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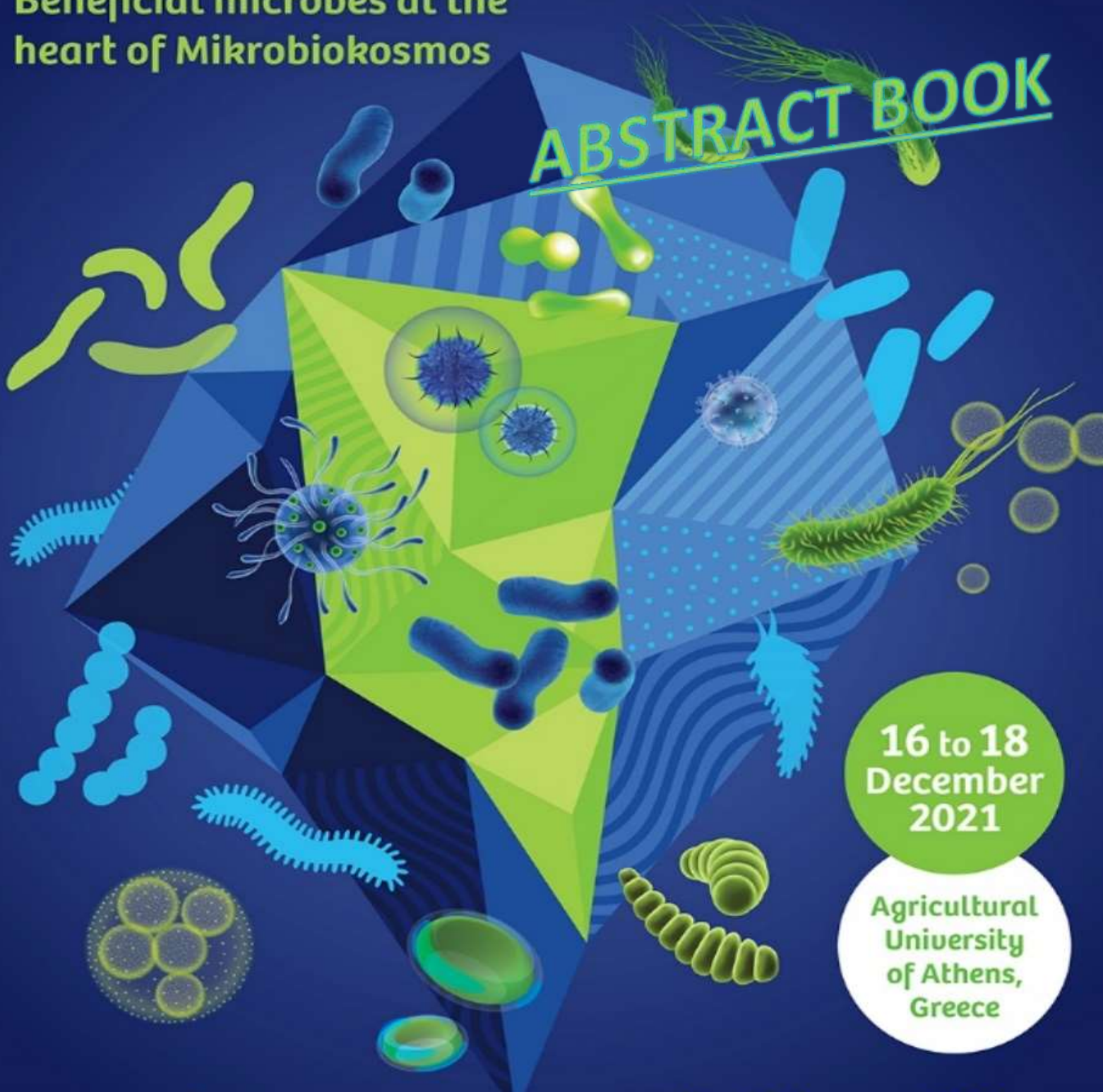
ABSTRACT BOOK



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OP_01

BEYOND ON-TARGET EFFECTS OF POTENTIAL AND ESTABLISHED NITRIFICATION INHIBITORS ON THE SOIL MICROBIOTA

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Dicyandiamide (DCD) and nitrapyrin (NP) are established nitrification inhibitors (NIs) used in agricultural settings. Recently, the fruit preservative ethoxyquin was proposed as a novel NI, acting through its oxidation derivative quinone imine (QI). Still the specific activity of these NIs on the different ammonia-oxidizing microbes (AOM), and mostly their off-target effects of on other soil microbiota components remains unknown. We determined the impact of DCD, NP and QI applied at two doses (low: recommended for agronomic use; high: multiple times higher), on the function, diversity, and dynamics of target (AOM), functionally associated (nitrite-oxidizing bacteria (NOB)), and off-target prokaryotic and fungal microbial communities in two loamy soils mainly differing in pH (acidic vs. alkaline).

This was achieved via monitoring inorganic N-pools, potential nitrification (PN) rates, amoA gene and transcripts abundance, the abundance of other phylogenetic marker genes (nxrB,16S rRNA, 18S rRNA) and amplicon sequencing of amoA, 16S rRNA and ITS. DCD was more persistent than NP and QI and showed a dose-dependent dissipation pattern with higher persistence in the alkaline soil.

QI was equally efficient to DCD and less efficient than NP in inhibiting nitrification in the acidic soil, while in the alkaline soil QI was less efficient than DCD and NP. This was attributed to the higher activity of QI towards AOA prevailing in the acidic soil, as inferred from q-PCR and RT-q-PCR measurements, unlike AOB whose abundance and transcriptional activity was less affected by both QI dose rates. Amplicon sequencing revealed a significant and uniform effect of all NIs on the AOB community in both soils, unlike AOA which were less responsive. Beyond on target effects, we observed a dose-dependent effect of the NIs on the dynamics of NOB in the alkaline soil, with Nitrobacter being more sensitive than Nitrospira.

QI was the only NI that induced significant changes in the composition of the bacterial and fungal communities that might imply for broader effects on soil ecosystem functioning. Our findings lay the ground for a comprehensive assessment of the effects of NIs on the soil microbial community, beyond the current focus on target AOM.

OP_02

PHYLOGENETIC COMPARISON OF THE RHIZOSPHERE-ASSOCIATED MICROBIAL POPULATIONS OF FOUR DIFFERENT ALFALFA GENETIC LINES

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Rhizosphere-associated bacteria (RAB) play an integral role to plant growth due to microbial processes, such as carbon and nitrogen fixation, potassium and phosphorus mineralization, and iron solubilization that enable nutrient availability to the plant. The RAB community seems to be influenced by plant root exudates that signal chemotaxis specific for the microbe-root association as demonstrated for plants of the legume family (Fabaceae) and nitrogen-fixing bacteria. Therefore, specific microbe-plant symbioses seem to be linked to specific plant types and growth requirements.

Alfalfa, a perennial, herbaceous legume, is of great economic significance as it provides excellent quality livestock feed, thus supporting the production of our primary food protein source. Understanding RAB communities and microbial function specializations in alfalfa roots is therefore beneficial for better crop maintenance and yield increase.

In order to study RAB communities that might 'specialize' in alfalfa root associations and perhaps specific high-yield genetic lines (Medicago cultivars), we investigated the microbial communities (16S rRNA gene) associated with the rhizosphere of four field-grown lines. These lines were selected out of a total of 17 different lines based on average forage yield from a total of 35 plants per line. Rhizosphere soil samples were acquired from selected individual plants of the two highest and two lowest yielding lines in two ways in order to account for sampling biases; (a) a spatially random selection of six plants per row for each line and (b) a selection of the four first and four last plants per row for each line. Soil samples from proximal uncultivated areas (bulk soil) were also acquired. Extracted soil DNA was amplified with Archaea- and Bacteria- specific primers and analyzed with Denaturing Gradient Gel Electrophoresis (DGGE) in order to compare phylogenetic diversity, and identify the main microbial populations present in the bulk and rhizosphere environments. In addition, a more comprehensive phylogenetic analysis of the bacterial communities was performed using Next Generation Sequencing in order to identify dominant bacterial taxa and estimate useful ecological indices such as richness and taxa diversity and relative abundance within each sample (alpha-diversity) but also between rhizosphere and bulk samples and between line genotypes (beta-diversity).

Keywords: Alfalfa, crops, root-associated, DGGE, MiSeq, 16SrR

OP_03

INVESTIGATING THE BACTERIAL COMMUNITIES IN THE BELOWGROUND COMPARTMENTS OF POTATO PLANTS

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Bacterial communities of the potato (Solanum tuberosum) rhizosphere have been studied in detail as part of the aim to improve plant productivity. However, less is known about the tuberosphere (soil attached to the tuber surface) bacteria and how they compare to the structure of the rhizosphere and bulk soil. In the present study, we conducted bacterial community analysis of the tuberosphere, rhizosphere and bulk soil, of two potato varieties (Kerr's Pink and Rooster) grown in two fields with different physicochemical characteristics. Following soil DNA extraction, the 16S rDNA libraries were sequenced on a Illumina platform. The diversity (Shannon index) and richness (Chao1) were similar between the three compartments of both varieties, indicating that alpha diversity was not affected by the soil compartment.

Non-metric Multidimensional Scaling (NMDS) analysis demonstrated that in both varieties the bacterial communities in the bulk soil and tuberosphere clustered together and they were different from the communities in the rhizosphere. These results were largely confirmed by ANOSIM testing. Furthermore, relative abundance analysis revealed that Proteobacteria, Firmicutes, Actinobacteriota and Acidobacteriota were the most abundant phyla and presented differences in the three soil compartments of both varieties.

Soil compartments had also a significant effect on arylsulfatase activity that was highest in the rhizosphere in both varieties. Likewise, the abundance of sulfonate utilizing bacteria was also highest in the rhizosphere which was significant for Rooster.

The results of this study show that different mechanisms are involved in the selection and patterns of assembly of bacterial communities in the rhizosphere and tuberosphere, putatively due to a lack of exudates stemming from the potato that is in contrast fueling the rhizosphere community.

This trend was confirmed when a functional comparison of the bacterial communities i. e. sulfur cycling was assessed, highlighting the importance of the unique rhizosphere habitat with its bacteria putatively involved in plant sulfur supply.

OP_04

ENDOPHYTIC BACTERIAL ISOLATES FROM HALOPHYTES & OLIVE TREES: A POTENTIAL SOURCE OF BENEFICIAL MICROBES FOR A SUSTAINABLE AGRICULTURE

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The need for alternative strategies of crop improvement is intensifying, due to global population increase, the subsequent required increase in yields of food crops to reach demand, climate change, and the deterioration of agricultural soils due to extensive use and increased salinity.

The close relationship with microorganisms is a plant mechanism to tolerate various stresses and facilitate their growth and development. Endophytes are non-pathogenic microorganisms that live within plant tissues for a part or for the duration of their life cycles. Endophytes have been shown to boost the immune system of host plant, and to protect their hosts from drought, low temperature, and salinity.

The Microbiology & Plant Biotechnology Lab at IMBB-FORTH and UoC, has embarked the study of root endophytic bacteria from olive trees and from a variety of halophytic plants, using a combination of culture-dependent methodologies and metagenomic methods of the entirety of the root endophytic microbiome.

To better understand the root endophytic microbiome, we have collected root samples from olive trees in Crete. Apart from cultivating and studying 252 endophytic bacterial isolates, we have performed a metagenomic analysis of the root endophytic community using 16S rRNA gene from multiple samples from single trees, across three seasons and from five agricultural fields .

Furthermore, to capture endophytic microbes with potential use in agriculture, we studied the culturable endophytic bacteria of crop wild relative halophytes. The potential of our isolates to improve crop adaptations to various stresses was investigated, using both in-vitro and in-planta approaches. In total, 115 endophytic isolates were identified. Endophytic isolates were identified by their 16S rRNA gene sequence and evaluated for their ability to: grow in-vitro in high levels of NaCl; inhibit the growth of three economically important phytopathogens and the human pathogen Aspergillus fumigatus.

Moreover, they were evaluated for their capacity to provide salt tolerance and promote host plant growth effect in-planta. Moreover, genomes of selected isolates were sequenced, and representatives of three novel species were identified: two Pseudomonas species and one Arthrobacter. Our studies provide proof-of-concept that endophytes can be used as “bio-inoculants,” for the enhancement of growth and stress tolerance in crops.

OP_05

THE MULTIPLE EFFECTS OF THE BIOCONTROL AGENT PSEUDOMONAS PUTIDA Z13 AGAINST BOTRYTIS CINEREA IN TOMATO FRUITS

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Grey mold, caused by Botrytis cinerea, is an important postharvest disease on fresh-market tomatoes. Although fungicide treatment has been one of the main methods for controlling gray mold, there is increasing international concern over the heavy use of fungicides on crops because of the possible harmful effects on human health and the emergence of pathogen resistance to fungicides. Therefore, there is a need to develop alternative disease control methods, such as the use of plant beneficial microorganisms.

The Pseudomonas genus encompasses various species with biocontrol activity against various plant pathogens. Recently, we reported the isolation and identification of strain Pseudomonas putida Z13, a potent biocontrol agent against Verticillium dahliae. Pseudomonas putida is a bacterium commonly used in environmental studies because it is able to degrade many aromatic compounds. It degrades cellulose and chitin, and also produces a large number of different antibiotics.

The aim of the present study was to evaluate the biocontrol activity of strain Z13 against Botrytis cinerea in tomato f ruits. The application of Z13 in tomato fruits reduced disease severity and incidence by 50% compared to controls.

This plant protective activity can be attributed to antibiotic production, since Z13 reduced the growth of B. cinerea in vitro and also to the priming of the plant defense responses. The qPCR analysis revealed that expression of the defense associated genes PR1 and WRKY70 was upregulated in the Z13 treated fruits; this upregulation was most prominent upon B. cinerea infection.

Therefore, Z13 may constitute a biocontrol agent targeting multiple pathogens in different plant species, as it has been shown in our experiments against V. dahliae and B. cinerea in eggplants and tomato fruits, respectively.

OP_06

GRAPEVINE WOOD MICROBIOME ANALYSIS IDENTIFIES KEY FUNGAL PATHOGENS AND POTENTIAL INTERACTIONS WITH THE BACTERIAL COMMUNITY IMPLICATED IN GRAPEVINE TRUNK DISEASE APPEARANCE

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Grapevine trunk diseases (GTDs) is a disease complex caused by wood pathogenic fungi belonging to Phaeomoniella, Phaeoacremonium, Fomitiporia, Eutypa and Botryosphaeriaceae. GTDs appear mostly in mature vines causing central, black necrosis and/or white rot leading to vine stunting and annual economic losses of 1.132 million euros. Even though GTDs have been studied and described over a century we still lack a good understanding of the fungi involved, their interactions and the factors controlling their assemblage. We determined the fungal and bacterial microbiome in wood tissues of asymptomatic and symptomatic vines of three main Greek cultivars (Agiorgitiko, Xinomavro, Vidiano), each cultivated in geographically distinct viticultural zones, using amplicon sequencing. Our analysis identified cultivar/biogeography (lumped factor) as the strongest determinant of the wood fungal microbiome ($p<0.001$, 22.7%), while GTD symptomatic condition showed a weaker but significant effect ($p<0.001$, 3.5%), being prominent only in Xinomavro. Several fungal ASVs reported as GTD-associated pathogens like Kalmusia variispora, Fomitiporia spp., Neosetophoma spp., and Phaemoniella chlamydosporα (most dominant in our study) were positively correlated with symptomatic vines in a cultivar/viticultural zone dependent pattern. Random Forest analysis pointed to P. chlamydosporα, K. variispora, A. alternata and Cladosporium sp., as highly accurate predictors of symptomatic vines (0% error rate). The wood bacterial microbiome showed similar patterns, with biogeography/cultivar being the main determinant ($p<0.001$, 25.5%), followed by the GTD status of vines ($p<0.001$, 5.2%). Differential abundance analysis revealed a universal positive correlation ($p<0.001$) of Bacillus and Streptomyces ASVs with asymptomatic vines, and network analysis identified a significant negative co-occurrence network between these bacterial genera and Phaemoniella, Phaeoacrominum, Seimatosporium suggesting a plant beneficial interaction between Bacillus/Streptomyces and GTD pathogens. We provide first evidence that GTDs favor the assembly of a wood fungal microbiome and a pathobiome whose composition shows cultivar and/or biogeography-dependent patterns. Specific members of this fungal wood microbiome could be used as a proxy to distinguish between healthy and diseased vines. Potential interactions between the bacterial and fungal wood microbiome in GTD-free vines would be further pursued in the quest for discovery of novel biocontrol agents.

OP_07

UNRAVELLING THE IMPACT OF CONVENTIONAL AND ORGANIC FARMING SYSTEM ON BLACK ASPERGILLI POPULATION STRUCTURE, MYCOTOXIGENIC CAPACITY AND MYCOTOXIN CONTAMINATION ASSESSMENT IN GREEK WINES, USING A NEW Q-TOF MS-MS DETECTION METHOD

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Aspergillus bunch rot, caused by several mycotoxigenic species of Black Aspergilli section Nigri is one of the most severe pre- as well as post-harvest diseases of grapevines, while contaminated grape products and derivatives with Aspergillus mycotoxins may have an important impact on consumers health. During a 2-year survey (2018-2019), the impact of conventional and organic farming systems on Black Aspergilli population structure in Greek vineyards was thoroughly investigated. In total, 300 isolates of Aspergillus spp. were selected and identified by amplicon sequencing. Four different Aspergillus section Nigri species (A. tubingensis, A. uvarum, A. carbonarius and A. niger) were identified as the casual agents of the disease in the sampled vineyards. A. uvarum and A. tubingensis were identified as the dominant species in the 2018 and 2019 samplings, respectively.

During both sampling years, higher frequencies of A. tubingensis and A. uvarum were found in the organic and conventional vineyards, respectively.

In vitro production of ochratoxin A and fumonisin B2, B3 and B4 was evaluated in two selective media. The analysis revealed a low frequency of mycotoxigenic isolates, mainly originated from conventional vineyards. Additionally, A. carbonarious was identified as the main OTA producer, whereas A. niger was the leading producer of FB2, FB4, and FB6. In total, 74 organically and conventionally produced wines were analyzed using a new Quadrupole Time-of-Flight Mass Spectrometry (QTOF MS-MS) analytical method developed to detect and quantify various mycotoxins (OTA, FB1, FB2 FB3, AOH, AME, and CIT) using a modified QuEChERS extraction protocol.

Interestingly, a low frequency of mycotoxin-contaminated wines was detected. However, fumonisins were identified as the main mycotoxins in Greek wines compared to OTA which was found in only one sample. This is the first study deciphering the impact of conventional and organic farming systems on Aspergillus section Nigri species in Greek vineyards, suggesting that cropping system may affect the species composition within the vineyard.

OP_08

CHARACTERISING AN EFFECTOR FROM THE FUNGAL PATHOGEN OF WHEAT, ZYMOSEPTORIA TRITICI

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Septoria tritici blotch (STB) is a severe wheat disease caused by the filamentous ascomycete Zymoseptoria tritici. During outbreaks, wheat yields can be reduced up to 40% (Eyal et al., 1987), while no wheat varieties are fully resistant to the pathogen (Torriani et al., 2015). The Z. tritici genome includes at least 90 effector genes, which encode small, secreted proteins (ZtSSPs). These effectors/ZtSSPs have a potential role in pathogenicity and virulence as they can modulate the host's defense and facilitate infection (Karki et al., 2021; Macho & Zipfel, 2015). However, only a limited number of such ZtSSPs have been characterised from Z. tritici. Previously, ZtSSP2; a small, conserved, cysteine-rich secreted effector from Z. tritici, was found to interact with a wheat ubiquitin ligase which plays a role in defense against STB (Karki et al., 2021). In this study, we further explored the role of ZtSSP2 in Z. tritici pathogenicity and virulence by generating gene replacement fungal knock-out strains (ΔZtSSP2_A; ΔZtSSP2_B; ΔZtSSP2_C). These ZtSSP2 knock-out mutants had significantly reduced disease symptoms and produced less fruiting bodies in wheat, compared to wildtype Z. tritici. Furthermore, yeast two-hybrid (Y2H) assays are ongoing to elucidate further potential interactions of ZtSSP2 with host proteins from wheat. In summary, our findings suggest that ZtSSP2 is key for STB virulence.

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OP_09

USING THE INTERACTION OF THE ENDOPHYTE FUSARIUM SOLANI STRAIN K WITH LOTUS JAPONICUS AS A PROXY TO ASSESS INTRACELLULAR TRANSLOCATION OF EFFECTORS OF ARBUSCULAR MYCORRHIZAL FUNGI

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Through enhancing nutrient and water acquisition, agricultural application of arbuscular mycorrhizal fungi (AMF) can tremendously enhance crop productivity, without compromising the integrity of the world's ecosystems. Nevertheless, usage of AMF in the field remains limited due to variable crop yield enhancement and fungal spore production, suggesting a previously unanticipated species specificity during AMF symbiosis. Microbial secreted proteins, so-called 'effector proteins', are crucial regulators of pathogenic and/or symbiotic outcome. Cytoplasmic effectors target host macromolecules in various ways, allowing evasion of the host immune response and modulation of host metabolism and physiology in such a way that a suitable niche is established.

Progress in elucidating the AMF effectome is hampered by diverse factors. Effector prediction remains notoriously difficult for plant-interacting fungi, due to rapid sequence evolution and a proposed role for unconventional, signal peptide-independent secretion mediated by unknown sequence motifs. Consequently, prediction of cytoplasmic effectors is subject to a consistent error rate, and unambiguous effector secretion- and up-take assays are required to confirm the biological relevance of effector candidates.

Moreover, as the reliability of conventional uptake assays is subject to debate, and AMF are genetically intractable, an unambiguous effector translocation assay is currently lacking for AMF. In order to overcome this bottleneck, we propose the use of Fusarium solani strain K (FsK) colonizing Lotus roots as a model system for AMF effector translocation. Similar to AMF, interaction of host plants with FsK is mutually beneficial and both fungi colonize the plant root in the cortical cells.

Moreover, infection with FsK also uses the so-called common symbiosis signaling pathway. Hence, FsK is a highly interesting model for studying AMF effector translocation in planta, and development of this proxy assay may form a crucial step in elucidating the AMF effectome, enhancing our understanding of the molecular players underlaying this ancient symbiosis.

OP_10

PRODUCTION OF POLYUNSATURATED OMEGA-3 FATTY ACIDS FROM FOREST AND AGRICULTURAL RESOURCES BY THE HETEROTROPHIC MARINE MICROALGAE CRYPTHECODINIUM COHNII

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Lignocellulosic biomass residues constitute an abundant, renewable source of polysaccharides that, when subjected to enzymatic hydrolysis, are transformed into monomeric sugars that serve as carbon sources for microbial fermentation processes. Many tons of forest and agricultural materials are annually made available as side streams of the industrial processes and these residual streams are usually under-valorized. Crypthecodinium cohnii is a heterotrophic microalgae that can grow on various substrates including organic acids and glucose, while accumulating high amounts of polyunsaturated omega-3 fatty acids (PUFAs), more specifically docosahexaenoic acid (DHA) [1, 2]. Since the demand for omega-3 fatty acids has increased significantly in recent years, their production through environmentally friendly alternatives, such as from microalgae, is considered a promising approach. In this work, beechwood, pine and wheat straw biomass were pretreated with a mild oxidative organosolv process (OxiOrganosolv) [3]. A repertoire of different organic solvents (ethanol, acetone, isobutanol, tetrahydrofuran) were used in aqueous mixtures as a liquid phase for the pretreatment, achieving efficient fractionation and recovery of the cellulosic and hemicellulosic sugar streams [2,3]. The sugar streams obtained, namely the hexose-rich solid pulp and the pentose-rich aqueous liquid fraction were enzymatically hydrolyzed and evaluated for their potential to support the growth and lipid accumulation of C. cohnii. The results showed that the strain is able to utilize both hexoses and pentoses, although there are several challenges in the valorization of the liquid fraction, thus achieving >70 wt% total lipid accumulation, while the oil had a high content of DHA (20-40%). The overall process demonstrated the utilization of non-edible biomass towards high added value food supplements in a sustainable and efficient manner.

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OP_12

DEGRADATION OF THE HIGHLY PERSISTENT FUNGICIDE THIABENDAZOLE BY A BACTERIAL CONSORTIUM IN A BENCHTOP BIOREACTOR.

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Thiabendazole (TBZ) is a fungicide used in the fruit-packaging industry. Its use constitutes an environmental threat due to its high off-target toxicity and persistence. We previously enriched from soil a bacterial consortium able to degrade TBZ and characterized its composition, identified the TBZ degrader (a Sphingomonas strain) and the associated metabolic pathway, and pinpointed roles of the rest members. Here, we evaluated the performance of the TBZ-degrading consortium in a 800 ml immobilized cell bioreactor receiving TBZ-contaminated effluent (250 mg/L) operated at a hydraulic retention time of 10 days for over 5 months. We monitored over time its removal efficiency, physical-chemical parameters, the abundance and diversity of total bacteria via q-PCR and amplicon sequencing, and horizontal transfer molecular markers.

We further performed a shotgun metagenome sequencing analysis at three time points for assessing the functional potential and the associated dynamics of the consortium under the operational bioreactor conditions.

The removal efficiency of the bioreactor remained stable throughout its operation, with 97% mean TBZ removal. The pH remained stable (influents: 7.16 ± 0.04; effluents: 7.12 ± 0.05), the mean influent EC was 12.8 mS cm-1 while effluent EC was 13.8 mS cm-1), and the COD removal efficiency was 79 ± 12%. Total Kjeldahl nitrogen removal was 80% and effluent ammonium was 2.3 mg L-1; nitrates were measurable at the effluents in the last 3 months with values 6.7 ± 1.9 mg L-1.

Total bacteria abundance did not significantly vary over the bioreactor operation period as did the horizontal gene transfer marker counts. β-diversity analysis of the amplicon sequencing data showed a stable post-establishment microbial community composition.

The Sphingomonas degrader showed an initial increase in its relative abundance during establishment. Bacteroidetes, Planctomycetes and Chloroflexi OTUs increased over time possibly linked to the establishment of conducive conditions for their growth in the bioreactor. No/low post-inoculum-establishment taxonomic and functional community shifts were observed. Collectively, these results demonstrated high performance stability and high efficiency of the microbial consortium in the immobilized cell bioreactor at the range of tested conditions, highly desirable traits for such biodepuration setups.

OP_13

CONSTRUCTION OF SHUTTLE VECTORS UTILIZING AN ENDOGENOUS ZYMOMONAS MOBILIS CRYPTIC PLASMID

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Zymomonas mobilis is an α-proteobacterium under development for the production of first and second generation bioethanol. The application of Z. mobilis in bioprocesses requires its metabolic broadening via genetic engineering and foreign gene introduction. For this purpose and in this work we created a series of shuttle vectors for Z. mobilis that make use of a 4.5-kb native plasmid from the wild-type strain NCIMB 11163, namely plasmid pZA1003 (GeneBank acc. no. CP001725). pZA1003 harbors genes encoding for a replication initiator protein homologous to those of other α-proteobacteria (RepB), a plasmid addiction toxin-antitoxin system (DinJ/YafQ type), a restriction-modification protein and mobilization proteins (MobA-MobD), resembling those of colicinogenic plasmids.

The shuttle vectors created are fusion products between pZA1003 and a 2.7-kb derivative of pBluescript II KS (+) that lacks the f1 ori. They are extremely stable in all Z. mobilis subsp. mobilis strains tested, except for the parental NCIMB 11163 that hosts the native pZA1003. Additionally, they are highly transferrable to E. coli and Z. mobilis recipients via TraP-mediated mobilization (10-1 and 10-5 transconjugants per recipient correspondingly) and stably co-exist with the broad-host-range plasmids pBBR1MCS and pSUP104 that have been hitherto preferred for gene introduction and maintenance in Z. mobilis. The shuttle vectors are offered full- or downsized such that they lack unnecessary backbone genes and/or the mob region, with or without the Z. mobilis pyruvate decarboxylase (pdc) promoter for desirable insert overexpression and, lastly, with each of three different antibiotic resistance marker genes suitable for selection in Z. mobilis or other proteobacteria.

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OP_15

THE POTENTIAL OF NOVEL FUNGAL ISOLATES FOR THE DEGRADATION OF NON-BIODEGRADABLE SYNTHETIC POLYMERS

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The explosive production of plastic goods and their pervasive disposal have rendered them ubiquitous in the environment. According to recent studies, only 9% of plastic waste generated during the last 65 years has been recycled, a fact that imposes the establishment of a new waste management plan.

Traditional plastic recycling processes demand high energy requirements and toxic reagents, while biodegradation is considered an eco-friendlier alternative, which can achieve depolymerization under mild reaction conditions. In the present work, fungal strains isolated from diverse polluted sites were screened for their ability to degrade synthetic polymers, namely polyethylene terephthalate, polyurethane and polyethylene. For this, screening tests were conducted in agar plates supplemented with respective oligomers as sole carbon source.

Subsequently, the most promising strains were grown in liquid cultures with the aforementioned carbon sources for 3 days at 30 °C and the culture supernatants were used as biocatalysts for the degradation of each plastic.

The yield of biodegradation was estimated by measuring plastic weight loss and variation in average molecular weights. The strains secreting the most efficient enzymes were identified based on their internal transcribed spacer (ITS) sequence.

The fact that fungi possess the enzymatic arsenal to degrade non-biodegradable synthetic polymers, makes microbial degradation a realistic alternative for the utilization of plastic waste. The success of this ongoing research may make this method a more viable solution, so our future goal is to point out, study and evolve the enzymes responsible for polymer degradation.

OP_16

ENHANCEMENT OF CHLORELLA VULGARIS BIOMASS PRODUCTIVITY THROUGH A NON-GMO STRAIN IMPROVEMENT APPROACH FOR FOOD AND FEED APPLICATIONS

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The demands of a growing population, estimated to reach 9 billion people in 2050, is raising concerns regarding the increasing food and feed needs worldwide[1-2]. In order to meet such requirements, it is of outmost importance to explore alternatives to conventional feedstocks, such as microalgae. Microalgae display a variety of interesting nutritional profiles, rich in bioactive compounds, with high protein or fatty acid contents, depending on the species[3-4]. However, only a few microalgal strains are currently approved for human consumption (EU2017/2470), namely Chlorella vulgaris. In addition, microalgal products are still far from having competitive market prices, due to the low productivities attained in industrial cultivation[5]. In this context, strain improvement approaches, such as random mutagenesis, might be essential to develop non-GMO microalgae mutants (DIRECTIVE2001/18/EC) to tackle these hindrances[6-7].

Likewise, this work focused on the development and isolation of novel food-grade non-GMO Chlorella vulgaris'mutants with improved growth performances, through chemical random mutagenesis.

This methodology allowed to isolate eleven mutants, from which two stood out, 200A and 200D, with growth performances 16% and 7% higher (2.21 ± 0.03 and 2.00 ± 0.05 g/L/day, respectively) than the wildtype (1.86 ± 0.09 g/L/day).

Chlorophyll and protein contents of mutants 200A (1.71 ± 0.07 g/100g and 23.78% ± 1.17, respectively) and 200D (1.74 ± 0.08 g/100g DW and 27.94% ± 1.23, respectively) were slightly inferior, comparing to the wildtype biochemical profile (2.00 ± 0.16 g/100g of chlorophyll and 29.66% ± 2.41 of protein).

