (3, 4). This is the first study on C12O gene from the metabolically versatile Sphe3, which was cloned and expressed in E. coli and successfully produced ccMA from catechol at faster rates in low-cost settings.

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PP114

Utilization of real-time monitoring technologies in a food Risk Assessment framework

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In recent years, risk assessors and other stakeholders of the food industry have shown an increased interest in risk-based food safety decision tools. Quantitative microbial risk assessment (QA) models customized for specific products or even for facilities and vertically integrated brands can support food business operators' decision-making. The ongoing development of sensors and on-line monitoring devices for QA-relevant environmental parameters can result in a "real-time" decision-making tool. Based on the above, the concept of a product-specific, real-time QA model (RT-QA) as a decision-making tool for the food industry is outlined.

In traditional QA, data obtained from existing literature or databases, fitted to appropriate probability distributions, are used to populate the QA model. The risk outcome, therefore, relates to the food-pathogen combination under study. In RT-QA, assigned distributions are optionally substituted by point estimates for a particular case under study. These point estimates are collected by on-line sensing devices. In the food industry, the transportation and storage phases are the first candidates where on-line temperature data can be amassed with the purpose of being used in the model. This is because temperature is paramount for the safety/quality of food, as well as easy to collect/utilize by use of thermocouples or temperature data-loggers. The risk outcome in this case refers to the particular product or lot under surveillance.

The real time tracking of hazard levels enables the progressive (re)evaluation of consumer risk for the product or lot under study. The final risk estimate retains its stochasticity and becomes less variable as single value inputs are integrated. Additionally, since the consumer risk is updated based on the elapsed stages, the necessity for corrective measures to attain the accepted level of risk can be decided. The implementation of a RT-QA approach is expected to support food business operators' decision-making and minimize the risk imposed on consumers.

Acknowledgement

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PP116

THE CO-EVOLUTION OF MITOCHONIAL AND NUCLEAR GENES ENCODING PROTEINS IMPLICATED IN THE CELLULAR RESPIRATION OF FUNGI

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Mitochondria are the energy-producing organelles in the cells of almost all eukaryotic organisms and derived from an α-proteobacterial endosymbiont in an archaeal ancestral host, according to the prevailing theory of endosymbiosis. Although mitochondria contain their own genome, the vast majority of genes – originally encoded in the endosymbiont – have either been lost or transferred to the nucleus. Thus, only a small number of genes remained within the organelle, comprising the mitochondrial genome. The mitochondrion can be functional, only when gene products that are involved in its function but encoded by nuclear genes interact with the mitochondrial gene products and form complete complexes. One such case is found in the assembly of the electron transport chain, consisting of Complexes I, II, III and IV and the ATP synthase or also known as Complex V. All these complexes constitute the third step of aerobic cellular respiration, i.e., the oxidative phosphorylation pathway. As the most efficient energy production mechanism in eukaryotic organisms, the need to maintain the communication and functional integrity of the gene products that compose it, despite their different genomic origin, is evident and renders the study of their coevolution – the main focus of this work – particularly interesting. Phylogenetic analyses of previously and newly annotated sequences of nuclear and mitochondrial subunits of all oxidative phosphorylation complexes in 102 species of fungi – belonging to Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Mucoromycota and Zoopagomycota phyla – shows a similar evolutionary history between mitochondrial and nuclear genes. In addition, comparative in silico analyses have shown putative genetic events – such as gene fusions, duplications and alternative transcripts – and have identified intron positions that are conserved between phyla and help further clarify evolutionary relationships between subunits. As such, this study provides insights into an extensive degree of co-evolution of genes involved in the same function (oxidative phosphorylation and ATP production) but located in different organelles. This is further supported by STRING database subunit associations.

Keywords: co-evolution; fungi; mitochondrion; nucleus; oxidative phosphorylation; phylogeny.

PP117

Food waste management for the production of animal feed

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The growth of environmental pollution raises the necessity for the development of new, feasible and sustainable technologies targeting the exploitation of the generated by-products in various sectors. Additionally, nowadays a significant issue has been emerged concerning the increased cost of feedstuffs, due to the elevated price of cereal grains and soybean products (Vasta and Luciano, 2011). Animal husbandry is considered as one of the fastest-growing sectors in agriculture, with most countries facing a shortage of required animal feed. Therefore, it is imperative to discover alternative sources for the production of high-quality animal feed, which could play a significant role in addressing this shortfall, with a parallel reduced cost. Municipal wastes form an intriguing case, since they can be adopted by this field in order to develop novel feedstuffs, due to their rich nutritional composition. The aim of this study concerns the biotransformation of municipal wastes into novel feedstuffs, with a parallel contribution to the reduction of environmental footprint as well as, the improvement of the nutritional composition of the developed fortified feedstuffs (Eliopoulos et al., 2022; Galanakis, 2012). Therefore, the combination of EU's directive on the landfill of waste (1999/31/EC) targeting the progressive reduction of biodegradable municipal waste sent to landfill sites to 35% of the 1995 disposal level by 2020 with the utilization strategies of latter wastes in livestock sector, confronts the disposal and pollution-associated problems and contributes to the need for environmental sustainability and conservation

Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

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PP118

Incorporation of pomegranate juice and its concentrate in sodium alginate for the production of "Smart" edible packaging for chicken minced meat

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Preservation of food through packaging has been a long-standing practice in the food industry. Traditional food packaging material, such as plastic and metal cause harmful consequences to ecosystem. As alternative method, natural material, like sodium alginate film have been used for the creation of biodegradable and sustainable food packaging. Alginate is a natural polymer extracted from brown seaweed and widely used in the food industry as a thickener, stabilizer, and gelling agent for film and edible coating production that contributes to meat preservation. Through the incorporation with natural extracts, such as pomegranate juice and its concentrate, sodium alginate film can enhance its functionality and extends product shelf-life. Pomegranate juice has been shown high levels of polyphenols, which provides antibacterial, antifungal properties and high antioxidant activity. Moreover, sodium alginate-pomegranate film has been demonstrated to possess pH-sensitive properties, which can enable the detection of pH changes in the packaged food through color.

In the present study, chicken minced meat was packed on sodium alginate- pomegranate fil and stored at 4°C. Based on the microflora growth during storage period, changes were noticed on the color of film depending on pH values. The mechanical properties of sodium alginate-pomegranate fil, such as tensile strength, young's modulus, and elongation at break were improved compared with control sodium alginate film. The addition of pomegranate juice and its concentrate combined with antimicrobial agents or plant extracts or without, worked as an inhibitor on spoilage growth, because of the low pH value of the package environment. More specific, an inhibition on fungi growth was observed. Also, the active film incorporated with pomegranate juice and its concentrate effectively delayed the lipid oxidation and prevented the formation of undesirable compounds that affects meat color and odor.

In conclusion, the use of sodium alginate fil with the inclusion of pomegranate juice and its concentrate has proposed as a potent smart packaging material, combining food preservation and food spoilage detection based on pH-sensitive film color changes.

This research was funded by the research program entitled 'Production of Processed meats by applying Novel Biotechnological Methods for increasing their shelf life, microbiological safety and nutritional value' (MIS number: 5033164), supported by the action 'Strengthening of small and medium-sized enterprises for research programs in the fields of agro-nutrition, health and biotechnology', co-financed by the European Union (European Regional Development Fund) and Greece, under the 'Operational Program Epirus 2014–2020' of the National Strategic Reference Framework.

PP119

The complex GH32 orchestra from Priestia spp. holds the key to better discriminate sucrose and fructan metabolism in bacteria

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β-Fructofuranosidases are part of the family glycoside hyolase 32 (GH32) enzymes and are well-known to hyolyze sucrose, as well as fructose-based oligo- and polysaccharides, called fructans. Bacteria able to metabolize fructans often harbor multiple GH32 members in their genome, Bacillus subtilis being one of the best studied models (Fouet et al., 1987; Martin-Verstraete et al., 1990; Pereira et al., 2001). In this study, multiple microorganisms from the lettuce phyllosphere were isolated, two belonging to the genus Priestia (previously Bacillus) and one identified as B. subtilis. Differences in the degradation pattern of inulin-type fructans in the extracellular fraction of these cultivates suggest activity of two putative inulinases (one more specific towards short-chain fructooligosaccharides; FOS) in Priestia spp., compared to only one in B. subtilis, in which inulin is solely hydrolyzed by the extracellular exo-fructosidase SacC. Available genomes of Priestia spp. were screened for GH32 members and compared to the model B. subtilis, confirming the presence of three distinct genes encoding predicted intracellular β-fructofuranosidases with high sequence identity to the single intracellular β-fructofuranosidase from B. subtilis; sucrose-6-phosphate hydrolase (SacA). We hypothesized that these enzymes differ in their substrate specificity, being either sucrose, sucrose-6-phosphate (Suc6P) or FOS. This was investigated by heterologous expression and subsequent characterization of these β-fructofuranosidases, referred to as SacAP1, SacAP2 and SacAP3. Our results show that SacAP2 is a broad exo-fructosidase, while SacAP1 and SacAP3 are only specific for sucrose. In addition, molecular docking analysis predicts that SacAP1, but not SacAP3, has a high affinity for Suc6P. Implications of these findings are discussed further in detail by comparing the amino acid sequences of SacAP1, SacAP2 and SacAP3 to other (already characterized) GH32 enzymes.

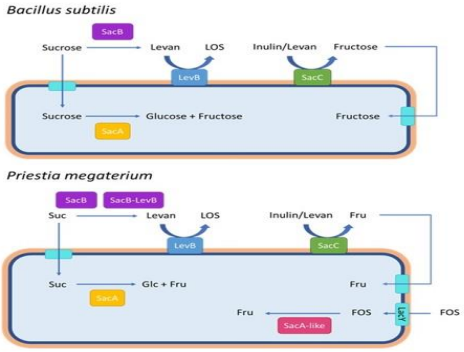

Figure 1. Sucrose and fructan metabolism in *Bacillus subtilis* and *Priestia megaterium*. In *B. subtilis*, sucrose can be transported intracellularly through the phosphotransferase system (PTS). SacP, the sucrose-specific PTS transporter, phosphorylates sucrose at the C6 of the glucose moiety upon import, which is subsequently hydrolyzed by a sucrose-6-phosphate hydrolase, also known as SacA. By contrast, fructans are exclusively degraded in the extracellular environment by either an endolevanase (LevB), only specific towards levan, or an exo-fructosidase (SacC), that releases fructose from both inulin- and levan-type fructans. The GH32-encoding genes in the genome of *P. megaterium* predicts the same mechanism, but three extra genes encoding predicted intracellular β-fructofuranosidases with high sequence identity to SacA (in the figure referred to as SacA-like) can be found. In this study, we show that one of them (SacAP2) is a broad exo-fructosidase, degrading not only sucrose but also fructooligosaccharides (FOS). Together with its neighboring gene encoding for a LacY proton/sugar family symporter, this suggest a role in intracellular import and hydrolysis of FOS in *P. megaterium*.

PP120

LESS IS MORE: DESIGN OF A MINIMAL ZYMOMONAS MOBILIS GENOME BY GENERATING TARGETED DELETIONS IN STRAIN ATCC 29191

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Global warming, climate change and the precariousness of non-renewable energy sources have rendered the research for alternative energy production, such as biofuels, more urgent than ever. The ethanologenic a-proteobacterium Zymomonas mobilis is a promising microorganism for the industrial production of bioethanol and other high-added value products, because of its ability to ferment simple sugars to ethanol and carbon dioxide to almost perfect yields. Strain ATCC 29191, the phenotypic centrotype of the species, has been isolated from fermenting Elaeis palm sap and has the smallest genome among sequenced Z. mobilis strains. Despite its small size, it exhibits robust sucrolytic activity and levan (polyfructan) production without considerable loss in ethanol yields and is, therefore, of interest to temperate environments where sugar-rich biomass substrates are abundant. Consequently, the ATCC 29191 genome constitutes an ideal starting point in order to study the effects of targeted genome reduction in Z. mobilis. In general, a genome free of dispensable sequences can confer various benefits, such as reduced metabolic burden, simplification of cellular and genetic processes and ease in genetic engineering applications. In this work, we used bioinformatic tools to identify the complement of redundant genes in ATCC 29191 and singled-out genomic islands that can be removed with site-specific recombination without compromising viability. As proof-of-concept, we are testing the efficacy of the Cre/loxP genomic recombineering system in the organism, and also attempt to cure the plasmids. Via the elimination of obvious redundant islands, the eminent goal will be the creation of a strain with at least 7% less genome compared to the parental strain and ca. 15% compared to industrial strains ZM4 and CP4. Furthermore, recognition and elimination of all possible dispensable genes could predictably double this amount.