These novel mutants with improved biomass productivities are very promising for Chlorella vulgaris industrial production. Nonetheless, further work is required to analyse mutants' growth performances and biochemical profiles on a larger scale.

OP_17

DISPERSAL OF AIRBORNE AQUATIC MICROORGANISMS: SETTLEMENT AND GROWTH ON DIFFERENT FRESHWATER COLONIZATION HABITATS

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In the present study we attempted to mimic aeolian ecosystems to examine how filters posed by regional characteristics can influence the settlement and growth of airborne microcolonizers of a common air source. Using a natural single source of aerosols we applied a combined microscopy and high-throughput amplicon sequencing approach to examine the diversity, settling and growth potential of air-dispersed microorganisms in water containers.

These containers represented newly formed aquatic colonization habitats of different trophic state and salinity. Heterotrophic microeukaryotes were favoured as initial settlers when nutrients were low, while autotrophs rapidly proliferated in the high-nutrient containers, possibly due to favourable germinating conditions for their preferred mode of dispersal with resting spores. Following the settlement of colonizers, we investigated two contrasting hypotheses: Are the different water colonization habitats harbouring the same microbial communities after establishment and growth periods?

Or, significant community dissimilarities appear during the growth period? The first scenario points towards a selection of best-fit cosmopolitan colonizers, regardless of habitat-specific characteristics.

Alternatively, confirmation of the latter would suggest a selection of settlers due to bottom-up controls combined with priority effects. Both microscopy and amplicon sequencing suggested that the structure of the microbial communities in the different colonization habitats were driven by nutrient content and salinity, showing clustering to similar bottom-up forces, and dissimilarities in colonization habitats with significant differences of environmental forces.

OP_18

BEHAVIOR OF AUTOCHTHONOUS MICROBIAL COMMUNITIES, WITHIN A DEEP-SEA OIL PLUME, UNDER IN SITU PRESSURE.

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The response to oil contamination by autochthonous deep sea microbial communities has been largely studied using decompressed seawater samples, which are either repressurized or incubated at atmospheric pressure. However, the disturbance of microbial communities due to decompression results in loss of biodiversity followed by unknown effects on the function of microbial communities and, consequently, on overall hydrocarbon degradation rates.

Here, we used a novel high-pressure sampling and experimentation system to collect and incubate deep water microbial communities (600 -1000 m, off southern Crete) maintaining the in situ pressure during the whole process.

The undisturbed microbial communities were subjected to constantly low levels of oil contamination in a high-pressure bioreactor, emulating the conditions within a deep-water hydrocarbon plume. The early and long-term response of the microbial community to the disturbance and the degradation of hydrocarbons under high pressure were studied over a period of several weeks. The results of this study contribute towards a mitigation plan for accidental oil releases in deep-sea environments, tailored to the Eastern Mediterranean Sea.

OP_19

THE EFFECT OF FOULING CONTROL COATING TYPE ON THE DEVELOPMENT OF MARINE BIOFILM COMMUNITIES IN THE SOLENT UK.

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Marine biofouling occurs upon adhesion and accumulation of biofilms on submerged structures [1,2] and constitutes a significant impediment of environmental and economic relevance in marine applications [3]. In the shipping industry, biofouling is partially combated through the application of protective fouling control/antifouling paints on vessel hulls that are divided into biocidal and non-toxic fouling release coating types [4]. Although the use of chemically-active biocides shows a good performance against biofouling, their efficacy against biofilms is often evident to a lesser degree, in addition to the toxicity effects they might impose for marine life. The present study [5] aimed at investigating the effect of coating type on the formation of marine biofilm communities. Microfouling was examined after a 4-month deployment of biocidal (Intersmooth® 7460HS SPC), fouling release (Intersleek® 900) and inert surfaces in Langstone Harbour UK; using Illumina NGS sequencing, targeting the prokaryotic 16S rRNA gene. The results demonstrated distinct biofilm community profiles between the fouling control coating treatments. The biocidal coating was dominated by Alphaproteobacteria (Loktanella, Sphingorhabdus, Erythrobacter) and Bacteroidetes (Gilvibacter), whilst other taxa belonging to Bacteroidetes (Portibacter) and Actinobacteria (Sva0996 marine group) proliferated on the fouling-release surface. The insights provided into biofilm selective attachment and biocidal tolerance on fouling control coatings are anticipated to extend understanding of key biofilm players with potential biocidal resistance and marine biofilm ecology, and contribute towards further development of high-performance environmentally acceptable antibiofilm strategies.

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OP_20

WHAT LIES BENEATH? MISSING LINKS IN THE EVOLUTION OF METHANE METABOLISM FROM THE DEEP SUBSURFACE.

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Methanogenesis in Archaea is among the oldest known metabolisms dating to at least 3.5 billion years and being in great part responsible for maintaining the planet's climate from their Archean heyday until today. Archaeal methane metabolism is inextricably linked to the Wood-Ljungdahl pathway of carbon fixation, itself dating to the Last Universal Common Ancestor. Recently discovered uncultured lineages, have sparked a controversy concerning the evolution of methanogenesis, particularly whether its original form was carbon dioxide reducing or methylotrophic, and how anaerobic methane and alkane oxidation fit in the picture. Such inferences are mostly founded on phylogenies of subunits of the main methanogenesis complexes, the methyl-CoM reductase (Mcr) and tetrahydromethanopterin:CoM methyltransferase (Mtr).

In this contribution we present our recent work on the ecology and evolution of methane and hydrogen metabolism. A comprehensive phylogenomic analysis demonstrated that the last methane metabolizing ancestor was an H_2/CO_2-reducing methanogen that possessed the Wood-Ljungdahl pathway, two closely related families of anaplerotic hydrogenases (Eha, Ehb) and the auxiliary genes belonging in the so-called "methanogenesis markers".

Methylotrophic methanogenesis and anaerobic methane oxidation emerged later and multiple times independently, through a combination of vertical inheritance and a patchwork of lateral gene transfers. Leveraging publicly available metagenomic datasets, we analyzed the taxonomy, metabolism, and biogeography of two novel order-level lineages that represent intermediate stages of methanogenesis loss.

To determine their taxonomic position, we developed WhereDoGGo? (Where Does my Genome Go?), a new pipeline that aims to alleviate biases in prokaryotic phylogenomic placements. These lineages, Hecatellales and Mnemosynellales, are globally distributed in the subsurface, acting as versatile intermediaries in anaerobic carbon cycling.

OP_21

SCRATCHING THE TIP OF THE GLOBAL ARCHAEAL DIVERSITY THROUGH MASSIVE INTEGRATION OF NGS DATA.

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The Archaea domain remains in large part unexplored due to difficulties encountered in isolation and cultivation in the lab. Our lack of knowledge is demonstrated by the underrepresentation of Archaea sequences in public databases like the Living Tree Project (3.5%) and SILVA (4%).
There are currently formally described 35 distinct families, 129 genera and 484 species in those databases. In this work, we utilize the accumulated sequence effort available in public repositories of NGS data to identify novel families, genera and species.

We integrated all Archaea reads from the 500.000 pre-processed samples available in the IMNGS platform. Those 935 millions reads were clustered in 15 million sample-wise 16S Archaea OTUS, originating from a multitude of different ecological niches and sequencing technologies. We taxonomically classified all the sequences in our dataset, aligned them and extracted the most represented region.

This homogenous, quality-filtered dataset was used as input into a novel taxonomic-informed clustering method that we developed, achieving better resolution than naive clustering.

The similarity limits for clustering, representative of the different taxonomic levels, were calculated de-novo for the selected region based on the Archaea sequences present in LTP. For every formed cluster, information related to the origin of the sequences included were kept allowing us to address the distribution of novelty across different environmental niches. Resulted OTUs were tested for additional evidences within metagenomic datasets of GTDB and IMG.

Our results show that nearly hundred thousand families, half a million genera and 2.8 million species of Archaea can be supported by existing sequences.
Over 16% of those predicted molecular species were also found in the set of available metagenomes. We estimate that the lower bound for Archaeal diversity, supported from our analysis, is around 1.5 million molecular species. Most of those novel Archaeal species originated from saline water environments, pointing to the potential of marine ecosystems for novel discoveries.

OP_22

BACTERIAL COMMUNITY TIME SERIES FROM GROUNDWATER SAMPLES

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The use of Next Generation Sequencing (NGS) has allowed the detailed study of microbial communities in aquatic environments. The present study examined the bacterial diversity of groundwater samples from wells in Northwest Romania.

A total of 42 samples were collected from three different water wells from July 2016 to July 2020, in order to investigate spatio-temporal changes. Samples were filtered and DNA extraction was performed, followed by sequencing of the 16s RNA genes with Illumina using universal bacterial primers.

Morisita similarities exceeded 70% for the year 2017 including all wells, while similar results were found for the period 2018-2020, demonstrating stability in bacterial community composition over time. After MDS analysis similar conclusions about the temporal variations of the bacterial diversity were obtained for each well separately.

Finally, taxonomic analysis at the genus level showed the prevalence of Leptospirillum genus in most wells and seasons. This genus is an acidophilic iron-oxidizing aerobic chemolithoautotroph and is common in aquifer samples.

Other species identified, clustered in uncultured Omnitrophica and Saccharibacteria that are also typical in freshwater aquifers. Overall results acquired from this study showed high temporal stability in terms of bacterial community composition and also highlighted groups that suggest good groundwater quality.

OP_23

FECAL AND SKIN MICROBIOTA OF TWO HOSPITILISED MONACHUS MONACHUS FEMALE YOUNG INDIVIDUALS

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Since the introduction of the holobiont/hologenome concept an ongoing explosion of the scientific effort in unraveling the microorganisms (microbiota) and their genomic features (microbiomes) associated with plants and animals is underway.

However, host-microbes associations in marine and especially protected mammals are still poorly studied. Here we analyzed the fecal and skin microbiome of two healthy orphan monk seal (Monachus monachus) pups of the same age during their 8-week hospitalization in one of the MOm rehabilitation centers.

The two individuals were kept in the same tank following similar feeding regimes. During their hospitalization, feces and skin samples were collected separately from both individuals nine and four times, respectively. Illumina sequencing using universal bacterial primers followed DNA extraction with standard procedures. Sequences data analysis was performed with MOTHUR and taxonomic classification was performed with the SILVA database.

The feces Shannon diversity index decreased slightly the first days and reached its maximum at the end, while it was always higher in the skin samples. Analysis of Similarities (ANOSIM) in OTUs abundance showed the emergence of three congruent groups ($p<0.005$; $R>0.85$) clearly separating feces and skin samples for both animals. Feces samples between the first period (0-19 days) were clearly separated from the second period for both animals as well. SIMPER analysis showed the prevalence of Enteorbacteriales, Enterococcales and Lactobacillales in the first period, Clostridiales and Fusobacteriales in the second period and Flavobacteriales and Pseudomonades in the skin microbiome.

These prevalent groups implied tank water influence in the skin microbiome while the clear time separation in the gut microbiome combined with the feed and medicine regimes, was clearly driven by the use of metronidazole, known for its antibacterial and antiparasitic activity.

OP_24

PROTOCATECHUATE 4,5-DIOXYGENASE: TRANSCRIPTIONAL ANALYSIS AND A NOVEL APPROACH FOR IN-SITU BIOTRANSFORMATION MONITORING IN THE NMR TUBE BIOREACTOR.

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Protocatechuic acid (PCA) is an important common intermediate derived from the catabolism of Polycyclic Aromatic Hydrocarbons (PAHs) which, in turn, derive from major environmental pollutants. One of the ways that PCA is further metabolized and funneled in the TCA circle is through 4,5-dioxygenation of the aromatic ring (meta-cleavage pathway) [1]. Studying key enzymes in biodegradation pathways is significant for bioremediation and for efficient biocatalysts development.

Transcriptional analysis of PCA 4,5-dioxygenase gene (pcaA) from Pseudarthrobacter phenanthrenivorans Sphe3 by RT-qPCR showed a higher mRNA transcript level upon PCA growth in comparison with the phenanthrene-grown cells that could be attributed to a different regulatory mechanism. The divergently directed LysR-type transcriptional regulator (LTTR) (pcaR), located upstream of the pca gene cluster in Sphe3 showed a minimal basal constitutive expression; however, in response to PCA or a metabolite thereof that act as inducers, PcaR can act as the transcriptional activator of the PCA meta-cleavage pathway in Sphe3.

pcaA was heterologously expressed in E. coli BL21 cells and the recombinant enzyme exhibits Michaelis–Menten kinetics. From a broad range of alternative substrates tested, only gallate seems to be recognized by PcaA.

The biotransformation products of PCA and gallate were identified and characterized, for the first time, through in situ biotransformation monitoring inside an NMR tube. The PCA reaction product was for the first time observed in the 1H NMR spectrum and demonstrated a keto-enol tautomerization as was furthermore confirmed by various 1D and 2D NMR techniques. The gallate reaction product was present only in the keto form.

The advantage of the present in situ monitoring approach of an enzymatic reaction by free enzyme in the NMR tube is that the complete characterization can be achieved by the combined use of various NMR experiments. Furthermore, the fact that the enzyme is free in the solution allows it to adopt its natural conformation, which is essential for a natural enzymatic biotransformation as a dynamic process.

1. Kamimura, N.; Masai, E. The protocatechuate 4, 5-cleavage pathway: Overview and new findings. In Biodegradative Bacteria; Nojiri,H., Tsuda,M., Fukuda,M., Kamagata,Y., Eds.; Springer: Tokyo, Japan 2014; pp. 207–226

OP_25

THE BEAUTY AND THE BEAST: MICROBIOME ANALYSIS OF HONEYBEES AND WASPS FOR POSSIBLE TRANSMISSION OF PATHOGENIC BACTERIA TO HUMANS DURING STINGING

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During the last decades, venom derived from Hymenoptera species has been applied for medicinal purposes due to its antimicrobial and other therapeutical properties. Furthermore, bee venom through sting-acupuncture is extensively used in Apitherapy. However, it has been suggested that stinging of honeybees and wasps may transmit bacterial pathogens to humans causing infections that could even be fatal for some susceptible individuals. In order to search if contact through stinging or even simple contact with bees and wasps could indeed consist a microbial hazard, the present research was conducted. More specifically, our main goal was to detect whether some clinically important bacteria for human (i.e., Staphylococcus aureus, Streptococcus pyogenes, Enterococcus feacalis/ faecium and Pseudomonas aeruginosa) could be transmitted during stinging procedure. To do this, 288 honeybees (Apis mellifera), 252 wasps (Vespula germanica), and 36 hornets (Vespa orientalis) were collected from apiaries, farms and towns, in Lemnos Island, Greece. For each insect, experiments were conducted in three different ways: 1) simulations of stinging were performed in the laboratory with live insects stinging directly the nutrient substrates; 2) insects were forced to sting sterilized leather (human skin simulation) and immediately afterwards the stung leather was transferred to the nutrient substrates; 3) the 3rd pair of insects' legs (back legs) which is always in contact with insects' victims during stinging incidents, were removed and were attached to the nutrient substrates. To isolate the targeted bacterial species, selective agar media were used as the nutrient substrates, while following incubation of the plates, a total of approximately 100 suspect colonies were recovered and identified through classical microbiological approaches. Results revealed none of the target bacteria or other pathogenic bacterial species in honeybees' experiments. However, almost half of the suspect colonies isolated from wasps and hornets belonged to important hygienic indicators (i.e., Enterococcus and coliforms), suggesting the contact of these insects with fecal origin materials. The simultaneous isolation from these samples of some important opportunistic pathogenic bacterial species (such as Proteus mirabilis, Enterococcus faecalis and Klebsiella pneumoniae), also known for multidrug resistant strains, is a reason of concern.

OP_26

FISH MYCOBACTERIOSIS: DIAGNOSTICS AND PREVENTION IN THE GENOMIC ERA

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Fish mycobacteriosis is a chronic progressive disease caused by ubiquitous acid-fast bacilli, identified as nontuberculous mycobacteria (NTM). NTM could affect cultured and wild fish worldwide and are associated with high levels of mortality, ranging from 10% to 100% of the infected fish. Rapid and accurate identification is critical for proper diagnosis and management of mycobacteria infections and for outbreak investigation, particularly considering the extraordinary number of species in the genus. While the immune responses against aquatic Mycobacterium sp. have been partly characterized, an effective vaccine against mycobacteriosis has not been developed. In this study, we report the isolation and characterization of two NTM namely M. pseudoshottsii and M. hippocampi, from meagre and European sea bass respectively, originating from aquaculture farms in Western Greece. The genome sequencing of the isolated NTM allowed the design of a PCR-based discriminative assay in one step. The assembled draft genome of M. pseudoshottsii had a length of 5,934,315 bp, with a GC content of 65.6% and for M. hippocampi the length was 6,251,150 bp, with a GC content of 66.7%. Moreover, we applied the integrative in silico approaches of reverse vaccinology on the isolated NTM to identify putative vaccine candidates for further experimentation. Two web-based tools (VaxiJen and Vaxign) and one locally installed program (VacSol) were used to screen and detect prioritized proteins as vaccine candidates. The selection was based on cell topology, secondary structure, presence of epitopes and non-homology to eukaryotic proteins. In silico prediction of vaccine candidates resulted in the selection of 70 proteins from M. pseudoshottsii and 89 from M. hippocampi. Eventually, from this set, we selected 11 genes that can be used for immunization against both Mycobacterium strains. The constructs were assembled by cloning these genes in the pcDNA 3.1 eukaryotic expression vector and their immunogenicity effects were estimated in zebrafish.

OP_27

UNVEILING THE BEHAVIOR OF ANAEROBIC DIGESTION MICROBIOME UNDER EXPOSURE TO LIPID SHOCK VIA -OMIC APPROACHES

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The structure and function of the anaerobic digestion (AD) microbiome in engineered ecosystems is far to be fully elucidated. The aim of this study was the in-depth understanding of the AD performance under a shock load induced by single-pulse of unsaturated Long Chain Fatty Acid.

Thus, a comprehensive analysis was performed, combining the biochemical monitoring of the process, metagenomics and metatranscriptomics analyses, to decipher the intricate roles of the individual microbes and the mechanism governing the process during the lipid shock. Triplicate mesophilic (37oC) continuously stirred tank reactors (1.5L working volume, 25 days hydraulic retention time) fed with cattle manure were used for the study.

Once steady-state conditions were achieved, the process was subjected to a single shock load by injecting 3 g Na-Oleate/L in the reactors. The biochemical parameters were recorded throughout the whole experimental period, while DNA and RNA were extracted at: i) steady-state before the shock, ii) 14 hours after the shock, and only DNA at iii) steady-state long after the shock. Process monitoring showed a stable performance, with biogas composition 60% CH4 and 40% CO2, an average methane yield of 200 mL CH4/g VS, and low concentrations of volatile fatty acids (VFA). Upon the shock, a mild deterioration of the process was observed determined by a slight decrease in biogas production and an increase in VFA concentration.

Metagenomic analysis unveiled that Methanoculleus bourgensis WMB155 was the most abundant archaeon, demonstrating that hydrogenotrophic pathway was the dominant mechanism for methane production.

Additionally, the key microbial members for the β-oxidation pathways were assigned to the genus Syntrophomonas. Of great interest, was the significant increase of the Nigerium sp. WMB168, a novel bacterium never been reported in biogas communities, the presence of which may be related to the increase of CO2 share in biogas and its ability for carbon fixation through the reductive tricarboxylic acid cycle.

Metatranscriptomic analysis revealed differentially expressed genes assigned to different COG categories. The categories “Lipid metabolism” and “Amino acid transport and metabolism” had numerous genes up-regulated, while genes involved in “Inorganic ion transport and metabolism” and “Translation, ribosomal structure and biogenesis” were mostly down-regulated.

OP_28

BIOCONVERSION OF CRUDE GLYCEROL INTO HIGH VALUE-ADDED PRODUCTS AND THEIR DOWNSTREAM PROCESS

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Bioconversion of crude glycerol derived from a biodiesel plant, produced by Used Cooking Oil, into high valued-added products, such as 2,3-bunanediol (2,3-BDO), 1,3-propanediol (1,3-PDO), ethanol and acetoin and the following downstream process are the main goals of the present study.

11 bacterial strains were screened for their ability to convert glycerol into the aforementioned products, while 5 different culture conditions were studied (variation of pH, O2 supply, glycerol's purity). Results showed that Klebsiella oxytoca ACA-DC 1581, Hafnia alvei ACA-DC 1196 and Citrobacter freundii NRRL B-2645 have a great potential for BDO and acetoin, PDO, ethanol production respectively.

Moreover, in K. oxytoca fed-batch culture BDO concentration reached almost 70 g L-1 (69,7 g L-1) having yield per glycerol consumed 45%.

Meanwhile, acetoin production (10 g L-1) was non negligible. Crude's glycerol purity was 73% and pH value 0.5, no pretreatment applied. BDO production from this wild strain is among the highest of contemporary literature.

The downstream process of these products especially BDO and PDO attracts a lot of interest as there is still a shortage in cost-effective methods with a high product recovery. In this study, salting-out extraction (SOE) was implied to separate BDO from fermentation broth. A solution of 25% Ethanol (extraction agent), 25% K2HPO4 (salting out agent) and 50% fermentation supernatant was agitated and rest for 5h, BDO's recovery reached almost 90%. Remaining glycerol was equally separated in both phases (0.1g in organic and 0.1 g in aqueous).

Glycerol's partition coefficient needs further investigation, as in most cases SOE systems haven't studied in this type of carbon source. In the near future, new downstream methods will be applied and modified in order to achieve the highest possible recovery of products.

OP_29

MAPPING THE KEY TECHNOLOGICAL AND PROBIOTIC CHARACTERISTICS OF INDIGENOUS LACTIC ACID BACTERIA ISOLATED FROM GREEK TRADITIONAL DAIRY PRODUCTS

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The aim of this work was to isolate indigenous lactic acid bacteria (LAB) from traditional Greek cheeses and characterize them using biochemical, technological, and probiotic assays, to develop novel specific bacterial cultures with multi-functional properties. Hence, 109 LAB isolates were isolated from 2 traditional fresh Greek cheeses and evaluated in vitro for gas production, proteolytic, lipolytic and haemolytic activity, exopolysaccharide production, enzymatic potential, ability to grow at 6.5% NaCl, at pH 4.4 and 9.6, at 10 and 42 °C and under anaerobic conditions. 48 isolates (assigned to Lactococcus lactis, Levilactobacillus brevis, Lactobacillus paracasei, Lactiplantibacillus plantarum, Leuconostoc mesenteroides, Leuconostoc sp., and Leuconostoc pseudomesenteroides) were selected according to the different biochemical and technological propertiesand further investigated for their probiotic potency (resistance to low pH and bile salts, bile salts hydrolase activity, antibiotic resistance and antimicrobial activity against pathogens).

The majority of the 48 isolates, showed high resistance to low pH and bile salts, while 2 isolates (L. lactis and L. mesenteroides) exhibited antimicrobial effect against Listeria monocytogenes in vitro.
Consequently, the 48 isolates were incorporated as adjunct cultures (6 log CFU/mL initial inoculum level) in yogurt production to examine their survival and the sensory characteristics of the final product. Yogurt was produced according to the traditional method using Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus as starter cultures (control) and with one of the 48 isolates per case. Then, sensory, microbiological and physicochemical analyses took place. It was evident that 19 isolates, including Lb. paracasei, Lc. lactis and Ln. mesenteroides exhibited notable organoleptic profile to the yogurt while their population was detected in high levels after 24 h of storage. The results of the present study are promising for the production of novel dairy functional products with distinctive organoleptic properties, using the multi-functional isolates with probiotic potential.

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OP_30

EFFECTS OF RICH IN B-GLUCANS PLEUROTUS OSTREATUS MUSHROOMS ON FAECAL BACTERIAL POPULATIONS AND INTESTINAL BARRIER IN AUTISTIC CHILDREN: AN IN VITRO STUDY

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Autism Spectrum Disorder (ASD) is a complex group of developmental disorders of the brain, typically characterized by deficits in social and communicative behaviors as well as repetitive patterns of behaviors. Despite its prevalence (affecting 0.1% to 1.8% of the global population), the pathogenesis of ASD remains poorly understood. Children with autism are reported to have more frequent gastrointestinal complaints, while alterations or dysbiosis of gut microbiota (GM) have also been observed. The potential interaction between GM and autism has not been fully elucidated. Treatment with dietary components, such as pro/prebiotics, has been postulated to regulate GM and improve gastrointestinal symptoms, but there is a lack of evidence for such approaches in autism, especially for prebiotics. This study aimed to investigate the effects of rich in β-glucans Pleurotus ostreatus mushrooms (candidate prebiotic) on GM composition, using faecal samples from autistic children in an in vitro batch culture fermentation system. Total bacterial load and selected members of GM were enumerated at baseline (0 h) and after 24 h fermentation by quantitative PCR. Furthermore, the effects of fermentation supernatants (FSs), derived from P. ostreatus mushrooms, on the expression levels of tight junctions' (TJs) genes in LPS-stimulated Caco-2 cells, were examined. Our results demonstrated the in vitro beneficial effects of P. ostreatus mushrooms on the GM composition of autistic children. Also, our data highlighted the potential preventive effect of P. ostreatus FSs against dysregulation of the intestinal barrier, through upregulation of tight junctions' genes, associated with the integrity and function of the intestinal barrier. To our knowledge, this is the first study to examine the effects of P. ostreatus mushrooms, in terms of GM composition and of expression levels of TJs genes in autistic children.

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OP_31

DISTRIBUTION OF SACCHAROMYCES CEREVISIAE VINEYARD STRAINS AT SMALL SPATIAL SCALE SUPPORTS THEIR CONTRIBUTION TO LOCAL WINE CHARACTER

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Recent studies have identified S. cerevisiae strains that are characteristic of different continents or habitats. Here we asked if this may be also the case at smaller spatial scale and whether regional genotypes may be associated with specific wine characteristics.
To this end, we analysed the genetic and phenotypic diversity of S. cerevisiae strains isolated from three major wine-producing areas of Greece (Santorini, Peza and Nemea).

More than 2.500 yeast isolates obtained from the final stage of spontaneous fermentations were genotyped through interdelta-PCR resulting into 372 distinct genotypes. The enological potential of 80 strains representing the prevailing genotypes in different regions were thoroughly evaluated.

Considering all factors analyzed, PERMANOVA analysis based on sums of squared distances revealed significant differences ($p<0.05$) in S. cerevisiae phenotypes over space. Santorini possessed the most differentiating S. cerevisiae phenotypes, attributed to their distinct kinetic behavior (including the lag phase, the rate and the duration of fermentation) and metabolite production (i.e., succinic acid, phenylethyl alcohol etc.).

Strains from Nemea and Peza shared higher phenotypic resemblance, although significance differences were observed in important technological parameters such as in the levels of total acidity and lactic acid. Present results show that regional strains of S. cerevisiae produce distinct wine phenotypes, thereby supporting microbial contribution in the terroir concept.

Selected S. cerevisiae strains with peculiar phenotypes could therefore represent starter cultures, contributing to the typicity and genuineness of local wines.

OP_32

DEVELOPMENT AND VALIDATION OF PREDICTIVE GROWTH MODELS FOR ESTIMATING THE IMPACT OF CLIMATE CHANGE ON PLANT-BASED MILK ALTERNATIVES

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Thermophilic spore-forming bacteria are related to spoilage of a wide range of non-refrigerated food products. Since the minimum temperature for growth of thermophilic bacilli is quite high, these products are currently considered as microbiologically stable. However, microbiological stability is based on the current distribution and temperature storage conditions.

Therefore, an environmental disturbance, such as temperature increase, which is expected due to climate change, may affect microbial stability. Hence, the aim of this study was to assess the effect of temperature increase on the spoilage of non-refrigerated food products and re-evaluate their microbiological stability.

For this reason, a model of Anoxybacillus flavithermus and Bacillus coagulans growth as a function of temperature was developed. In this model, cardinal temperature values, along with the maximum specific growth rate of Anoxybacillus flavithermus DSM 21510 and Bacillus coagulans DSM 1 were estimated, by studying the temperature range between 30 and 70 and 30 and 60 ℃, respectively.

Each model was validated in one plant-based milk alternative, as a representative of non-refrigerated dairy food products, under static and non-isothermal conditions.

This study is of a great importance since the developed models will allow for the assessment of the effect of climate change on the microbiological stability of plant-based milk alternatives, under different temperature scenarios.

OP_33

EVALUATION OF SUB-LETHAL INJURY AND VNBC STATE IN LISTERIA MONOCYTOGENES USING FLUORESCENCE ACTIVATED CELL SORTING

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Exposure of Listeria monocytogenes to sub-lethal stresses, related with food processing, may induce sub-lethal injury and Viable-But-Non-Culturable (VBNC) state that is stochastically expressed at single-cell level, with varying potential of subsequent recovery.

The objectives of the present study were: i) to outline the proportion of culturable, injured, VBNC and dead cells using flow cytometry and CFDA/PI staining, ii) to identify the different physiological states of the sub-populations with fluorescence activated cell sorting and iii) to evaluate the resuscitation capacity of sorted cells on agar (colonial growth) versus broth (planktonic growth).

Acidic conditions (acetic and hydrochloric acid adjusted to pH 2.5-3.0, 20oC for 5h) and peracetic acid (PAA) (20, 30, 40 ppm, 20oC for 3h) were used to evaluate induction of sub-lethal injury and VBNC state of L. monocytogenes Scott-A.

To define injured (CFDA+/PI+) and VBNC (CFDA+/PI-) cells, flow cytometry coupled with CFDA (metabolically active) and PI (dead) staining was used. Stressed CFDA+/PI- cells were sorted on Tryptic Soy Agar supplemented with 0.6% Yeast Extract (TSAYE) in order to evaluate culturability and determine VBNC cells. Resuscitation capacity was monitored by visual inspection (macroscopic colony observation) on TSAYE and by optical density measurement on TSBYE for 5 days at 37oC.

L. monocytogenes CFDA+/PI- sorted cells remained culturable after exposure to hydrochloric acid (pH 2.5-3.0). PAA (20, 30, 40ppm) and acetic acid (pH 2.8) induced VBNC state. Exposure to 20ppm PAA for 180min at 20oC resulted in 6.56% of CFDA+/PI- sorted cells being resuscitated on TSAYE, after incubation for 24h at 37oC.

Increasing incubation time to 72h led to 29.64% recovery of CFDA+/PI- sorted cells. CFDA+/PI- sorted cells did not resuscitate after incubation for 180min of exposure to 30 ppm PAA at 20oC, while 30min of exposure at 40ppm PAA resulted in 10.91% recovery on TSAYE. Acetic acid-treated cells (pH 2.8) following sorting showed 5.95% VBNC induction after 4h incubation at 20oC and 19.08% after 5h. Sub-lethally injured (CFDA+/PI+) acetic acid-treated cells (pH 2.8) recovered in 69.79% after 5h of incubation at 20oC.

Assessing heterogeneity and dormancy states in L. monocytogenes sheds light into risks of underestimation of a product's real microbial status.