PP121

A beneficial fungal endophyte triggers RNA silencing to an intronless but not to an intron-containing host reporter gene

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Fusarium solani strain K (FsK) is a non -pathogenic, endophytic fungus, previously isolated from the roots of tomato plants. It is a beneficial organism that confers resistance to biotic and abiotic stressors and also promotes plant growth. It can also colonize the roots of Nicotiana benthamiana and the whole body of Lotus japonicus. There is growing evidence that during interactions of plants with pathogenic fungi, there is bi-directional movement of small RNAs. However, the mechanisms of trans-kingdom RNAi during symbiotic relationships, are poorly understood. Previous work from our laboratory revealed that FsK encodes the core RNAi proteins (AGO1-2 and DCL1-2) and the machinery is functional. The goal of this experiment is to study small RNA transmission from a beneficial fungal endophyte to its host. To monitor if small RNA transmission takes place during the interaction of FsK with its host, we used gfp expressing Nicotiana benthamiana lines inoculated with an FsK transformant containing a transgene that targets host GFP. The effect of colonization levels of the root system by the endophyte was also tested. The efficiency of silencing mediated by FsK was monitored for a period of nine weeks, both visually under ultraviolet light as well as quantitatively by PCR. Finally, bisulfite sequencing was performed to assess the methylation levels of plant GFP.

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PP122

LAURUS NOBILIS AND ROSA CANINA PLANT EXTRACTS AS POTENT FUNCTIONAL REGULATORS OF FOOD MICROBIOME

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Although plant extracts have been used as biopreservatives for centuries, only recently have they gained scientific and industrial interest as growth stimulators of beneficial microbes. Thus, their addition at the optimum concentration may result in functional regulation of the food microbiome. In this vein, aqueous and ethanolic extracts from Laurus nobilis and Rosa canina plant material cultivated in the Epirus Region in Greece were prepared and their phytochemical profiles were unveiled by LC-triple quadruple and LC-QToF mass spectrometry. Subsequently, the growth stimulatory activity of the extracts on the wild-type Lacticaseibacillus rhamnosus OLXAL-1 (isolated from olives), L. casei ATCC 393, Lactiplantibacillus plantarum DSM 20174, Levilactobacillus brevis DSM 20054, Lactobacillus. delbrueckii DSM 20074 and the commercial Lacticaseibacillus rhamnosus GG was assessed by monitoring cell growth in the presence of the extracts at concentrations ranging 0.25–10 mg (dry matter)/mL. Moreover, the Minimum Inhibitory (MIC) and Minimum Bactericidal Concentrations (MBC) against the probiotic strains and foodborne pathogens were determined. The ethanolic extracts contained more quantities of flavonoids, while the aqueous extracts were richer in flavonoid glycosides, phenolic compounds and organic acids. L. nobilis aqueous extract stimulated the growth of L. rhamnosus strains at 1 mg (dry matter)/mL, L. plantarum and L. delbrueckii at 0.5 mg (dry matter)/mL, as well as of L. casei and L. brevis at 0.25 mg (dry matter)/mL. Similarly, R. canina aqueous extract stimulated the growth of all strains [at 2.5 mg (dry matter)/mL for L. brevis and at 0.25 mg (dry matter)/mL for the rest strains]. In contrast, no stimulatory activity was recorded by the ethanolic extracts. MIC of the aqueous end ethanolic extracts of both plants ranged 1.56-25 mg (dry matter)/mL and 6.25-25 mg (dry matter)/mL, respectively, for the pathogenic bacteria and 25-50 mg (dry matter)/mL for the probiotic strains. Interestingly, the foodborne pathogens tested were more sensitive to the extracts than the probiotic strains. Our results demonstrated for the first time that the L. nobilis R. canina aqueous extract stimulated the growth of beneficial microbes, while both aqueous and ethanolic extracts inhibited the growth of pathogens, indicating their potential use as functional regulators of food microbiome.

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PP123

Medium chain length polyhyoxyalkanoates (mcl-PHA) model compounds for the discovery of novel PHA depolymerases

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PHAs are naturally made microbial polyesters that have been commercialized as biodegradable plastics. However, it has been shown that these materials are not so easily biodegraded in natural environments [1]. PHA depolymerases are key PHA degrading enzymes and their identification and characterization is of great interest and importance. Currently, screening is done on polymeric substrates using techniques such as clear zone assays on agar or weight loss measurements. Results obtained using these different methods cannot be directly compared, since they depend highly on the polymer used, PHA granules preparation and assay conditions [2].

In order to design a more specific test for the determination of PHA depolymerase activity, we synthesized 3-hyoxyalkanoate monomers (3-HA monomer) and 3-hyoxyalkanoic acid dimers (3-HA dimer) and their respective p-nitrophenyl esters, allowing for spectrophotometric determination of their activity [3]. Compounds were characterized using N and FTIR. Para-nitrophenyl labeled substrates were then used in the enzymatic activity assay with the benchmark polyhyoxyoctanoate (PHO) depolymerase from Pseudomonas fluorescens GK13 expressed in Escherichia coli CodonPlus-RIPL hosts. This activity was compared to recombinantly expressed leaf-branch compost cutinase (LCC cutinase) and polyethyleneterephtalate (PET) hyolyzing esterase from Ideonella sakaiensis (IsPETase). Our initial results revealed increased specificity of PHO depolymerase towards newly synthetized substrates, suggesting their suitability for specific screens and isolation of new mcl-PHA depolymerases, as well as in high throughput screening assays designed for guiding their directed evolution.

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PP124

ELUCIDATING THE EVOLUTION OF EARLY DIVERGING FUNGI: THE ROLE OF THE MITOCHONIAL GENOME

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Early diverging fungi (EDF), the oldest living fungal species, are known for their variable interactions with plants, animals, bacteria and other microbes, as the majority of them are either parasites or endosymbionts. Their study is essential for clarifying the precise fungal route from aquatic to terrestrial lifestyle, providing information regarding the first evolutionary events in the kingdom of fungi. However, the classification and phylogeny of EDF lineages is still unresolved since related studies are limited. Mitochondrial (mt) DNA is an alternative significant tool for phylogenetic purposes, as it has a different evolutionary rate compared to the nuclear DNA. The necessary genes for the process of oxidative phosphorylation, tRNA and rRNA genes, that are located in mt genomes, can be used as molecular markers in taxonomic, phylogenetic and evolutionary analyses. In this work, we present a comparative phylogenetic study of 62 mt genomes that portrays the evolutionary relationships of the main EDF phyla, i.e. Cryptomycota, Aphelidiomycota, Sanchytriomycota, Chytridiomycota, Blastocladiomycota, Zoopagomycota, Mucoromycota.
Since the mtDNA of most EDF was not characterized, a manual annotation for the majority of them was necessary in order to proceed.

A concatenated matrix of the 14 mt genes implicated to the oxidative phosphorylation was used, in order to construct phylogenetic trees by employing both Neighbor- Joining and Bayesian methods. Synteny, intron analysis and diversity of intergenic regions are also included in this study.

Ancestral intron insertion positions in cox1, cox3, cob and nad5 genes have been determined. Although the sequences of the 14 genes were quite conserved as expected, their synteny presented great variability and syntenic units could be mainly found within species in the same genus. This diversity contributes to gene shuffling which is further supported by the presence of various G4 quauplexes in many mt genomes. Therefore, important conclusions can be awn for the mitogenomic evolution of these early diverging fungal phyla.

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PP127

Speed up Mosquito Control: Innovative Approaches to Identify Invasive Species and Detect Vector-Borne Viruses

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Invasive mosquito species have become a major public health concern worldwide due to their potential to cause harm to human health, the economy, and the ecosystem. These species are particularly known for transmitting viruses such as Zika (ZIKV), Dengue (DENV), Chikungunya (CHIKV) and West Nile (WNV), which can cause a range of symptoms, from mild fever to more severe complications or even death. The invasive species Aedes albopictus has already been confirmed to be present in Greece and Europe, while Aedes aegypti has not yet been well established in the continent. Both species look very similar, making it challenging to distinguish between them based on their morphology alone. Accurate identification of these species and the viruses they may carry is a keystone for effective control and disease prevention. The current study introduces two novel and rapid assays: an ELISA-based approach to differentiate the mosquito species Ae. albopictus and Ae. aegypti (MQ-kit), and a DNA-hybridization-based technique designed to detect the presence of viruses transmitted by mosquitoes (VectorChip). To determine the protein to be used in designing the ELISA assay, a differential gene expression analysis was conducted based on 45 publicly available RNA-seq data that included males and females of both species at adult and teneral stages. The results indicated that 172 and 241 genes were expressed differently in Ae. albopictus and Ae. aegypti respectively. Proteins encoded by those genes were mostly related to ribosomal and structural molecule activity. The highly differentially expressed gene was selected from each species and undergo qPCR validation using appropriate primers before the antibody synthesis. With regards to VectorChip, the genomes of ZIKV, DENV, CHIK and WNV were retrieved from the NCBI database followed by the design of 70-mer length probes that can hybridize with the target viruses. After the in-silico validation of the specificity of the designed probes, 50 probes including 10 probes for each virus with five for each of the four DENV types (type1, type2, type3 and type4) were selected for the experimental test using a Southern hybridization. The experimental validation of both assays is currently in progress and the results will be presented

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PP128

EVALUATING THE EFFECTS OF NOVEL PROBIOTIC STRAINS IN HEALTHY MICE

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Gut microbiota is a complex and dynamic community which maintains a symbiotic relationship with the host and participate in several processes. In the past years, specific changes in the gut microbial community have been associated with a number of human diseases; notably probiotic administration has been shown to have beneficial effects in such diseases. However, little is known regarding the effects of probiotics on healthy individuals. To this end, we are studying the effect of two novel probiotic strains namely Lactiplantibacillus pentosus and Lactococcus lactis in healthy mice following a 6-week intervention by analyzing the gut and fecal microbial community along with a series of biochemical tests and histological evaluation.

PP129

Novel Greek Streptomyces strain with antimicrobial activity against multi-drug resistant pathogens

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There has been an alarming rise in microbial drug resistance over the past decades, making it one of the most critical global health concerns. Misuse and overuse of antimicrobial drugs has caused the prevalence of resistant pathogens, leading to increased healthcare costs, treatment failure, and mortality. In this context, discovering novel bioactive compounds is of major importance. Streptomyces represent the most prominent genus of bacteria able to produce bioactive compounds with antimicrobial activity and with many medical applications. Greek habitats, mainly due to their Mediterranean location and distinct microclimatic conditions, harbor a plethora of streptomycete strains with antimicrobial potential. This work aimed to explore the metabolic repertoire of Greek Streptomyces and to address the emerging problems of multiple antibiotic resistance. For this purpose, streptomycetes from the Athens University Bacterial & Archaea Culture Collection (ATHUBA), some of which have been isolated from unique environments (caverns, volcanoes, thermal springs, etc.), were studied for their ability to produce antimicrobial compounds. We selected multi-drug resistant pathogens as targets, isolated from Greek hospital clinical samples, that were prioritized as emerging hazards for public health both at national and global level. 100 Streptomyces strains were tested against multi-drug resistant Klebsiella pneumoniae and Candida auris cultivated antagonistically in solid cultures. Streptomyces sp. ATHUBA 263 strain caused strong pathogen inhibition. Antimicrobial activity-guided fractionation was performed for the isolation of the corresponding bioactive compounds in culture supernatants by using semi-preparative HPLC analysis coupled with diode array UV-VIS detection. In silico analysis of secondary metabolite biosynthetic gene clusters in whole genome sequence generated via PacBio of the selected strain, led to the identification of 29 clusters related to secondary metabolite biosynthesis, some of which may be responsible for the antimicrobial activity against Klebsiella pneumoniae and Candida auris. These results suggest that Streptomyces sp. ATHUBA 263 strain can potentially produce novel bioactive compounds, that could be used as part of the arsenal to combat the urgent threat of multiple antibiotic resistance.

PP130

Understanding the formation and inactivation of biofilms of the pathogenic microorganism Legionella pneumophila

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Legionella pneumophila, the causative agent of Legionnaires' disease, constitutes one of the main colonizers of public buildings' water systems. The ability of the microorganism to form biofilms results in its improved ability to survive, and further spread inside the water supply systems. Subsequently, controlling biofilm formation can be considered a cornerstone in order to confront L. pneumophila effectively. Implementation of effective control measures in colonized water settlements is proven to be of great importance due to the widespread of the pathogen in such systems. One of the most common techniques is water chlorination. There is, however, a dearth of knowledge regarding the formation, as well as the inactivation, of L. pneumophila biofilms, and therefore the effectiveness of potential control measures. The objective of the present study was the assessment of the effect of some of the most relevant factors on the formation of mono-species L. pneumophila biofilms, as well as the effect of different sodium hypochlorite concentrations on their inactivation. Initial cell concentration of planktonic cells in the water, water pH, incubation conditions, and the presence of ions were the factors under study. Stainless steel coupons emerged in sterile bottled natural mineral water were used for biofilm formation. Regarding biofilm formation, the results indicated that the majority of the factors studied showed statistically significant effects. The initial cell population level was found to have the most effect. With regards to biofilm inactivation, considerable L. pneumophila population reductions were observed even at low sodium hypochlorite concentrations. The findings of the present study can help better elucidate the underlying mechanisms of formation and inactivation of L. pneumophila biofilms, and further contribute to the effective control of the pathogen in water systems

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PP131a

A panoramic view of the genomic landscape of the genus Streptomyces

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We delineate the evolutionary plasticity of the ecologically and biotechnologically important genus Streptomyces, by analysing the genomes of 213 species. Streptomycetes genomes demonstrate high levels of internal homology, whereas the genome of their last common ancestor was already complex. Importantly, we identify the species-specific fingerprint proteins that characterize each species. Even among closely related species, we observed high interspecies variability of chromosomal protein-coding genes, species-level core genes, accessory genes and fingerprints. Notably, secondary metabolite biosynthetic gene clusters (smBGCs), carbohydrate-active enzymes (CAZymes) and protein-coding genes bearing the rare TTA codon demonstrate high intraspecies and interspecies variability, which emphasizes the need for strain-specific genomic mining. Highly conserved genes, such as those specifying genus-level core proteins, tend to occur in the central region of the chromosome, whereas those encoding proteins with evolutionarily volatile species-level fingerprints, smBGCs, CAZymes and TTA-codon-bearing genes are often found towards the ends of the linear chromosome. Thus, the chromosomal arms emerge as the part of the genome that is mainly responsible for rapid adaptation at the species and strain level. Finally, we observed a moderate, but statistically significant, correlation between the total number of CAZymes and three categories of smBGCs (siderophores, e-Polylysin and type III lanthipeptides) that are related to competition among bacteria.