OP_34

AN IN SILICO GENOMIC AND METABOLIC ATLAS OF LACTOBACILLUS REUTERI DSM 20016 IN RELATION TO BIOSYNTHESIS OF BENEFICIAL PRODUCTS FOR HUMAN HEALTH

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Probiotics are microbial strains that are known to provide health benefits to the host when administered. Lactobacillus reuteri is a well-documented lactic acid bacterium that is present on numerous sites within the human body, and is currently on the market as a probiotic supplement. The strain investigated here was L. reuteri DSM 20016, which has been found to produce various useful metabolites, including an antimicrobial compound, called reuterin. There has been some effort to characterise the metabolome and genome. However, these reports tend to focus on the produced health effects of metabolites in this strain and closely-related strains, rather than on the characterisation of the biosynthetic mechanisms. By investigating these pathways and understanding the mechanisms behind the health benefits, inflammatory diseases, cancers, and diarrhoeal diseases could be treated within the human host.

The strain was explored using metabolomic and proteomic tools, and databases, including Kyoto Encyclopedia of Genes and Genomes (KEGG), STRING, BLAST similarity search, and UniProt. There was a specific focus on pathways and regions known to produce beneficial metabolites in closely-related strains. These genes were then compared to those within L. reuteri DSM 20016 to formulate a gene list.
This study located over 200 key genes which were involved in known human health benefit pathways, including: short chain fatty acid (SCFA) synthesis, amino acid metabolism, production of reuterin, GABA which could be involved in the gut-brain axis and immunoregulatory compound, histamine. The pathways for acetate, propionate and lactate were well documented, with some evidence of a pathway available for butanoate production. These SCFAs can produce immunomodulatory effects within the human host, influencing inflammation and promoting a pro-apoptotic and anti-proliferative effect.

A viable histamine pathway was documented, suggesting L. reuteri DSM 20016 may regulate the immune system. Whilst antimicrobial reuterin was found to be able to reduce enteric bacterial competition. Other key mechanisms comprise the amino acid catabolism and synthesis, digestive enzymes and vitamin synthesis pathways. This search also highlighted cell wall proteins that could influence aggregation and immunomodulation. The ability to influence the immune system would make this strain potentially useful in treating inflammatory-related disorders and proliferative cancers.

OP_35

MONITORING THE BIOPROTECTIVE POTENTIAL OF LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL DAIRY PRODUCTS AGAINST LISTERIA MONOCYTOGENES IN YOGURT

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The aim of the current study was to assess the effectiveness of 2 multi-functional lactic acid bacteria (LAB) strains for the control of Listeria monocytogenes during production and storage of Greek yogurt. For this purpose, milk was inoculated using commercial starter-culture without (control) or with the addition of 2 multi-functional strains (8 log CFU/g) as mono- and mixed cultures (Leuconostoc mesenteroides-FMX3, Lactococcus lactis-SMX2) with technological and probiotic properties and the standard procedure of yogurt manufacture was followed.

In parallel, the milk was also inoculated with 2 strains of L. monocytogenes (4 log CFU/g) as mono- and mixed cultures (L1, L2 and Lmix) and the final product was stored at 4°C for 30 days.

The yogurts were evaluated throughout storage in terms of microbiological, pH and sensorial analyses (non-inoculated samples), while the presence of the strains in the product was determined using RAPD-PCR. Results showed that after production, the population of Listeria strains (L1, L2 and Lmix) decreased by ~1 log CFU/g in the yogurt with FMX3 and by ~0.5 log CFU/g in the yogurt with SMX2, while the pathogens' population in control cases remained stable.

However, during storage Listeria population continued to decrease in yogurts with the multi-functional strains, while by the end of storage the pathogens' population showed a significant decrease (2 to 3 log CFU/g, depending on the case). In contrast, Listeria population decreased by 0.2 to 0.7 CFU/g in control samples.

No differences were observed in the LAB population until the end of storage (30th day), while the yogurt samples with the mix culture of LAB strains exhibited lower pH values and better sensory characteristics.

Finally, RAPD-PCR showed that the LAB strains were maintened in high percentages during storage. The results of the study encourage the use of novel LAB strains as bioprotective cultures in order to develop dairy products with enhanced safety and functional properties.

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OP_36

IN VITRO FERMENTATION OF PLEUROTUS ERYNGII MUSHROOMS BY HUMAN GUT MICROBIOTA: META-TAXONOMIC ANALYSIS, ANTI-GENOTOXIC AND METABOLOMIC PROFILING OF THE FERMENTATION PRODUCTS

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Edible mushrooms are known for their health-promoting properties since ancient years. They contain a variety of bioactive compounds, including β-glucans, that possess immunomodulatory and anti-cancer activities. Their non-digestible dietary fibers content has been shown to have a beneficial effect on intestinal well-being, acting as substrate for growth and function of intestinal bacterial populations.

Mushrooms produced by a Pleurotus eryngii strain of Greek origin were fermented in vitro by fecal slurry of asymptomatic volunteers aged >60 years old (three male, two female), for 24 hours. In parallel, fermentations without any additional carbon source were carried out and used as negative controls. Fermentation supernatants (FSs) were collected, and their anti-genotoxic properties were investigated in human whole blood cells, obtained from non-smoking volunteers (two male, two female), using Lymphocyte Cytokinesis - block Micronucleus Assay. The global metabolic profile of FSs was assessed using 1H NMR spectroscopy and metabolites resonances were assigned employing the Chenomx NMR Suite, 2D NMR spectroscopy, Metabominer platform and literature data. Finally, we established a meta-taxonomic approach to examine the in vitro fermentation-induced changes in gut microbiota communities. Amplicon-based Next Generation Sequencing of the DNA which correspond to the hypervariable regions of 16S ribosomal RNA was applied.

P. eryngii FSs were found to protect lymphocytes against the damage induced by Mitomycin C, a known genotoxic agent. In addition, the metabolomic and metataxonomic profiles showed significant variations as a result of mushroom's in vitro fermentation. Multiple regression analysis was employed for the assignment of anti-genotoxicity to specific constituents of the multi-component mixture of the metabolome. The identified metabolites, associated with the anti-genotoxic effects of the fermentation, will be further evaluated as for their correlation with changes of gut composition at family, genus or even species level.

OP_37

LOGGERHEAD SEA TURTLES (CARETTA CARETTA) AND THEIR MICROBES: CHARACTERIZING ORAL AND CLOACAL MICROBIAL COMMUNITIES

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Microbial communities of wild animals are being increasingly investigated as they can provide data for understanding host's biology and promote conservation. Loggerhead sea turtles (Caretta caretta) are a keystone species in marine ecosystems and are considered vulnerable by the IUCN Red List, which led to growing efforts in sea turtle conservation by rescue centers around the world.

Here we describe oral and cloacal microbiome of Mediterranean C. caretta by 16S rRNA gene sequencing to compare the microbial communities of wild subjects versus turtles in, or after, rehabilitation in the Adriatic Sea rescue centers and clinics.

Our results show that the oral microbiome is more susceptible to the environmental shifts than the cloacal microbiome, and that it does retain a portion of the microbial taxa regardless of the shift from the wild and into rehabilitation.

Proteobacteria and Bacteroidetes dominated both oral and cloacal microbial communities, while the novel Kiritimatiellaeota phylum was prevalent in cloacal samples.

Unclassified reads were abundant, which indicates high incidence of yet undiscovered bacteria of the marine reptile microbiomes.

We provide the first insights into the oral microbial communities of wild and rehabilitated loggerhead sea turtles, and establish the framework for quick and non-invasive sampling of the oral and cloacal microbiome, useful for expansion of the sample collection in wild loggerhead sea turtles.

This investigation on the effects of captive environment on the gut-associated microbial community provides a baseline for studying the impact of husbandry conditions on turtles' health and how it can potentially affect loggerheads' survival upon return into the wild.

OP_38

EFFECT OF SOIL DRYING ON THE EMISSION OF VOLATILE ORGANIC COMPOUNDS (VOCS) FROM CYPRUS VINEYARDS

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Soil volatile organic compounds (VOCs) are important molecules that are affecting soil ecology and function and several studies suggest that in regional and global scale they may affect atmospheric chemistry.

In Eastern Mediterranean Region, climate change unequivocally already affected the productivity and the ecology of agricultural soils.

Previous studies mainly reported VOCs fluxes in surface soils of Mediterranean region in different ecosystems. Soil water availability is affecting both biological and physical processes of soils which in turn are affecting VOCs emissions. In the current study, we examined how soil drying influences the VOCs exchange rates in two different vineyard soils using the headspace solid-phase micro-extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) method.

The main VOCs identified in low ppbv values were nonanal, hexane, heptane, and toluene.

Drying had a significant effect on VOCs concentration and the emission rates of the different compounds were negatively associated with soil water holding capacity.

Multivariate analysis also showed that soil and water content had a significant impact on the composition of VOCs identified. Soil chemical properties have been affected by water availability and partially explained the VOCs emissions from the soils tested.

OP_39

TREATMENT OF URBAN WASTEWATER BY A CONSTRUCTED WETLAND SYSTEM: ELIMINATION OF ENTERIC VIRUSES, ANTIBIOTIC RESISTANT BACTERIA AND RESISTANCE GENES.

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Pathogenic microorganisms in the effluents of wastewater treatment plants (WWTPs) entail a potential risk to public health, as they are responsible for many waterborne diseases. Among them, viruses have been recorded in high concentrations, even when bacterial indicators are detected in low levels.

Also, the spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in recent years has emerged as a major problem for human health.

The overuse of antibiotics, in combination with the inability of the conventional WWTPs to fully eliminate them, has led to the proliferation of ARB and to the dispersion of antibiotic resistance in microbial communities.

The aim of the study was to examine the capability of economic and eco-friendly treatment to encounter the challenge to offer water safety. In this perspective, a constructed wetland system (CW) receiving domestic primary wastewater was evaluated as alternative wastewater treatment systems over its potential to eliminate enteric viruses (Adenoviruses and Enteroviruses), ARB and ARGs. In addition, the concentration of target ARGs in different types of genetic material was estimated and the alterations of antibiotic resistance profile of bacteria (Escherichia coli and Enterococci) were examined.

A multi linear regression model was developed in an effort to estimate the role of the resistance genes to population's reduction after the exposure to the selected antibiotics. Results demonstrated that CW could achieve satisfactorily removal rates for viruses, over 90%, compared to conventional WWTPs.

Moreover, despite the fact that bacteria exhibited a complex behavior regarding their tolerance to antibiotics, the CW could contribute to the elimination of the ARB and ARGs. For example, the removal rates of blaTEM, sul2 and tetA genes were 91%, 77% and 86%, respectively. Generally, ARGs continued to be present in the effluent, while no clear pattern of bacterial resistance profile was observed.

Finally, the crucial role of bacteriophages in the dissemination was observed as the ARGs appeared abundant in phages fractions.

Compared to conventional treatment methods, the examined system provided promising results. However, public health risks still remain, especially if the water is intended for reuse, mainly due to the presence of viruses in the final effluents.

OP_40

BIOAUGMENTATION OF ANIMAL FECES AS A MEAN TO MITIGATE ENVIRONMENTAL CONTAMINATION WITH ANTHELMINTIC BENZIMIDAZOLES

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Gastrointestinal nematodes constitute a major problem for the production of grazing animals. Anthelmintic compounds are widely used to control these infestations. The limited and partial metabolism of anthelmintic compounds in animals result in their excretion in feces and urine which, through their use as manures, reach agricultural soils.

There they could persist entailing a risk for the contamination of other environmental compartments. Removal of anthelmintics from manures prior to their application could prevent contamination of agricultural soils.

However, no manure curation methods are in place. In this study we examined the efficiency of bioaugmentation as a method to remove anthelmintics from feces. Specifically, we tested the capacity of bacterial consortium able to degrade thiabendazole, a benzimidazole anthelminthic, to degrade two others widely used benzimidazole anthelmintics, albendazole and fenbendazole. Prior liquid culture tests with a wider range of benzimidazole anthelmintics showed that the consortium can degrade efficiently beyond thiabendazole also other benzimidazoles like albendazole and ricobendazole, both carrying low molecular weight substituents in their benzimidazole moiety.

However, it was less effective against benzimidazoles with bulky substituents like mebendazole, flubendazole, fenbendazole. We further investigated the bioaugmentation ability of the consortium in feces fortified with 5 and 50 mg kg-1 of thiabendazole, albendazole and fenbendazole. To determine the contribution of the indigenous manure microbial community to benzimidazole degradation we determined their dissipation in fumigated and non-fumigated fecal samples augmented with the consortium.

Bioaugmentation enhanced the degradation of all three compounds with DT50 of thiabendazole, albendazole and fenbendazole being reduced from 77.8-115, 10-13 and 43.8-58 days to 35.7-88.5, 7.4-9.7 and 41.6-42.2 days respectively. The bioaugmentation efficiency was further accelerated in fumigated samples, in the absence of the indigenous microbial community.

The latter contributes to anthelmintic degradation as suggested by the significantly lower DT50 values in fumigated vs non-fumigated, non-bioaugmented feces. Our findings suggest that bioaugmentation could be an efficient tool for the in situ detoxification of contaminated manures minimizing exposure of agricultural soils to these compounds.

OP_41

FATE OF MICROPLASTICS IN WASTEWATER TREATMENT PLANTS AND THEIR INTERACTIONS WITH ANTIBIOTIC RESISTANT GENES

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Wastewater treatment plants (WWTPs) are known to be one of the main and most important sources of microplastics (MPs) discharge into the environment.

The present work investigated the occurrence of microplastics in several stages (inlet, anaerobic digestion, activated sludge and effluent) of a WWTP located in Chania, Greece.

Two sampling periods were performed, the first sampling took place in the winter and the second took place in summer of 2020, during which, numerous cases of Covid-19 were recorded in Greece.

At the same time, the presence of 4 antibiotic resistance genes (qnrA, ampC, tetA and sul2) within the plastisphere of microplastics collected from the activated sludge and effluents was studied. MPs were detected in all samples collected from all the stages of the WWTPs in the two sampling periods.
Fragments were the dominant shape of MPs while the dominant size of MPs was smaller than 200 μm.

An increase in the concentration of MPs were detected in the effluents and activated sludge during the summer period. MPs in the effluents were mainly high density polyethylene (HDPE) while the majority of MPs in the inlet was polyvinyl chloride (PVC).

The concentration of antibiotic resistant genes within the plastisphere displayed different patterns in terms of sampling period and MPs origin.
This study suggests that WWTPs can act as source and sink of MPs while the MPs can serve as sinks of specific antibiotic resistant genes in WWTPs.

OP_42

PILOT SCALE APPLICATION OF A HERBAL BIOCIDE FOR THE PROTECTION OF CAVES BELONGING TO NATURAL AND CULTURAL HERITAGE SITES

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The present study concerns the serious issue of biodeterioration of the caves belonging to natural and cultural heritage sites due to the development of various microorganisms. A series of 18 essential oils (EO) extracted from various Greek plants were evaluated in vitro against 35 bacterial and 31 fungi isolates (isolated from a Greek cave). In continuance, eight (8) representative bacterial isolates were further used to evaluate the minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values of the most effective EOs.

Following the findings of the in vitro study, a new commercial product was developed by utilizing the most effective EO in a formulation capable to be applied by spraying on cave walls.

In continuance, the new product was applied in situ in Peania Cave (Koutouki) located in Attica, Greece at two different time points (16 months apart). The effectiveness of the product was evaluated against total viable counts (TVC), total bacterial counts (TBC), Cyanobacteria, Enterobacteriaceae, coliforms, yeasts and moulds.

According to the in vitro study results, two EO of Origanum vulgare were the most effective, followed by that of Satureja thymbra. The MIC ranged between 0.015-0.157 and 0.013-0.156 (v/v) for the bacterial and fungal isolates respectively, depending on the case.

Therefore, the Origanum vulgare EO was utilized as the main antimicrobial combound in the the new product formulation and tested by spraying the cave walls. The biocide application showed a similar antimicrobial effect at both application times. The population of TVC and TBC was reduced by 98-99%, of Cyanobacteria by 99-100%, of Enterobacteriaceae and coliforms by 95 και 98%, respectively (detected only at the 2nd pilot experiment measurements) and yeasts/ moulds by 76-86%.

The current study demonstrated that conventional biocides may be replaced by herbal biocides with significant prospects for commercial exploitation.

OP_43

STARVATION-INDUCED CELL FUSION AND HETEROKARYOSIS FREQUENTLY ESCAPE IMPERFECT ALLORECOGNITION SYSTEMS IN AN ASEXUAL FUNGAL PATHOGEN

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Generation of genotypic and phenotypic diversity, through genetic recombination in meiosis, is of utmost importance for the survival and adaptability of species over evolutionary timescales. In asexual fungi genetic recombination is thought to be mediated by an alternative mechanism, termed the parasexual cycle. This involves anastomosis between genetically different hyphae and/or conidia, which can lead to hetokaryosis and nuclear fusion followed by mitotic recombination during ploidy reduction (i.e., haploidization). However, fusion between different individuals and establishment of viable heterokaryons are believed to be largely prevented in nature by non-self recognition systems, a phenomenon described as heterokaryon incompatibility.

Here, we investigated the extent and mechanisms of cell fusion and heterokaryosis in the important asexual plant pathogen Verticillium dahliae. We used live-cell imaging and genetic complementation assays of tagged V. dahliae strains to analyze the extent of non-self vegetative fusion, heterokaryotic cell fate and nuclear behavior. Under starvation, non-self fusion of germinating spores occurs frequently regardless of the previously assessed vegetative compatibility of the partners. Supposedly “incompatible” fusions often permit nuclear transfer, establish viable heterokaryotic cells, and support the growth of mosaic mycelia. In these heterokaryons nuclei can engage in fusion or transfer of genetic material, and formation of diploid cells was confirmed by flow cytometry and genetic analysis. In parallel, we developed an efficient CRISPR/Cas9-mediated system to study the role of autophagy during heterokaryosis. In cells with GFP-tagged Atg8, we documented an autophagy-dependent nuclear degradation pathway which was responsible for the reduction of the nuclear copy number in heterokaryons upon cell fusion. Deletion of the core autophagic genes atg1 and atg8 impaired nucleophagy and as a result a significant fraction of fused cells (~25%) contained more than one nucleus. In addition, we observed that autophagy was strongly induced before the manifestation of the incompatibility reaction, but functionally analysis of atg1 revealed that autophagy is not required for cell death but actually protects some “incompatible” fusions from destruction.

Our results demonstrate an imperfect function of somatic incompatibility systems in V. dahliae that frequently tolerate the establishment of heterokaryons and potentially the initiation of the parasexual cycle even between vegetative incompatible strains.

OP_44

INSIGHTS ON THE SPECIFICITY OF BACTERIAL PURINE PERMEASES REVEALED FROM PHYLOGENETIC AND MUTATIONAL ANALYSIS OF A RECONSTRUCTED ANCESTRAL HOMOLOG

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Ancestral sequence reconstruction (ASR) is a powerful strategy for studying the etiological relation of protein sequence, structure and function. We used ASR to analyze structure-function and specificity relationships in NAT/NCS2 (Nucleobase-Ascorbate Transporter/ Nucleobase-Cation Symporter-2) family. We performed phylogenetic analysis of the bacterial NAT/NCS2 homologs and reconstructed AncXanQ, the putative common ancestor of a monophyletic group represented by the xanthine-specific XanQ of E. coli. AncXanQ was analyzed functionally by extrachromosomal expression in E. coli K-12 and shown to be a rather promiscuous transporter which transports both xanthine and guanine and recognizes a wide range of analogs. However, homology modeling showed that AncXanQ conserves all binding-site residues of the xanthine-specific XanQ. To understand the difference, we subjected AncXanQ and XanQ to rationally designed mutagenesis. Our results show that five residues outside the binding site are linked with the difference in specificity. Mutation of one of them (S377G) enlarges the specificity of XanQ towards an AncXanQ profile, whereas combination of S377G with mutations in at least one of the other positions (G27S or T312S) restores the xanthine-specific profile. In AncXanQ, G377S can restrict specificity only in combination with mutations at the other four positions. Molecular Dynamics (MD) indicates that S377G tilts transmembrane helix 12 resulting in rearrangement of F376 relative to F94, a phenyl directly involved in substrate binding, in the XanQ binding pocket, whereas G27S strengthens a H bond network that runs through the core (binding-site carrier) domain of the transporter and affects the positioning of two other binding residues, F322 and Q324. Thus, sequence changes outside the binding site (S377G, G27S) may influence the binding site through long-range interactions and these conformational effects may explain the specificity-related roles of S377G (allowing recognition/transport of additional purine substrates) and G27S (restoring the specificity for xanthine in the G27S/S377G mutant).

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OP_45

PHAEODACTYLUM TRICORNUTUM DICER PROCESSES HETEROCHROMATIC SMALL RNAS FROM EPIGENETICALLY REPRESSED AUTONOMOUS RETROTRANSPOSONS

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Diatoms are prominent Stramenopile microalgae responsible for near half of the marine productivity. RNA interference (RNAi) is a mechanism of regulation of gene expression mediated by small RNAs (sRNAs) processed by the endoribonuclease Dicer (DCR).

To date, the mechanism and physiological role of RNAi in diatoms are unknown. We mined recently available diatom genomes and transcriptomes for key RNAi effector proteins and retraced their phylogenetic history. DCR-knock out lines in the model diatom species Phaeodactylum tricornutum were generated by CRISR-Cas9 approach.

We analyzed their mRNA and sRNA populations and studied their acclimatory response to nitrate starvation.

Our results uncovered a diversification of key RNAi effectors in diatoms whose distribution across species suggests the presence of distinct RNAi pathways in these organisms. PtDCR is indispensable for the processing of 26-31 nt long double-stranded sRNAs originating from retrotransposons covered by repressive epigenetic marks suggesting the presence of a RNA directed methylation pathway in this diatom species. Finally, PtDCR-KO lines presented a compromised recovery post nitrate starvation supporting a role for RNAi in the acclimation to nutrient limitation.

Our results shed light on the mechanism and role of RNAi in diatoms broadening our understanding on the evolution of RNAi in eukaryotes.

OP_46

CRISPR/CAS9 ABLATION OF S. AUREUS P1 TRNAGLY GENE AFFECTS REDOX BALANCE, NUCLEOTIDE BIOSYNTHESIS AND TRANSPORT AND INTERCONNECTS RIBOSWITCH-MEDIATED REGULATORY CIRCUITS

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Staphylococcus aureus is a major gram-positive human pathogen responsible for severe nosocomial antibiotic resistance, worldwide. Recently, we have shown that mainstream antibiotics that target protein synthesis in S. aureus can modulate transcription which is controlled by structure-specific T-box riboswitches which bind tRNAs as ligands (1). A specific glyS T-box riboswitch which controls transcription of the sole glycyl-tRNA synthetase (GlyRS) synchronizes both translation and cell wall formation upon recognition of P (proteinogenic) tRNAGly and NP (non-proteinogenic) tRNAGly, respectively (2). However, nothing is known on the role of expression of each tRNA species in the synchronization of both pathways and possibly other important pathways that are functionally interconnected. We successfully eliminated P1 tRNAGly in the S. aureus RN4220 strain for the first time, using an appropriate CRISPR/Cas9 genome editing tool. Subsequent analysis of the edited strain using whole transcriptome NGS, revealed important but not extensive expression profile alterations. Gene enrichment ontology analysis showed changes in the redox status of the edited strain which was correlated with alterations in the phosphate pentose pathway, the purine metabolism pathways and several pathways that contribute to tRNA post-transcriptional modification. In addition, we observed modulation of the ABC transporters and FMN biosynthetic pathway which is extensively regulated by riboswitches. Our results show that tRNAs are important regulators of bacterial homeostasis and reveal new layers of RNA-mediated regulation through the interconnection of different types of riboswitches.

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OP_47

ESTRADIOL AND PROGESTERONE MODULATED GROWTH, ADHESION AND BIOFILM PROPERTIES OF CANDIDA ALBICANS

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Since, it was reported that microorganisms have receptors for host hormones, researchers are focused on "microbial endocrinology" to understand the communication between hormones and microorganisms during infection [Lyte and Cryan, 2010; Lyte and Freestone, 2014]. It is well known that during the infection, host's body has become the microbe's environment [Lyte and Cryan, 2010; Lyte and Freestone, 2014; Plotkin and Viselli, 2000; Engelsöy et al., 2021] and host's hormones are part of this environment. Previous studies suggested that hormones sensed and responded by microorganisms modulate growth and gene expressions associated with metabolism, virulence and/or antimicrobial susceptibilities [Plotkin and Viselli, 2000; Lyte and Cryan, 2010; Hosoda et al., 2011; Lyte and Freestone, 2014; Ho et al., 2017; Salgado-Lora et al., 2020; Liu et al., 2020; Engelsöy et al., 2021].

Candida albicans is a member of the healthy human microbiota of vagina, skin, mouth, respiratory and intestinal system can cause opportunistic infections. In this preliminary study, the roles of estradiol and progesterone on growth, adhesion and biofilm properties of C.albicans ATCC 90028 strain' were determined under experimental conditions mimicking host' conditions.

C.albicans was grown in breast cancer cell line (MCF-7) with /without two different concentrations of estradiol (0.1 and 0.4ng/mL) and progesterone (2 and 20ng/mL).

Growths were measured spectrophotometrically in two-, four-, six- and 24-h periods. Adhesive yeast counts were determined in infected MCF-7 cells lysed and inoculated on Saboured dextrose agar. Biofilm was evaluated by microtiter plate assay. One and two- way ANOVA Dunnett's multiple comparisons tests were performed for statistical analyses.

The growth of C.albicans in MCF-7 cells was found to be statistically increased during first four hours ($p \leq 0.01$); on the other hand, when the incubation prolonged to 24 hours, it was statistically significantly decreased ($p<0.0001$).

Moreover, hormones reduced adhesion of yeast except for the presence of 20 ng/mL progesterone; which was statistically significantly (p: 0.01). Interestingly, biofilm formation statistically significantly increased in the presence of all hormones at all concentrations at both 24 and 48 hours ($p<0.0001$).

These findings suggest that C.albicans sensed hormones and regulated self-growth and virulence properties during infectious process.

OP_48

SAMPLING THE FLUX SPACE OF MICROBIAL METABOLIC NETWORKS FOR (UN)BIASED ASSESSMENT OF THE POSSIBLE FUNCTIONAL STATES: THE EXAMPLE OF SARS-COV-2 ON THE HUMAN ALVEOLAR MACROPHAGE METABOLIC NETWORK

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Genome-scale metabolic network reconstruction is a mathematically structured representation of an organisms' metabolic network, including biochemical, genetic, and genomic information [1]. Commonly used constraint-based methods depend on an objective function, leading to biased analyses, and return a sole (Flux Balance Analysis) or just the minimum and maximum solutions (Flux Variability Analysis). Contrary, flux sampling can be performed either with or without an objective function and covers all the possible flux values a reaction may get, by estimating a probability distribution for each flux [1].

Here, we present applications and potential of flux sampling using the dingo* Python library we build, implementing the Multiphase Monte Carlo Sampling (MMCS) algorithm [2]. dingo is able to sample on the flux space of metabolic networks of the highest dimensions accessible today (Recon3D). An example of the usage of dingo is presented: using the iAB-AMØ-1410** human alveolar macrophage metabolic network, flux sampling was performed, maximizing the human biomass objective. Subsequently, the biomass objective function of SARS-CoV-2 [3] was added in the model and flux sampling was performed again, asking for the biomass function to be maximized. Our findings are in line with those of Renz et al. [3] where guanylate kinase (GK1) was suggested as a potential drug target for COVID-19. Figure 1 highlights how GK1 is increased when SARS-CoV-2 is maximized contrary to Tyramine Sulfotransferase (TYMSULT) that is not affected.

Building on current approaches for metabolic modeling of microbial communities, dingo will support constructing a community model from a list of models assuring the exchange of fluxes between both individuals and their environment but also among them. This way, dingo will enhance inference of microbial interactions and design of synthetic communities.

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* https://github.com/GeomScale/dingo/ ** https://www.ebi.ac.uk/biomodels/MODEL1011090001

OP_49

VISUALIZING 3D MULTILAYERED NETWORKS INTERACTIVELY WITH ARENA3DWEB

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Efficient integration and visualization of heterogeneous biomedical information (e.g., host-microbiome interactions, implicated biological pathways, potential inhibiting drugs) in a single view is a key challenge. We present Arena3Dweb [1], the first, fully interactive and dependency-free, web application which allows the visualization of multilayered graphs in 3D space. With Arena3Dweb, users can integrate multiple networks in a single view along with their intra- and inter-layer connections.

For clearer and more informative views, users can choose among a plethora of layout algorithms to apply on a set of selected layers either individually or in combination. Users can also align networks and highlight node topological features, whereas each layer as well as the whole scene can be translated, rotated and scaled in 3D space. User-selected edge colors can be used to highlight important paths, while node positioning, coloring and resizing can be adjusted on-the-fly. In its current version, Arena3Dweb supports both weighted and unweighted graphs and is written in R, Shiny and JavaScript.

To avoid confusion, while Arena3Dweb has been inspired by the Arena3D [2] standalone version, it is a new application which has been written from scratch using different technologies. Compared to Arena3D, Arena3Dweb comes with richer functionality and navigation options. The most advanced feature in Arena3Dweb is the ability to apply layout algorithms on a subset of selected nodes, or layers or on the whole network. This enables generating views with drastically fewer line crossovers and makes it easier to detect feature patterns. Furthermore, as a web server application, Arena3Dweb can run on any browser and attract a much broader audience beyond the biomedical field.

Availability: http://bib.fleming.gr:3838/Arena3D

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OP_50

MICROBIAL RESOURCE RESEARCH INFRASTRUCTURE (IS_MIRRI21, HORIZON 2020): DEVELOPMENT AND LONG-TERM SUSTAINABILITY OF NEW PAN-EUROPEAN RESEARCH INFRASTRUCTURES

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The Microbial Resource Research Infrastructure is a pan-European research infrastructure that focuses on the preservation, study, provision and valorization of microbial resources and biodiversity. MIRRI provides a single-entry point called Collaborative Work Environment (CWE) to facilitate access to microbial resources, facilities and data in order to preserve and exploit the microbial diversity across Europe.

MIRRI is composed of over 50 microbial Biological Resource Centres, Culture Collections and research institutes possessing over 400,000 microbial strains and over 200,000 genetic and biological resources. Services available through the CWE include:

- Microbial deposits (public, patent and safe)
- Microorganism isolation, preservation and cultivation
- Molecular identification
- Phenotypic characterization
- Molecular characterization, molecular typing and phylogenetic analysis
- High-throughput sequencing services
- Screening, tests and bioassays
- Taxonomic database tools
- Consultancy, training and contract research
- Expert Clusters for legal issues
- Expert Clusters for microbial taxonomy and cultivation
- Expert Clusters for IT and Data Management
- Advanced online courses in microbiology
- Training courses in partner organisations

In Greece the institutions taking part in MIRRI include the Culture Collections of the National and Kapodistrian University of Athens, the Dairy Collection of Agricultural College of Athens and the Benaki Phytopathological Institute Collection. MIRRI and its CWE is a unique opportunity for collaborations with other researchers or partnership with other institutions.