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PP132

Evaluation of biofilm formation on oenological surfaces by new isolated Brettanomyces bruxellensis strains

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Quite a number of microorganisms have been reported to be present on grape surfaces and in different phases of winemaking process. Among these microorganisms, there are beneficial species, such as Saccharomyces cerevisiae, but also spoilage ones that can ruin wine quality. The most fearful wine spoilage yeast belongs to the species of Brettanomyces bruxellensis. The development of B. bruxellensis in wine can change the sensory properties of wine due to the production of undesirable aromas. The present work focuses on the biofilm-forming ability of B. bruxellensis, derived from Greek wines, on stainless steel surfaces. Seventeen wines from different regions of Greece were collected and subjected in molecular analyses and identification at species level. RAPD (Random Amplified Polymorphic DNA) genomic fingerprinting with the oligo-nucleotide primer M13 was used, combined with Matrix Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF) technique. Strain differentiation of B. bruxellensis different strains was achieved by rep-PCR fingerprinting method with the oligo-nucleotide primer GTG5. For the biofilm formation assay, stainless steel coupons were placed in test tubes containing sterilized Ringer solution and pure cultures of the different B. bruxellensis strains, were inoculated at an initial population of approximately 107 CFU/mL. The tubes were incubated at 28 °C for 3 hours to allow attachment of the yeast cells onto the coupons surface. Biofilm growth was evaluated with the bead vortexing method. The molecular analysis revealed the presence of the spoilage species in 17,6% of the tested wines while four different strains of B. bruxellensis were identified. Additionally, based on the phenotypic analysis, strain effect was observed for the attachment and biofilm formation capacity of the spoilage yeast.

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PP133

ENDOMETRIAL MICROBIOTA AND IN VITRO FERTILIZATION

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INTRODUCTION: Genital microbiota and its impact on the success of in vitro fertilization (IVF) has generated immense interest in recent years, nevertheless there is conflicting evidence regarding the association of endometrial microbiome and pregnancy rates. This study sought to determine the endometrial microbiota composition and its possible association with the reproductive outcome of IVF.

MATERIALS-METHODS: Endometrial samples were collected using the embryo transfer catheter tip from 50 women undergoing IVF. Microbial DNA was extracted, and samples were analyzed by next generation sequencing technology. Nine hypervariable regions of the 16S rRNA gene (V2-4, V6-9) were amplified and sequenced using the Ion 16S Metagenomics Kit and Ion Torrent technology. Results were analyzed using the 16S Metagenomics workflow on the Ion Reporter software.

RESULTS: Various dysbiotic endometrial microbiota profiles composed of Bifidobacteriaceae, Clostridiales, Prevotellaceae, Enterobacteriaceae, Corynebacteriaceae, Staphylococcaceae, Ruminococcaceae, Micrococcaceae were seen in women with reproductive failure. The presence of Enterobacteriaceae was the only independent dysbiotic biomarker that was associated with infertility (p=0.0175). Even though Lactobacillus sp. were present in both groups (79,2% successful vs 69,2% unsuccessful), the abundance of lactobacilli was higher in women with reproductive outcomes of live birth (p=0,031).

DISCUSSION: Our findings indicate that there is a significant level of diversity in the microbial profile of endometrial samples and endometrial microbiome (EM) may be a potential contributor to implantation failure and/or pregnancy loss. Nevertheless, further insight into the mechanisms through which microbiome may impact embryo implantation is needed in order to improve the predictive value of endometrial metagenomic analysis in IVF outcome.

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PP135

GENOMIC ANALYSIS, PROBIOTIC PROPERTIES AND ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM RAW SHEEP MILK AGAINST MASTITIS-CAUSING AGENTS

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Introduction: Staphylococci and Streptococci are the main pathogens causing mastitis in ruminants followed by Escherichia coli. Use of antibiotics is the most common practice to deal with mastitis-causing bacteria. An alternative approach is the use of lactic acid bacteria as probiotic cultures to improve udder health and prohibit pathogens colonization.

Purpose: The objectives of this work were the isolation of lactic acid bacteria from raw sheep milk, the determination of their genetic diversity and evolution, and the identification of their probiotic potential.

Methods: Raw sheep milk samples were collected from the bulk tank of a commercial dairy sheep farm (Lacaune) over a period of one year. Four samples were collected at each visit and cultured on 3M Petrifilm Lactic Acid Bacteria Count Plate and M17 agar (at 22oC and 37oC). Colonies were isolated, cleaned and subjected to whole-genome sequencing and screening for probiotic properties (hydrophobicity, aggregation, and antagonistic activity). Genetic analysis was performed to all strains.

Results: The isolates were divided into clusters and taxonomic assignment was performed. The functional and probiotic properties of lactic acid bacteria were also determined which associated with the phenotypic results.

Significance: Development of a bacterial culture for potential use as probiotic to fight ovine mastitis in conventional far.

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PP137

Investigation of the biological actions of selected probiotics on breast cancer cells in vitro

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Breast cancer is the most common cancer in women worldwide and the leading cause of cancer-related death in both sexes. The human microbiome is a complex system of various probiotic strains that in adequate amounts benefit the host in multiple ways. Probiotics interact with each other as well as with the host organism and play a crucial role in the function of different systems, including the immune system, they produce a variety of metabolic enzymes and molecules with antioxidant and anti-inflammatory effects. Recently, many studies have revealed a potential role of certain probiotic strains in the prevention and treatment of different types of cancer types. Here, we aimed to study the anti-carcinogenic effects of a variety of probiotic strains in breast cancer. More specifically, we investigated the effects of 11 probiotic strains (Lactobacillus plantarum (3001), Lactobacillus plantarum (3002), Lactobacillus rhamnosus (C44), Lactobacillus acidoplhilus (LA85), Lactobacillus rhamnosus (LR206), Lactobacillus bulgaricus (LB42), Lactobacillus helveticus (LH76), Lactobacillus casei (LC89), Streptococcus thermophilus (ST81), Bifidobacterium lactis (Bla80), Bifidobacterium longum (BL21)) on proliferation, apoptosis, cell cycle and migration of breast cancer cell lines in vitro. Some of these strains were commercially available and some were isolated either from dairy products or from the human gut epithelium. In summary, our results revealed that 5 probiotic strains (3001, C44, LB42, LC89, Bla80) had a strong inhibitory effect on breast cancer cells' viability. Flow cytometry analysis for cell cycle and apoptosis offered preliminary evidence on the underlying mechanisms. Some strains also inhibited significantly cancer cell migration. Overall, our data argue that certain probiotics have potent anti-cancer activity and may be beneficial to human health.

PP138

Modification of tomato hormonal pathways during a tripartite plant-microbe-arthropod interaction

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Aculops lycopersici (tomato russet mite, TRM) is an important pest, leading to considerable yield losses in tomato crops. This indicates that protective measures must be taken to mitigate the damages caused by the mite. Thus, the beneficial role of fungi such as Fusarium solani strain K (FsK), which acts protectively against root and foliar pathogens, and herbivores in tomato, as well as alleviating the response of the plant to ought, is worth exploring. In the present study tomato plants were grown in the presence or absence of FsK or TRM or both. Sampling was made after 2, 7 and 14 days after infestation with TRM on control plants or plants colonized by FsK. RNA was collected from leaves and a whole transcriptome analysis was conducted. RNA-seq data, combined with hormonal metabolism data from the database TomatoCyc and verified by qPCR, resulted in the retrieval of differentially expressed genes during the tripartite interaction of the plant-microbe-arthropod that are involved in hormonal homeostasis.

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PP140

Development and validation of a stochastic model for the effect of temperature on the growth kinetics of Bacillus cereus strains in pasteurized milk

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Bacillus cereus is a spore-forming bacterium, that can grow in milk and milk products even if they have undergone the heat treatment of pasteurization. Furthermore, its pathogenic strains produce toxins capable of causing food poisoning, while its non-pathogenic strains can cause deterioration and organoleptic rejection of milk.

The present study ai to describe quantitatively the effect of storage temperature on the growth of Bacillus cereus strains and provide stochastic predictions for growth and risk of toxin production in pasteurized milk. Thus, it will potentially be a very useful tool for risk-based decision support of dairy companies.

The effect of temperature on the growth rate of Bacillus cereus was studied in Brain Heart Infusion (BHI) using the Bioscreen optical density measurement system. Strain variability was assessed by studying the kinetics of 30 strains isolated from a Greek milk industry plant. Initially, the theoretical minimum, optimum and maximum temperature (°C) for growth (Tmin, Topt, Tmax) parameters and the maximum specific growth rate (μmax) at optimum conditions, parameters of the secondary model Cardinal Model with Inflection (CMI), were estimated. Then, a stochastic predictive model for the effect of temperature on the growth kinetics of Bacillus cereus in pasteurized milk, considering strain variability, was developed. The model was further validated at both static and dynamic temperature conditions. The results showed that the developed model can accurately predict the growth of the pathogen and can be used as an effective based for a risk assessment model.

Acknowledgments

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PP140a

Developing a new yeast selection method for wine fermentation starters

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Climate change leads to even more hostile and stressful conditions for the wine microorganism and consequently issues with fermentation rate progression and off-character formation are frequently observed. The objective of the current research was to classify a great collection of yeast isolates from Greek wines based on their technological properties with oenological interest. Towards this direction, fourteen spontaneously fermented wines from different regions of Greece were collected for further yeast typing. The yeast isolates were subjected in molecular analyses and identification at species level. RAPD (Random Amplified Polymorphic DNA) genomic fingerprinting with the oligo-nucleotide primer M13 was used, combined with Matrix Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF) technique. Additionally, S. cerevisiae isolates were characterized at strain level by interdelta-PCR genomic fingerprinting. Yeasts strains were examined for their growth kinetics in grape must medium as well as for their fermentative capacity in Assyrtiko grape must. A free sorting task was applied to categorize the samples according to their organoleptic similarities. All yeast isolates were scrutinized for their sensitivity to killer toxin, production of non-desirable metabolites such as acetic acid and H2S, β-glucosidase production and resistance to the antimicrobial agents; SO2. Qualitative data were statistically treated by homogeneity of variances, one sample Kolmogorov-Smirnov and off between-subjects effects tests. According to our results, among the 190 isolates, S. cerevisiae was the most dominant species (83,5%) while some less common non-Saccharomyces species were identified in minor abundancies. Statistically significant differences were observed between the different levels of H2S production in terms of sample origin and yeast species. Hierarchical Cluster Analysis (HCA) revealed the presence of four yeast groups based on phenotypic fingerprinting. Additionally, strain level typing reported 20 different S. cerevisiae strains from which 65% indicated fermentative capacity and led to y wines. HCA based on sensory evaluation results clearly discriminated against the produced wines. These results confirm the proposed preliminary selection of yeasts, as strains exhibiting abnormal fermentations with off-odor characteristics were clustered in the less favorable groups, and vice versa. This study proposed a fast preselection of wine autochthonous yeast with oenological potential using a simple phenotypic-based methodology.

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PP140b

PROGRESSING ULTRAGREEN, ENERGY-EFFICIENT BIOBASED DEPOLYMERIZATION OF POLY(ETHYLENE TEREPHTHALATE) VIA MICROWAVE-ASSISTED GREEN DEEP EUTECTIC SOLVENT AND ENZYMATIC TREATMENT

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Effective interfacing of energy-efficient and biobased technologies presents an all-green route to achieving continuous circular production, utilization, and reproduction of plastics. Here, we show combined ultragreen chemical and biocatalytic depolymerization of polyethylene terephthalate (PET) using deep eutectic solvent (DES)-based low-energy microwave (MW) treatment followed by enzymatic hydrolysis. DESs are emerging as attractive sustainable catalysts due to their low toxicity, biodegradability, and unique biological compatibility. A green DES with triplet composition of choline chloride, glycerol, and urea was selected for PET depolymerization under MW irradiation without the use of additional depolymerization agents. Treatment conditions were studied using Box-Behnken design (BBD) with respect to MW irradiation time, MW power, and volume of DES. Under the optimized conditions of 20 mL DES volume, 260 W MW power, and 3 min MW time, a significant increase in the carbonyl index and PET percentage weight loss was observed. The combined MW-assisted DES depolymerization and enzymatic hydrolysis of the treated PET residue using LCC variant ICCG resulted in a total monomer conversion of ≈16% (w/w) in the form of terephthalic acid, mono-(2-hydroxyethyl) terephthalate, and bis-(2-hydroxyethyl) terephthalate. Such high monomer conversion in comparison to enzymatically hydrolyzed virgin PET (1.56% (w/w)) could be attributed to the recognized depolymerization effect of the selected DES MW treatment process. Hence, MW-assisted DES technology proved itself as an efficient process for boosting the biodepolymerization of PET in an ultrafast and eco-friendly manner.

PP141

Effect of storage temperature on the production of volatile metabolites in chicken meat of similar microbiological quality, based on the microbial counts

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The determination of volatile compounds (VOCs) at different storage temperatures could provide further information for a more accurate and reliable quality assessment. This study aimed to the investigation of any correlation between temperature and volatile metabolites produced during storage of chicken meat, testing samples of similar microbial load. Chicken thigh and breast fillets (n=240) were aerobically stored at different temperature conditions (0, 5, 10 and 15 °C) for specific time intervals and were microbiologically analysed for the determination of aerobic plate counts (APC), Pseudomonas spp., B. thermosphacta, Lactic Acid Bacteria and Enterobacteriaceae. Solid phase microextraction (SPME) combined with GC– was used for the estimation of VOCs throughout storage, while PLS – Discriminant Analysis (XLSTAT, 2018) was performed for the discrimination of samples stored under different temperatures based on volatile compounds' production.

Samples stored at different temperatures were classified to the correct temperature at a percentage higher than 85%, while the accuracy was even better (>90%) for the discrimination of 0 and 5 vs 15 °C. A more thorough examination of the results showed that the abundance of specific compounds which play an important role in spoilage was differentiated in samples with the same microbial populations, stored at different temperatures. Indicatively, 1-heptanol was found at higher levels at 10 and 15 °C (30 and 70x10^6 AU) compared to 0 and 5 °C (<3x10^6 AU) in samples of similar APC (7.0 log CFU/g). Moreover, a similar trend was observed in butanal-3-methyl and acetoin in samples of 6.0 log CFU/g total aerobes.
Different storage temperatures can result in a differentiated volatile profile and different type of spoilage or spoilage rate, even if the total microbial counts are found to be at similar levels.
This work has been funded by the project DiTECT (861915).

PP142

INACTIVATION BEHAVIOR OF SALMONELLA UNDER ACIDIC FOOD PRESERVATION STRESS: INDIVIDUAL CELL HETEROGENEITY AND POPULATION DYNAMICS (HETERO - INACTIVATION)

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Food safety is a fundamental issue for consumers and food industry and, lately, the need for a new consideration for the preservation techniques targeting on high quality and mild processing is highlighted. For moving towards this trend, a detailed and accurate description of the parameters affecting microbial responses is needed. Most of the models in predictive microbiology are developed on a deterministic basis describing the behavior of microbial populations as a whole, without considering the individual cells and their heterogeneity in the resistance to a lethal stress. Deterministic models providing point estimates seem to be inadequate to satisfactorily manage food safety. If the consequences of unacceptable levels of a surviving pathogen in a food after processing are grave, the knowledge only of the mean population decline is unlikely to be a sufficient basis for processing design. For this Hetero – inactivation will study Salmonella single cell inactivation phenotypic behavior upon exposure to low pH conditions and will develop stochastic modelling approaches for the description of inactivation behavior. It is expected that the exploration of single cell inactivation behavior will increase the accuracy in risk assessment models and will lead to the development or improvement of risk-based processing designs and food safety management systems. Hetero-inactivation focuses on a holistic approach for microbial inactivation at the single cell so, in this respect, will study the underlying molecular mechanisms and the heterogeneity in gene expression governing the differential survival characteristics of single cells in homogeneous populations. The linking of phenotypic behavior with gene expression will lead to a new generation of "white-box" models and will help to exploit any mechanism to destabilize microbial homeostasis. The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "3rd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 7610)

PP144

Microbial stability of home-made dried fruits and vegetables

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Consumers tend to show a preference towards minimum or unprocessed foods since chemical preservatives or highly processed foods often relate with health issues of diet imbalances. Among foods, snacks, breakfast ingredients or energy bars containing dried fruits and vegetables are among the highest in preference. In contrast to the branded commercial dried products, homemade dried fruits and vegetables are often lacking proper handling before, during and after their preparation, thus possessing some risks mainly of microbiological nature.

In this study, fruits and vegetables were dried by using a commercially available home drier, without any additional treatment in order to monitor their microbiological stability and infer about the possible risks. Multiple samples from apple, banana, kiwi, pear, carrot, eggplant and zucchini were dried in three different temperatures (40, 50 and 60°C) for 24 and 48h. Weight (moisture loss), water activity and total viable counts (by plotting on the surface of plate count agar petri dishes) were recorded at the beginning and after 24h and 48h of ying. In total, 63 samples were used.

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Weight of various fruits and vegetables was reduced in the range from 18 to 49% during the first day and between an additional 6 to 31% within the second day. Water activity (aw) in all samples showed a constant op rate (0.009 to 0.015 per hour) with minimum variation due to the ying temperature, starting from values of 0.96 to 0.98 and reaching 0.23-0.60 after 48h. Total viable counts (TVC) ranged from 0.33 and up to 3.06 log cfu/g at the beginning of the experiment in 40°C, reaching values between 1.11 and 5.76 after 48h indicating that a rich microflora survived during the process. In contrast, a similar initial TVC microbial load reached from 0 to 4.02 log cfu/g at the end of the experiment.

In contrast to brand products, homemade dried fruits and vegetables without any (pre)treatment, seem to retain or even increase their microbial load resulting at least to a shorter shelf life, a doubtful quality and even possess threats for the consumers health if pathogens survive the process or cross contaminate the product

PP145

DETERMINATION, CHARACTERIZATION AND BIOPROTECTIVE PROPERTIES OF THE MICROFLORA OF NATURALLY FERMENTED TRADITIONAL GREEK YOGURT FROM SHEEP AND COW MILK OF DIFFERENT ARTISANAL DAIRY PRODUCERS

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The components of the microflora of six traditional cow or sheep milk yogurt samples, produced by three different Greek manufacturers in region of Thessaly, were determined by culture dependent and independent techniques. All yogurt samples were produced (since many decades) via natural fermentation, i.e. repetitive inoculations with yogurt of previous production, without use of starter cultures.

Viable yogurt fermentation microorganisms and microbial contaminants were enumerated on M17 and MRS agar acidified to pH 5.5 at different incubation temperatures (25oC, 37oC and 42oC). Total lactic acid bacteria (LAB), Enterobacteriaceae, Yeasts and Molds, Enterococcus and S.aureus, were analyzed on day 1, 7, 12 (end of shelf life), within 24 h after manufacture, the 7th day of storage and at the end of their shelf life (11th day), in order to monitor the change in microbial populations during storage at 4 °C.

In total, 54 bacterial isolates were identified by 16S rDNA gene sequencing, using universal primers 27F (50-AGAGTTTGATCMTGGCTCAG-30) and 1492R (50- GGTTACCTTGTTACGACTT-30) and sequenced via the Sanger dideoxy termination method. Sequences were assembled into a single sequence via MEGA X version 10.1.6 software and Gene Runner version 6.5 software and subjected to a BlastN (Megablast) search in the 16S rRNA Database-GENEBANK for species identification. The majority of the isolates were assigned to the genera Lactobacillus, Streptococcus, Enterococcus, Staphylococcus and Bacillus. Specifically, the isolates belonged (with decreasing order of abundance) to the following species: Streptococcus thermophiles, Lactobacillus delbrueckii subsp. bulgaricus, Staphylococcus epidermidis, and Bacillus sp.(Bacillus amyloliquefaciens / Bacillus velezensis, Bacillus tropicus / Bacillus nitratireducens / Bacillus luti / Bacillus albus / Bacillus cereus, Bacillus stercoris / Bacillus subtilis) and Enterococcus faecium.

Also, isolates that belonged to Lactobacillus, Streptococcus and Bacillus were tested for their antibacterial activity against the growth of Staphylococcus aureus, Listeria monocytogenes, Salmonella typhimurium, Escherichia coli.

This study revealed that the microbiota of naturally fermented artisanal Greek yogurt includes both LAB and non-LAB bacteria (e.g. Bacillus species) participate in yogurt fermentation and survive during the acidification process. Potentially bioprotective bacteria with antimicrobial activity are included in this microbiota and could be exploited in food bioprotection.

PP146

Effect of 12-week probiotic consumption immobilized on oat flakes on blood and urine biomarkers and human microbiome

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Probiotic consumption has a positive influence on human health, as it seems to affect the composition of the gut microbiota; however, through this modulation, immunological biomarkers and lipemic and glycemic profiles seem to be improved. In a randomized placebo-controlled design with two arms, 60 healthy participants were assigned to consume 6 g of oat flakes daily in combination with a meal of their choice. Specifically, 30 of them were consuming oat flakes with immobilized probiotics and the other common oat flakes (placebo). Blood, urine, and fecal samples were collected at baseline and at 6 and 12 weeks after the intervention.
According to study design, primary outcomes are expected to improve fasting blood glucose, insulin resistance and triglycerides blood levels, while immunological biomarkes (IL-6, IgA) and the absorption of vitamins, along with fecal microbiome changes will be examined.

PP147

MULTISPECTRAL IMAGING (MSI) FOR THE DISCRIMINATION OF DIFFERENT MICROORGANISMS FORMING BIOFILMS ON STAINLESS STEEL SURFACES

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In recent years, food industry is interested in the implementation of fast, non-invasive techniques to acquire information about the microbiological status of foods and surfaces. Herein, the efficiency of I analysis to detect biofilm formation and discriminate different microorganisms forming it, was investigated. Biofilm formation was achieved by inoculating 78 sterilized coupons (3cm*1cm*0.1cm) with the desired microorganism (10 Lacticaseibacillus casei, 10 Lactiplantibacillus plantarum, 10 co-culture of L. casei and L. plantarum, 10 Salmonella enterica serovar Enteritidis, 10 Salmonella enterica serovar Typhimurium, 10 co-culture of S. ser. Enteritidis and S. ser. Typhimurium, 6 Pseudomonas fragi, 6 P. putida and 6 co-culture of P. putida and P. fragi) followed by immersion in TSB broth for six days at 15 °C. The broth was renewed every second day and the coupons were rinsed with ringer solution between renewals and after the incubation period was over. Control coupons (n=47) were incubated in ringer solution for six days at the same temperature. All coupons were left to y for 10 minutes before images' acquisition using VideometerLab instrument. Partial Least Squares Discriminant Analysis (PLS-DA) was used to discriminate the different cases, using an independent dataset to validate model's performance (test set). PLS-DA model exhibited satisfactory performance in separating control from biofilm samples with 95% accuracy for the internal validation and 89% accuracy for the test set validation. Control samples could be separated from LAB, Salmonella and Pseudomonas biofilm samples, with 90% accuracy for the internal validation and 75% accuracy for the test set validation, regardless of the microbial group. Excluding control samples from the analysis, LAB, Salmonella and Pseudomonas biofilms were fully discriminated achieving 100% accuracy for both the internal and the external validation. These results are quite satisfactory and can be extremely important for future research on detecting biofilms on stainless steel surfaces.

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PP148

Physicochemical and microbiological characteristics of yogurts fortified with apple pulps

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Fortification of food products, like yogurt, with natural antioxidants leads to functional food production and is considered a new approach to food technology. Fruits, like apples, are rich in bioactive compounds with high nutritional value and can be used for this purpose. Apple pulp can be a valuable resource that can be utilized, in line with sustainability and circular economy, in order to contribute to high-added value products. In line with this trend, the present study was conducted to evaluate the effect of the addition of apple pulp on the physicochemical and microbiological characteristics of yogurts during 28 days of refrigerated storage. Four yogurt formulations with different apple pulp amounts (0%, 5%, 10%, and 15% w/w) were prepared. There were no significant differences in pH and acidity values of all formulations. Increase of apple pulp showed an increase in water-holding capacity and a slight increase in moisture content. Moreover, a slight decrease in pH values and an increase in titratable acidity during storage were observed for all yogurts. Regarding the effect of apple pulp on yogurt starter culture viability, a slight decrease in viable counts was noted while the amount of apple pulp increased. Despite that, in all cases, starter culture viable counts were above 10^7 CFU/g even until the end of storage time. Apart from the need of studying more characteristics, yogurt fortified with apple pulp can be suggested as a possibility for development a new sustainable functional product.