OP_51

A BENEFICIAL ENDOPHYTIC FUNGUS DELIVERS RNAI MOLECULES TO PLANTS

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Fusarium solani strain K (FsK) is a soil-borne beneficial endophytic fungus. The mechanistical details governing the interaction between this endophyte and its host are still elusive, but a growing body of evidence suggests that RNA interference (RNAi)-related processes underlie the onset of symbiosis.

In order to characterize the core RNAi machinery of FsK, we performed next-generation sequencing and identified that the endophyte encodes two Dicer and two Argonaute genes.

By transforming the FsK with a hairpin GFP construct (FsK-hpGFP) and performing small RNA sequencing, we could record the abundant accumulation of 20-25 nt GFP siRNAs in FsK-hpGFP. Importantly, by colonizing GFP-expressing Nicotiana benthamiana plants (Nb-GFP) with FsK-hpGFP, we could record the onset of systemic RNAi of the GFP transgene in Nb-GFP, demonstrating that the endophytic fungus transmits RNAi molecules to its host.

Our data suggest that trans-kingdom RNAi phenomena may govern symbiotic relationships to a greater extent than previously thought.

OP_52

GENOMIC MUTATIONS IN VIBRIO SPECIES CAN DRIVE ACQUIRED RESISTANCE AGAINST LYTIC BACTERIOPHAGES INVOLVING A QUORUM-SENSING MEDIATED MECHANISM.

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Interest in bacteriophage research has been revived during the last decade in an attempt to better understand their interaction with antibiotic-resistant bacteria, and benefit from it.

Nevertheless, bacteriophage application often referred to as phage therapy, is hampered by the development of phage-resistant bacterial strains. Vibrio species have been proven a valuable tool to study the orchestrated metabolic response of gram-negative bacteria, against abiotic and biotic challenges.

Previously, we showed that Vibrio alginolyticus is able to diminish the expression of specific receptors and transporters in its membrane and potentially abort infection by lytic bacteriophages. Here we show that this process is also in line with a metabolic adaptation strategy involving quorum sensing receptor genes LuxP and LuxN, as well as LuxS and LuxM auto-inducer biosynthesis genes and finally AphA and HapR, two cell density regulatory proteins. Marine Vibrios alter the expression patterns of these genes achieving widespread metabolic reprogramming.

This adaptation process was also linked with genomic mutations of phage-resistant strains. Whole-genome sequencing of bacteriophage - resistant strains, revealed mutations in genomic regions, responsible for organizing and orchestrating a complex metabolic response such as transcriptional regulator UhpA.

This phenomenon appears to be phage specific, a fine-tuned metabolic engineering, imposed by the different phages the bacteria have interacted with, updating their role in microbial marine ecology.

Aknowledgements:

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OP_53

ASSESSING THE E. COLI STRESS RESPONSES BY RNA-SEQ FOLLOWING THE APPLICATION OF HIGH-POWER ULTRASOUND

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Bacterial resistance due to different treatments have been quite a huge threat in both food industrial and medical fields. It is thus important that the antimicrobial mechanism of actions for bacteria due to different disinfection environments, such as ultrasound, are investigated and understood. This study aimed at unravelling the mechanism of action of ultrasound as a pH reduction enhancer by assessing the effects of (sequential) high power ultrasound treatments (26kHz) on E. coli K-12 MG1655 and its isogenic mutant gadW by RNA-SEQ.

The isogenic mutant ΔgadW was chosen, considering that the GAD system is known to play an important role in acid tolerance of bacteria while knowing that ultrasound enhances the production of (hydroxyl) radicals and decreases the pH of the processed medium. According to the obtained RNA-SEQ results a total of 1825 genes were expressed following ultrasound treatments, playing different roles in the cell. The expression of these genes were associated to DNA damage, cell membrane integrity and also metabolic effects. The studied strains also showed different Differential Expressed Genes (DEGs), with some genes being directly responsible for defence mechanisms, while others playing an indirect effect due to cell damage. Furthermore, a heat map analysis of the results indicated a gradual decrease in expression of the genes as we move from just one cycle of ultrasound treatment to a sequential treatment.

This response was less pronounced in the gadW than the WT.

Thus, it can be concluded that gadW is playing an important role in general stress resistance. In addition, E. coli K-12 builds a self protection mechanism by increasing the expression of genes involved in the respiration for increased growth, production of flagellum and pili, whilst also activating various protection mechanisms such as sigma S and E.

Overall, it can be concluded that high power ultrasound is a technology that triggers reactive oxygen species production and increases acidity resulting in a number of different defence mechanisms which directly link to E. coli resistance.

OP_54

THE IMPACT OF PLASMA MEMBRANE LIPID COMPOSITION ON FLAGELLUM-MEDIATED ADHESION OF ENTEROHEMORRHAGIC ESCHERICHIA COLI

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Animal pathogenic enterobacteria are a major source of foodborne outbreaks. Among them, Enterohaemorrhagic Escherichia coli (EHEC) O157:H7 is known to lead to severe diseases like haemolytic uraemic syndrome. The adhesion on tissue, which is the first step initiating the colonisation, is mediated by cell surface appendages and organelles, including flagellum. Recently, EHEC flagellum has been described to recognise the phospholipids of plant plasma membranes. This finding stressed the need to investigate further the molecular mechanisms of the flagellum interaction with plasma membrane lipids.

Here, we use biomimetic membrane models and cell lines to decipher the impact of lipid content of the plasma membrane on enterohemorrhagic E. coli flagellum-mediated adhesion. The flagellum interaction with membrane lipids was studied using Giant Unilamellar Vesicules (GUVs), as biologically relevant cell membrane model. Bacterial adhesion on GUVs was dependent on the presence of the flagellar filament and its motility. By testing different phospholipid head groups, the nature of the fatty acid chains, or the liposome curvature and based on molecular dynamics simulation, we found that lipid packing is a key parameter to enable bacterial adhesion. Using HT-29 cells grown in the presence of polyunsaturated fatty acid or saturated fatty acid, we found that α-linolenic acid reduced adhesion of wild-type EHEC but not of a non-flagellated mutant. Finally, our results reveal that the presence of flagella is advantageous for the bacteria to bind to cholesterol-enriched lipid microdomains.

All together, these findings demonstrate that plasma membrane adhesion via bacterial flagella play a significant role for an important human pathogen [1]. The results generated in the present study give the first elements for a comprehensive elucidation of the mechanism of the bacterial flagellum interaction with plasma membrane lipid. A precise understanding of what are the parameters that controls the interaction at the molecular level represents the first step toward a new insight on the mechanisms of pathogen persistence. These results pave the road for designing new solutions to prevent the bacteria attachment on living tissues.

[1] Cazzola H. et al. (2020) mSphere. 16;5(5):e00702-20

OP_55

PREGO (PROCESS, ENVIRONMENT, ORGANISM), MINING LITERATURE AND -OMICS (META)DATA TO ASSOCIATE MICROORGANISMS, BIOLOGICAL PROCESSES, AND ENVIRONMENT TYPES

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To elucidate ecosystem functioning, it is fundamental to recognize what processes occur in which environments (where) and which microorganisms carry them out (who). These what-where-who associations are scattered in the scientific literature and in public repositories. PREGO aims to extract such associations and make them available via a one-stop-shop web platform.

To this end, PREGO combines text mining and data integration techniques to mine data and metadata from the scientific literature (PubMed abstracts) and metagenomic resources (MGnify, MG-RAST, JGI).

Microorganisms, biological processes and environment types mentioned in free text (abstracts, abundance tables, metadata) are identified and mapped to structured entities from established community resources (NCBI Taxonomy, Gene Ontology, KEGG orthology, Environment Ontology (ENVO)).

Comention-in-text and co-occurrence in data/metadata analyses are then performed to extract such what-where-who associations and assign a level of confidence to each of them. In its current version, PREGO has processed 31.5Mi PubMed abstracts, 60K MGnify, 32K MG-RAST samples and 17.5K JGI isolate genomes.

This combination has resulted into a knowledge base in which 91K microbial taxa (bacteria, archaea and unicellular eukaryotes) are associated with distinct entities out of of 900 ENVO terms, 9K Gene Ontology Biological Process terms, and 2K KEGG Orthology terms. From this network of pairwise associations there are 44K microbial species and 10K microbial strains that have 1Mi associations with Environments, 650K associations with Biological Processes and 15Mi associations with KEGG orthology terms.

Users have access to microorganisms and their associations through a simple web interface with a search field to query for microorganisms of interest. PREGO returns interactive visualizations, based on KEGG modules for different aspects of metabolism (e.g Energy metabolism, Carbohydrate metabolism etc.) and ENVO hierarchies for each queried taxon. Associations are also presented as ranked lists of entities associations which are available for bulk download.

PREGO (prego.hcmr.gr) aspires to aid researchers to easily interpret experimental results by exploring processes likely to occur in a sample, or an experimental design or by exploring recommended environments in which microorganisms are likely to be found or a metabolic process to occur.

OP_56

CHRONOS: A MACHINE LEARNING BASED PIPELINE FOR THE DESCRIPTION AND PREDICTIVE MODELING OF MICROBIOME TRANSITIONS OVERTIME.

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Traditionally, microbial time series analysis follows the values of individual taxa overtime and attempts to assign etiology on observed patterns. This approach assumes a homogenous base reacting in unison to external effectors. These assumptions are not always fulfilled especially on complex natural systems like the microbiome of human gut. Humans with otherwise uniform demographic or dietary backgrounds can have distinct microbial profiles that are referred to as enterotypes. We suggest an alternative approach based on the following premises.

At any time, communities organize in distinct clusters of similar composition as an adaptation of the internal restrictions to external forces. Those intrinsic subsets of communities have differential inertia and individual species response to the same external effects. Nevertheless, the progress of those communities is largely deterministic given same external conditions. Therefore, tracking the transition of communities rather than individual species can help us understand the ecological processes and allow prediction of future states by knowledge of current state and applied effect. We implement those ideas into Chronos, an analytical pipeline written in R language.

Chronos takes as input a microbial composition table (e.g., OTU table) and associated meta data. For every time point it detects the intrinsic microbial profile clusters and describe them in term of composition.

It then produces a transition matrix with the cluster that every sample belong to at every time point. The matrix is then used to train a Markov chain model that is utilized to evaluate the predictive power from knowing the cluster/state of a microbial community at one time point in relation with the cluster/state at a future time point.

For every transition, external effectors are modeled using Multinomial Logistic Regression to determine their influence and hence predictability of final state. We applied Chronos pipeline on available data from growing infant's microbiome and could show that the community composition as early as 3 months of age can be predictive of the state at the second year by including the knowledge of feeding with breast milk or infant formula. Chronos is freely available at https://github.com/Lagkouvardos/Chronos.

OP_58

GUT MICROBIAL COMMUNITY PROFILE AND SPECIFIC FAECAL METABOLOMIC SIGNATURE OF HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

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Hematopoietic stem cell transplantation (HSCT) is considered as the most effective form of tumour immunotherapy for many hematologic disorders. Although advances in transplant management have greatly improved outcomes, patients are at high risk of several complications including graft-versus-host disease (GvHD). Mounting evidence highlights the emerging role of the gut microbiota (GM) to profoundly impact hematopoietic stem cell transplantation and its subsequent complications. Moreover, HSCT is followed by major changes in metabolomics profiles that could impact transplant outcomes. In the present study, we investigated changes in GM composition and faecal metabolic profiles among transplant patients and healthy controls. The intestinal microbiota was characterized by next generation sequencing (NGS), while gas chromatography–mass spectrometry was employed to perform untargeted analysis of faecal metabolites. We found lower relative abundances of Actinobacteria, Firmicutes and Bacteroidetes and higher abundance of Proteobacteria after HSCT. Particularly, GvHD microbiota was characterized by lower relative abundance of the short chain fatty acid producing bacteria Feacalibacterium, Akkermansia, Veillonella and Lachnospiraceae, and an increase in multidrug-resistant bacteria belonging to Escherichia/Shigella and Bacteroides. Network analysis showed that the bacterial community of GvHD patients was linked to a higher number of positive interactions of Blautia and a significant mutual-exclusion rate of Citrobacter. Regarding changes in the metabolic profile, the faecal metabolome of the transplant group was dominated by lipids when compared with the healthy individuals. Overall, 76 metabolites were significantly altered within transplant recipients, of which 24 were selected as potential biomarkers. Furthermore, the most notable altered metabolic pathways included the TCA cycle, butanoate, propanoate, and pyruvate metabolisms, steroid biosynthesis, and glycolysis/gluconeogenesis. Specific biomarkers and altered metabolic pathways were correlated to GvHD onset. Overall, our results indicated significant shifts in gut microbiota structure and faecal metabolites characterizing HSCT.

POSTER PRESENTATIONS

PP_001 : BIOME: APPLICATION OF EMERGING BIOTECHNOLOGICAL METHODS IN ORGANICALLY GROWN VEGETABLE CROPS

PP_002: REVERSAL OF FRUITBODIES PRODUCTION INCAPACITY IN DEGENERATIVE STRAINS OF THE ENTOMOPATHOGENIC FUNGUS CORDYCEPS MILITARIS THROUGH THE USE OF INSECT-ENRICHED SUBSTRATES

PP_003 : OCCURRENCE OF PHAEOMONIELLA CHLAMYDOSPORA, CAUSAL AGENT OF PETRI DISEASE, IN DIFFERENT STAGES OF GRAPEVINE PROPAGATION MATERIAL AND PATHOGEN BIOMASS FLUCTUATION FROM THE NURSERY TO THE FIELD.

PP_004: THE EFFECT OF NITROGEN FERTILIZATION AND NITRIFICATION INHIBITORS ON COMAMMOX NITROSPIRA IN A LOAMY RICE SOIL

PP_005: TOXICITY OF PESTICIDES ON SOIL MICROBES: AMMONIA-OXIDIZING MICROORGANISMS AS NEW BIOINDICATORS FOR REGULATORY USE

PP_006 : DEVELOPMENT AND APPLICATION OF FORMULATED ENDOPHYTIC FUNGUS FOR NOVEL PLANT GROWTH STRATEGIES

PP_007: EFFECT OF PHOTOPERIOD AND TEMPERATURE ON GROWTH AND BIOMASS COMPOSITION OF THE MARINE MICROALGA TETRASELMIS STRIATA

PP_008: EXPLORING THE EFFECT OF PLANT VARIETY AND DEVELOPMENTAL STAGE ON THE COMPOSITION AND DIVERSITY OF THE OLIVE TREE MICROBIOME

PP_009 : **WITHDRAWN**

PP_010 : A SUBSET OF TOMATO TRANSCRIPTION FACTORS IS HIGHLY RESPONSIVE UPON PEPMV INFECTION

PP_011 : COMPARATIVE EVALUATION OF THE EFFICACY OF QUINONE IMINE, DICYANDIAMIDE (DCD), NITRAPYRIN, AND 3,4-DIMETHYLPYRAZOLE PHOSPHATE (DMPP) TO INHIBIT NITRIFICATION UNDER DIFFERENT TEMPERATURE AND PH CONDITIONS

PP_012: HOW CULTIVARS, TERROIR AND VINIFICATION STRATEGIES AFFECT MICROBIAL SUCCESSION IN THE VINIFICATION OF THE GREEK CULTIVARS RODITIS AND SIDERITIS

PP_013 : BIOLOGICAL CONTROL OF COLLETOTRICHUM ACUTATUM, CAUSAL AGENT OF OLIVE ANTHRACNOSE

PP_014: IN VITRO EVALUATION OF 10 ESSENTIAL OILS AGAINST ASPERGILLUS CARBONARIUS AND THEIR EFFECTS ON OCHRATOXIN A RELATED ACLAEA GENE EXPRESSION

PP_015: EVALUATION OF EPIPHYTIC GRAPE YEASTS FOR THE CONTROL OF ASPERGILLUS CARBONARIUS AND OCHRATOXINS IN GRAPES

PP_016: THE TRANSCRIPTIONAL EFFECTS OF BACILLUS VELEZENSIS K165 VOLATILE ORGANIC COMPOUNDS ON ARABIDOPSIS THALIANA AND THEIR PLANT PROTECTIVE ACTIVITY AGAINST BOTRYTIS CINEREA

PP_017 : VINEYARD SOIL BACTERIAL COMPOSITION AND ITS RELATION TO GEOGRAPHIC LOCATION

PP_018: EVALUATION OF INTEGRATED PEST MANAGEMENT (IPM) STRATEGIES FOR THE CONTROL OF ALTERNARIA LEAF BLIGHT IN CARROTS

PP_019: MOLECULAR PHYLOGENETICS OF SIX MARINE MICROALGAE STRAINS FROM GREEK TRANSIENT WATERS

PP_020: A STUDY OF THE BACILLUS VELEZENSIS K165 MEDIATED RESISTANCE AGAINST VERTICILLIUM DAHLIAE, BOTRYTIS CINEREA AND HYALOPERONOSPORA ARABIDOPSIDIS AND THE ROLE OF HISTONE ACETYLTRANSFERASES IN BIOCONTROL

PP_021: EARLY TRANSCRIPTIONAL RESPONSES OF ARABIDOPSIS TO VERTICILLIUM DAHLIAE INFECTION AND ENHANCEMENT OF PLANT IMMUNITY BY DEAD CELLS OF THE PATHOGEN

PP_022: BACILLUS VELEZENSIS K165 VOLATILE ORGANIC COMPOUNDS ACTIVATE CHITIN PERCEPTION PATHWAY TO ENHANCE PLANT DISEASE RESISTANCE AGAINST VERTICILLIUM

PP_023: BIOLOGICAL CONTROL OF ASPERGILLUS CARBONARIUS AND BOTRYTIS CINEREA IN GRAPEVINE BERRIES

PP_024: GENETIC CHARACTERIZATION AT THE SPECIES AND SYMBIOVAR LEVEL OF SEED BORNE RHIZOBIA ISOLATED FROM POT-GROWN COMMON BEAN NODULES.

PP_025: MICROBIAL DIVERSITY DURING THE SPOILAGE OF EDIBLE FRESH BROWN ALGAE

PP_026: CHARACTERIZATION OF FUNGI ASSOCIATED WITH OLIVE FRUIT ROT AND OLIVE OIL DEGRADATION IN CRETE, SOUTHERN GREECE

PP_027: THE EFFECT OF FEEDING INTENSITY ON MICROBIOME COMPOSITION IN AN INTEGRATED KOI CARP-SAGE RECIRCULATING MULTI-TROPHIC PRODUCTION SYSTEM

PP_028: GENETIC DIVERSITY OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS STRAINS ISOLATED FROM TOMATO CROPS IN GREECE

PP_029: QUALITY ASSESSMENT OF SEA BREAM AT DYNAMIC TEMPERATURE CONDITIONS THROUGH CONVENTIONAL AND INNOVATIVE TECHNIQUES

PP_030: DIFFERENT FACTORS ARE OPERATIVE IN SHAPING VINE MICROBIOME ACROSS DIFFERENT GEOGRAPHICAL SCALES: BIOGEOGRAPHY, CULTIVAR OR TERROIR?

PP_031 : CHARACTERIZATION OF THE SEABREAM BACTERIOME FROM WILD AND AQUACULTURE

PP_032 : THE NATIONAL SOIL GENE BANK OF THE REPUBLIC OF CYPRUS: THE IMPORTANCE OF SOIL GENETIC RESOURCES

PP_033 : **WITHDRAWN**

PP_034 : CHARACTERIZATION OF THE CAUSATIVE AGENT OF RED SKIN LESIONS (RED SPOTS) IN SEA BREAM

PP_035 : MICROBIAL COMMUNITIES SUCCESSION OF SEA BREAM FILLETS STORED UNDER VARIOUS CONDITIONS

PP_036 : PLANT GROWTH PROMOTING RHIZOBACTERIA AS POTENTIAL BIOAGENTS AGAINST FUNGAL PATHOGENS

PP_037: THE EFFECT OF SUSTAINABLE INNOVATIVE FEED ON GROWTH PERFORMANCE, WELFARE STATUS AND EXTREME TEMPERATURE RESILIENCE OF KOI CARP

PP_038 : MICROBIAL COMMUNITIES OF FISH FROM LAKE KARLA

PP_039: APPLICATION OF FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY AND ELECTRONIC NOSE (E-NOSE) TO THE RAPID ASSESSMENT OF SEA BREAM FILLETS QUALITY.

PP_040: INSIGHTS INTO THE EFFECTS OF AGRONOMICAL MANAGEMENT PRACTICES IN ASPERGILLUS INCIDENCE AND CARPOSPHERE'S MICROBIAL COMMUNITIES OF GRAPEVINE (CV. SYRAH)

PP_041: A NOVEL TETRA-PRIMER ARMS-PCR FOR THE DETECTION OF BCPOS5 GENE MUTATIONS CONFERRING RESISTANCE TO ANILINOPYRIMIDINE FUNGICIDES IN BOTRYTIS CINEREA AND USE OF CRISPR/CAS9 EDITING TO GENERATE RESISTANT MUTANTS

PP_042: DEVELOPMENT OF NOVEL CULTIVATION METHODOLOGIES FOR MUSHROOMS PRODUCTION BY THE ENDANGERED SPECIES PLEUROTUS NEBRODENSIS

PP_043: EXPLOITATION OF LOCALLY AVAILABLE LIGNOCELLULOSIC BY-PRODUCTS FOR THE CULTIVATION OF THE TROPICAL MILKY WHITE MUSHROOM CALOCYBE INDICA

PP_044: WOLBACHIA - MEDFLY INTERACTIONS IN NOVEL GENOMIC BACKGROUNDS

PP_045: IN VITRO ANTAGONISTIC ACTIVITY OF RHIZOSPHERIC AND ENDOPHYTIC BACTERIA AGAINST SOIL BORNE PHYTOPATHOGENIC FUNGI.

PP_046: **WITHDRAWN BY AUTHOR**

PP_047: ENDOPHYTIC STRAIN B. HALOTOLERANS HIL4 IS A PROMISING BIOLOGICAL CONTROL AGENT OF GRAY MOLD IN FRUIT WITH PLANT GROWTH PROMOTING POTENTIAL

PP_048: GENOME MINING AND METABOLOMIC ANALYSIS OF THE ENDOPHYTE BACILLUS HALOTOLERANS CAL.L.30 PRESENTED SEVERAL SECONDARY METABOLITES THAT CONTRIBUTE TO POSTHARVEST BIOCONTROL OF BOTRYTIS CINEREA

PP_049: DEVELOPMENT OF CERATITIS CAPITATA VIENNA 8 GENETIC SEXING STRAINS HARBORING WOLBACHIA FOR IIT AND COMBINED IIT-SIT APPLICATIONS

PP_050: MICROBIOLOGICAL QUALITY AND SAFETY OF SALMON SALMO SALAR, SCALLOPS PECTEN MAXIMUS AND SEAWEED ALARIA ESCULENTA CO-CULTURED IN AN INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) SYSTEM

PP_051: ENDO-ROOT MICROORGANISMS FROM ALOE VERA CULTIVATIONS IN LACONIA, GREECE WITH GROWTH PROMOTING PROPERTIES.

PP_052 : **WITHDRAWN BY AUTHOR**

PP_053: THE BIOCONTROL POTENTIAL OF TWO BACILLI RHIZOBACTERIA THROUGH THE PRODUCTION OF SURFACTINS AND FENGYCINS

PP_054: EFFECT OF LABORATORY ADAPTATION PROCESS ON THE BACTERIAL COMMUNITY ASSOCIATED WITH THE MEDITERRANEAN FRUIT FLY, CERATITIS CAPITATA

PP_055: MINING THE POTENTIAL OF ENDOPHYTES AS THE NEXT GENERATION PLANT PROTECTION PRODUCTS: ISOLATION AND BIOACTIVITY ASSESSMENT OF THE OLIVE TREE ENDOPHYTE BACILLUS SP. PTA13 LIPOPEPTIDES

PP_056: INSIGHTS ON THE SUBSTRATE SPECIFICITY OF AA9 AND AA16 LPMOS FROM THERMOTHELOMYCES THERMOPHILA

PP_057: INTEGRATION OF BIOCATALYSIS AND CHEMICAL PROCESSES FOR THE VALORIZATION OF LIGNOCELLULOSIC BIOMASS TOWARDS THE PRODUCTION OF 5-HYDROXYMETHYLFURFURAL

PP_058: VALORIZATION OF SUPPLEMENTED COMPOST THROUGH COLONIZATION OF AGARICUS BISPORUS AND A. SUBRUFESCENS MUSHROOMS

PP_059: BACTERIAL BIOSYNTHESIS OF LARGE COMBINATORIAL LIBRARIES OF CYCLIC OLIGOPEPTIDES AND DIRECT FUNCTIONAL SCREENING FOR DISCOVERING INHIBITORS OF PATHOGENIC PROTEIN AGGREGATION

PP_060: GENOME EDITING TOOLKIT OF ENDOPHYTIC FUSARIUM SOLANI STRAIN K USING CRISPR/CAS9

PP_061: VALORIZATION OF LIPID FERMENTATION WASTEWATER FOR MACERATION WATER FOR THE CULTIVATION OF GANODERMA MUSHROOMS

PP_062: COMPARATIVE STUDY OF AGARICUS AND PLEUROTUS STRAINS FOR THEIR EFFICIENCY TO PRODUCE IMPORTANT BIOCHEMICAL COMPOUNDS

PP_063: TARGETED ENZYMATIC HYDROLYSIS: TOWARDS A MORE EFFICIENT UTILIZATION OF CORN FEEDSTOCK AS ANIMAL FEED

PP_064: NOVEL DEEP-SEA SAMPLING AND EXPERIMENTATION SYSTEM FOR HYDROCARBON BIOREMEDIATION MONITORING UNDER IN SITU CONDITIONS

PP_065: CHARACTERIZATION OF A NOVEL B-1,3-1,4-GLUCANASE FROM THE THERMOPHILIC BACTERIUM SPIROCHAETA THERMOPHILA DSM6578

PP_066: **WITHDRAWN BY AUTHOR**

PP_067: DYNAMIC ANALYSIS OF BLOOD MICROBIOME IN PATIENTS WITH SCHIZOPHRENIA

PP_068: **WITHDRAWN BY AUTHOR**

PP_069: BIOCATALYTIC ENHANCEMENT OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF GREEN MARINE ALGAE EXTRACTS

PP_070: BIOTECHNOLOGICAL VALORIZATION OF SECOND CHEESE WHEY BY OLEAGINOUS YEASTS FOR PRODUCTION OF CELL BIOMASS AND METABOLITES

PP_071: SUPTOXR AND SUPTOXR2.0: SPECIALIZED ESCHERICHIA COLI STRAINS FOR RECOMBINANT MEMBRANE PROTEIN PRODUCTION AT HIGH YIELDS

PP_072: COMPARATIVE GENOMIC ANALYSIS OF IPRODIONE-DEGRADING BACTERIA REVEALS GENETIC ELEMENTS AND INTERACTIONS INVOLVED IN THE DEGRADATION OF IPRODIONE

PP_073: DISCOVERY OF NEXT-GENERATION LEAD ANTIBIOTICS TO COMBAT ANTIMICROBIAL RESISTANCE BY COMBINING A BIOSYNTHETIC BACTERIAL PLATFORM AND HIGH-THROUGHPUT SCREENING METHODOLOGIES

PP_074: STRUCTURAL AND BIOCHEMICAL INSIGHTS INTO A CE16 ACETYL ESTERASE FROM THERMOTHELOMYCES THERMOPHILUS.

PP_075: ISOLATION AND CHARACTERIZATION OF CARBAZOLE DIOXYGENASE DRIVING THE TRANSFORMATION OF THIABENDAZOLE

PP_076: MAGNETIC MICROALGAE PRODUCTION FOR IMMOBILIZATION ONTO MAGNETIC SURFACES

PP_077: DISSECTING THE FUNCTIONAL ROLE OF MICROBES IN BIOREACTORS PRODUCING BIOMETHANE USING GENOME CENTRIC METATRANSCRIPTOMICS

PP_078: GENETIC AND FUNCTIONAL ANALYSIS OF THE ZYMOMONAS MOBILIS STRAIN CP4 PLASMIDS

PP_079 : **WITHDRAWN BY AUTHOR**

PP_080: SECRETOME ANALYSIS OF TWO GREEK BASIDIOMYCETE WILD STRAINS GROWN ON CORN STOVER REVEALED TWO NOVEL LACCASES WITH SUPERIOR PROPERTIES.

PP_081: OVERVIEW AND BENCHMARKING OF AMPLICON-ORIENTED BIOINFORMATICS TOOLS, FOR PREDICTING FUNCTIONAL FEATURES OF MICROBIAL COMMUNITIES, AND THEIR COMPARISON WITH SHOTGUN METAGENOMIC DATA ANALYSIS

PP_082: ENDOPHYTIC FUNGI OF CANNABIS SATIVA AND THEIR POTENTIAL BIOTRANSFORMATION PROPERTIES

PP_083: BIODEGRADATION OF ENVIRONMENTAL POLLUTANTS: A BIOINFORMATICS APPROACH

PP_084: ISOLATION, CHARACTERIZATION AND APPLICATION OF A MYCOSPHAERELLA TASSIANA FUNGAL ISOLATE FOR THE REMOVAL OF IMAZALIL FROM AGRO-INDUSTRIAL EFFLUENTS

PP_085: UPGRADE OF AGRO-INDUSTRIAL WASTES INTO PROTEINACEOUS ANIMAL FEED BY SOLID STATE FERMENTATION

PP_086: COMPARISON OF HYDROCARBON-DEGRADING CONSORTIA FROM SURFACE AND DEEP WATERS OF THE EASTERN MEDITERRANEAN SEA: CHARACTERIZATION AND DEGRADATION POTENTIAL

PP_087: INITIAL REASSESSMENT OF THE DIVERSITY OF THE GENUS AGARICUS (AGARICACAE, BASIDIOMYCOTA) IN GREECE BY USING MORPHOANATOMIC AND PHYLOGENETIC APPROACHES

PP_088: DIVERSITY, PHYLOGENY AND CHEMICAL CHARACTERIZATION OF TUBER SPECIES FROM GREECE – ESTABLISHMENT OF FUNGAL INOCULA TO FOSTER TRUFFLE CULTIVATION ON NATIVE PLANTS

PP_089 : **WITHDRAWN BY AUTHOR**

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PP_001

BIOME: APPLICATION OF EMERGING BIOTECHNOLOGICAL METHODS IN ORGANICALLY GROWN VEGETABLE CROPS

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Contemporary agriculture faces great challenges imposed by global environmental changes forcing it to undergo radical transformations that will allow it to maintain its viability by transiting to more sustainable systems. A promising biotechnological approach that effectively addresses these challenges is to exploit the microorganisms that thrive in the rhizosphere (root microbiome). The microbiome may exert strong effects on crop growth and productivity, the response of crops to biotic and abiotic stressors, and eliminating the adverse effects of agriculture to the environment by reducing inputs (water, nutrients, biocides). Despite its potential benefits, practical applications of crop inoculation with microorganisms are hindered by many challenging limitations including i) the lack of a robust methodological framework that allows for the identification of microorganisms with a key role in microbiome assembly and maintenance, ii) a limited knowledge on the effect of environmental factors in the microbiome assembly, and iii) a limited understanding of the mechanisms by which plant species recruit microorganisms for populating their rhizospheric environment. The BIOME project, aiming to increase the crop productivity and to improve the sustainability of agroecosystems, attempts to increase current state-of-art knowledge of the crop microbiome and translate it into innovative tools of agroecosystems management (practical applications, updated production processes, and commercial products). Specifically, progress has been made in the development of a methodological framework allowing the identification, isolation and culture of “key” microorganisms that favor the growth of crops under stress conditions and protect them against pathogens. Agronomical practices and environmental variables are under consideration to “engineer” the root microbiome toward this end. Furthermore, BIOME is endowed with state-of-the-art genome editing approaches intending to decipher what makes a plant able to recruit beneficial microorganisms.