PP149

Application of ATR-FTIR spectroscopy as a PAT tool for discriminating among Tuber species, their origin and maturity status

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The genus Tuber F.H. Wigg. (Ascomycota, Pezizales) produces hypogeous ascomata ('truffles') famous for their unique organoleptic properties. Although truffles are widely traded and consumed, there is no reliable, fast and non-destructive methodology available for discriminating among species, samples' geographic origin and maturity status; this results in incidents of trading misidentified and/or immature products. Process analytical technology (PAT) has been applied during the last decade for determining the origin and composition of various food items. Fourier transform infrared (FTIR) spectroscopy is one the most widely employed PAT tools for such purposes, and it was successfully applied for the discrimination of various Pleurotus species by using either mushroom or mycelium samples (Bekiaris et al. 2020; Zervakis et al. 2012); however, no pertinent applications have been reported so far for truffles. In the frame of this study, the FTIR spectra of 58 Tuber specimens, deriving from eight geographic regions of Greece and assigned to three species (i.e., T. aestivum, T. macrosporum and T. magnatum), were recorded using attenuated total reflectance FTIR (ATR-FTIR) spectroscopy, and a spectral library was created. Principal component analysis (PCA) was performed on recorded ATR-FTIR spectra, and led to the discrimination of truffle species, while at the same time it allowed grouping of the obtained spectra for each species on the basis of the samples' maturity status (immature, semi-mature, mature) and geographic origin.

Key words: Tuber; Ascomycota; truffle; FTIR; ascomata maturity; taxonomy

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PP150

Directed fermentation of black olives cv. Konservolia by functional cultures in lab and industrial scale

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Nowadays, the driven fermentation of table olives is gaining ground to avoid food loss. The goal of this project was to control the fermentation of black table olives cv. Konservolia (harvested from Fthiotida region), not only with the use of microorganisms with several technological characteristics (e.g., salinity and acid tolerance) but with a specified functional character (i.e., probiotic ile). To this affair, two lactic acid bacteria (LAB) (L1 and L2) and two yeasts (Y1 and Y2) with probiotic profile were used alone or in combinations as starter cultures for the fermentation of black olives cv. Konservolia. The microbiological and the physicochemical profile of the olives were monitored during the fermentation process which lasted 90 days. Moreover, the ability of the starter to survive the fermentation process was monitored by RAPD-PCR. In addition, sensory evaluation was performed to reveal the best starter (single isolate or microbial combination) for black olive fermentation. According to the obtained results, the LAB population was maintained at high levels, with no significant differences between the different samples (7.0-7.2 log CFU/g when the starter L1 was used and 6.8-7.0 log CFU/g when inoculated with L2). In the case of brine's pH value, a slightly higher (ca. 4.2) value was reached in the microbial combination cases than in single LAB cultures (3.8-4.0). Following the monitoring of the survival of the starter cultures, it was shown that L1 was able to survive at higher levels. This is of importance if the higher scores of these samples is considered, suggesting L1 strain as functional culture for black table olives cv. Konservolia. These results allowed us the selection of L1 strain and apply it successfully in industrial scale fermentation.

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PP151

Effect of organic herbs on the quality and safety of chicken broth

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Modern lifestyle requires quick solution for the preparation of meals at households. Driven from these consumer's habits, products like meat, chicken and vegetables bouillons in cube or powder form are placed in the market. In this study, we aimed to produce a chicken broth with organic herbs and to monitor their effect on the microbiological, organoleptic and physicochemical quality during storage at different temperatures. For this purpose, chicken broth was prepared by boiling under-utilized portions of the chickens' carcasses with organic herbs. Different amounts and mixtures of organic oregano, thymus, summer savory, crithmum were evaluated. The samples with the higher values of organoleptic assessment were considered for further experiments and stored at four temperatures (2 to 15°C). Chicken broth without organic herbs was used as control. In addition, samples were artificially contaminated by Salmonella Enteritidis to estimate the effect of organic herbs in chicken broth on Salmonella growth and subsequently, the product's safety. In different time intervals, microbiological analysis, pH measurement and organoleptic evaluation were performed, while the concentration of NaCl and total nitrogen and protein content were also determined in fresh chicken broth. Fourier transform infrared spectroscopy (FT-IR) in combination with partial least squares (PLS-R) was used to estimate the storage time. According to the organoleptic characterization, the organic herbs were found to prolong the storage time of broth. The microbiological, organoleptic and physicochemical quality of the chicken broth was acceptable, while Salmonella growth was suppressed at low temperatures and by the presence of organic herbs. A good correlation of storage time with the FT-IR spectra was achieved through the PLS-R models, considering that FT-IR combined with partial least squares could be a promising rapid method to estimate the storage time of the new product. In conclusion, a new chicken broth with organic herbs was produced with good microbiological, physicochemical and organoleptic characteristics.

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PP152

Microbiological quality and safety assessment of various chicken meat products as influenced by manual processing operations

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Chicken meat is generally recognized as major reservoir for both spoilage and pathogenic bacteria originating from the animal and slaughterhouse environment microbiota during processing. This study evaluated the microbiological quality and prevalence of important pathogens in various chicken meat products as influenced by manual cutting, deboning and further processing operations. A total of 250 samples (n=5, 10 batches) from 5 chicken products namely, breast with skin, thigh with skin, breast fillet, thigh fillet and marinated thigh souvlaki were collected directly after packaging from a local poultry industry. Samples were microbiologically analyzed for the: 1) enumeration of total viable counts (TVC), Pseudomonas spp. and Escherichia coli and 2) presence of Salmonella spp., Campylobacter spp. and Listeria monocytogenes. Marinated souvlaki presented the highest TVC and pseudomonads populations (5.31±0.45 and 4.94±0.53 logCFU/g, respectively) which could be attributed to further processing, i.e. dicing and marination. For the majority of the rest products, TVC and pseudomonads levels did not differ significantly in samples with skin compared to fillets. E. coli was enumerated at acceptable levels (Commission Regulation (EC) 2073/2005) in samples of most batches and was found to be slightly higher in thigh (2.58 ± 0.41 and 2.42 ± 0.52 logCFU/g with skin and fillets, respectively) and souvlaki (2.27 ± 0.45 logCFU/g) followed closely by breast (2.18 ± 0.62 and 1.90 ± 0.54 logCFU/g with skin and fillets, respectively). Campylobacter was the most prevalent pathogen followed by Salmonella (found in 10.4% and 6.4% of samples, respectively), while L. monocytogenes was not detected. Specifically, Campylobacter was detected in three batches of thigh fillets, two batches of breast with skin and fillets, and one batch of thigh with skin and souvlaki. The occurrence of Salmonella was highest in breast with skin (4 out of 10 batches) followed by souvlaki (2 out of 10 batches), while in the rest meat products, it was detected in one batch. Results indicate that with the exception of souvlaki that had undergone additional processing operations, all other chicken products presented similar microbial populations. The presence of pathogens highlights the need for improving sanitation strategies.

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PP153

Survey: Microbiological quality of fish from retail

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The level of microbiological population of fish and fish products is a determinative parameter of quality and shelf life. The aim of the present study was to (i) determine microbiological level of different fish species available in the retail and (ii) to focus on the microbiological quality of gilthead seabream from the supermarkets since it holds about half of the fish production in Greece. In that framework for part one salmon, seabream, seabass, cod, tuna, trout, and mackerel were purchased from the retail market. In total 120 samples were collected and analyzed microbiologically for the enumeration of Total Aerobic Counts (TAC), Pseudomonas spp., Enterobacteriaceae and Vibrio spp.. Total aerobic counts ranged from 1.70 to 6.53 log CFU/g similar were the microbial levels of Pseudomonas spp., Enterobactriaceae and Vibrio spp. were <4.7 log CFU/g and <3.6 log CFU/g, respectively. The second part focused on the purchase of seabream fillets, which were obtained from several selling points. The packaged and non-packaged samples with different use-by-dates, from different retail stores were collected. Moreover, samples that were filleted at the lab were also analysed. The collected samples were subsequently stored at different temperatures (2 and 4 °C) for specific time intervals (2-3 days after the expiration date of the sample) and analysed microbiologically for the determination of TAC. Fifty-six (56) of these fillets were packaged in modified atmosphere packaging (MAP) conditions (original packaging) while 70 were aerobically packaged. The initial microbial population of samples from bulk (4.48±0.46 log CFU/g) was higher compared to the initial population of fish fillets from the MAP packages (3.83±0.72 log CFU/g). The microbial population until use by date was <7.08 log CFU/g for all examined samples for MAP. The samples stored aerobically had a high population before the time point use by date. In the case of higher storage temperature (i.e., 4°C) microbial population reached higher than 8.00 log CFU/g

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PP154

Occurrence and contamination levels of ochratoxin A and aflatoxins in corn-based products from markets in Greece

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Global corn consumption within 2021-2022 exceeded 1.15 billion metric tons, rendering corn the most consumed grain worldwide. Mycotoxins are naturally occurring fungal metabolites frequently encountered in corn and corn-based products, that constitute a significant hazard in the food sector due to their adverse effects on human health. This study aimed to evaluate the safety of corn-based products available in the Greek market with regards to the biological hazard of ochratoxin A (OTA) and total aflatoxins (AFtotal; sum of AFB1, AFB2, AFG1 and AFG2). A total of 160 corn-based products, namely pudding powder, corn starch, corn flour, corn flakes, canned sweet corn, frozen corn, corn cakes and raw corn kernels, were obtained from the market. The occurrence of OTA and AFtotal was assessed with High-Performance Liquid Chromatography equipped with a fluorescence detector (HPLC-FLD). Pretreatment of the samples was performed through immunoaffinity columns (IACs) to assure the detection of OTA and AFtotal even in negligible concentrations reinforcing the capability of HPLC. For the analysis of aflatoxins, a post-column derivatization with an aqueous iodine solution was additionally performed. OTA was detected in 24 out of 160 samples analyzed (corresponding to 15.0%) at levels ranging from 0.23 to 24.80 μg/kg. Although most of the samples complied with the Commission Regulation (EU) 2023/915, two corn cakes and one corn flour sample exceeded the permitted levels (3.0 μg/Kg for OTA). Contrarily, AFtotal was found in 6 out of 160 samples (corresponding to 3.8%) at a concentration range from 0.42 to 7.37 μg/kg. Two corn flour and one corn cake samples were contaminated with aflatoxins above the EU regulation limits (2.0 μg/Kg for AFB1 and 4.0 μg/Kg for AFtotal). In addition, co-occurrence of OTA and AFtotal was found in one corn flour and two corn cake samples. The results of this study reveal that most of the corn-based products traded in the Greek market comply with EU regulations. However, more efforts should be made towards mycotoxin mitigation in the far and their early detection in foods through the development of advanced, sensitive, accurate and rapid methods.

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PP155

EVALUATION OF THE GREEK PDO ANEVATO CHEESE WITH METAGENOMICS AND VOLATOLOMICS

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Anevato is a traditional Greek white soft cheese produced from sheep or goat milk or mixtures of them. The objective of the present study was the identification of the microbial ecosystem of the Anevato cheese. The microbiome of the cheese was characterized by culture-based microbiological analysis and shotgun metagenomics and its volatilome was also determined. The main microbial populations were lactic acid bacteria (LAB) and yeasts, but in lower abundance coliforms, Enterobacteriaceae, Staphylococcus spp., Escherichia coli and Pseudomonas spp. were also identified. The shotgun metagenomics allowed us to analyze the microbiome of the Anevato cheese at the species level. Thus, the main LAB species were, Lactococcus lactis, Streptococcus thermophilus, Lactococcus raffinolactis, Lactobacillus helveticus, Lactiplantibacillus plantarum, Streptococcus parauberis, etc. At lower abundances, yeast species were also identified as Kluyveromyces lactis and Saccharomyces cerevisiae. Furthermore, differences in the volatile compounds for the different samples of the Anevato cheese were determined. Overall, our research provided information about the quality characteristics and the starter cultures that could be used for the production of this cheese type.

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PP156

Assessment of the microbiota of industrial dry sourdoughs through MALDI-TOF and metagenomics

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The presence of lactic acid bacteria (LAB) and yeast communities in sourdoughs has been identified across diverse environments worldwide. Although there are many reports in the literature discussing the microbial diversity of yeasts and LAB of homemade sourdoughs from several regions, relatively limited information regarding industrial sourdoughs is available. Our study was conducted to provide more information about the starters used in industrial y wheat sourdoughs and evaluate their suitability for the production of sourdoughs at home. Overall, twelve manufactured samples from four brands were collected from supermarkets or manufactures and analyzed for their microbiological populations and physicochemical properties. Moreover, total DNA was extracted and the microbiota of the samples was investigated at the species level utilizing metagenomics. Taxonomic microbial identifications were also conducted through the use of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF). The results showed different microbial combinations of LAB and yeasts among the samples. LAB species, such as Pediococcus pentosaceus, Lactobacillus plantarum, Enterococcus faecium, Pediococcus acidilactici were detected, whereas most of the samples were dominated only by the yeast Saccharomyces cerevisiae alone or in combination with Wickerhamomyces anomalus

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PP157

Development of novel biomarkers as time-temperature indicators of freshness or spoilage of fresh, vacuum-packed prawns and squid

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Fish and seafood represent a valuable component of human diet and agricultural economy, but they are also highly perishable types of food, due to the presence of many nutrients, high moisture and close to neutral pH, which favor bacterial growth and spoilage. Vacuum packaging is one way of extending the otherwise very short shelf life of fresh seafood, but sometimes the storage conditions (temperature abuse) and mishandling of fresh seafood can reduce the expected shelf life, even in a vacuum packed product. Time temperature indicators have been developed in recent years to predict the actual shelf life of perishable foods, based on the initial microbial contamination level and the existing storage conditions.

In this framework, a novel biomarker (indicator) was developed to predict spoilage of fresh prawns and squid in vacuum packaging, using a gel of Nutrient agar with methyl blue stain, inoculated with certain amounts of Pseudomonas fluorescens and incorporated into a separate compartment of the seafood package that was then used for vacuum-packaging. The packed seafood was then stored under refrigeration (4ºC and 8ºC for up to 6 days. The populations of Psychrotrophic Plate Count (PPC), Pseudomonas, Bacillus, lactic acid bacteria (LAB), yeasts and molds were counted during storage and compared with a discoloration of methyl blue–Nutrient agar into a yellow colored Nutrient agar, as a result of proteolysis and alkaline pH shift, caused by the growth of P. fluorescens in the gel matrix.

The results showed that, in comparison to Bacillus and LAB, Pseudomonas and the total count of PPC were better associated with spoilage of fresh prawns and squids. Indeed, when the total psychrotrophs of fresh seafood reached or exceeded a population of 1.000.000 cfu/g and spoilage was detectable organoleptically (as off-odor), this coincided with a pH shift from (6.3-6.6) to (7.2-7.6) and a subsequent color change from blue to yellow in the stain (methyl blue) of the gel matrix. The effectiveness of this novel spoilage indicator can be exploited in designing and manufacturing new, low-cost time-temperature indicators for use in food packaging and prevention of food losses due to microbial spoilage.

PP158

Cytotoxic effect of selected cell-free probiotic supernatants on colon cancer cell lines

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Colorectal cancer remains one of the leading causes of death worldwide and despite the several improvements which have been made in this field, there is an urgent need for better therapeutic approaches, mainly due to the adverse effects of the commonly used anti-cancer drugs. Probiotics have revealed their beneficial effects on intestinal inflammation as well as on the prevention and treatment of gastrointestinal cancer in vivo and in vitro studies. Although the underlying molecular cancer-preventing mechanisms of probiotics have not yet been fully clarified, studies have revealed that probiotics inhibit cell proliferation and induce apoptosis in different types of cancer cells. The aim of this study was to investigate the cytotoxic effect of cell-free supernatants (CFS) of selected probiotics (Lactobacillus plantarum HBUAS52094, Lactobacillus plantarum 6093, Bifidobacterium lactis Bla80, Lactobacillus casei LC89, Lactobacillus bulgaricus LB42, Streptococcus thermophilus St81, Bifidobacterium longum Bl 21, Lactobacillus acidophilus La95, Lactobacillus rhamnosus C44, Lactobacillus helveticus LH76, Lactobacillus rhamnosus LR206), isolated from different sources (dairy products and human colon), on the colorectal cancer cell lines Caco-2 and HT-29. HT-29 and Caco-2 cells were treated with different concentrations of CFS ranging from $2.5x10^7$ to 10^9 cfu/ml at 48 hours while the cell viability was assessed by 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. Our results demonstrated that all the examined CFS, mainly in high concentrations (>10^8 cfu/ml), had an inhibitory effect on cancer cell viability. Our findings revealed that the examined CFS exhibit anti-cancer effects on the Caco-2 and HT-29 cell lines and support that the examined probiotics may have a beneficial role in the treatment of colorectal cancer.

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PP159

EVALUATION OF DIFFERENET ENCAPSULATION METHODS OF LACTOBACILLUS PLANTARUM 2035 TO ENHANCE ITS VIABILITY IN BEER ENVIRONMENT

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Probiotics are associated with the delivery of several health benefits to the host. In general, functional foods are raising the interest of the food industry more and more in the last decades, with one of the main challenges to be, in exerting probiotics' health effects, the maintenance of high viability (> 106 CFU/g) during food processing, storage, and consumption. However, as beer typically contains hop iso-α-acids, ethanol, and low pH, which prevent the growth and survival of probiotic lactic acid bacteria, the use of new strategies like encapsulation is crucial to retain their survival. In this study, encapsulation of a probiotic strain of Lactobacillus plantarum in alginate beads (ALG), as well as complex coacervates (whey protein isolate and gum arabic; WPI-GA), were evaluated in a beer environment. In all cases, the storage at high temperatures (20oC) resulted in a rapid reduction of cell viability. This reduction was higher in free cells (FC; no viable cells after 14 days), followed by ALG and WPI-GA (viable cells even after 28 days). In all temperatures, the increase of ethanol content (5.4% to 10% v/v) resulted in lower viabilities. At 4oC no viable cells were determined in FC (reduction of 9 log CFU at 5.4% v/v ethanol) while using encapsulated cells retained viability (reduction of 5 log CFU). Encapsulation in WPI-GA resulted in better viability than ALG even when beer with high ethanol content (10% v/v) was used. In general, the use of encapsulated cells provided adequate protection of probiotics up to 21 days of storage at 4oC. Last but not least, there were no important fluctuations in the pH of the beer during storage. The results suggest that beer could be a vehicle for probiotic delivery under appropriate conditions of encapsulation. However, more studies are needed to further increase the viability of probiotic cells.

Keywords: probiotics; L. plantarum; beer; encapsulation; functional foods

PP160

Application of alternative preservation methods for fruits and production of novel highly preservable products

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The perishable nature of fruits has led to the application of drying to prolong their shelf life. However, despite the increasing trend in the market for such products, innovation and technologically advanced fruit drying techniques are lacking. It has been confirmed that in the Region of Peloponnese the innovative process of freeze-drying is not applied to an agricultural holding or a fruit processing unit. The gap is filled by the present Action of the Operational Group “Innovation in Fruits Preservation” through the utilization of the knowledge that ITAP has acquired in drying technologies such as dehydration at low temperatures and lyophilization. Through the utilization of this knowledge and the pilot application of modern fruit drying techniques in OEBEA, fruit producers and processors will produce new value-added products and make more efficient use of PDO fruits of the Region (e.g., Tripoli PDO “Pilafa” Delicious apples). The transportation cost of the new products will decrease significantly due to their reduced weight and will line-up with the modern standards of holistic management since water resources will be saved. Also, it will be possible to utilize 2nd & 3rd grade shorted products that until now have brought reduced inputs to the farmers, or even costs. Finally, a plan will be delivered after the end of the project to ensure the continuity of the pilot actions and relevant investments for the benefit of producers. The latter will help them to invest in the knowledge they will acquire and either equip their own farms with similar methods or take advantage of the network that will be created to act in a coordinated manner for larger investments. The result of the present collaboration will lead to the extension of the lifetime of these products and the creation of new ones, giving the possibility to fruit producers and preservation companies to increase their income and to gain new shares in the market.

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PP161

Production of wine with enhanced varietal aroma from cv. "Moschofilero"

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The wine sector is a key economic and social pillar of the Peloponnese Region. However, the sector faces challenges such as a decrease in income and employment as a result of structural problems, such as the lack of innovative entrepreneurship or the connection with research institutes. Today, there is a spread of a small number of grape varieties, mainly of French origin, while the industry relies on a definite number of yeast starter cultures, leading to the evenness of wine aroma. This practice contrasts with the new trend for wines with special organoleptic characters, typical of the region of origin. The indigenous yeasts, especially non-Saccharomyces (NS) species, are important in shaping the aromatic profile and enhancing the typicality of wines. The Peloponnesian vineyard has a characteristic yeast microbiota that can be used to improve local wines. The goal of the project is to integrate an innovative wine production process for the benefit of both producers and consumers. It also aims to foster cooperation between beneficiaries in order to take advantage of new technology and boost producers' and businesses' competitiveness. By utilizing native NS yeasts, a novel Moschofilero wine will be developed with an enhanced varietal bouquet. For this purpose, selected strains will be used, and new, appropriate fermentation protocols will be applied. The novel wine is anticipated to have an improved organoleptic profile, characteristic of the region of origin, and increased added value, highlighting the uniqueness of local wines.

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PP162

Fungal mycobiota isolated from Kefalonian cheeses

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Cheese can be a source for the development of different microorganisms. Fungi comprise significant microorganisms for the industry since they play a crucial role in cheese production. The most frequently isolated genus from cheeses is Penicillium followed by Aspergillus. In the present work, fungal biota, with special reference to the genus Penicillium, was studied in 11 samples of commercial cheeses produced on the island of Kefalonia, Greece. They were identified and studied for their phenotypic characteristics. In 8 of the cheeses analyzed (73%) fungi were isolated. Penicillium was identified in 45% of the samples, Mucor spp. in 27%, Alternaria in 18%, Aspergillus in 9%, and Rhizopus in 9%. The presence of these microorganisms in cheeses may be due to poor hygiene conditions during cheese production and may cause spoilage problems. On the other hand, these cheeses can also be a source of these microorganisms with possible technological characteristics for cheese production. This work provides insight into the fungal mycobiota of Kefalonian cheeses and their importance for both consumers and cheese industry. More research is needed to identify the technological characteristics of these isolates and their possible applications in cheese production

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PP164

Bacterial communities of fresh mussels stored at 2 and 4oC

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Mussels are among the most popular seafood worldwide and the rise in consumption is highly linked to their nutritional value (e.g., high content of Omega-3) and health benefits (e.g., prevent skin diseases). However, their quality deteriorates rapidly mainly due to microbial growth and activity even when they are stored under chilled temperature conditions. To reveal the microorganisms most likely to cause quality loss in such products, 16S metabarcoding was used to monitor bacterial communities in mussels (Mytilus galloprovincialis) without shell in bags with seawater, as they were stored at 2 and 4oC. Microbial population changes were also recorded. Several bacterial genera e.g., Leuconostoc, Acinetobacter and Corynebacterium were found to compose the microbiota of mussels at Day 0 (beginning of shelf-life), while Psychrobacter and Pseudoalteromonas were the dominant genera at Day 6 (end of shelf-life). At this point, Total Viable Counts (TVC) reached levels of about 6 log cfu/g at both temperatures, while Pseudomonas and H2S producing bacteria were the most dominant microorganisms. Indeed, the use of 16S metabarcoding revealed bacterial genera e.g., Psychrobacter and Pseudoalteromonas that are involved in spoilage of seafood. Microorganisms associated with hygiene conditions in pre- or post-harvest stage e.g., Acinetobacter, were also revealed. Moreover, fish spoilage, including mussels, usually occurs when the APC or specific spoilage organisms (SSO) reach the level of 7–8 log cfu/g. In this work, their populations were considerably lower. This might have occurred due to a combination of microbial and biochemical (enzymatic/autolytic) changes during mussels' storage as both have been reported as causes of quality loss of crustaceans and mollusks several times in the past. The findings of this work could be used to enforce preventive measures towards hygiene and quality of chill-stored mussels.

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PP165

Developing stable functional ingredients for the food industry containing immobilized cells of a wild-type plant-based presumptive probiotic strain on high-fiber content supports

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Introduction: Nowadays, an upsurge of interest in developing functional foods containing probiotic microorganisms enriched with prebiotic fibers is witnessed. To induce the health benefits, functional foods should contain an adequate amount of living cells (above 7 logcfu/g), a requirement that constitutes a real bottleneck for the food industry, considering the cell susceptibility to food processes and storage. To overcome such obstacles, cell immobilization is recommended. In this vein, we examined the development of stable functional food ingredients containing immobilized cells of a wild-type Lactococcus lactis strain isolated from mushrooms that was previously evaluated in vitro for potential beneficial characteristics.

Methods: Cell immobilization of Lactococcus lactis on oat flakes and banana flour was investigated and the effect of trehalose and glucose used as cryoprotectants (at 10%) during freeze-drying on viable cell counts was assessed during storage at room and refrigerated temperatures for 90 days.

Results: Cell loads remained >7 logcfu/g in wet immobilized cells stored for 1 month at 4°C, while at room temperature, cell levels were significantly decreased and ranged ≥ 5.55 logcfu/g after 15 days of storage. Cell populations of freeze-dried cells remained > 7.5 logcfu/g during storage at 4°C for 3 months, and >7 logcfu/g at room temperature after 2 months. However, wet and freeze-dried free cells exhibited lower survival rates during storage at both room temperature and at 4°C compared to immobilized cells. Furthermore, the use of cryoprotectants had a positive impact on cell viability, leading to significantly higher cell loads after freeze-drying and during storage for 90 days at both room temperature and 4°C (> 7.5 and >8 logcfu/g, respectively), compared to the control.