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PP_002

REVERSAL OF FRUITBODIES PRODUCTION INCAPACITY IN DEGENERATIVE STRAINS OF THE ENTOMOPATHOGENIC FUNGUS CORDYCEPS MILITARIS THROUGH THE USE OF INSECT-ENRICHED SUBSTRATES

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The entomopathogenic fungus Cordyceps militaris (Hypocreales, Ascomycota) has attracted great interest due to its ability to produce biomass rich in valuable bioactive compounds, e.g., cordycepin, ergosterol, mannitol and several polysaccharides, which present (among others) hypoglycemic, hypolipidemic, anti-inflammatory, antitumor, antimicrobial, antioxidant and immuno-protective activities. Although artificial cultivation of C. militaris is feasible, there are many factors that influence considerably the yield and the quality of the ascomata formed. The most important is culture degeneration usually expressed by phenotypic instability and reduced sporulation in most entomopathogenic fungi. In C. militaris, degeneration causes reduced or abnormal fruitbody formation resulting in important economic losses at the industrial scale. In this work we experimented with degenerative commercial strains that were unable to produce fruitbodies in standard rice-based solid substrates. Degenerative strains CmCan1DK and CmCan2DK were grown in basal nutrient media that were sequentially supplemented with (a) 80% + 0%, (b) 70%+10%, (c) 60%+20%, (d) 40%+40% and (e) 0%+80% w/v of rice and dried Bombyx mori (Bombycidae) or Tenebrio molitor (Tenebrionidae) commercial pet/animal feed pupae and larvae, respectively. While both strains efficiently colonized most of the aforementioned substrates, only the insect-enriched cultures were able to form ascomata. The highest production in fruitbodies and cordycepin was observed in the 60%+20% w/v rice and dried T. molitor treatment (i.e., 50 ± 10 g w.w./unit and 1750 ± 100 μg/g d.w., respectively). Our results are consistent with the entomopathogenic nature of C. militaris and could contribute in providing a viable and economically feasible solution to be readily implemented at the industrial-scale cultivation of this fungus.

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PP_003

OCCURRENCE OF PHAEOMONIELLA CHLAMYDOSPORA, CAUSAL AGENT OF PETRI DISEASE, IN DIFFERENT STAGES OF GRAPEVINE PROPAGATION MATERIAL AND PATHOGEN BIOMASS FLUCTUATION FROM THE NURSERY TO THE FIELD.

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Grapevine trunk diseases (GTDs) pose a huge threat to grapevine cultivation worldwide. Phaeomoniella chlamydospora, associated with young grapevine decline syndrome, is the most abundant fungal species along with Phaeoacremonium minimum, and its occurrence in vine nurseries and young vines is widely reported. The aim of this study was to investigate the occurrence and the fluctuation of Pa. chlamydospora biomass at different stages of propagation material produced during the propagation process.

Dormant cuttings and different rootstock-scion combinations of grafted unrooted or rooted vines planted for one year in the field, were sampled. Plant tissue was collected from the basal end of the dormant cuttings, the rootstock and grafted union of the grafted vines. Total gDNA was extracted from the collected tissues and qPCR assays using TaqMan chemistry were carried out for the detection of the pathogen and the determination of its biomass. Pa. chlamydospora was detected in 65% and 80% of dormant cuttings originated from rootstocks 41B and R110, respectively, and in 50% and 65% of dormant cuttings originated from the cvs Agiorgitiko and Asirtiko, respectively.

The mean biomass of Pa. chlamydospora for all dormant cuttings was 3,94 pg/100 ng of plant DNA. Concerning the grafted unrooted vines, the highest biomass value was detected in the combination Agiorgitiko-41B, which was significantly different from the other rootstock-scion combinations, while no significant differences were found between the other combinations of grafted rooted vines. In addition, the mean fungal biomass for all rootstock-scion combinations was 1,21 pg/100 ng of plant DNA and 62,14 pg/100 ng of plant DNA for grafted unrooted and rooted vines, respectively, indicating a 30-fold increase of pathogen's biomass from the nursery to the field.

Considering the above but also according to the literature, Pa. chlamydospora is detected in dormant cuttings deriving from nursery mother vines. The huge increase of the pathogen's biomass from the grafted unrooted vines before planting to the grafted rooted vines planted for one year in the field, shows that the propagation process plays a crucial role in the dissemination and development of Petri disease.

PP_004

THE EFFECT OF NITROGEN FERTILIZATION AND NITRIFICATION INHIBITORS ON COMAMMOX NITROSPIRA IN A LOAMY RICE SOIL

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The conventional perspective of two-step nitrification, mediated by ammonia-oxidizing archaea (AOA) and bacteria (AOB), and nitrite-oxidising bacteria (NOB) was radically challenged by the recent discovery of comammox Nitrospira, being able to perform the complete oxidation of ammonia to nitrate within an individual cell. Still, the response of commamox Nitrospira to nitrification inhibitors (NIs) and their relative contribution in soil nitrification under different fertilization regimes remains unknown, impeding our ability to predict the efficacy of NIs in agroecosystems. To address these issues, we established two incubation studies using a loamy rice-soil, where both clade A and B comammox Nitrospira had been detected. Experiment 1 examined the responses of inorganic N-pools, potential nitrification rates, and ammonia-oxidizing microbial (AOM) populations to different forms (ammonium sulphate (AS) vs. urea (U)) and rates (0, 5, 50, 200, and 500 mg NH4+- N kg-1 dwt soil) of N-fertilization. Experiment 2 used the optimum combination of N source and concentration for stimulating AOM growth and activity in soil and studied the effect of three commercial NIs widely used in agriculture, DCD, nitrapyrin, and DMPP, compared to a potential novel NI, ethoxyquin (EQ), on the function and dynamics of AOM, using similar microbial endpoints.

Nitrification activity significantly increased with increasing N rates, by both N forms. AOA and AOB populations increased in response to the two highest AS and U rates, whereas a higher but transient response of comammox Nitrospira was observed only for the highest AS rate. DCD and DMPP were more effective than nitrapyrin and EQ in inhibiting nitrification, an effect mostly driven by their selective activity against AOB, prevailing in this soil. Growth of AOA and comammox Nitrospira significantly increased following inhibition of AOB by DCD and DMPP, suggesting that competition between AOA/comammox Nitrospira and AOB is a key factor restraining the former groups in agricultural soils. Comammox clade A were more abundant than clade B and more responsive to NIs addition.

Our findings indicate the potential contribution of comammox Nitrospira in autotrophic ammonia-oxidation in agricultural soils amended with inorganic N-fertilizers and emphasize the importance of microbial interactions on the efficacy of NIs in agroecosystems.

PP_005

TOXICITY OF PESTICIDES ON SOIL MICROBES: AMMONIA-OXIDIZING MICROORGANISMS AS NEW BIOINDICATORS FOR REGULATORY USE

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Pesticides are major environmental pollutants regulated by a stringent EU framework. This is based on toxicity tests which are quite well established for aquatic organisms and terrestrial macro-organisms, but not for soil microorganisms. The assessment of pesticides toxicity on soil microbiota still relies on outdated mineralization tests which fail to identify effects on key microbial functions and microbial diversity. Furthermore, current scientific knowledge is limited to assessment of pesticide effects at single microbial groups, while effects at ecosystem level along the soil food-web remain unknown. Recent benchmarking research has pointed to ammonia-oxidizing microbes (AOM) as ideal microbial indicators of agrochemicals toxicity. AOM mediate the transformation of NH_4^+ to NO_2^-, the rate-limiting step of nitrification, a key process for N cycling and a major contributor of N_2O (greenhouse gas) under oxic soil conditions. AOM includes autotrophic ammonia - oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and comammox bacteria, the latter perform the whole nitrification process in one step.

The different AOM groups exhibit niche differentiation in soil mainly driven by differences in ammonium affinity and pH optima. Beyond their key functional role in soil, their sensitivity to external perturbations, and the availability of tools to measure their activity and abundance, made AOM ideal indicators of the effects of abiotic stressors on the soil microbial community.

We aim to determine the ecotoxicological response of AOM as microbial indicators of pesticides toxicity through a tiered-based approach encompassing (i) the development and standardization of pioneering in vitro tests, as a first conservative step (tier I) for assessing the toxicity of pesticides on phylogenetically and ecophysiologically distinct soil AOM; (ii) the development of advanced experimental lab and field tests to assess the toxicity of pesticides on natural soil assemblages of AOM, as a more realistic toxicity assessment step; and (iii) the development of an ecosystem-level toxicity assessment for exploring the impact of pesticides on microorganisms from different trophic-levels within the soil food-web (predator - prey).

Overall, our work is expected to provide a new risk assessment scheme and ecotoxicity tools for the realistic estimation of the impact of pesticides on non-target soil microbes.

PP_006

DEVELOPMENT AND APPLICATION OF FORMULATED ENDOPHYTIC FUNGUS FOR NOVEL PLANT GROWTH STRATEGIES

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Most beneficial endophytic fungi are part of the microbial communities of plants and are directly related to promoting plant health and resistance to the abiotic stresses and facilitating their nutrition. Fusarium solani K (FsK) strain is such a fungus which is capable of conferring resistance to pathogenic fungi of both roots and leaves and protecting tomato plants from the negative effects of reduced water availability. It can therefore be used as a microbial inoculum in agriculture. The characteristics of these inocula can be greatly improved by encapsulation systems and suitable properties of the biopolymers.

In this study, we aimed at the selective propagation of finely dispersed Fusarium solani K (FsK) strain mycelium in submerged culture and encapsulation in calcium alginate/starch beads to protect the fungus during drying, enable growth in different soils and cultivation media and promote endophytism in tomato plants.

We found that a combination of culture conditions promoted selective formation of finely dispersed mycelium reflected by 4.5-fold decreased pellet diameters, 10-fold increased mycelial biomass concentrations and low blastospore contents of 52 ×106 mL-1 after 48 h.

Encapsulation of mycelium enhanced survival under drying by 29.14%. Co-encapsulated starch served as a nutrient source for growth mediain with best results on sterile and non-sterile peat substrate with 3.99 mm and 4.28 mm radial mycelial growth, respectively.

This study provides the first evidence that survival of FsK mycelium can be substantially improved by encapsulation and that encapsulated FsK is able to grow out of beads in non-sterile soils. These results may provide the basis for future work on increasing inoculum shelf-life and efficiency and extend of endophytism by formulation technologies.

PP_007

EFFECT OF PHOTOPERIOD AND TEMPERATURE ON GROWTH AND BIOMASS COMPOSITION OF THE MARINE MICROALGA TETRASELMIS STRIATA

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Microalgae are an exceptional source of polyunsaturated fatty acids, vitamins, proteins, carbohydrates and amino acids that can improve fish growth. Their biomass nutritional value is determined by these nutrients and therefore, parameters affecting growth and biomass composition (pH, growth media, temperature and photoperiod) are often adjusted.Tetraselmis striata was cultivated in drilling waters (salinity 29 ‰) obtained from the commercial fish farm Plagton S.A..

Previous studies showed that the microalga displayed optimum growth using 0.2 gr L-1 of the commercial fertilizer Nutri-Leef (30%-TN, 10%-P, 10%-K) together with an inorganic carbon source (0.18 gr L-1, $NaHCO_3$) at a pH value of 8. Under these optimized conditions, the effects of photoperiod and temperature on growth and biomass composition were evaluated. Initially, the photoperiod effect was studied at a temperature of 25 ±1^{o}C. Tetraselmis was cultivated in constant light (24:0, L(Light):D(Dark)) and then 20:4, 18:6, 12:12 L:D exposure. The results revealed that biomass was significantly affected by light absence. The highest productivity of 93.7 mg L-1 d-1 was noted at 24:0 L:D but gradually reduced as the dark periods lengthened. Protein contents ranged between 50.3 to 49.9%, while only at 12:12 L:D were lower at the value of 42.9 %.

Lipid contents were 23.5 to 29.7%, while carbohydrate contents were ranged from 14.6-19.7%. Pigments also exhibited higher accumulation rates under 24 h light photoperiod (5.1%) and thus continuous illumination was selected as the optimum growth condition. The growth temperatures of 19±1^{o}C and 28 ±1^{o}C were then studied. Recorded biomass productivity values were 69.3 and 55.0 mg L-1 d-1 respectively, while protein (43.6-40%) and pigment (3.4-2.7%) contents reduced slightly under these conditions.

Thus, optimum growth and biomass composition for Tetraselmis is achieved at constant light and at 25^{o}C. High quality biomass important for fish growth can be produced under these conditions.

PP_008

EXPLORING THE EFFECT OF PLANT VARIETY AND DEVELOPMENTAL STAGE ON THE COMPOSITION AND DIVERSITY OF THE OLIVE TREE MICROBIOME

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Plant - associated microbial communities are key determinants of plant health and productivity, which may aid in nutrient availability and uptake, enhanced tolerance to abiotic and biotic stress, and increased biodiversity. Olive (Olea europaea L.) represents a hallmark crop of the Mediterranean basin, accounting for 95% of the cultivated olive area worldwide. Although several studies investigated the microbiome of olive trees, we lack a holistic view of its microbiome in the different plant compartments, its seasonal patterns along the growing season and the occurence of a core microbiome.

In this work, we determined the prokaryotic, fungal and arbuscular mycorrhizal fungal (AMF) microbiome in below- (rhizosphere soil, roots) and above-ground (phyllosphere and carposphere) plant parts of two selected Greek olive varieties in five developmental stages along a full fruit-bearing season, using amplicon sequencing.

To address this aim, we followed two sampling strategies: (i) to determine whether the above- and below-ground plant compartments support distinct microbial (bacteria, fungi, arbuscular mycorrhizal fungi - AMF) communities and (ii) to address whether the microbiome of the different plant compartments exhibits different seasonal patterns along the olive tree fruit-bearing period season. In both cases, the samples (rhizosphere soil, roots, leaves and fruits) were derived from two emblematic Greek varieties cultivated at two experimental fields located in Crete ("Koroneiki") and Thessaloniki ("Chondrolia Chalkidikis").

Samples were collected at five selected developmental stages along the growing season. The microbiome diversity was determined by amplicon sequencing of the 16S rRNA gene (bacteria), ITS2 region (fungi) via Illumina Hiseq 2x250 bp paired-end analysis and of the small ribosomal subunit (AMF) via Illumina Miseq 2x300 bp paired-end analysis. Results will be presented and discussed at the conference.

Our analysis verified that above- (phyllosphere, carposhere) and below-ground (soil, roots) plant parts supported distinct microbial communities. In both varieties, the microbial community assemblage in the different plant-compartments was prone to seasonal changes and expands our knowledge on the role of the host-plant variety and the climate changes in a near future.

PP_010

A SUBSET OF TOMATO TRANSCRIPTION FACTORS IS HIGHLY RESPONSIVE UPON PEPMV INFECTION

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Plants have developed a compendium of well-tuned surveillance systems, including sophisticated genetic defense mechanisms, to restrain pathogens. Plant defense response involves transcriptional reprogramming that occurs in a highly synchronized manner mainly governed by transcription factors (TFs) to promote elegant biological changes. However, the host responsive TFs against pathogenic viruses remain still largely unknown.

Pepino Mosaic Virus (PepMV) has emerged a major threat of tomato globally due to the insufficient protocols of disease control. Herein, we show that a Chilean-derived PepMV strain remains highly virulent and significantly modifies the expression pattern of a subset of tomato TFs together with a network of co-expressed genes.

This group comprises of thirteen TFs showing a similar expression pattern upon PepMV and Tomato Yellow Leaf Curl Virus (TYLCV) tomato infections. Remarkably, the expression pattern of three TFs, namely MTEF1, JA2L and ZF2 including their co-expressed Differentially Expressed Genes (DEGs), was reversed when tomatoes were infected by non-viral phytopathogens.

This distinct defense response signifies an evolutionary diverse mechanism against various phytopathogenic agents. Functional analysis of the three TFs and their DEGs showed that PepMV activates the cyclic electron flow around Photosystem I. Moreover, the Salicylic Acid (SA)-mediated defense signaling was stimulated contrary to the deactivation of Jasmonic Acid (JA) cascade. This type of response supports the widely appreciated notion that one pathway represses the other and SA signaling predominantly combats viral infections. Our analysis reveals a dynamic reprogramming of plant defense mechanisms upon viral infection to implement an array of barriers preventing pathogen invasion and spread.

PP_011

COMPARATIVE EVALUATION OF THE EFFICACY OF QUINONE IMINE, DICYANDIAMIDE (DCD), NITRAPYRIN, AND 3,4-DIMETHYLPYRAZOLE PHOSPHATE (DMPP) TO INHIBIT NITRIFICATION UNDER DIFFERENT TEMPERATURE AND PH CONDITIONS

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Dicyandiamide (DCD), nitrapyrin (NP), and 3,4-dimethylpyrazole phosphate (DMPP) are nitrification inhibitors (NIs) used in agriculture. However, their efficacy in regulating soil N transformations is highly variable across soils and may depend on soil and climate variables which affect both the soil persistence of NIs and the composition and activity of the members of the nitrifying microbial community. Recently, ethoxyquin was proposed as a novel NI, acting through its major transformation product quinone imine (QI). Still the environmental and agronomic performance of these NIs under diverse edaphic and climatic conditions, remains unknown. We investigated how key abiotic factors, such as soil pH and temperature, modified the effect of DCD, NP, DMPP and QI on nitrification, through a microcosm-based study comparing two loamy soils mainly differing in pH (acidic vs. alkaline) under two incubation temperatures (12.5 vs. 25°C), after urea application. This was achieved via monitoring inorganic N-pools, potential nitrification (PN) rates, the abundance of amoA gene, and amplicon sequencing of amoA. To relate our results to the level and duration of the microbial exposure to these compounds, the dissipation of the NIs in soil, was also determined. NIs persistence decreased with increasing temperature, with all compounds being more persistent in the alkaline soil. A clear reduction of the nitrification activity at the lowest tested temperature (12.5°C) was observed only in the acidic soil, implying for a higher temperature effect on ammonia-oxidizing archaea (AOA) prevailing in this soil. Soil pH and temperature interactions determined the efficacy of NIs in inhibiting nitrification. At both incubation temperatures NIs did not appear to impose an accumulation of NH4+-N in the two soils but induced a significant and persistent reduction of PN rates and NO3–-N concentrations mainly in the alkaline soil. A higher activity of DCD and QI against AOA, and of DMPP against AOB was observed only at 25°C, in the acidic and the alkaline soil, respectively. Results on the effects of NIs on the community composition of ammonia-oxidizers are pending. Our findings have practical implications for the improved utilization of stabilized ammonium fertilization strategies under different soil and climate conditions.

PP_012

HOW CULTIVARS, TERROIR AND VINIFICATION STRATEGIES AFFECT MICROBIAL SUCCESSION IN THE VINIFICATION OF THE GREEK CULTIVARS RODITIS AND SIDERITIS

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Wine production is a process strongly mediated by the activity of microorganisms with commercial vinification relying largely on the inoculation with a limited number of Saccharomyces cerevisiae strains. These ensure high quality wine production but hinder the unique local identity of wines produced globally.

Recent studies have shown that the native vine microbiome (the sum of microorganisms colonizing the inner and outer parts of vines) is shaped by plant genotype, geography, microclimate and human practices, all contributing to the establishment of a microbial terroir identity and furthermore the vinification microbiome identity. The exploitation of the vine microbiome to produce high quality local wines instead of relying to allochthonous inocula has been proposed years ago, however we are still lacking autochthonous microbial strains with high oenological value.

Moreover, besides Saccharomyces we know little about the presence and contribution of other microorganisms, yeasts, fungi and bacteria, that are present in the vinification process to the chemical profile of the produced wines. In this context, we aimed to identify the composition of the prokaryotic and fungal microbiome along the vinification process through amplicon sequencing of the 16S rRNA gene and the ITS2 region in Illumina Hiseq.

To achieve this, we established a series of pilot vinification's using grapes collected from two emblematic cultivars of the viticultural zone of Aigialeia (Greece) Roditis and Sideritis. For each cultivar we collected samples from two terroirs. Grapes from each cultivar x terroir combination was subjected to three different vinification processes: (a) spontaneous carried out by the autochthonous microbiota (b) spontaneous carried out by the autochthonous microbiota but controlled with preservatives added and (c) inoculated with commercial inocula.

According to our results, NMDS and pairwise-PERMANOVA analysis showed that fungal and bacterial vinification microbiome can be distinguished and separated among the studied varieties. Bacterial microbiome showed to be more variety depended (p-value 0.001) than fungal microbiome (p-value 0.02), also, variety/terroir associations were more promented for bacteria (p-value 0.001).

Nevertheless, differential abundance analysis for both fungi and bacteria highlighted strains that seem to be terroir/variety depended and contribute to the local character of the wines produced.

PP_013

BIOLOGICAL CONTROL OF COLLETOTRICHUM ACUTATUM, CAUSAL AGENT OF OLIVE ANTHRACNOSE

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Olive Anthracnose (OA) is considered to be the most important fungal disease of olive fruits worldwide. Several species complexes of the genus Colletotrichum have been indicated as responsible for OA in different countries, including C. gloeosporioides and C. acutatum species complex. Systemic fungicides have been effective under field conditions but there is a high risk of the pathogen to develop resistance. Furthermore, public concerns about potential risks of fungicides on the environment and human health have been arisen. Thus, the discovery of alternative and sustainable control strategies are necessary. The use of plant resistance-inducers (PRIs) or biological control agents (BCAs) could contribute to this direction. The aim of this study was to investigate whether several biological Plant Protection Products (bioPPPs) were able to reduce OA disease incidence and severity or induce plant's defence. All experiments were conducted on cv. Kalamon. Disease assessment trials were carried out in planta, with application of the bioPPPs on fruits followed by artificial inoculation with C. acutatum. Subsequently, the most effective bioPPPs were applied on young olive trees, in greenhouse conditions, in order to test their ability to induce olive plant's defence system. Total RNA was extracted from leaves, and the expression levels of ten olive defence genes were evaluated with RT-qPCR method. The bioPPPs Mevalone®, Serenade®, Sonata®, Trianum® and Remedier® were able to reduce significantly disease severity and also to induce several plant genes in high levels. Considering the above, biological control could be a part of an Integrated Pest Management (IPM) program, for the control of OA.

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Further information: https://dromoielias.gr/en/

PP_014

IN VITRO EVALUATION OF 10 ESSENTIAL OILS AGAINST ASPERGILLUS CARBONARIUS AND THEIR EFFECTS ON OCHRATOXIN A RELATED ACLAEA GENE EXPRESSION

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Ochratoxin A (OTA) is a mycotoxin that mainly occurs on grapes and is being produced by Aspergillus carbonarius, which is a hazard for human and animal health. Chemical and physical treatments have been proven inefficient at removing the OTA from grapes and wine without effecting their organoleptic properties. The increasing use of pesticides in vine cultivation during the last decade, combined with the low maximum residue levels (MRLs), demands to find more alternative applications of natural antimicrobials to control sour rots and ochratoxin A contamination in vineyards. Essential oils (EOs) contain diverse bioactive compounds, that prevent mold growth and their toxic metabolite production, while they are not phytotoxic and biodegradative. The aim of this study was to evaluate the impact of 10 EOss, (cinnamon, thyme, mint, lavender, marjoram, tea tree, rosemary, sage, citronella and geranium) on growth of A. carbonarius (strains Ac29 and 5010) and OTA production. Experiments of fungal growth and OTA production were performed on Malt Extract Agar (MEA) and Czapek Yeast Extract Agar (CYA) solid cultures and the impact of EOs was measured after 7 days of incubation at 25oC. Further investigation on the effect of the most efficient E.Os was carried out by the relative expression of the AclaeA gene, by using Real Time PCR on synthetic grape medium (SGM) liquid cultures.

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PP_015

EVALUATION OF EPIPHYTIC GRAPE YEASTS FOR THE CONTROL OF ASPERGILLUS CARBONARIUS AND OCHRATOXINS IN GRAPES

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Yeasts are considered ideal biological control agents, as they are able to survive in a wide range of environmental conditions, grow rapidly with simple nutritional requirements, colonize plant surfaces relatively easy even under prolonged dry conditions and do not secrete toxic substances for humans. Yeasts have been widely used against plant pathogens at pre- or post-harvest level. The purpose of this study was to discover effective endemic yeasts as biocontrol agents against the black rot of grapes. Aspergillus carbonarius, the causative agent of this disease, adversely affects the organoleptic characteristics of the wine and produces carcinogenic ochratoxins. In this study, we tested various grapevine yeasts form the collection of Laboratory of Phytopathology (AUA) as well as new isolates from different grapevine varieties and regions in Greece. Several yeast strains were tested in vitro, for their ability to inhibit the production of conidia in the ochratoxigenic strain Ac-29 of A. carbonarius on a solid Yeast Malt Agar medium. Antagonism and ochratoxin inhibition bioassays were also carried out on detached grape berries. Yeasts with high antagonistic properties against A. carbonarius were selected for further field trials on two Greek grape white varieties (Malagousia and Savatiano). The results of the comparative study of the different yeasts against A. carbonarius and ochratoxins will be presented. The discovery of endemic yeast strains that are acclimated and adapted to the local environment and local flora and fauna and can effectively inhibit the ochratoxin production may lead to the creation of novel biocontrol products.

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PP_016

THE TRANSCRIPTIONAL EFFECTS OF BACILLUS VELEZENSIS K165 VOLATILE ORGANIC COMPOUNDS ON ARABIDOPSIS THALIANA AND THEIR PLANT PROTECTIVE ACTIVITY AGAINST BOTRYTIS CINEREA

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In recent years, evidence supporting the idea that plants respond strongly to microbial volatile organic compounds (mVOCs) has grown. Most of the research carried out so far has investigated the impact of microbial VOCs on the model plant Arabidopsis thaliana.

This has revealed that, without physical contact, microorganisms are able to drastically alter plant root system development, plant physiology, hormonal pathways, and biomass production. mVOCs can also function as a direct source of nutrients for plants, induce resistance to pathogens in plants, affect plant secondary metabolite production, directly inhibit plant pathogens and induce soil fungistasis and suppressiveness. In the present study, we investigated the transcriptional effects of the biocontrol agent Bacillus velezensis K165 VOCs on Arabidopsis thaliana plants and their plant protective activity against Botrytis cinerea.

The in planta experiments were performed in a novel dual compartment device, where the plants are placed in soil in the upper part of the device and the bacterial cells are applied in the lower part, ensuring the spatial separation of plant roots from K165 cells. The results of RNAseq analysis revealed that the most affected group of the upregulated Arabidopsis genes by the K165 VOCs belonged to the classification “response to stimulus”, according to the AGRIGO database.

This group comprised the 25% of the upregulated genes. Interestingly, a 13% of the genes classified in the “response to stimulus” category are involved in chitin perception. Therefore, we suggest that K165 VOCs may prime plants against fungal pathogens.

Indeed, the pathogenicity experiments revealed that K165 VOCs reduced B. cinerea disease severity and incidence and triggered induced systemic resistance upon infection, since the expression levels of PR1, PDF1.2 and VSP2 were higher in the K165/B. cinerea plants compared to controls.

PP_017

VINEYARD SOIL BACTERIAL COMPOSITION AND ITS RELATION TO GEOGRAPHIC LOCATION

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Soil microbiomes are essential to viticulture and winemaking where diverse bacteria and fungi play a pivotal role in the vine productivity, the grape composition and health and wine quality.

However, little is known about how the external factors (vineyard location and soil characteristics) influence the diversity of the microbial communities. In this study, we used high throughput 16s rRNA gene sequencing to investigate the potential relationships between vineyard locations and the soil microbial community composition of a Cypriot vine variety, Xynisteri, from four different vineyards locations in Cyprus.

Results showed that significant differences in soil bacterial community composition were associated with vineyard locations, whose soils showed variances in pH, organic matter, active carbon and nitrogen.

This indicates that geographic distance plays an important role in shaping the composition of vineyard soil-associated bacterial communities, which might contribute to the local identity of wine.

PP_018

EVALUATION OF INTEGRATED PEST MANAGEMENT (IPM) STRATEGIES FOR THE CONTROL OF ALTERNARIA LEAF BLIGHT IN CARROTS

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Alternaria dauci, a necrotrophic plant pathogenic fungus, is responsible for Alternaria leaf blight, the worldwide major foliar disease in carrot production. The disease commonly occurs when carrots are cultivated during moderate temperatures and the leaves are exposed to prolonged periods of wetness due to rainfall, dew, or sprinkler irrigation. Severe epidemics have been reported to reduce yields by 40-60%. Under high disease pressure, no single control measure is sufficient to manage the disease adequately. The disease management relies on the combination of the application of synthetic plant protection products (PPPs) with the use of partial resistant varieties and monitored by disease prediction models. In this direction, a total of 7 biological PPPs, 5 commercial synthetic PPPs, and one formulation that combines a synthetic fungicide with biostimulants were used to create 15 IPM modules. The efficacy of these IPM modules against Alternaria leaf blight in carrots were evaluated under field conditions including two reference IPM modules and three treatments using different bio-PPPs. For the pathogenicity trials, an intermediate disease resistant carrot variety (Bolero) and a susceptible one (Presto) were used that were inoculated with a spore suspension of A. dauci. All tested IPM modules and bio-PPP treatments were able to control successfully Alternaria leaf blight in the field. Efficiency seems to depend on the carrot genotype. Higher protection was observed with the partial resistant genotype. These results indicate that IPM strategies that combine biological and synthetic fungicides can effectively control Alternaria leaf blight in the field.

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PP_019

MOLECULAR PHYLOGENETICS OF SIX MARINE MICROALGAE STRAINS FROM GREEK TRANSIENT WATERS

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Microalgae represent an unexplored reservoir of biodiversity with great potential in various applications. However, their classification using traditional approaches is often hampered by the complexity of their morphological and structural characteristics.

On the contrary, molecular markers have been used for fast and practical classification with the usage of proper genes, such as tufA (Vieira et al. 2016). The AthU-Al (Athens University Algae) culture collection of the NKUA preserves strains isolated from various Greek coastal lagoons.

The strains AthU-Al Mes5 and AthU-Al Mes17 belonging to the species Tetraselmis verrucosa f. rubens were classified using several approaches including the molecular markers 18S rDNA and rbcl (Chantzistrountsiou et al., 2016). In the present study, the aforementioned strains alongside with AthU-Al Dun30, AthU-Al Dun31, AthU-Al Dun32 and AthU-Al T_3_1 were studied using both nuclear ITS region and 18S rDNA and plastid rbcl and tufA molecular markers.

For a more in-depth analysis and for a better resolution of molecular assignation, tufA and rbcL markers which were successfully determined for all tested strains, were selected to generate a phylogenetic tree based on their concatenated sequences.