Discussion: Our strategy was to develop stable functional ingredients for the food industry containing a wild-type plant-based presumptive probiotic strain at the recommended cell loads to confer a health effect. Our results suggested the protective effect of cryoprotectants during freeze-drying and the positive impact of cell immobilization on maintenance of high cell loads during storage. However, further investigation on application of the immobilized probiotic cells in real food systems is required

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PP166

Effectiveness of probiotic immobilized on lyophilized banana powder on patients with Irritable Bowel Syndrome

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Changes in the composition of the gut microbiota lead from a state of symbiosis to a state of dysbiosis between the microbiota and the host, which is associated with a variety of gastrointestinal diseases, such as Irritable Bowel Syndrome (IBS). Research data indicate the association of probiotic consumption with the improvement of IBS symptoms. In a randomized placebo-controlled design with two arms, 30 patients with IBS Diarrhea (IBS-D) and IBS Unclassified (IBS-U) will be enrolled and they will be assigned to consume 7 g of lyophilized banana powder dissolved in water daily for eight weeks. Fecal samples will be collected at baseline and at 4 and 8 weeks for studying fecal microbiome changes, while at the same timepoints, the participants will complete questionnaires related to their general and immediate anxiety. Moreover, on a weekly basis, volunteers will be asked to complete questionnaires related to the type of bowel movements using the Bristol Stool Form Scale questionnaire and questionnaires related to their eating habits during the study

PP167

DISCRIMINATION OF BIOFILM CELLS FROM PLANKTONIC AND OF DIFFERENT MICROBIAL GROUPS USING FT-IR

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Using spectroscopic methods combined with multivariate data analysis could overcome the disadvantages of traditional microbiological methods, which are laborious and time-consuming, giving retrospective results. Fourier Transform InfRared Spectroscopy (FT-IR), for instance, is a rapid, low cost, noninvasive analytical technique, which has been successfully tested coupled with Data Science for various applications in the food sector (i.e. food crime, microbiological quality). The aim of this study, was to discriminate biofilm from planktonic cells and among different microbial groups via FT-IR. Analysis was conducted on planktonic cell suspensions grown for one and six days in Tryptic Soy Broth (TSB) at 15 °C and biofilm cell suspensions which were collected by bead vortexing from stainless steel coupons (3cm*1cm*0.1cm) immersed in TSB for six days at 15 °C. The broth where the coupons were incubated in, was renewed every second day. The microorganisms used were Salmonella enterica ser. Enteritidis, Salmonella enterica ser. Typhimurium, Lactiplantibacillus plantarum, Lacticaseibacillus casei, Pseudomonas putida and Pseudomonas fragi; for each were retrieved both biofilm and planktonic cells. 1 ml from each kind of suspension was placed on a zinc selenide (ZnSe) crystal to obtain the FT-IR spectrum. In total 189 samples were collected; 117 spectra of biofilm cells and 72 spectra (from one and six days) of planktonic cells suspensions. These 189 samples consisted equally of 63 spectra of Salmonella, Lactobacillus and Pseudomonas. The dataset was split so as 70% to be used for training (interval validation) and 30% was used for the testing (external validation). PLS-DA was applied on FT-IR data and was attained 97% accuracy for the internal validation and 100% accuracy for the test set for the discrimination of planktonic cells from biofilm cells. Salmonella, Lactobacillus and Pseudomonas suspensions can be separated with 98% accuracy for the internal validation and 91% accuracy for the test set. The model exhibited great potential for the discrimination of biofilm and planktonic cells, as well as the distinction among genera in a mixture of biofilm and planktonic cells.

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PP168

Tracking of Salmonella in sesame edible seeds imported in Greece

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Salmonella spp. is one of the most common causes of foodborne illness in humans. Although salmonellosis is related to foodstuffs of animal origin, gastroenteritis outbreaks linked to the consumption of plant origin food products are increasing in various parts of the world. Nowadays, non-typhoidal Salmonella has been related to enteric disease linked to spices and nuts including sesame. The aim was to evaluate the presence of Salmonella spp. in raw sesame (edible seed) before being imported to Greece from non-European countries, mostly from Africa and Asia. A total of 2944 different batches of sesame samples were tested for the presence of Salmonella spp. during the period 2013-2022. Samples were analyzed according to accredited ISO 6579-1:2017 until May 2021, and afterwards the detection was continued with VIDAS-AFNOR validated method Certificate No BIO 12/32-10/11. Confirmatory and identification tests to species, subspecies and serovar level were performed by the accredited for serotyping National Reference Laboratory for Salmonella of the Hellenic Ministry of Rural Development and Food. Salmonella was detected in 628 out of 2944 different batches for the whole period time. In brief, 191 isolates were identified as Salmonella enterica subsp. enterica, 10 as Salmonella enterica subsp. salamae, 7 as Salmonella enterica subsp. diarizonae, 1 Salmonella enterica subsp. houtenae, 1 Salmonella enterica subsp. arizonae and 8 Salmonella Bongori. The most frequently encountered (in different batches) serotypes in the group of Salmonella enterica subsp. enterica designated by their antigenic formula included Salmonella Agona (6), Aterdam (5), Bergen (5), Isangi (7), Johanesburg (7), Karamoja (6), Kentucky (7), Mbandaka (10), S. Millesi (6), Orion (5), Poona (5), Ruivu (5), Senftenberg (11), Telelkebir (5) and Tennessee (10). The study indicated that raw sesame edible seeds constitute a potential hazard for human consumption

PP169

Volatile metabolites analysis coupled with machine learning for the rapid quality assessment of chicken meat

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The determination of volatile compounds (VOCs) in tandem with machine learning could contribute to a rapid and reliable quality assessment. This study aimed to the investigation of applying gas chromatography-mass spectroscopy (GC-) and e-nose in combination with different machine learning algorithms to estimate the microbial load in chicken fillet, regardless of the part of the carcass originating from. Chicken thigh and breast fillets (n=240) were aerobically stored at different temperature conditions for specific time intervals and were microbiologically analyzed for the determination of aerobic plate counts (APC). Solid phase microextraction (SPME) combined with GC– and e-nose analysis were also performed for the estimation of VOCs. Different machine learning regression models (Partial Least Square, Multilinear, Bayesian, k nearest neighbors, Support Vector Machines, Random Forests and Extra Trees regression) were generated and validated to assess the correlation between GC-, e-nose and microbial data. Both GC- and e-nose showed satisfactory models' performance using specific algorithms. Tree-based algorithms were more efficient in predicting the microbial populations with both analytical technologies as indicated by the performance indices on the validation dataset (slope a; 0,98 and 0,83, R2; 0,94 and 0,69, RE; 0,27 and 0,63 for the GC- and e-nose, respectively). This work has been funded by the project DiTECT (861915)

This work has been funded by the project DiTECT (861915)

PP170

Bioprotective effect of probiotic lactic acid bacteria against Salmonella enterica in orange juice

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Introduction: Non-sterile fresh juices have been identified as the vehicle of foodborne pathogens such as Salmonella in several outbreaks. On the other hand lactic acid bacteria (LAB) can exert strong antagonistic activity against many pathogenic and spoilage microorganisms. In parallel probiotic lactic acid bacteria confer several health and nutritional benefits in foods. The aim of this study was to examine the effect of two probiotic strains as free and encapsulated against Salmonella during orange juice storage.

Methods: Orange juice (pH 3.8) was inoculated with 104 CFU/mL of a strain cocktail of Salmonella enterica and with a mixed culture of commercial probiotic strains; Lacticaseibacillus casei Shirota and Lacticaseibacillus rhamnosus GG at initial population 108 CFU/mL in free and encapsulated form with whey protein isolate (WPI) /gum arabic (GA). Juice inoculated only with the pathogen was used as control. All samples were stored at 5, 10 and 25oC for up to 7 days and microbiological (enumeration in XLD agar and enrichment procedure) and pH analyses were performed.

Results: Results showed that during fruit juice storage at 5 and 10oC in the presence of LAB, Salmonella counts reduced by 0.5 log CFU/mL in 24h and by 3 log CFU/mL after 4 days while in the control samples no pathogen reduction was observed in 24h and only 1.5 log CFU/mL reduction after 5 days of storage. In contrast at 25oC in samples containing probiotics, Salmonella counts dropped by 1.5 log CFU/mL in 24h and at 2 days the pathogen was detected only after enrichment while in the control samples the pathogen survived for 4 days. The pH of the juices containing the probiotic strains was slightly reduced to 3.5, whereas remained stable in the control samples. Concluding, the presence of the probiotic lactic acid bacteria accelerated the reduction of Salmonella in fruit juice with the reduction being more evident at 25oC.

Significance: The use of probiotic lactic acid bacteria in fruit juices can have a beneficial effect in human health and bioprotection against foodborne pathogens.

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PP172

Potential of Fourier transform infrared spectroscopy for the rapid monitoring of ochratoxin A and aflatoxin B1 in foods

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Aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) have gained global attention and concern due to their toxicity and widespread occurrence in various food and feed commodities. Currently, conventional analytical methods applied to determine mycotoxins are time-consuming and require complex sample preparation. Fourier transform infrared spectroscopy (FTIR) has emerged as a promising alternative for the rapid and non-destructive monitoring of these toxins. This study evaluated the potential of FTIR for the rapid quantification and detection of AFB_1 and OTA in foods. AFB_1 (n=26) and OTA (n=46) standard solutions were prepared with MeOH/CH3COOH (99:1 v/v) and MeOH/purified H2O (50:50 v/v) solvents, respectively, at various concentrations ranging from 0.1 to 10.0 ppb. Samples were subjected to quintuplicate FTIR measurements. Spectra for AFB_1 (n=130) and OTA (n=230) were acquired over the wavenumber range of 4000-450 cm^{-1} using a diamond ATR crystal plate. Four distinct spectral regions, including the entire spectra, were analyzed for each mycotoxin to identify the optimal wavelength range that provides the most informative data. Partial Least Square (PLS) regression models were calibrated and externally validated by partitioning each dataset into a training (60%) and test (40%) set. The generated models were also refined by selecting the most important wavenumbers using the regression coefficients (b-coefficients). Model performance was assessed through the root mean square error (RE) and the coefficient of determination (R^2). The AFB_1 model based on spectral data from the wavenumber region of 3750-2550, 1850-850 cm^{-1} achieved the highest performance when externally validated, with R^2 and RE values of 0.812 and 1.069, respectively. Accordingly, the OTA models based on spectral data from 3650-2700, 1800-900 cm^{-1} region provided satisfactory predictions, with an R^2 value of 0.885 and an RE value of 0.917. Feature selection further increased the performance of OTA model (R^2 = 0.906 and RE = 0.829) These findings suggest that FTIR has the potential to be an effective tool for the rapid and accurate qualitative and quantitative determination of mycotoxins in various food matrices.

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PP173

Predicting the survival of probiotic Lactiplantibacillus plantarum in fruit juice as a function of pH and storage temperature

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Probiotic foods receive market interest as health promoting functional foods. However, the severe conditions often employed during food processing and storage might lead to important losses in viability of probiotic cells. Microencapsulation is considered as the most efficient method that protects probiotics against external adverse environment. The aim of this study was the development of mathematical models to describe the combined effect of storage temperature and pH on viability of encapsulated and non- encapsulated cells of Lactiplantibacillus plantarum in fruit juice.

A complex microencapsulation system, comprised of whey proteins and gum arabic, was developed to protect the cells of the probiotic. Survival kinetics experimentations were conducted for both free and encapsulated cells at various pH (2.6-3.6) and static temperatures (5-15°C) for the estimation of the kinetic parameters. A mathematical model based on the Weibull distribution was used to fit the survival curves of the microorganism. The estimated data of the time to first log reduction were expressed as a function of temperature and pH.

Results indicated that microencapsulated bacteria showed higher viability than free probiotic bacteria especially in more acidic conditions. Kinetic parameters for both encapsulated and free cells in different conditions were estimated and used for the development of a mathematical model for predicting the survival of encapsulated and non-encapsulated cells of Lactiplantibacillus plantarum in fruit juices with different pH and storage conditions.

Microencapsulation systems are of high importance considering the viability of probiotic bacteria, especially in more severe environments. The developed models could be further used for the prediction of the shelf life of fresh fruit juice while maintaining the viability of probiotics.

The proposed model is expected to support the FBOs in selecting an effective expiration date, leading to the maximum exploitation of the product's shelf life, containing the desired concentration of probiotics.

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PP175

Comparative genomics of Bacillus cereus isolates responsible for sweet curdling of milk

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Spore-forming bacteria belonging to the Bacillus cereus group (Bacillus cereus sensu lato) are widespread in the environment, many of which are food pathogens or responsible for food spoilage1,2. Sweet curdling is the coagulation of milk without the production of acid. It is usually caused by the proteases of the spore-forming bacteria of the Bacillus cereus group3,4. In the present study, we used comparative genomics to identify the mechanism underlying these events. Bacillus cereus sensu lato isolates from pasteurized milk that were known to form sweet curdling were grown in liquid cultures, and their genomic DNA was isolated using the Quick-DNA HMW MagBead Kit. Libraries were constructed with the Nextera XT DNA Library Preparation Kit and sequenced using the Illumina MiSeq platform along with the MiSeq Reagent Kit v3 (600-cycle). The analysis was performed on the PATRIC platform, using the tools "Comprehensive Genomic Analysis" and "Comparative Systems". The results highlight genes and transcriptional factors that may play a role in this mechanism suggesting new insights for further research on this topic. These findings, together with transcriptomics analysis, will contribute to a better understanding of the underlying molecular process of sweet curdling defects, as well as the development of new approaches for detecting early spoilage events.

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PP177

Modeling the survival of two probiotic strains encapsulated in a whey protein isolate - gum arabic coacervate matrix under harsh conditions

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Introduction: Microencapsulation is an efficient method that protects microorganisms against adverse conditions apparent during food manufacturing/storage. This work studied the effectiveness of an encapsulation system to increase the survival of two probiotic strains under low pH values during storage of a food-model.

Methods: Lacticaseibacillus casei Shirota and Lacticaseibacillus rhamnosus GG (inocula of 8 log CFU/mL) was encapsulated in a whey protein isolate and gum arabic (WPI-GA) coacervate matrix. The encapsulated cells were added in a food-model (Tryptic Soy Broth without glucose) to study their survival under pH conditions of 2.0, 2.5, 3.0, 3.5, 4.0 during storage at 4oC and 10oC. Furthermore, non-encapsulated (free) cells were studied as controls. All microbial data were employed as an input in the modified-Weibull model (GInaFiT-version:1.6) to describe their survival kinetic parameters.

Results: Results showed that the encapsulated cells exposed to pH 2.0 and 2.5 decreased gradually to reach 3 log CFU/mL after 12 days of storage, in contrast to free cells that showed an immediate 2 log reduction and were not detected from the 2nd day and onwards. After 24h of storage at both temperatures, the encapsulated cells showed a 0.5 log CFU/ml population reduction, in contrast to free cells that declined by 6.5-7.0 log CFU/ml. At the end of storage at both temperatures, population of the encapsulated cells was 3.0 log CFU/ml, in comparison to free cells that were not detected (<1.0 log CFU/ml). At pH 3.0 and 3.5, population of the encapsulated cells was 3.0-3.5 log CFU/ml, while free cells population was 1.0 log CFU/ml lower. At pH 4.0, population of the encapsulated and free cells was maintained at 7.0 and 6.0 log CFU/ml, respectively, throughout storage. The developed modified-Weibull model for the survival data of the probiotics indicated a good fit since high R2 (0.99) and low RMSE values were found at most of the cases examined. Encapsulated cells provided significantly higher δ-values than non-encapsulated cells, indicating enhanced survival.

Significance: Encapsulation with WPI-GA can be efficient in protecting probiotics in adverse environments.

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PP178

Survival of Lactic acid bacteria and pathogens in a fermented product made from soy beverage during cold storage

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The present work studied the adaptation and acid-producing capacity of lactic acid bacteria in a plant-based milk substitute. More specifically, the behavior of selected lactic acid bacteria (LAB) from the collection of the Dairy Laboratory, ACA-DC: St. thermophilus ACA-DC 0020, Lb. bulgaricus ACA-DC 0081, Lb. plantarum ACA-DC 2582 and Lb. acidophilus ACA-DC 4002 in a soy ink was observed, followed by a trial preparation of a corresponding soy-yogurt dessert with the additional use of a stabilizer (modified starch) and sucrose. The prepared soy yogurts were tested for total microbial population of LAB, pH, acidity, water holding capacity, solid residue, and fat and protein content. Moreover, the viability of three pathogenic bacteria: Listeria monocytogenes, Staphylococcus aureus, and Escherichia coli, was studied in the above-fermented product. Three experimental tests were performed by inoculating the prepared soy yogurts with a high population (~105 cfu/ml) of the above pathogenic strains and testing for their survival over the storage period at 7 oC. The population of LAB was maintained at high levels, with an initial population at the level of 8 logarithmic cycles, which did not appear to have any reduction over 28 days. Regarding the survival of pathogen microorganisms, the results showed relative stability in their population, except for Escherichia coli, for which a 2-log decrease was observed. At the same time, for the other two, the reduction was lower and not exceeded 1 log cycle. Furthermore, the relatively high pH value of 4.9 of soy yogurt was kept constant until the first 2 weeks of storage, showing a op of ~ 0.3 units until the end of the storage period. The changes in acidity were smaller in the first week and more pronounced later in the last two weeks. The product was not characterized by a high water holding capacity, but followed a relatively stable and slight downtrend in the samples over the weeks, while the stabilizer did not indicate any particular effect on it.

PP178a

DESIGN AND DEVELOPMENT OF FUNCTIONAL STRAINED YOGHURT WITH PROBIOTIC CULTURES ENCAPSULATED IN PREBIOTIC MATRICES

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INTRODUCTION: Despite their proven benefit on the gut health, the addition of probiotics in food products remains a challenge for the food industry due to their short viability and vulnerability during the production process. Microencapsulation of probiotics in prebiotic matrices may enhance their viability during processing and through the intestinal tract too. AIM: This study aims to design and develop a functional dairy product with microencapsulated probiotic cultures in prebiotic matrices, in laboratory and pilot scale. METHODS: Strained yoghurt samples were developed in laboratory scale using probiotic cultures isolated for Greek dairy products, human colon, and/or commercially available cultures. A stability study was conducted for 15 days (4 ºC) examining appropriate criteria (pH, organoleptic characteristics, probiotic cultures viability, product's shelf-life). Hedonic test was performed for the acceptance of the product (taste, structure, color, odor etc.). RESULTS: A commercial culture of *Bifidobacterium lactis, obtained* by China, encapsulated in whey protein: inulin matrices, presented simultaneously the best shelf-life, probiotics' viability and organoleptic acceptance and was selected to be upscaled in pilot scale. CONCLUSION: The acquired *Bifidobacterium lactis* encapsulated in appropriate matrices, could be used as innovative ingredients in functional food products, presenting sufficient viability. The strained yoghurt with microencapsulated probiotic cultures was organoleptically accepted. Future studies would be interesting to examine its potential biological action.

Key words: probiotics, Bifidobacterium lactis, microencapsulation, viability, dairy products, functional food products

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PP178b

Fate of *Salmonella enterica* in orange juice and in consequent simulated human gastrointestinal system in the presence of free or encapsulated probiotic lactic acid bacteria

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Pathogens such as *Salmonella* have been detected in fresh juices, causing a threat to the consumers. This study aimed to examine *Salmonella enterica* survival in juices containing lactic acid bacteria (LAB) during storage and consequently in simulated human gastrointestinal system (GIS). Orange juice was inoculated with 1.7 log CFU/mL *Salmonella* enterica (control) and with 8 log CFU/mL of probiotics *Lacticaseibacillus casei* Shirota and *Lacticaseibacillus rhamnosus* GG (free and encapsulated cells). Encapsulation matrix was whey-protein-isolate/gum-arabic. Samples were stored at 4°C and 12°C for 5 days. Every 24h the static *in vitro* digestion protocol was applied in all samples mimicking juice consumption, i.e., samples were diluted (1:1 vol/vol) with simulated gastric fluid (SGF) containing pepsin, $CaCl_2$ and HCl (pH=3) and incubated under agitation (2h, 37°C) and accordingly, the gastric chyme was diluted (1:1 vol/vol) with simulated intestinal fluid (SIF) containing bile salts, pancreatin, $CaCl_2$ and NaOH (pH=7.5) and incubated at same conditions. At time=0 (immediate after storage), and every 1h of each stage of GIS, microbiological (MRS agar and ISO:6579-1:2017 *Salmonella* enrichment protocol) and pH analyses were performed. Results showed that after 5 days of storage at both temperatures, LAB (free and encapsulated) were maintained at 8 log CFU/mL, whereas *Salmonella* declined by 1 log CFU/mL in juices containing free LAB, in contrast to no reduction in the control and the juices containing encapsulated LAB. During GIS simulation, in juices with free LAB cells, the pathogen was not detected after 1h in SGF, while in control samples it was 1.4 log CFU/mL and in juices with encapsulated LAB, 1 log CFU/mL. After SIF treatment, *Salmonella* in control was 1.2 log CFU/mL, whereas in juices containing free and encapsulated cells was not detected. Finally, free and encapsulated LAB were 4 and 7 log CFU/mL after SIF treatment, respectively. In conclusion, microencapsulation can protect the probiotic LAB cells against GIS stress conditions and maintain high population levels, while their release in gut can eliminate Salmonella.

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Keywords: orange juices, probiotics, microencapsulation, pathogens, static in vitro digestion protocol

PP178_c

Natural Fruit Juices Enriched With Probiotic Bacteria And Other Biofunctional Constituents In Encapsulated Form

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Encapsulation of probiotics is applied to ensure the functionality and viability of the added cultures in the stressful environment of foods and the adverse conditions during processing, handling, storage and consumption of these products. The project "FUNJUICE" aimed to develop new biofunctional natural fruit juices containing encapsulated probiotic bacteria and other biofunctional components (vitamin D and omega3 fatty acids), which will not affect their quality and organoleptic characteristics. Furthermore, the project expected to add value to these products by preventing pathological conditions in humans, promoting health and maintaining quality of life. Whey protein was selected as encapsulating material to enhance the protein content of the products. The shelf life of these products was determined, and an easy-to-use prediction software was developed for the estimation of viability of probiotics in juices and in other products. Through "FUNJUICE": a) a variety of encapsulation systems (whey protein isolate-Arabic gum coacervate, potato protein-pectin coacervate, etc.) were studied along with the structured release systems, b) the effectiveness of the developed encapsulation systems to increase the viability and control of probiotic cell metabolism, and maintain the added biofunctional components during processing, distribution and storage of juices were assessed, c) mathematical models to predict the survival of encapsulated probiotics in juices were developed and validated to develop a prediction software that can be used to efficiently design products and determine their shelf life and d) the viability of encapsulated probiotics and other biofunctional ingredients and their effect on health were estimated in three clinical trials (short, medium and long term) with healthy adults or with patients in high cardiometabolic risk. The results showed that the examined encapsulated systems can be efficient in protecting probiotics in adverse environments apparent during food manufacturing/storage. Finally, preliminary results of the clinical trials showed that the addition of vitamin D3 and probiotic bacteria to juice had beneficial effects on postprandial glycemia, on the reduction of body weight, blood pressure and blood lipids.

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Keywords: juice, microencapsulation, clinical trials, probiotic bacteria

PP178_d

Effect of microencapsulation on the survival of probiotic bacteria in model food and in orange juice during heat treatment

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Microencapsulation is considered as an effective technique to prolong probiotic survival during exposure to harsh conditions (i.e., heat treatment). The aim of this study was to examine the survival of encapsulated cells after heat treatment. *Lacticaseibacillus casei* Shirota and *Lacticaseibacillus rhamnosus* GG were encapsulated (inocula 9 log CFU/mL) in a whey protein isolate-gum arabic coacervate matrix (WPI:GA). Consequently, the encapsulated cells were added in a food-model (TSB without glucose) or in orange juice and heat treatment was applied for 50, 55, 60°C for 0, 1, 5, 10, 15 min. Free cells were used as controls. The experimental data (counts in log CFU/mL) were fitted using different modified Weibull model to describe the survival kinetics of bacterial population after treatment. Results showed that after treatment at 50°C, the population of encapsulated cells was maintained stable (9 log CFU/mL) for each holding time at both broth and orange juice, whereas treatments in higher temperatures resulted in a higher population decline, to reach final populations of 5 log CFU/mL. Free cells decreased (<3 log CFU/mL), after treatment at 55, 60°C for 15 min. The modified Weibull model provided a good fit to the survival data of the bacteria (R^2>0.960). As regards δ rate, lower rate indicates higher death rate of bacteria, consequently, the lowest δ-values were obtained for the samples heated at 60°C for 15 min. Also, since δ parameter corresponds to the time of the first decimal reduction of the population, the encapsulated cells provided the highest survival rates, especially at 50°C, where no decimal reduction was observed during heat treatment. It can be concluded that the WPI:GA coacervate can be efficient in protecting the sensitive probiotic cells during heating at mild temperatures.

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Keywords: heat treatment, Weibull model, probiotic cells, microencapsulation

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Application of novel olive fruit processing methods and technologies for the high-efficiency production of olive oil and olive paste with improved quality and nutritional characteristics

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Greece holds the 2nd place in olive oil and table olives production, the 2nd place in EU exports for both products, and the 2nd and 4th place in table olives and olive oil exports outside of the EU, respectively (IOOC, 2022). These data indicate that there are high margins to optimize the exports and the profit if the aforementioned products are standardized and the added value is increased. Thus, the objective of the project is the application of novel technologies and processing methods to produce olive oil and olive paste (including PDO) that reinforce and feature the unique products characteristics, ensuring in parallel the hygiene and safety standards. The new method that is being applied is the cold forging hydraulic press (CFHP) with novel hydraulic system equipment to produce olive oil and olive paste and the enzymatic processing of the raw olive paste. The CFHP mild treatment offers high added value olive products with high concentrations of nutritional compounds and polyphenols. The Project's actions are the following: a) production of olive oil and olive paste from raw olive drupe of 3 Greek varieties (including PDO), b) production of olive paste products from fermented table olives of 2 Greek varieties (including PDO), and c) assessment of selected processing by-products as soil improvers, aiming to exploit the olive drupe to the maximum.

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Keywords: olive oil, olive paste, fermented olive paste, novel processing methods