In conclusion, herein we report the molecular classification of six microalgal strains based on four single DNA-barcoding markers alongside with a new suggestion for the combined usage of genes tufA and rbcl.

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PP_020

A STUDY OF THE BACILLUS VELEZENSIS K165 MEDIATED RESISTANCE AGAINST VERTICILLIUM DAHLIAE, BOTRYTIS CINEREA AND HYALOPERONOSPORA ARABIDOPSIDIS AND THE ROLE OF HISTONE ACETYLTRANSFERASES IN BIOCONTROL

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The use of microbes for the biological control of plant diseases during the last decades has been well studied along with the molecular aspect of plant- biocontrol agent (BCA) interactions. Nevertheless, the epigenetic effect of the BCAs on plants is a relatively new research field to be explored. Recently, we showed that treatment of Arabidopsis thaliana with the BCA Bacillus velezensis (previously Paenibacillus alvei) K165 confers inherited resistance to the soilborne pathogen Verticillium dahliae in K165-treated plants and their offspring. The observed plant protection was attributed to histone acetylation of genes participating in lignin biosynthesis and immune responses; resulting in lignin accumulation and induction of the jasmonate/ethylene pathway. In the present work, we studied the role of K165 in the Arabidopsis-Botrytis cinerea/Hyaloperonospora arabidopsidis/Pseudomonas syringae pv tomato (Pst) interactions in K165-treated wild type plants and their offspring. It was revealed that K165 protected the plants against B. cinerea and H. arabidopsidis; while, the offspring of the K165-treated plants were as susceptible as the controls. Furthermore, the performance of pathogenicity experiments in in Arabidopsis mutated in genes of the histone acetyltransferase (HAT) families: GNAT-MYST (HAG) and CBP (HAC), showed their implication in the K165 mediated disease resistance.

PP_021

EARLY TRANSCRIPTIONAL RESPONSES OF ARABIDOPSIS TO VERTICILLIUM DAHLIAE INFECTION AND ENHANCEMENT OF PLANT IMMUNITY BY DEAD CELLS OF THE PATHOGEN

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The soil-borne fungus Verticillium dahliae is causing a devastating vascular disease in more than 200 species of dicotyledonous plants. The pathogen attacks susceptible plants through the roots, colonizes the plant vascular system, and causes the death of aerial tissues.

The use of a model plant-pathogen system could accelerate the discovery and understanding of the molecular mechanisms underlying Verticillium resistance. In this study we used Arabidopsis as a model plant to (i) identify transcriptome changes occurring during infection byV. dahliae and (ii) examine the effect of co-application of dead and alive cells of the pathogen on plant immunity. To answer our first question, we performed RNA-sequencing in Arabidopsis at 36 h afterinfection with V. dahliae.

Already at this early time point of the interaction, there was upregulation of 340 defense-related genes in Arabidopsis.

Around 10% of the upregulated genes are involved in the perception and response to the hormone abscisic acid, suggesting its involvement in plant defense responses against V. dahliae.

It's noteworthy thatduring infection byV. dahliae there was downregulation of genes involved in the plant energy generation mechanisms. That suggests an active "growth-defense trade off", since the plant is not investing in resources that could be used for growth, rather generates energy-demanding defense responses against the fungal pathogen. Finally, and to answer our second question, we found that the application of V. dahliaedead cells in eggplants and Arabidopsis resulted in enhanced protection against V. dahliae, since the disease severity and pathogen colonization were lower in the plants treated with V. dahliaedead cells compared to controls.

PP_022

BACILLUS VELEZENSIS K165 VOLATILE ORGANIC COMPOUNDS ACTIVATE CHITIN PERCEPTION PATHWAY TO ENHANCE PLANT DISEASE RESISTANCE AGAINST VERTICILLIUM

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The rhizosphere is an area of intricate microbe-microbe interactions that can determine plant growth and health. There is growing evidence that beneficial microorganisms use volatile organic compounds (VOCs), in addition to classical antibiotics, to inhibit the growth of their microbial competitorsand also protect plants against various pathogens.

Here, we studied the contributionof VOCs emitted by the biocontrol agent Bacillus velezensis K165 (K165)on the protection ofArabidopsis thalianaagainst Verticilliumdahliae(Vd).

Using a novel dual compartment devicewe tested the in planta role of the K165 emitted VOCs against Vd.In this device, the plants are placed in soil in the upper part of the device and the bacterial cells are applied in the lower part, ensuring the spatial separation of plant roots from K165 cells.

Exposure of A. thalianato the K165 VOCs resulted in reduced pathogen colonization of the plant tissues compared to control plantsand also reduced disease severity, as demonstrated by qPCR analysis of the endophytic Vd DNA levels and pathogenicity experiments. Subsequently, RNAseq analysis of K165, E. coli, Vd, K165/Vd, E. coli/Vd and mock treated plants revealed the involvement of the CERK (chitin receptor) triggered defense responses in the K165 VOCs induced plant protection.

These results were further confirmed by pathogenicity experiments performed on the A. thaliana mutants cerk1, lyk5 and myb15, which participate in the CERK triggered immunity. More specifically, K165 VOCs lost their biocontrol activity in the examined mutants against Vd., since disease severity and pathogen colonization were not reduced compared to controls. Therefore, K165 VOCs enhance Arabidopsis resistance against Vd via a pathway involving CERK1, LYK5 and MYB15.

PP_023

BIOLOGICAL CONTROL OF ASPERGILLUS CARBONARIUS AND BOTRYTIS CINEREA IN GRAPEVINE BERRIES

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Grapevine bunch rot is mainly caused by Botrytis cinerea and Aspergillus carbonarius and constitutes one of the most significant problems in grapevine production.

Grape berries are more prone to bunch rot at the ripening stage, when the use of fungicides is limited due to the legislation for chemical residues. Therefore, bunch rot pathogens result in important economic losses to the producers, also affecting the quality of the wine. It is evident that the development of biocontrol agents against the main pathogens of bunch rot could be an ideal disease management strategy. In the present study, we examined the plant protective activity of the biological control agents, Paenibacillus alvei K165, Blastobotrys sp. FP12 and Arthrobacter sp. FP15 against B. cinerea and A. carbonarius on grapes. The in vitro experiments showed that strain K165 reduced significantly the growth of both fungi, while FP15 restricted the growth of A. carbonarius and FP12 was ineffective. Following the in vitro experiments, we conducted in planta experiments on grape berries. It was shown that K165, FP12 and FP15 reduced A. carbonarius rot severity by 81%, 57% and 37%, respectively, compared to control; whereas, in the case of B. cinerea, the only protective treatment was this of K165, which reduced disease severity by 75% compared to controls. The transcriptomic analysis of the genes encoding the pathogenesis related proteins PR2, PR3, PR4 and PR5 indicated the activation of multiple defense responses involved in the biocontrol activity of the examined biocontrol agents.

PP_024

GENETIC CHARACTERIZATION AT THE SPECIES AND SYMBIOVAR LEVEL OF SEED BORNE RHIZOBIA ISOLATED FROM POT-GROWN COMMON BEAN NODULES.

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Although, Phaseolus vulgaris (L.), namely as common bean, is considered a promiscuous legume host since it forms nodules with diverse rhizobial species and symbiovars, compared to other leguminous crops, is a poor nitrogen fixer pulse. In order to improve the reduced BNF ability, maximize nodulation and nitrogen fixation in common bean and achieve optimal biofertilization, the selection of efficient, competitive, and well-adapted rhizobial strains in different edaphoclimatic zones is considered as a good sustainable agricultural practice. In this study the classic way of rhizobia isolation from field-grown common bean nodules was not followed, as previously, but another.

Actually indigenous rhizobia from pot-grown common bean nodules were isolated in laboratory conditions. Aiming to seed born rhizobia (SBR) from local common bean varieties of different geographical areas of Greece (Prespes, Domokos, Viotia, Kavala, Tripoli, Larisa and Lesvos island), sterile substrate and seeds were used. The genetic diversity of thirty-one seed born rhizobia was assessed by BOX-PCR and the phylogenetic affiliation was assessed by multilocus sequence analysis (MLSA) of three housekeeping and symbiosis-related genes. Five representative isolates with distinct BOX-PCR fingerpriniting patterns were subjected to phylogenetic analysis which revealed that all strains belonged to Rhizobium sp.

A part of them assigned to R. tropici, some of them were closely related to a putative new genospecies which is provisionally named as Rhizobium sp. I and others to two putative still unknown novel genomic lineages. Most strains belonged to symbiovar phaseoli carrying the α- and γ-a alleles of nodC gene, while some of them belonged to symbiovar tropici. All strains were able to re-nodulate their original host, indicating that they are true microsymbionts of common bean.

PP_025

MICROBIAL DIVERSITY DURING THE SPOILAGE OF EDIBLE FRESH BROWN ALGAE

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Concerning the marine macroalgae for human consumption , there are only a few studies investigating their microbial diversity, and the microbial growth and their shelf life. This study investigates the microbiological quality of the two most frequently cultivated brown algae in Europe Alaria esculenta and Saccharina latissima during their storage as well as to elucidate the main microbial groups that are present during spoilage.

Fresh samples of A. esculenta and S. latissima harvested from Scotland and A. esculenta harvested from Ireland in the year 2020 were assessed for the presence of microorganisms, when stored under isothermal conditions (0, 5, 10, and 15 °C) for specific time intervals. During storage, microbiological analyses were performed for the enumeration of Total Viable Counts (TVC), Pseudomonas spp., Enterobacteriaceae, Bacillus spp. and fungi.

The presence of food pathogenic bacteria Salmonella, Listeria monocytogenes and Vibrio spp. was also investigated. Additionally, bacterial colonies from the general growth medium (Marine Agar) were isolated from all temperature conditions throughout storage and subjected to partial 16S rDNA sequencing.

The initial TVC of A. esculenta from Scotland was 3.2 log CFU/g, while microorganisms reached the level of 7.0 log CFU/g on day 4 at 5 °C. The specific spoilage microorganisms Bacillus spp. and Pseudomonas spp. were found at levels similar to TVC, particularly at lower storage temperatures, while the initial TVC of A. esculenta from Ireland was 3.0 log CFU/g, which reached also 7.0 log CFU/g on day 4 at 5°C. The microbial population of S. latissima was close to the enumeration limit (< 7.0 log CFU/g), with no specific growth pattern to be observed.

DNA sequencing results revealed the presence of Psychrobacter, Cobetia and Pseudomonas species in A. esculenta cultivated in Scotland, whereas Pseudoalteromonas and Psychrobacter species were predominant during the spoilage of A. esculenta harvested from Ireland. Psychrobacter and Micrococcus species were identified during the spoilage of S. latissima. Differences of the dominant bacteria were observed among different storage temperatures, algae and geographical origin.

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PP_026

CHARACTERIZATION OF FUNGI ASSOCIATED WITH OLIVE FRUIT ROT AND OLIVE OIL DEGRADATION IN CRETE, SOUTHERN GREECE

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In November 2019, a severe outbreak of fruit rot was observed in olive orchards in Crete, southern Greece. Symptoms appeared primarily on fruits and stalks, resembling those caused by anthracnose.

Typical symptoms were fruit rot, shrinkage and mummification, associated commonly with stalk discoloration and fruit drop. Disease incidence was estimated up to 100% in some cases and an unprecedented increase in olive oil acidity reaching up to 8% (percentage of oleic acid) in severely affected olive groves was recorded. Thirty-two olive groves were then surveyed, and samples of fruit, stalk, leaf and shoot were collected. Visual, stereoscopic and microscopic observations revealed several fungi belonging to the genera Alternaria, Botryosphaeria, Capnodium, Colletotrichum, Fusarium and Pseudocercospora.

Fungal infection in fruits was commonly associated with concomitant infestation by the olive fruit fly Bactrocera oleae along with increased air temperature and relative humidity conditions that prevailed in October-November 2019. Twenty representative fungal strains isolated from symptomatic fruits and stalks were characterized by morphological, physiological and molecular analyses. By rDNA-ITS region and EF1-α gene sequencing analysis, these isolates were identified as Alternaria spp., A. infectoria, Botryosphaeria dothidea, Colletotrichum boninense sensu lato, Fusarium lateritium, F. solani species complex and Stemphylium amaranthi. Pathogenicity tests on punctured fruits revealed that all isolates were pathogenic; however F. solani isolates along with B. dothidea were the most virulent and wounds were necessary for efficient fungal infection. Moreover, as little as 10 spores of F. solani were sufficient of causing significant infection in punctured fruits. Fusarium solani was also capable of infecting olive fruits in the presence of B. oleae, with no additional wounding, in artificial inoculation experiments.

Moreover, it was capable of colonizing and affecting olive blossoms. Further analyses in olive oil extracted from fruits artificially inoculated with F. solani indicated a significant increase in oil acidity, K232, K270 and peroxide value, whereas total phenols content was significantly decreased. To the best of our knowledge, this is the first report of Fusarium solani associated with olive fruit rot and olive oil degradation worldwide.

PP_027

THE EFFECT OF FEEDING INTENSITY ON MICROBIOME COMPOSITION IN AN INTEGRATED KOI CARP-SAGE RECIRCULATING MULTI-TROPHIC PRODUCTION SYSTEM

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Integrated multi-trophic aquaponic (IMTA) system is a sustainable system that combines aquaculture and hydroponics, forming a symbiotic relationship between fish, plants, and microbes. In IMTA systems, nutrients originate mainly from the fish feed and water inputs with the help of beneficial microorganisms.

In order to better understand the role of microbes in this association, we studied the bacterial community composition in relation to different feed inputs, by utilising next generation sequencing. To this purpose, two different feed scenarios were tested in koi carp (Cyprinus carpio var Koi, L., 1758) - sage (Salvia officinalis) integrated recirculating production system conditions. An 83% higher nutrient input through administrated feed is assured in V1, compared to V2, throughout the entire 12 weeks experimental period.

Physiological measurements, showed that the sage biomass gain records better values in V2 (2.56 kg/m2), compared to V1 (1.99 kg/m2) until the 1st cut of leaves biomass (the first 6 weeks), while between 1st and 2nd cut (the last 6 weeks), a 6.33 kg/m2 biomass gain was recorded for V1 and 5.40 kg/m2 for V2. Moreover, the nitrogen compounds removal rate emphasizes better values at V2, compared to V1, situation which may indicate a need of upscaling the aquaponics modules surface of V1 integrated system in order to assure an optimum water treatment process.

Alterations in the microbiome structure were assessed by sequencing reads of V3-V4 16S rRNA regions obtained from Illumina NovaSeq 6000. Samples were collected from two compartments of the designed ITMA system. Actinobacteria, Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were mostly monitored, which also constitute the core microbiome in both water and root plants.

The correlation between the bacterial community distribution, plant growth and nutrient uptake, as related to the proposed feed input variants is discussed. This study is the first to report on the bacterial community in an integrated IMTA system including koi carp and sage plants which also evaluates the beneficial effect of fish feeding intensity on the water quality and plant growth performance in the proposed system.

PP_028

GENETIC DIVERSITY OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS STRAINS ISOLATED FROM TOMATO CROPS IN GREECE

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One of the most destructive threats for commercial tomato crops production worldwide, is the bacterial canker disease. The disease is caused by the vascular bacterium Clavibacter michiganensis subsp. michiganensis (Cmm).

Cmm strains have been isolated from open-field and greenhouse cultures in several regions in Greece during outbreaks between 2003 to 2018. They were identified using KOH test, immunofluorescence and polymerase chain reaction with specific primers (PSA 8/R, RZ_ptssk 10/11).

Pathogenicity was confirmed on tomato seedlings. Ninety-three identified Cmm strains were selected for genetic diversity study based on multi locus sequence analysis (MLSA). The MLSA scheme consisted of four housekeeping genes (atpD, ppk, kdpA and sdhA) which were partially amplified and sequenced.

The sequence polymorphism analysis showed 4.07% variable sites in concatenated sequence of 4 genes (2283bp) and the 93 strains were differentiated into 38 haplotypes.

Our results show high diversity within the population, indicating clearly that Cmm was introduced in Greece from different sources during the last fifteen years that most probably results from seed importation. The phylogenetic clustering of the Cmm strains at most cases was not in line with their geographic distribution. Thus, we could hypothesize that the outbreaks of Cmm in the studied regions arose mainly from infected seeds.
However, in some cases, strains isolated from the same region at the same or at different years were grouped in the same cluster.

This finding could be possibly attributed to the transmission of Cmm strains from one culture site to the neighboring one, or by the use of infected seeds lots with similar strains. Our study gives the first insights on the genetic diversity, the origin of the outbreaks and spread of Cmm in Greece. Preventive measures should be taken to avoid the introduction of infected seeds in the nurseries and consequential spreading of Cmm to the tomato farms.

PP_029

QUALITY ASSESSMENT OF SEA BREAM AT DYNAMIC TEMPERATURE CONDITIONS THROUGH CONVENTIONAL AND INNOVATIVE TECHNIQUES

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Fluctuation of temperature during storage, transportation and handling affect the growth of microorganisms, resulting in spoilage of fish products.

Hence, the objective of this work was to investigate the quality of cultured sea bream during storage under dynamic temperature conditions using conventional microbiological techniques (plate counting) and sensory analysis (odor and skin color) as well as electronic nose (e-nose), a novel and non-invasive sensor.

For this purpose, two independent experiments were performed on aerobically packaged whole sea bream and sea bream fillets as well as in sea bream fillets in modified atmosphere packaging (26.3% CO_2, 13.5% O_2, 60.2% N_2) stored in three different dynamic temperature profiles. Results showed that Pseudomonas spp., H_2S producing bacteria and Brochothrix thermosphacta were the dominant spoilage microorganisms in all storage conditions.

According to sensory evaluation, fillets in air and MAP were rejected when total viable counts reached to 8.2 and 7.7 log CFU/g at 96 h and 120 h, respectively. Likewise, spoilage in whole fish was observed at 144 h based on TVC counts (7.4 log CFU/g). Partial Least Squares Discriminant Analysis (PLS-DA) was also used to classify volatile data of e-nose into clusters, separating and grouping fresh and non-fresh fish samples.

PP_030

DIFFERENT FACTORS ARE OPERATIVE IN SHAPING VINE MICROBIOME ACROSS DIFFERENT GEOGRAPHICAL SCALES: BIOGEOGRAPHY, CULTIVAR OR TERROIR?

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Vine microbiome interacts with soil type, local environmental conditions, cultivars and agricultural practices, all together contributing to the establishment of vine terroir, a proof of quality and geographical identity of the produced wines.

Today we know little about the composition of the vine microbiome in local Greek varieties and the factors shaping its composition. In this frame we asked the following questions (a) which factors shape the composition of the vine microbiome and how these operate at different geographical scales (viticultural zone vs nation level) (b) which is the composition of the vine microbiome in these Greek varieties.

To provide answer, we collected grape and leaf samples at harvest (year 2019) from four Greek cultivars Vidiano, Agiorgitiko, Roditis and Sideritis at different geographic scales. Samples from the first two cultivars were collected from three vineyards in Thessaloniki, Athens and Crete, while for Roditis and Sideritis samples were collected from two vineyards per cultivar constituting different terroirs in the viticultural zone of Aigialeia. Samples were analyzed via Illumina amplicon sequencing. We noted that different factors affected the composition of the vine microbiome depending on the geographical scale of the study. Hence for Vidiano and Agiorgitiko biogeography (at national scale) was the main factor controlling the composition of the vine microbiome especially of the fungal component, with cultivars showing a weak effect. When looking at the microbiome with the same viticultural zone we noted that cultivar (Roditis and Sideritis) becomes a strong determinant of the vine microbiome, with terroir also contributing to a lower extent only for the fungal microbiome.

The latter was dominated by Ascomycetes (mostly Sordariomycetes and Dothideomycetes), with Saccharomycetes being a minor component. The bacterial microbiome was dominated by α-proteobacteria, γ-proteobacteria, Actinobacteriota. Archaea showed a low representation mostly belonging to Nitrososphaeria (ammonia oxidizers) and Methanobacteria (methanol consumers). DA analysis identified microorganisms specifically associated with certain cultivars, often yeasts, suggesting a possible role in vinification and the distinct character of local wines. Analysis of samples of the 2020 harvest is in progress to verify these observations and determine the effect of vintage on vine microbiome.

PP_031

CHARACTERIZATION OF THE SEABREAM BACTERIOME FROM WILD AND AQUACULTURE

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Fish intestines harbor large and diverse populations of bacteria which play an integral role in host health by stimulating development of the immune system, aiding in nutrient acquisition and outcompeting opportunistic pathogens. Microbial colonization of fish larvae originates from the eggs, the surrounding water and the first feed. Most studies have shown that this gut microflora varies among fish species, and changes with life stage and habitat. However, a relatively stable gut microbiota is established within the first 50 days of life for many species. Captive breeding and rearing of fish involve the manipulation of multiple factors, including the environment, social interactions, and diets.

Changes in gut microbiota composition attributed to captive-state have been frequently reported. Our study is focused on the detailed characterization of the gut microflora of Sparus aurata from wild and aquaculture populations, with the use of high-throughput amplicon sequencing of the 16S rRNA gene.

In more detail, we examined two wild populations from Tholi and Messolonghi and two populations from aquaculture farms from Vonitsa and Astakos. For all populations two developmental stages were examined.

Sequence analysis showed the formation of distinct bacterial communities between the wild and aquaculture populations, with significant differences in bacterial diversity and taxa composition. In all populations examined, the most dominant genera were those of Acinetobacter, Aeromonas, Bacillus, Enterobacter, Pseudomonas, Staphylococcus and Vibrio. Significant differences were also found between the two wild populations and the two aquaculture populations as well as between the different developmental stages. Understanding the natural importance of the seabream bacteriome is challenging but studying the dynamics over time it will assist us to elucidate the potential role of these bacteria.

PP_032

THE NATIONAL SOIL GENE BANK OF THE REPUBLIC OF CYPRUS: THE IMPORTANCE OF SOIL GENETIC RESOURCES

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The exploitation of soil genetic resources is a critical component of the overall strategy for sustainable agriculture and food security globally. Soil microbes are key players in ecosystem process through the control of biogeochemical cycles but also directly and indirectly influencing plant, animal, and human welfare. In addition, soil microbial communities thereby represent a major component of terrestrial ecosystems and a tremendous gene pool, which is of invaluable importance for environmental quality and public health.

In this context, the National Infrastructure «MAGNET» was initiated in 2019 by the Agricultural Research Institute in Cyprus through the establishment of a new center for Environmental Microbiology and Biotechnology. MAGNET's vision is to provide high impact environmental and economic benefits to Cyprus's bio-economy and biosciences, by promoting advanced innovative microbial solutions for agriculture to optimize crop yields and quality in a climate changing environment and provide a more sustainable industry impact profile, ultimately resulting in new opportunities to protect the environment.

The unique state-of-the-art National Infrastructure Center can collect, analyze and understand the structure, function, activities and dynamics of soil microbial communities generating solutions to societal challenges of the country and the region. In this presentation we are showing the first National Soil Genetic Resource Bank and an overview of the its activities and the importance of microbial communities in vineyards and cowpeas are presented.

PP_034

CHARACTERIZATION OF THE CAUSATIVE AGENT OF RED SKIN LESIONS (RED SPOTS) IN SEA BREAM

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Aquaculture in Greece has contributed to the direct production of consumer goods, with the main cultivable species being sea bream, and specifically the species Sparus aurata. The emergence of infectious diseases may cause epidemics of known or unknown pathogenesis resulting in product loss, due to increased mortality or quality deterioration, eventually leading to severe financial losses.

One such pathogenic condition is the red spot syndrome. Its symptoms include one or more red skin lesions on the body of the fish. Its mortality is rare, but it leads to the degradation of the product rendering it unmarketable. Despite the impact of the disease, the responsible pathogen remains undetermined. For this reason, we examined three groups of fish depending on the severity of the symptoms: group 0 (healthy fish, negative control), group 1 (slight skin damage), and group 2 (large extent of skin lesions). Both culture dependent and molecular approaches were used to identify the responsible pathogen. For the cultivation approach, fish skin homogenates were grown on solid media.

Bacterial isolates were characterized morphologically and identified by sequencing the full 16S rRNA gene. On the other hand, the molecular approach was based on DNA extraction from skin samples, PCR amplification of a 460 bp fragment of the 16S rRNA gene, and next-generation sequencing with the Illumina MiSeq technique. The bacterial profiles that resulted from the analyses gave us an estimate of the pathogen responsible for the occurrence of the syndrome. They were also compared with reports from other researchers who had observed and studied similar symptoms in other fish species.

PP_035

MICROBIAL COMMUNITIES SUCCESSION OF SEA BREAM FILLETS STORED UNDER VARIOUS CONDITIONS

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The aim of this work was to study the microbiota succession of sea bream fillets, a vital exportable foodstuff of the Greek commerce, during storage at various temperatures (0oC, 4oC and 8oC) and atmospheres (air and MAP). Metabarcoding analysis indicated an environmental-based initial microbiota and a similar profile in all but one treatment at the end of shelf-life, since Pseudomonas spp., was the key player in specimens stored on air, as well as under MAP at 4oC, while Carnobacterium spp., predominated in samples stored at 0oC under MAP. These findings shed light regarding the significance of preservation conditions and temperatures on the fillets' spoilage microbiota changes during storage, which affects the spoilage mechanism and consequently the fillets' shelf-life.

PP_036

PLANT GROWTH PROMOTING RHIZOBACTERIA AS POTENTIAL BIOAGENTS AGAINST FUNGAL PATHOGENS

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Agrochemicals apart from exhibiting often insufficient activity as pesticides or fertilizers, they also can be hazardous for humans and environment. Plant growth promoting rhizobacteria (PGPR) constitute a potential alternative for enhancing crop yields and alleviating biotic and abiotic stresses. The present study aimed to evaluate PGP strains previously isolated from the rhizosphere of plants grown under harsh environments as potential biological agents. A total of 73 strains were evaluated in vitro against seven fungal pathogens. i.e. Alternaria alternata, Botrytis cinerea, Rhizoctonia solani, Verticillium dahliae, Sclerotinia sclerotiorum, Pythium ultimum, Fusarium oxysporum f.sp. radicis - lycopersici. Strains were also screened for the production of hydrolytic enzymes (amylase, protease, cellulose, chitinase), hydrogen cyanide and ammonia.

Dual culture bioassays were also conducted in order to determine antifungal activity due to volatile production of the tested strains. The most efficient strain was further evaluated in pot experiment as antifungal agent against Sclerotinia sclerotiorum, while its impact on physiological and agronomic characteristics of the host plants, lettuce, was also assessed.

According to the results from the in vitro assays, 19 bacterial strains were found active against one or more fungal pathogens. Almost all strains were able to produce NH3, three strains were able to produce HCN, while only 5 strains were not able to produce hydrolytic enzymes. Results from the subsequent in vivo trials showed that lettuce plants treated with strain Es4 via root irrigation showed higher resistance against S. sclerotiorum compared to foliar application of the same bacterial strain. Plant growth characteristics and photosynthesis were also enhanced after bacterial treatment. The above results indicate that the PGP strain can serve as an effective biological agent. More research is needed in order to elucidate its mode of antifungal activity and to specify the most efficient application procedure.

PP_037

THE EFFECT OF SUSTAINABLE INNOVATIVE FEED ON GROWTH PERFORMANCE, WELFARE STATUS AND EXTREME TEMPERATURE RESILIENCE OF KOI CARP

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The present study aims to identify the effect of innovative fish feed (IF), based on alternative and sustainable protein sources, on koi carp (Cyprinus carpio var Koi, L., 1758) growth performance, welfare status and extreme water temperature resilience. Thus, 4 experimental variants were performed, as follows: V1-IF additivated with turmeric, V2-IF additivated with beetroot, V3-IF with no additives, V4-commercial feed. Fish growth performance indicators registered the best results at V4, followed by V1, V3 and V2. The welfare status analysis indicates V1 IF as most suitable for koi carp, followed by V2, V3 and V4. At the end of the experimental period (8 weeks), fish exemplars from each of the tested variants were exposed, for 6 hours, to the following temperatures: 17 °C (LT), 25 °C (NT) and 35 °C (HT).

The welfare status analysis performed after the extreme temperature exposure test recommends additivated IF as it offers better resilience, compared to commercial feed and non-additivated IF. Temperature stress increase the reactive oxygen species (ROS) production, that subsequently cause oxidative stress. Therefore, the effect of extreme temperature exposure, on the induction of oxidative stress and the alteration of gene expression, in correlation with different protein sources, were evaluated in fish tissues sampled from V1 IF and V4 variants. Our data showed that the temperature stress affected not only the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione-S-transferase (GST), but also the transcriptional levels of their respective genes. However, the mRNA induction patterns were not in accordance with those of antioxidant enzyme changes in all tissues. Additionally, the transcriptional expression of stress-related genes including heat shock protein 70kDa (Hsp70), insulin like growth factor 1 (Igf1) were also assessed. Moreover, the compositions and concentrations of amino compounds were analysed using a Waters Acquity UPLC-System.

The current results not only recommend an innovative fish food (IF) suitable for koi carp, but also could provide valuable new insights into oxidative stress mechanism induced by the extreme temperature exposure that affect fish health and could be used for selection of biomarkers to monitor the fish adaptation to the specific stress.

PP_038

MICROBIAL COMMUNITIES OF FISH FROM LAKE KARLA

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Biological hazards such as foodborne pathogens from animal farms, pastureland, cropping systems, etc., can enter lakes and contaminate fish. Tile drains or artificial subsurface drainage or wastewater discharges that result from animal or human activity, can serve as means of transporting pollutants from the agricultural field or industrial area to the surface water environment and further into lakes. The aim of this study was to reveal the most important bacterial genera in fish Cyprinus carpio from Lake Karla taken from three site points (A, B and C) communicating with the surrounding land. Fish tissue of 25g was taken (from three fish individuals as a pool sample) for 16S amplicon metagenomic sequencing.

The metabarcoding analysis was performed with the Illumina protocols by MR DNA (Shallowater, TX, USA). Sequencing was applied on a MiSeq Illumina platform according to manufacturer's protocols. Bioinformatic analysis was performed using the MR DNA pipelines to cluster and present the sequences into operational taxonomic units (OTUs).

The findings showed that Pseudomonas (66,33%) and Calochaete (16,08%) were the most abundant genera in fish from point A Calochaete (38,39%), Corynebacterium (21,7%) and Propionibacterium (18,16%) in fish from point B, moreover, Erwinia (44.1%) and Serratia (34,46%) in fish from point C. To conclude, 16S NGS revealed microorganisms that are associated with the lake's water e.g., Calochaete cimrmanii and others e.g., Serratia fonticola and Corynebacterium aurimucosum that come from various sources of microbial contamination from the land.

Additionally, the above-mentioned genera of all points of the lake contain many species that are potential human pathogens. Our findings will be used for increasing public awareness on the importance of the quality of water in lakes, that directly affects the quality of the lake's fish products.

PP_039

APPLICATION OF FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY AND ELECTRONIC NOSE (E-NOSE) TO THE RAPID ASSESSMENT OF SEA BREAM FILLETS QUALITY.

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The objective of the study was to develop PLS-R models by using the innovative methods of Fourier transform infrared (FTIR) spectroscopy and electronic nose (e-nose) to assess the spoilage of sea bream (Sparus aurata) fillets. Therefore, the fish fillets stored at 0, 4, 8 and 12 oC under air or modified atmosphere packaging (MAP) conditions (33% CO2, 19% O2, 48% N2), were examined for the enumeration of Total Viable Counts (TVC). Meanwhile, the spectral data from FTIR and the e-nose chemical volatile profile were recorded during the storage of the fillets. The PLS-R models developed on FTIR spectral data provided satisfactory performance in the estimation of TVC, as the coefficients of determination (R2) were 0.98 and 0.94, while root mean square error of calibration (RMSE) were 0,43 and 0,87 for fish fillets stored aerobically and under MAP, respectively. However, PLS-R models based on e-nose data exhibited less satisfactory performance, as R2 were 0.34 and 0.17, while RMSE were 1.77 and 1.43 for fish fillets stored aerobically and under MAP, respectively. Hence, the FTIR analysis is effective for a rapid evaluation of the quality of sea bream fillets in contrast to the technique of e-nose in which more data are needed.

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PP_040

INSIGHTS INTO THE EFFECTS OF AGRONOMICAL MANAGEMENT PRACTICES IN ASPERGILLUS INCIDENCE AND CARPOSPHERE'S MICROBIAL COMMUNITIES OF GRAPEVINE (CV. SYRAH)

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Aspergillus bunch rot is one of the most important pre- as well as post-harvest diseases of grapevine. Disease development may lead to great quantitative losses, while contributing to qualitative deterioration of grape products due to the production of a great variety of mycotoxins , such as ochratoxins and fumonisins that impose a risk for consumer`s health.

This study aimed to evaluate several agronomic practices (irrigation, defoliation and bunch density) to manage the incidence of Aspergillus on grapes. High/Low bunch density (HBD, LBD) of the grape bunch was achieved with the formation of vines during pruning season (HBD: 4-5 buds/vine; LBD: >8 buds/vine), while defoliation treatment was achieved with total leaf removal of the vines till the bunch line before berries formation. Additionally, in irrigation treatment, selected vines were irrigated three times during the growing season.

Aspergillus incidence on grape berries was recorded on each maturity stage (Pea-sized berry, Veraison, and Harvest), while carposphere's total fungal communities were assessed via amplicon sequencing analysis. Between Pea-sized berry and Veraison, the incidence of Aspergillus spp. showed low frequencies (0 to 2.5%), and no significant differences were noticed among treatments.

However, at the harvest stage, Aspergillus spp. incidence increased and ranged from 1.8 to 93.6%. Significant differences were observed between treatments. In detail, in non-defoliated, HBD, and Irrigated treatments, the frequency of Aspergillus spp. was significantly higher compared to the defoliated, LBD, and non-irrigated treatments ($p<0.05$).

Amplicon sequencing analysis revealed that many members of fungal community, including Aspergillus spp., exhibitied differences in their abundance between treatments and maturity stage.

A carefull look on Aspergillus members showed that harvest they exhibited higher abundances in Irrigated, non-defoliated, and HBD treatment than their pair-mock treatments.

This study showed that the application of agronomical management practices might significantly impact the reduction of Aspergillus Bunch rot and paves the way for a comprehensive study of the effect of these practices in carposphere's microbiome communities.

PP_041

A NOVEL TETRA-PRIMER ARMS-PCR FOR THE DETECTION OF BCPOS5 GENE MUTATIONS CONFERRING RESISTANCE TO ANILINOPYRIMIDINE FUNGICIDES IN BOTRYTIS CINEREA AND USE OF CRISPR/CAS9 EDITING TO GENERATE RESISTANT MUTANTS

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Despite the long-standing use of anilinopyrimidine fungicides (APs) against Botrytis cinerea, their mode of action and resistance mechanisms have only recently been elucidated. It is now evident that the molecular target of this specific class is of mitochondrial origin and specific resistance mutations G408V, L412V and L412F in Bcpos5 gene are the most common in the field.

To confirm the presence and frequencies of these mutations in the Greek fields, a sampling of B. cinerea isolates (n=255) was conducted throughout mainland Greece. The isolates were characterized as APs-resistant or -sensitive using the discriminatory dose of 7ppm cyprodinil, according to a standard phenotyping protocol.

Furthermore, an adapted Tetra-primer ARMS PCR was developed to effectively identify the two most common field mutations (L412F, L412V). The PCR yielded a 702bp band for all isolates while, the presence of a 470bp or 252bp band corresponded to F and V substitutions, respectively.

To confirm the presence of the third mutation, the PCR products with a single band (702bp) were further digested by enzyme MlyI yielding 467bp and 235bp bands in the G408V mutants. The results were verified by Sanger sequencing.

The developed Tetra-primer ARMS PCR method was applied to a set of 82 B. cinerea isolates collected from strawberry and tomato crops and revealed that L412F, L412V and G408V were occurring at frequencies of 69%, 7% and 1.5% respectively.

To further characterize AP-resistance mutations, B.05 reference strain was transformed with L412F and L412V using the CRISPR/Cas9 technique and homologous recombination.

The transformants were resistant to cyprodinil and are used to subsequent studies. The results show that the aforementioned method could be extensively used in resistance monitoring patterns in B. cinerea in a fast, cheap and efficient way.

Moreover, it is crystal-clear that CRISPR/Cas is an extremely effective tool concerning molecular genetics whereas protocols based on B. cinerea transformation are more than efficient.

PP_042

DEVELOPMENT OF NOVEL CULTIVATION METHODOLOGIES FOR MUSHROOMS PRODUCTION BY THE ENDANGERED SPECIES PLEUROTUS NEBRODENSIS

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Pleurotus nebrodensis is an endemic species of the alpine zone of Sicily and southern Greece which grow naturally on root and stem residues of Prangos ferulacea (Apiaceae). It was the first mushroom recognized as critically endangered by the International Union for Conservation of Nature [IUCN]. Few individuals reach maturity each year, and although its successful cultivation is considered of major importance for reducing the human pressure on wild populations due to harvesting, ex situ mushroom production is practically non-existent. In the frame of this project, strains of diverse geographic origins were initially evaluated in terms of their crop parameters and then, directed matings among selected homokaryons were performed to finally produce 25 new hybrid strains. Among them, the hybrid strain PnbH17 showed significantly better performance in respect to all cultivation parameters and was hence qualified for further research. Finally, the effects of various substrates based on locally available lignocellulosic residues and of adding a casing layer were studied. Results revealed that the duration of the incubation period ranged from 13 to 21 days and earliness (time from inoculation to primordia formation) from 15 to 30 days when different substrates were evaluated. The sum of mushrooms yield of the first two flushes was relatively low in substrates consisting of wheat straw or beech sawdust (108 to 171 g/kg of substrate), and substantially higher in olive leaves or in mixtures of wheat straw and grape marc (299 and 303 g/kg of substrate, respectively). Moreover, a high variation in biological efficiency values (fresh weight of fruitbodies to dry weight of substrate; values ranging from 22 to 106%) was observed among the substrates examined. Regarding Furthermore, the addition of the casing layer delayed the appearance of mushrooms by up to 6 days, but it increased biological efficiency in all substrates from 2 to 23%. In conclusion, the new hybrid PnbH17 exhibits satisfactory production performance and could be further exploited for the commercial cultivation of Pleurotus nebrodensis mushrooms.

PP_043

EXPLOITATION OF LOCALLY AVAILABLE LIGNOCELLULOSIC BY-PRODUCTS FOR THE CULTIVATION OF THE TROPICAL MILKY WHITE MUSHROOM CALOCYBE INDICA

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Calocybe indica, commonly known as the “milky white mushroom”, is an endemic species of tropical India, which grows saprotrophically on substrates rich in organic matter. Its cultivation requires temperatures of 30 – 35 oC in contrast to most other mushroom species for which the optimum temperature for mushroom production lies within the range of 10 – 20 oC. Therefore, it is an ideal candidate for cultivation during the warmer months of the year when production of most other mushrooms ceases or is drastically reduced. The aim of this study was to optimize the conditions for C. indica mushroom production, including the evaluation and possible exploitation of locally available lignocellulosic substrates. C. indica was cultivated in substrates consisting of wheat straw (WHS) or beech sawdust (BSW) supplemented with either wheat bran and soya or in high ratio mixtures with grape marc (GMC). All substrates were tested with or without the application of a casing layer based on peat moss. The addition of GMC led to a reduction in the time required for mushroom appearance by 1 to 9 days compared to WHS or BSW, while no significant differences were observed in the length of the crop cycle (62 – 69 days; i.e., time required from inoculation to the harvest of the 3rd flush). Furthermore, biological efficiency (fresh weight of fruitbodies to dry weight of substrate) significantly increased in WHS by reaching 153%, which is among the highest values recorded for cultivated mushrooms. It is noteworthy that the application of the casing layer enhanced biological efficiency by up to 70%. The estimation of the effect of the nature/composition of substrates on the nutritional value and functional properties of mushrooms is in progress to determine the best substrate/strain combination for the cultivation of C. indica

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PP_044

WOLBACHIA - MEDFLY INTERACTIONS IN NOVEL GENOMIC BACKGROUNDS

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The Mediterranean fruit fly, Ceratitis capitata (medfly) (Diptera: Tephritidae), is considered a naturally Wolbachia-free insect since all colonized populations screened so far have been found negative. Few reports from Latin America pointing to Wolbachia being naturally present in the area have not been verified. In the last two decades, an artificial relationship of medfly with Wolbachia has been established, with the transinfection of Rhagoletis cerasi Wolbachia strains wCer2 and wCer4. This relationship has proven stable, with complete unidirectional and bidirectional cytoplasmic incompatibility (CI). Studies up to now suggest that this association comes with a significant cost mainly in hatching, along with other phenotypes that can be quite plastic in different genetic backgrounds.

To study this recent association in more detail, we performed a series of crosses to place Wolbachia in different genomic backgrounds, along with introgression experiments that resulted in an estimated genomic replacement of more than 95%, thus leaving Wolbachia as the main driving factor of any differences observed. The resulting strains have been stable for up to 100 generations since their development. The two main phenotypes previously observed, meaning complete unidirectional and bidirectional CI and reduced hatching, are evident in the new strains. Our data suggest differences among the two Wolbachia strains since wCer2 comes with a smaller cost in hatching and a better ability to stabilize in the new backgrounds, compared to wCer4. The Wolbachia-infected strains presented here are ideal models to study the coevolution of medfly and Wolbachia in this recently established association.

PP_045

IN VITRO ANTAGONISTIC ACTIVITY OF RHIZOSPHERIC AND ENDOPHYTIC BACTERIA AGAINST SOIL BORNE PHYTOPATHOGENIC FUNGI.

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There are numerous microorganisms in the soil, that are organized in communities, and contain bacteria and fungi that are beneficial for plants.

Microorganisms that colonize the root and rhizosphere in particular, form mutualistic and symbiotic associations with plants and influence them positively as Plant Growth Promoters, mainly by making essential substances as nutrients and hormones readily available, and by contributing to the plant's immune response.

Bacteria can act as biocontrol agents against soil borne fungal phytopathogens in two main ways.

The biocontrol trait could be the result of a direct mechanism of the interaction between the bacteria and the pathogenic fungi or an indirect result that involves triggering the plant's immune response system.

Among the most typical direct actions of biocontrol bacteria are the production of liquid or volatile antifungal compounds, the production of (chitino)lytic enzymes that target the fungal cell walls and the antagonism for mineral resources, like Fe.

In the present study we investigated in vitro the biocontrol activity of rhizosheric and endophytic bacteria isolated from the rhizosphere and roots of crop plants at the island of Amorgos, against the soil borne pathogenic fungi Rhizoctonia Solani, Botrytis Cinerea, Sclerotinia sclerotiorium, Verticillium Dahliae and Fusarium Oxysporum Lycopersici.

Tests were conducted by comparing the growth of each phytopathogenic fungus, in petri dishes on PDA medium, with or without the presence of the bacteria isolates (dual culture method).

Most of the bacteria isolated were assigned to Pseudomonas genus, while one isolate was identified as Bacillus velezensis, a bacillus well known for its biocontrol traits. All the tested phytopathogens were inhibited or delayed in growth by some isolates, however only Bacillus velezensis caused complete inhibition to all the tested phytopathogens.

Our results suggest that the use of bacteria as biocontrol agents against pathogenic fungi is promising and further investigation on mechanisms of action and in planta trials are needed.

PP_047

ENDOPHYTIC STRAIN B. HALOTOLERANS HIL4 IS A PROMISING BIOLOGICAL CONTROL AGENT OF GRAY MOLD IN FRUIT WITH PLANT GROWTH PROMOTING POTENTIAL

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Bacterial strain Hil4 was isolated from the leaf endosphere of the medicinal plant Hypericum hircinum. It exhibited strong antifungal activity against Botrytis cinerea under in vitro conditions. Whole genome sequencing revealed that it belongs to Bacillus halotolerans species. Using genome mining approaches, numerous secondary metabolite biosynthetic gene clusters were located, such as cyclic lipopeptides, as well as genes involved in plant growth promotion, colonization and elicitation of plant defense. Ethyl acetate extracts of agar diffusible metabolites secreted either from singly grown bacterial culture or dual culture of the bacterial strain with B. cinerea on solid growth medium were bioactive against the phytopathogen. Further analysis of the single culture extract with UHPLC-HRMS identified antimicrobial substances and ISR elicitors that are produced constitutively, such as cyclic lipopeptides, siderophore, antibiotics as well as azelaic acid. The isolate also proved efficient in minimizing disease severity of gray mold on detached table grape berries as well as cherry tomatoes. Finally, it positively influenced the biomass and root architecture of Arabidopsis thaliana Col-0 in vitro as well as Solanum lycopersicum var. Chondrokatsari Messinias in vitro after seed biopriming. Overall, B. halotolerans strain Hil4 is a promising biological control agent against gray mold, as well as a promising plant growth promoting agent.

PP_048

GENOME MINING AND METABOLOMIC ANALYSIS OF THE ENDOPHYTE BACILLUS HALOTOLERANS CAL.L.30 PRESENTED SEVERAL SECONDARY METABOLITES THAT CONTRIBUTE TO POSTHARVEST BIOCONTROL OF BOTRYTIS CINEREA

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Bacteria of the genus Bacillus have extensively been studied for their ability to biosynthesize and secrete multiple secondary antimicrobial metabolites, making them excellent candidates to use as biological control agents (BCAs). The endophytic bacterial strain Bacillus halotolerans Cal.l.30 isolated from the pharmaceutical plant Calendula officinalis is able to control mycelial growth and spore germination of several phytopathogenic fungi. We have focused our experiments at the phytopathogenic fungus Botrytis cinerea under in vitro and ex vivo conditions. A preventive formulation of Cal.l.30 on grape berries revealed excellent suppression of gray mold disease caused by the postharvest pathogen B. cinerea, in comparison to control experiments. A strong biological control activity against B. cinerea was also observed after inoculating grape berries with the bacterial cell free supernatant. Genome sequencing and genome mining of Bacillus halotolerans Cal.l.30 revealed 10 biosynthetic gene clusters (BGCs) encoding for secondary metabolites with diverse antimicrobial properties such as fengycin, surfactin, mojavensin, bacilysin, bacillaene, bacillibactin, kalimantacin and subtilosin. Further analysis indicated that strain Cal.l.30 is capable to biosynthesize and secrete agar-diffusible secondary metabolites (ADSM) when singly grown on a solid surface. Finally, UHPLC-HRMS chemical analysis detected different types of secondary metabolites, including the lipopeptides fengycin and surfactin, four isoforms of the polyene diamide polyketide bacillaene, the intermediate metabolite of bacilysin biosynthetic pathway, L-dihydroanticapsin, the siderophore bacillibactin and the dicarboxylic acid azelaic acid.

PP_049

DEVELOPMENT OF CERATITIS CAPITATA VIENNA 8 GENETIC SEXING STRAINS HARBORING WOLBACHIA FOR IIT AND COMBINED IIT-SIT APPLICATIONS

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The Mediterranean fruit fly, Ceratitis capitata (medfly) (Diptera: Tephritidae) is a cosmopolitan agricultural pest of high economic importance.

Decades of research led and coordinated by the Insect Pest Control Laboratory of FAO/IAEA, have made this insect a model species for sterile insect technique (SIT) applications, based on the development of the VIENNA 7 and 8 genetic sexing strains (GSS) that facilitate male-only releases.

Medfly is considered a species that is not naturally infected with Wolbachia.

Taking into account that one important Wolbachia-induced phenotype can be cytoplasmic incompatibility (CI), during the last two decades, the medfly strains 88.6, S.10.3, and 56S2 have been developed by artificial transfer of Wolbachia wCer2 and wCer4 strains using Rhagoletis cerasi as donor.

Indeed, these strains have been proven stable and induce 100% unidirectional and bidirectional CI, showing that development of the incompatible insect technique (IIT) is feasible for medfly.

A major concern for the implementation of IIT is the accidental release of fertile females along with the males, making sustainable application of the method problematic. To minimize such risks, IIT for medfly can a) be combined with the already available VIENNA 8 GSS to eliminate the possibility of releasing females and, b), can be combined with low irradiation doses that lead to 100% female sterility with no severe impact on males' fitness, a methodology recently proposed for mosquitos and known as the 'combined IIT-SIT approach'.

In the present study, wCer2 and wCer4 have been introduced through crosses to the wp tsl strain that is used for the construction of all VIENNA 7 and 8 GSS, thus giving the Cc(wp tsl)[wCer2] and the Cc(wp tsl)[wCer4] strains. By crossing males from Vienna 8 with females from the newly developed strains, two new VIENNA 8 GSS harboring Wolbachia have been developed, named VIENNA 8 [wCer2] and VIENNA 8 [wCer4]. Initial evaluation of these strains showed that they are genetically stable, and they induce 100% unidirectional and bidirectional CI.

Further evaluation of these strains at a larger scale and additional generations is needed, along with experiments to establish the minimal irradiation dose that induces 100% female sterility.

PP_050

MICROBIOLOGICAL QUALITY AND SAFETY OF SALMON SALMO SALAR, SCALLOPS PECTEN MAXIMUS AND SEAWEED ALARIA ESCULENTA CO-CULTURED IN AN INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) SYSTEM

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Although advantages and limitations of Integrated Multi-trophic Aquaculture (IMTA) have been widely reported, few studies have focused on potential effects of IMTA on the microbiological quality and safety of the end products. In this study, the spoilage potential and the presence of pathogenic bacteria in salmon Salmo salar, seaweed Alaria esculenta and scallops Pecten maximus co-cultured in an IMTA system were investigated.

Alaria esculenta, Atlantic salmon and scallops were co-cultivated at a pilot scale IMTA site in Ireland, operated by the Marine Institute and samples of different harvest periods (June 2020 and March 2021) were sent to the Agricultural University of Athens for microbial testing. The received samples were stored at different temperatures for specific time intervals, whilst microbiological analysis was performed on the day of their arrival at the lab and at certain days of storage. The populations of Total Viable Counts (TVC), Pseudomonas spp., lactic acid bacteria, yeast and moulds, Bacillus spp., B. thermosphacta and bacteria of the Enterobacteriaceae family were estimated while, the presence of Vibrio spp., Salmonella and Listeria monocytogenes was also investigated.

Salmons were of acceptable microbial quality (TVC < 7.0 log CFU/g) and safe for human consumption for up to 12 and 8 days stored at 0 and 4 °C, respectively, while the microbial load was not differentiated between the two harvest periods. The respective time period for the seaweed samples was 5 and 7 (or more) days at 5 °C for 2020 and 2021 harvest, respectively, while scallops were fresh for at least 5 days at 4 °C. It should be noted that bacteria of Enterobacteriaceae family which are considered as hygiene indicator, were at low levels - even below enumeration limit - in all species, while all the examined pathogenic bacteria were absent both at the beginning and at the end of storage.

Although further research is intended regarding quality and safety of IMTA products, these preliminary findings provide significant information about the overall microbiological quality of these products.

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PP_051

ENDO-ROOT MICROORGANISMS FROM ALOE VERA CULTIVATIONS IN LACONIA, GREECE WITH GROWTH PROMOTING PROPERTIES.

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Plants synthesize numerous secondary metabolites to adapt to specific environments, to communicate with soil microorganisms and neighbor plants and to cope with biotic and abiotic stresses. Many of these compounds have essential value in human health and constitute unique sources of pharmaceuticals and food additives. Soil microorganisms which form beneficial associations with plants, such as plant growth promoting rhizobacteria (PGPR) or fungi (PGPF) and arbuscular mycorrhiza fungi (AMF) can act as potent elicitors of the biosynthesis of bioactive compounds and enhance the accumulation of high-value secondary metabolites in plants. Aloe vera plants contain numerous bioactive compounds, with antitumor, immunostimulating and anti inflammatory effects. The role of endorhizal microorganisms in biosynthesis of such plant-derived compounds is under-investigated. In the present study, Aloe vera roots were collected from twenty-four different organic farming fields in Lakonia, Greece and endoroot microorganisms (i.e. bacteria, fungi, AMF) were isolated, to investigate their putative role as promoters of plant growth and bioactive compounds biosynthesis. In total, forty-three bacterial and twenty fungal isolates were obtained, based on their macroscopic phenotypic differences in pure culture. Additionally, the collected roots were used in trap cultures in pots for propagation of existing AMF. In total, three putative AMF isolates were collected, based on their spores phenotypic differences, as examined under the stereoscope. The isolated bacteria and fungi were subjected to various in vitro tests for plant growth promoting traits and the results will be presented at the conference.

PP_053

THE BIOCONTROL POTENTIAL OF TWO BACILLI RHIZOBACTERIA THROUGH THE PRODUCTION OF SURFACTINS AND FENGYCINS

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A crucial part in the colonization of root systems by rhizobacteria, is their ability to antagonize other harmful microorganisms. During this important process, soilborne pathogens are directly oppressed through the production of metabolites with antimicrobial action by the rhizobacteria. Bacteria of the genus Bacillus produce lipopeptides belonging to the families of surfactins, iturins and fengycins. These compounds play an important role during the process of root colonization and the plant's defense responses (Ongena & Jacques, 2008). In this study, the Plant Growth Promoting Rhizobacteria (PGRP) B. subtilis MBI600 and B. amyloliquefaciens subsp. plantarum D747 were studied for their ability to produce surfactins and fengycins. Moreover, the potential for enhanced production of these lipopeptides was investigated by inoculating Botrytis cinerea in a B. subtilis MBI600 liquid culture. After bacterial growth in specific media for 72 hours, the samples were acidified and a second set of them was also extracted using an ethyl acetate/methanol mixture (4:1) in order to obtain the lipopeptides. B. subtilis MBI600 was also cultivated in the presence of B. cinerea for 72 hours in Potato Dextrose Broth (PDB), and the lipopeptides were isolated following the aforementioned extraction procedure. The identification of the lipopeptides was carried out using a LC-QTOF HRMS system. The results revealed that both rhizobacteria were able to produce a significant number of surfactins. Additionally, when B. subtilis MBI600 was cultured in the presence of the plant pathogenic fungus, the number of surfactins increased, showing a very promising biocontrol aspect. In order to reinforce the aforementioned results, gene expression analysis of srfB and feng was performed. Results demonstrated an increase of transcription levels in time and supported the elevated amounts found in the presence of the pathogen. These findings enhance the ability of B. subtilis MBI600 to act as a biopesticide mainly because the production of these lipopeptides is directly associated with antagonism and inhibition of growth of plant pathogens. Additionally, recent studies underline the correlation of lipopeptides with successful root colonization and reinforcement of host resistance potential.

PP_054

EFFECT OF LABORATORY ADAPTATION PROCESS ON THE BACTERIAL COMMUNITY ASSOCIATED WITH THE MEDITERRANEAN FRUIT FLY, CERATITIS CAPITATA

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The Mediterranean fruit fly, Ceratitis capitata (Diptera: Tephritidae) is one of the most damaging pests of fruits and vegetables worldwide and a model for the development of the Sterile Insect Technique (SIT). Laboratory adaptation process used in SIT programs can influence the dynamics of the associated symbionts which negatively impact the fly's quality and performance. In this study, we investigated changes in the bacterial community's composition and diversity due to the laboratory adaptation process, using 16S rRNA amplicon sequencing. Analyzed DNA samples were isolated from the gastrointestinal tract of individual flies originating from a domesticated wild population and a Vienna-8 GSS population, which were both reared under laboratory conditions for 14 generations (F0-F13). Overall, the bacterial community seems to be affected significantly by the origin of the initial population. Klebsiella was the dominant genus in the wild population, followed by Pantoea, and Citrobacter, while the Vienna-8 GSS were mainly dominated by members of Providencia, Pluralibacter, and Klebsiella. The bacterial profile of both populations changed significantly during the adaptation process, where samples from the first generations formed different profiles compared to those from the later generations. In the wild population, Klebsiella was dominant across most of the generations. Acetobacter, Serratia and Pluralibacter exhibited high relative abundance in the first two generations and decreased significantly afterwards. On the contrary, Providencia and Morganella were absent from the first generation with their relative abundance increasing in subsequent generations. In the Vienna-8 GSS population, Providencia exhibited high relative abundance in the first three generations and decreased significantly later. Pluralibacter was detected in all generations with a peak in the seventh and nineth generation, while Klebsiella was relatively stable. Furthermore, for both populations, bacterial communities displayed significant differences between different developmental stages (larva, 1-day, 5-day, and 15-day adults), while they remained mostly unaffected between male and female flies. In addition, the operational taxonomic unit co-occurrence and mutual exclusion networks of the wild population showed that most of the interactions were classified as co-presence, while in the Vienna-8 GSS population, the number of mutual exclusions and co-presence interactions were equally distributed.

PP_055

MINING THE POTENTIAL OF ENDOPHYTES AS THE NEXT GENERATION PLANT PROTECTION PRODUCTS: ISOLATION AND BIOACTIVITY ASSESSMENT OF THE OLIVE TREE ENDOPHYTE BACILLUS SP. PTA13 LIPOPEPTIDES

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The exploitation of alternative sources of bioactivity for applications in plant protection is demanding in order to provide solutions to the issues that the agrochemical sector is facing. Recently, endophytic microorganisms (EM) have become the focus of the research on natural products' discovery, mainly due to the fact that they synthesize a vast array of compounds, with unique physicochemical properties, structures, and bioactivities. Within this context, applying a bottom-up approach, an olive tree endophytic Bacillus sp. PTA13 was isolated and assessed as a potential plant protection agent that could be exploited in customized plant protection strategies of olive tree. The isolate exhibited bioactivity against the pathogenic fungus Colletotrichum acutatum that causes the devastating disease olive anthracnose. Next, its bioactive metabolites, belonging to the chemical group of lipopeptides (LP), were extracted and fractionated applying liquid-liquid extraction, size exclusion chromatography, and centrifugal partition chromatography. Several major LP of the surfactin, bacillomycin and fengysin families and minor LP of the gageotetrin and bacilotetrin families, were isolated, identified, and their antimicrobial properties were demonstrated. Additionally, the toxicity of the initially obtained LP extract to Colletotrichum acutatum was studied applying GC/EI/MS and 1H NMR metabolomics. Based on the analyses, metabolites-biomarkers of the applied LP extract's toxicity were discovered. Among those, various fatty acids, L-proline, and phenylacetate were the metabolites with the highest leverage on the observed toxicity. Phenylacetate is a well-known metabolite associated to fungal pathogenicity, an observation indicative of the antipathogenic activity of the applied LP. The obtained results confirm the potential of olive tree EM as alternative plant protection agents to the currently applied biological control agents and conventional plant protection products. Furthermore, as a proof-of-concept, the proposed pipeline is highly foreseen to become a primary plant protection strategy, integrating well with the concept of sustainability and the European Green Deal roadmap.

Keywords: endophytic microorganisms, fengycins, natural products, olive anthracnose, plant protection, surfactins

PP_056

INSIGHTS ON THE SUBSTRATE SPECIFICITY OF AA9 AND AA16 LPMOS FROM THERMOTHELOMYCES THERMOPHILA

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The discovery of lytic polysaccharide monooxygenases has profoundly changed the way in which we view the enzymatic conversion of polysaccharides, in particular recalcitrant materials such as cellulose. These enigmatic "boosting" proteins catalyze oxidative cleavage of glycosidic bonds, which suggests that LPMOs are central players in biomass conversion [1]. LPMOs are currently grouped into six CAZy auxiliary activities (AA) families (AA9–11, AA13–15), based on a bioinformatics analysis of their amino acid sequences similarities, while it is continuously expanding with the novel AA16 and AA17 families [2, 3]. The fungal AA9 family includes enzymes active not only on insoluble cellulose and soluble cello-oligosaccharides, but also on polysaccharides containing β-1,4-linked glucose units such as xyloglucans, glucomannans and β-glucans [4]. There is no doubt that the LPMOs, which are remarkably abundant in nature, still hold many unanswered questions. One of the most exciting of these relates to the possible existence of other functionalities, i.e., functionalities that have not previously discussed. LPMOs seem well suited to act on a wide variety of interfaces and it is likely only a matter of time before novel LPMO substrates (other polysaccharides, various recalcitrant protein fibers, lignin, or perhaps plastics) will be discovered. Here we present two novel LPMOs from Thermothelomyces thermophila, a cellulose-active AA9 C1 LPMO which also shows a non-common activity on beechwood and birchwood xylan and an AA16 LPMO exhibiting a strong xylanolytic activity, which is demonstrated for the first time for this recently discovered family. AA16 can oxidatively cleave recalcitrant substrate of insoluble wheat arabinoxylan as also beechwood xylan and birchwood xylan, while it is unable of recognizing cellulose based substrates. Mechanistic studies of these two novel LPMO catalytical systems can give new insights into the main molecular determinants driving LPMO activity toward cellulose and hemicellulose-containing substrates.

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PP_057

INTEGRATION OF BIOCATALYSIS AND CHEMICAL PROCESSES FOR THE VALORIZATION OF LIGNOCELLULOSIC BIOMASS TOWARDS THE PRODUCTION OF 5-HYDROXYMETHYLFURFURAL

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Lignocellulosic biomass can be utilized as a raw material for numerous processes within the biorefinery to substitute the continually depleting fossil fuel reserves toward the production of value-added chemicals.

One such route includes the conversion of sugars derived from biomass to furanics such as 5-hydroxymethylfurfural (HMF) and furfural (FA), that serve as monomers for the production of biobased polymers. These furan compounds may be produced from hexose and pentose streams respectively, by the acid catalyzed dehydration of sugars. In particular, HMF can be synthesized from glucose and fructose with the aid of an acidic catalyst.

Glucose is a more attractive feedstock due to its higher abundance and lower cost compared to fructose, however, harsher reaction conditions with higher environmental impact are required.. Hence, establishing an environmentally friendly process necessitates the introduction of an isomerization step in which glucose is transformed to fructose.

In this work, we have developed a chemo-enzymatic route for the conversion of beechwood biomass to HMF, starting with a mild oxidative organosolv pretreatment to remove lignin, leaving behind a sugar-rich solid fraction for the production of furan derivatives.

By integrating a high-gravity enzymatic saccharification at 20% w/w solids loading and an efficient enzymatic isomerization, a concentrated fructose syrup is obtained (104.5 g/L fructose and 2.3 g/L xylulose) which is subsequently used for HMF production.

The results show that over 50% of the initial glucose content is transformed to fructose, reaching 64 g fructose/ 100 g cellulose. For the subsequent step, different homogeneous catalysts are evaluated as potential candidates for the efficient dehydration of sugars to furans, targeting the maximum glucose conversion to HMF. Among them, formic acid proved the best catalyst, reaching an HMF yield of 44.6% with 55.8% selectivity.

The HMF produced can function as a substrate for 2,5-furandicarboxylic acid (FDCA) synthesis or its oxidative intermediates, therefore suggesting an alternative eco-friendly pathway towards polymer synthesis.

PP_058

VALORIZATION OF SUPPLEMENTED COMPOST THROUGH COLONIZATION OF AGARICUS BISPORUS AND A. SUBRUFESCENS MUSHROOMS

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Agaricus mushrooms are well known for their edibility and their nutritional and medicinal value. Agaricus bisporus is considered as one of the most commercialized mushrooms, on the contrary A. subrufescens is an exotic mushroom, difficult to be cultivated, so it is produced in small quantities in Europe and not at all in Greece. A. bisporus AMRL 209 and A. subrufescens AMRL 235 were tested for their ability to grow in pasteurized compost (wheat straw, manure, gypsum) (control substrate) and when sources of nitrogen, calcium salts and oils (sunflower oil; commercial, corn oil; commercial, peptone, yeast extract in 2%, w/w and CaSO4·2H2O; agricultural gypsum and CaCl2 in 3%, w/w) were added, in order to assess substrate colonization efficacy and time. Evaluation included measurement of the growth rate (Kr; mm/day) and the mycelial biomass concentration (estimated via the glucosamine content; mg/g, d.w.) at full colonization in glass tubes (200x28 mm). Results showed that A. bisporus presented higher Kr compared to A. subrufescens in all applications. Also, all applications led to further increase of growth rate compared to control substrate (4.26 mm/day; A. bisporus, 2.39 mm/day; A. subrufescens), except for the one with CaSO4·2H2O (4.17 and 2.37 mm/day, respectively). The highest Kr was achieved in substrate with addition of corn oil and CaCl2 for A. bisporus (4.66 mm/day) and in substate with sunflower oil for A. subrufescens (3.39 mm/day). Similarly, A. bisporus presented higher biomass concentrations compared to A. subrufescens for most of the tested applications. The highest biomass concentrations were achieved in the substrate supplemented with sunflower oil for both species (820.06 mg/g; A. bisporus, 581.69 mg/g; A. subrufescens). However, only oils' addition had positive effect on biomass concentration production. Consequently, it seems that corn and sunflower oil affected mycelium ability to colonize substrate in terms of both time and concentration. Overall, the potential for the commercial production of Agaricus spp. mushrooms on supplemented compost (substrate) seems particularly promising in financial and environmental terms.

PP_059

BACTERIAL BIOSYNTHESIS OF LARGE COMBINATORIAL LIBRARIES OF CYCLIC OLIGOPEPTIDES AND DIRECT FUNCTIONAL SCREENING FOR DISCOVERING INHIBITORS OF PATHOGENIC PROTEIN AGGREGATION

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Protein misfolding and aggregation are defining features of a wide range of human conditions, such as Alzheimer's disease, Parkinson's disease and cystic fibrosis, which have been collectively termed protein misfolding diseases (PMDs). The vast majority of these remain to date incurable and impose a very high socio-economic burden on humanity. To address this unmet medical need, we have developed a novel integrated bacterial platform for the discovery of potential therapeutics against PMDs. In this system, Escherichia coli cells are genetically engineered in order to perform two simultaneous tasks: (i) produce combinatorial libraries of more than 200 million drug-like, head-to-tail cyclic oligopeptides using protein-splicing technology and (ii) enable the identification of the bioactive cyclic peptides that correct the problematic folding and/or inhibit the aggregation of disease-associated misfolding-prone proteins (MisPs) using a genetic assay that links the folding of the target MisP with a fluorescent phenotype. In this way, the bioactive cyclic peptide hits can be identified in an ultrahigh-throughput manner using flow cytometric cell sorting, thus significantly decreasing the overall cost, time and complexity of early drug discovery for PMDs. Herein we present the implementation of this strategy against a model PMD, Alzheimer's disease, which is associated with the aggregation of the amyloid-β peptide (Aβ42). This procedure resulted in the discovery of more than 400 putative aggregation inhibitors, two of which were further tested in vitro and in vivo and found to potently inhibit the aggregation of Aβ42 at sub-stoichiometric ratios. Finally, through a combination of deep sequencing and site-directed mutagenesis we demonstrate how this system can accelerate the determination of structure-activity relationships and define consensus motifs required for high bioactivity in the discovered molecules.

PP_060

GENOME EDITING TOOLKIT OF ENDOPHYTIC FUSARIUM SOLANI STRAIN K USING CRISPR/CAS9

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Endophytic filamentous fungi play an important role in agriculture by colonizing plants and eliciting pathogenic or beneficial responses to their host. Fusarium solani strain K (FsK) is a beneficial endophyte that is able to colonize a diverse range of plant species and provide defense against drought and salt stress in tomato (Solanum lycopersicum) (Kavroulakis et al., 2007). Very little is known on the fungal genes that are involved in the establishment of beneficial associations with plants and in the manipulation of the host responses. In addition, tools to silence or knock out fungal genes of interest for functional analysis in a high-throughput manner do not exist. Here, we develop a genome editing toolkit for the non-model endophyte FsK using CRISPR/Cas9 technology. To achieve this, we utilize Synthetic Biology principles and the FungalBraid modular cloning platform to generate the desired vectors for transformation of the fungus (Hernanz-Koers et al., 2018). We create a toolkit containing several endogenous and heterologous promoters, terminators, reporters and Cas9 and dCas9 genes with different codon-optimization, to maximize flexibility and Cas9 expression. For a proof-of-concept experiment, we aim to disrupt GFP, from a transgenic FsK strain that has the reporter integrated into its genome. With this toolkit, we aim to streamline the creation of gene knockout or silencing FsK lines to help answer long-standing questions, and possibly apply it to different non-model filamentous fungi, of which the genome is known.

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PP_061

VALORIZATION OF LIPID FERMENTATION WASTEWATER FOR MACERATION WATER FOR THE CULTIVATION OF GANODERMA MUSHROOMS

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Fungi are known for their capability to degrade lignocellulosic materials during solid-state fermentations for the production of valuable biomass.

In this study, six different types of residues (rice husk-wheat straw; RW, coffee residue-wheat straw; CW, olive crop-wheat straw; OW, rice husk- beech wood shavings; RB, coffee residue- beech wood shavings; CB, olive crop- beech wood shavings; OB) were wetted with lipid fermentation wastewater (FW) from Rhodosporidium toruloides fungi culture and evaluated regarding the growth of Ganoderma applanatum, G. resinaceum and G. lucidum mushrooms. Initially, physicochemical analyzes were performed on the raw materials and substrate C:N ratio was defined to 20-30. After substrate preparation in large glass-tubes, sterilization and inoculation with grain-spawn of the mushroom strains, incubation took place at 26 ±0.5 °C.

Measurements of mycelium linear growth rates (Kr, mm / d) and the production of total biomass (glucosamine content) were recorded. Results showed that the addition of FW had not significant effect on the growth rate. G. resinaceum substrates showed the maximum Kr values (mean Kr=6.29 mm/d) at RW and RB compared to the other substrates (CW, CB; Kr=4.68 mm/d; OW, OB; Kr=4.92 mm/d). On the contrary, G. applanatum presented the highest values on OW and OB (6.32 mm/d) and the lowest on CW and CB (3.37 mm/d). The maximum Kr for G. lucidum was detected on OW (5.08 mm/d), value much higher than these of the other substrates (CW, CB Kr=3.17 mm/d; RW, RB Kr=2.88 mm/d). Determination of the biomass concentration indicated that addition of FW had positive effect and that in substrates containing wheat straw it was higher than substrates with beech wood shavings.

In particular, for G. resinaceum the addition of FW has contributed to a two-fold biomass increase (443.46 mg/g d.w.) and for G. lucidum a three-fold one (636.376 mg/g d.w.) at RW compared to others substrates. On the other hand, G. applanatum, produced the maximum biomass (160.99 mg/g d.w.) at olive crop-based substrate (OW and OB). In conclusion, various alternative substrates were successfully used in Ganoderma cultivation, while lipid fermentation wastewater was employed as maceration water with promising results in a zero-waste released approach.

PP_062

COMPARATIVE STUDY OF AGARICUS AND PLEUROTUS STRAINS FOR THEIR EFFICIENCY TO PRODUCE IMPORTANT BIOCHEMICAL COMPOUNDS

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The production of various biochemical compounds, such as proteins, glucans and glucanases from the mycelium of four Basidiomycetes species during their liquid batch culture in shaking flasks, was studied. The strains used in this research, Agaricus bisporus, A. subrufescens, Pleurotus eryngii and P. ostreatus, were grown for 26 days in defined media based on glucose under controlled conditions (temperature and stirring speed).

The referred strains were evaluated for their ability to consume glucose and produce mycelial mass and endopolysaccharides (IPS). Results showed that on the 26th day of cultivation, P. ostreatus had consumed most of the glucose (99%) and produced the maximum biomass among the studied strains (16.75 g/l P. ostreatus, 12.67 g/l A. bisporus, 11.58 g/l P. eryngii and 5.73 g/l A. subrufescens). Although P. eryngii consumed low amounts of glucose and produced reduced amounts of biomass, its IPS concentration was the maximum (3.81 g/l) in comparison to the rest of the strains. Other biochemical ingredients such as proteins, glucans and glucanases were also evaluated. All used strains showed a similar pattern in total protein production, with A. bisporus having the highest percentage of total proteins on the 21st day of cultivation (36% w/w), followed by P. ostreatus (34.4% w/w), P. eryngii (22.14% w/w) and A. subrufescens with the smallest percentage (10.67% w/w).

All studied strains exhibited an increase in glucans production during cultivation, an important bioactive ingredient for pharmaceutical applications, with A. bisporus reaching the highest value (8.05 w/w) on the 21st day of the culture period, followed by P. ostreatus on the 26th day (6.73 w/w|). It was also noticed that during the last stages of culture, when the carbon source in the nutrient medium had been totally consumed resulting in increased mycelium production as in the case of P. ostreatus and A. bisporus, the enzymatic glucanase activity was significantly increased, indicative of hydrolysis of produced glucans as an alternative glucose source for fungal growth. It seems therefore that various mushroom strains are good biomass and metabolic compounds producers during liquid-state cultivations, even if no carposomes are produced.

PP_063

TARGETED ENZYMATIC HYDROLYSIS: TOWARDS A MORE EFFICIENT UTILIZATION OF CORN FEEDSTOCK AS ANIMAL FEED

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Corn is among the most widely grown crops worldwide and its associated agroindustrial byproducts are, therefore, of high abundance. Corn bran ——being one of those—— is made from the outer layer of corn kernels and, apart from cellulose, it is mainly composed of highly branched hemicellulose that is additionally complexed with lignin.

Although corn bran is already being utilized in animal feed, the recalcitrant nature of its hemicellulosic fraction renders it an inefficient carbon source, since animals are incapable of harvesting its full potential.

On the contrary, microorganisms of both bacterial and fungal origin have been shown to code for an arsenal of lignocellulolytic enzymatic machineries that could be exploited for biotechnological purposes.

The focus of this study is the investigation of the synergistic effect of major hemicellulase activities upon addition of specific accessory enzymes. To this end, selected xylan-debranching enzymes are recombinantly expressed in suitable hosts and their potential towards the in vitro hydrolysis of highly branched corn arabinoglucuronoxylan is being assessed. Ultimately, an optimized enzyme mixture will be designed that will not only increase the yield of the released sugars from corn bran, but also reduce the overall process cost. Moreover, hydrolysis with enzymes presenting selected specificities will enable the release of prebiotic xylo-oligosaccharides and antioxidant components from corn feedstock.

PP_064

NOVEL DEEP-SEA SAMPLING AND EXPERIMENTATION SYSTEM FOR HYDROCARBON BIOREMEDIATION MONITORING UNDER IN SITU CONDITIONS

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During the Deepwater Horizon accident in the Gulf of Mexico in 2010, excessive subsea dispersant application and high pressures led to the confinement of hydrocarbons, creating an oil plume at depths of 1000–1200 m. The rapid and vigorous response of the indigenous microbial populations removed a major fraction of hydrocarbons in the plume. So far, the collection of deep-sea microbial communities involved depressurization of the sample during retrieval and in most cases, subsequent incubation experiments were carried out at atmospheric pressure. A novel sampling apparatus, was designed for the collection of indigenous microbial populations from the deep Eastern Mediterranean Sea, maintaining in situ pressure throughout the entire process of retrieval and experimentation to determine microbial oil degradation. The High Pressure (HP) Sampler collected seawater between 600 to 1000 m depth. A known volume of the collected sample was then transferred via a piston pump, without pressure disruption, into a HP-Reactor, at 10 MPa pressure and was incubated with crude oil at plume concentration for 77 days at in situ temperature (14oC). For the first part, Iranian light crude oil bioremediation was monitored for 35 days, and then the effect of dispersant addition (1:25 v/v COREXIT 9500) was observed until day 77. The results showed a high capacity of the indigenous microbial community for alkane degradation regardless of dispersant application; however, the removal of aromatic compounds was highly dependent on oil dispersion.

PP_065

CHARACTERIZATION OF A NOVEL B-1,3-1,4-GLUCANASE FROM THE THERMOPHILIC BACTERIUM SPIROCHAETA THERMOPHILA DSM6578

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A metagenome analysis of the microbial community of Thermopotamos, a thermal site enriched in plant biomass in Aidipsos Greece, revealed the free-living, thermophilic and anaerobic genus Spirochaeta as a key degrader of plant biomass residues. Indeed, the CAZyome profile of the genus contains several enzymes capable of the complete hydrolysis of cellulose and hemicellulose. Among them, we identified a putative hydrolase, StLich12_64B, comprising a catalytic domain, which belongs to the glycoside hydrolase family 12 (GH 12), and a carbohydrate-binding module of the family 64 (CBM 64). The enzyme is membrane-associated and it does not share any sequence similarity with any characterized enzyme. In order to characterize StLich12_64B biochemically, the corresponding gene was amplified from the genome of S.thermophila DSM 6578 and was overexpressed in E. coli cells. Size exclusion chromatography indicated that the enzyme is homotetramer. Regarding its activity against different substrates, the enzyme appeared to be β-1,3-1,4-glucanase (EC 3.2.1.73) acting on barley and lichenan but it also presented a minor endo-β-1,4-glucanase activity (EC 3.2.1.4) against sodium carboxymethyl cellulose (CMC) and filter paper. The optimal activity of the enzyme was observed at 80 °C and pH 6, while it retained 100% of its activity upon 12 h incubation at temperatures up to 75 °C. In conclusion, the biochemical characteristics of StLich12_64B renders it as a promising biocatalyst appropriate for several high-temperature biotechnological applications, such as in the areas of brewing industry, animal feed manufacture and biofuel production.

Key words: β-1,3-1,4-glucanase, thermophiles, Spirochaeta thermophila, GH 12, CBM 64, plant biomass degradation

PP_067

DYNAMIC ANALYSIS OF BLOOD MICROBIOME IN PATIENTS WITH SCHIZOPHRENIA

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Schizophrenia (SZ) is a serious mental illness that affects approximately 0.5 to 1% of the world's population. Despite the previous research efforts the disease pathogenic mechanisms remain elusive whereas identification of novel biomarkers and drug targets are indispensable towards more personalized disease prognosis, monitoring, prevention and treatment. Recent research has revealed a crucial role of the gut microbiome in psychiatric disorders, including SZ, mainly mediated through the complex interactions of the gut-brain axis. Although most microbiome studies on SZ have focused on the gut microbiome, new evidence supports that under dysbiosis microbes can overpass the gut barrier and translocate to the blood circulation of SZ patients, thus providing an easily accessible source for analysis of microbiota-host interactions. In the present work we aim to explore the blood microbiome of SZ individuals and examine the impact of conventional anti-psychotic drugs to microbial composition. The blood of 20 SZ patients from the First Psychiatric University Clinic of Eginition Hospital was collected before and after one month-treatment with antipsychotic medication and microbiome composition was analyzed by 16S ribosomal RNA gene amplicon sequencing. All downstream bioinformatic analyses were performed using the QIIME 2 platform. Raw data were first subjected to quality control and denoising to filter out low quality sequences and reads corresponding to contaminant species. Then, data were clustered to operational taxonomic units and taxonomy classification was assigned. Alpha-diversity indices indicating microbiota taxa richness and evenness within each sample were calculated. Beta-diversity demonstrating microbiota structure dissimilarities among groups were also computed. Statistical analyses were then performed to compare the obtained metrics between patients groups -before and after treatment- and negative controls (no template water). The results demonstrated that blood microbiome of SZ patients contained a wide range of bacterial taxa mostly from Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes phyla. Diversity data from patients were significantly different from negative controls strongly supporting microbiome specificity. Preliminary analysis did not identify any statistical significant effect of treatment on the microbial structure of SZ patients. Further analysis of pairwise comparisons as well as downstream functional exploration are in progress.

PP_069

BIOCATALYTIC ENHANCEMENT OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF GREEN MARINE ALGAE EXTRACTS

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Biomass derived from marine macro- algae considered to be a rich source of valuable nutrients and bioactive phytochemicals with applications in food, pharmaceutical and cosmeceutical industries. In particular, species of the genus Ulva have been proved to metabolize biomolecules such as polysaccharides, lipids, proteins, phenols and terpenoids, among others. Enzymatic biocatalysis is widely applied for the targeted modification of natural products with a view to enhance their biological activities. In the present work, aqua and organic extracts of green marine algae were prepared and studied for their properties. Various enzymes such as oxidoreductases and hydrolases were used for the enzymatic modification of the bioactive compounds of the extracts. The enzyme inhibitory activity related to dermal aging of extracts before and after their enzymatic treatment were evaluated, as well as, their anti-oxidant and anti-microbial activity. All the extracts exhibited notable biological activities, which in many cases were improved after their enzymatic modification.

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PP_070

BIOTECHNOLOGICAL VALORIZATION OF SECOND CHEESE WHEY BY OLEAGINOUS YEASTS FOR PRODUCTION OF CELL BIOMASS AND METABOLITES

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The rapid increase in waste generation, worldwide, is forcing the scientific community to find sustainable solutions reducing the volume of the produced residues and utilizing these materials under eco-friendly processes. The reduction of wastes in combination with their valorization for the production of new products of added-value or biogas benefits both the environment and the economy. Dairy and cheese-making industry have always been pillars of the Greek economy. Cheese-making practice, however, leads to the production of cheese whey, a by-product with a very high organic load. Various ways of reducing the pollutant load, such as the aerobic treatment, the anaerobic digestion, the usage as feed, the filtration to separate whey protein concentrates and lactose fractions, and the cultivation of useful microorganisms into the lactose fraction, in order to produce added-value metabolites have been reported in the literature.

The cultivation of oleaginous yeasts on agro-industrial residues and the production of microbial lipids, which can be used as starting materials for the synthesis of "2nd generation" biodiesel and various types of oleochemicals, is considered as one of the most important approaches of Lipid Biotechnology, in terms of circular economy and sustainable development.

Here, 8 yeast strains were evaluated for their growth on lactose used as carbon source, and five of these strains grown on this substrate were inoculated into the complex substrate, second cheese whey (SCW). All of these strains were grown on SCW, producing mainly yeast biomass, microbial lipids but also interesting metabolites such as polysaccharides. On SCW, Cryptococcus curvatus ATCC 20509 produced the highest total dry weight (TDW) (=22.7g/L) that contained 3.4 g/L of cellular lipids, followed by Cryptococcus curvatus NRRL Y-1511, (TDW=16.9 g/L, lipids 2.3 g/L). A new, not extensively studied strain namely Papiliotrema laurentii NRRL Y-2536, produced significant quantities of TDW (20.4 g/L) and also, interestingly, secreted quantities of exopolysaccharides. The highest TDW and lipid productivity, 0.23 g/L/h and 0.04 g/L/h respectively, were recorded for the strain Cryptococcus curvatus ATCC 20509. Cellular lipids of all microorganisms contained in variable quantities the fatty acids Δ9C18:1, C16:0, C18:0 and Δ9,12C18:2, constituting perfect candidates for the synthesis of 2nd generation biodiesel.

PP_071

SUPTOXR AND SUPTOXR2.0: SPECIALIZED ESCHERICHIA COLI STRAINS FOR RECOMBINANT MEMBRANE PROTEIN PRODUCTION AT HIGH YIELDS

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Membrane proteins (MPs) are basic components of cell membranes where they perform highly important functions such as maintenance of structural integrity, signal transduction, transportation of ions and molecules through the lipid bilayer etc.The crucial role of MPs is highlighted by the fact that in both prokaryotes and eukaryotes they are encoded by 20-30% of all genes and due to their key location and their multiple functions, they constitute more than half of all known targets for drug development. As a result, there is a huge need for access to large amounts of MPs in order to expedite the discovery of new pharmaceuticals that target such proteins, through the detailed characterization of their structure and function. The required quantities of MPs are typically produced recombinantly in heterologous hosts such as Escherichia coli (E. coli), because of their low natural abundance and to facitliate their genetic modification in order to create more stable and easily detectable proteins. In case of heterologous expression though, recombinant MP production in bacteria is accompanied with severe cytotoxicity for the host, making their use particularly difficult. Towards this direction, in previous work we managed to face this need and we engineered a genetically modified E. coi strain, SuptoxR, which overexpresses the gene, encoding for RraA, an inhibitor of the mRNA-degrading activity of E.coli RNase E. This strain has been proven particularly effective in suppressing the toxicity, which is often generated during the process of MP overexpression, while at the same time, it markedly increased the cellular accumulation of recombinant MPs of prokaryotic and eukaryotic origin. Herein, we evaluate a set of homologous RraA proteins from bacteria and plants for their ability to suppress MP-induced toxicity and enhance the productivity of recombinant MPs. This process led to the identification of several homologous suppressors, and enhancers of MP production. Intriguingly, some of them were found capable of enhancing bacterial MP production more effectively than the E.coli RraA of SuptoxR. Based on these results, we have developed second-generation SuptoxR strains, termed SuptoxR2.0, which can achieve even further enhanced levels of MP production in E.coli.

PP_072

COMPARATIVE GENOMIC ANALYSIS OF IPRODIONE-DEGRADING BACTERIA REVEALS GENETIC ELEMENTS AND INTERACTIONS INVOLVED IN THE DEGRADATION OF IPRODIONE

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Iprodione is a widely used fungicide with negative impact on off-target organisms. Previous studies revealed accelerated biodegradation of iprodione by Paenarthrobacter strains, showing a particular specialization in its degradation. The suggested pathway involves an amidase (IpaH), a deacetylase (DdaH) and another hydrolase (DuaH) transforming iprodione to 3,5-dichloroaniline. Here, we assembled and analyzed the genomes of three iprodione-degrading cultures, derived from our previous studies. Then, we employed comparative genomic analysis to gain mechanistic and ecological insights on the mechanism of biodegradation of iprodione by three iprodione-degrading bacterial cultures. Illumina and Nanopore sequencing followed by assembly and post assembly ANI/AAI based classification, resulted in two assembly cases: 1) one culture being pure with a single circular chromosome-sized contig of verified Paenarthrobacter pedigree; 2) the other two cultures were nearly identical and contained 2 chromosome-size contiguous DNA pieces each, which were classified as Paenarthrobacter and Microbacterium. All cultures carried three plasmid-sized contigs each. Assembly number 1 had all genes of interest (ipaH, ddaH, duaH) residing on the chromosome-sized (4,697,865bp) circular contig, yet, their sequences had low similarity with their known homologues. One interesting finding about assembly 2 was the equal participation of the Microbacterium chromosome-like contig-set with the Paenarthrobacter circular chromosome-sized contig as revealed by the contig coverage analysis. Suspect degradation genes were spread in the plasmids and the chromosomes of the components of assembly 2: two plasmids carried nearly identical amino acid sequences with the previously experimentally characterized IpaH and DdaH; the Paenarthrobacter chromosome-like contig carried a high identity DuaH, one low similarity IpaH and two low similarity DdaH putative homologs; the Microbacterium chromosome-like contig-set contained one low similarity IpaH coding gene. Such evidence along with literature reports about symbiotic relations might suggest an iprodione biodegradation enhancement due to the presence of both microorganisms in a syntrophic interaction. Pangenome analysis of the Paenarthrobacter chromosomes analyzed in this study showed a very narrow core genome and a very large pan-genome. Follow up studies will disentangle the role of the different genetic elements reported on the degradation of iprodione via transcriptomic analysis, isolation and heterologous expression.

PP_073

DISCOVERY OF NEXT-GENERATION LEAD ANTIBIOTICS TO COMBAT ANTIMICROBIAL RESISTANCE BY COMBINING A BIOSYNTHETIC BACTERIAL PLATFORM AND HIGH-THROUGHPUT SCREENING METHODOLOGIES

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The discovery of the first antibiotic back in 1928 revolutionized health-care. Today, the declining rate in which new effective antimicrobials are being developed in conjunction with the excessive use of antibiotics have led to the pressing problem of antibiotic resistance (AR). AR is the ability of microorganisms to resist compounds to which they were initially sensitive to and WHO has declared it as one of the biggest threats of global health. If not addressed, AR is expected to kill more than 30 million people until 2050. In this work, we aim to discover cyclic-oligopeptides with potential antimicrobial activity based on the fact that cyclic-peptides are well-known antibiotic candidates due to their unique biochemical properties. Towards this goal, we exploit the biosynthetic ability of our previously developed bacterial platform which allows us to produce billions of different cyclic oligopeptides, giving us access to a huge combinatorial protein space. To be able to interrogate more than 108 different oligopeptide sequences, we apply two different high-throughput screening methodologies in order to identify potential antimicrobials against Escherichia coli and Staphylococcus aureus resistant strains. The first high-throughput methodology is a bacterial self-screening platform in which engineered E. coli cells inducibly produce the oligopeptides. After deep-sequencing of the peptide-encoding plasmids carried by induced and uninduced cell populations, we analyze and compare the readouts of oligopeptide coding sequences. Sequences that are present in the uninduced sample but have depopulated the induced sample are likely to encode for peptides that kill the host and have potential antimicrobial properties. The second high-throughput methodology we apply is a FACS-Based Functional Screening via Microfluidic co-encapsulation. In each of the billions droplets produced, we encapsulate E.coli cells producing a single peptide sequence along with the target organism Staphylococcus aureus. If the peptide produced kills the target, cell death is detected by staining and Fluorescent Activated Droplet Sorting is performed to collect the droplets that contain the encoding sequences of the potential antimicrobials. By exploiting our biosynthetic bacterial platform and applying the aforementioned high-throughput screening methodologies to interrogate millions of unique cyclic- peptides, we aim to accelerate the discovery of new lead antimicrobials.

PP_074

STRUCTURAL AND BIOCHEMICAL INSIGHTS INTO A CE16 ACETYL ESTERASE FROM THERMOTHELOMYCES THERMOPHILUS.

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Biotechnological utilization of hemicellulose, the second most abundant component of lignocellulosic biomass, is a growing field towards circular bioeconomy. However, enzymatic biodegradation of hemicellulose is hindered by its structural diversity. One of the most common decorations are acetyl substitutions of the xylan backbone. Thus, the importance of acetyl xylan esterases (AXEs) on hemicellulose decomposition is well established. The discovery of CE16 acetyl esterases introduced a novel specificity that targets 3-O-acetylated xylopyranose residues substituted at O-2 position by 4-O-methyl-glucopyranosyluronic acid, which is one of the most recalcitrant units of the polymeric xylan. However, the mode of action of CE16 esterases is not yet fully elucidated.

In this work, a 45 kDa CE16 from Thermothelomyces thermophilus, TtCE16, was heterologously expressed in Pichia pastoris, and the protein was purified to homogeneity through affinity chromatography. TtCE16 was physicochemically and biochemically characterized, exhibiting activity on acetylated xylooligosaccharides (XOS).

The esterase performs optimally at pH 6 and 45 ˚C, exhibiting a KM of 35 ±3 μM on 4-Nitrophenyl-acetate. Its activity on acetylated XOS was compared to AXEs of known specificities from CE2 and CE6 families. Moreover, the cooperative activity between a commercially available GH10 xylanase, and TtXyn30A, a GH30 xylanase from T. thermophilus, together with TtCE16 was investigated upon hydrolysis of polymeric and pretreated lignocellulosic substrates. In order to interpret the biochemical findings at a molecular level, structural studies have also been implemented. TtCE16 has been crystallized in space group P3121 and X-ray diffraction data have been collected to 1.9 Å resolution. The structure of TtCE16 is currently under determination, applying the molecular replacement technique. The combination of biochemical and structural findings will shed light on the unique functional characteristics of this novel biocatalyst.

PP_075

ISOLATION AND CHARACTERIZATION OF CARBAZOLE DIOXYGENASE DRIVING THE TRANSFORMATION OF THIABENDAZOLE

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Thiabendazole (TBZ) is a benzimidazole fungicide used for the control of postharvest fungal infestation of fruits. Monitoring programs in Europe showed that thiabendazole is commonly detected in fruits at levels entailing an unacceptable risk for consumers, and it constitutes one of the most common pollutants of surface water systems in the Mediterranean region. Therefore, there is a need for immediate action to limit its dispersal. Previous work on our lab demonstrated the efficient degradation of the fungicide TBZ by a bacterial consortium and showed the key role of Sphingomonas in its degradation. Meta-transcriptomic and meta-proteomic analysis suggested that Sphingomonas activate a carbazole dioxygenase operon during the initial cleavage of TBZ. Carbazole is an N-heterocyclic aromatic hydrocarbon, structurally similar to TBZ. The objective of this study is to confirm the role of carbazole dioxygenase, a multicomponent enzyme consisting of terminal oxygenase (CarAa), a ferredoxin (CarAc), and a ferredoxin reductase (CarAd) unit, on the transformation of TBZ. Each enzymatic component was overexpressed in Escherichia coli strain BL21 (DE3) with histidine tag and was purified. The functionality of the purified proteins in the degradation of thiabendazole is currently tested at in vitro assays and the results will be reported at the conference. This multicomponent enzyme will be the first one reported able to detoxify TBZ. The application of this enzyme in appropriate formulations would be an innovative approach for the removal of thiabendazole from agro-food produce and the environment.

PP_076

MAGNETIC MICROALGAE PRODUCTION FOR IMMOBILIZATION ONTO MAGNETIC SURFACES

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Microalgae are presented as valuable and rich source of oils, proteins, polysaccharides and bioactive compounds for a wide range of biotechnological applications including human and animal nutrition, cosmetics, pharmaceuticals, green energy production and nutrient removal from wastewater (bioremediation). Nowadays, immobilization of algal cells has attracted a great deal of interest in the photo-bioreactors construction. Immobilized algal cells can be used for continuous production, applications as bio-catalysis, effluent treatment to remove nutrients, metals and industrial pollutants and carbon capture.

The target of this work is the production of protoplasts from different microalgae cells, their transformation with magnetic nanoparticles, immobilization on magnetic surfaces and the growth of immobilized algal cells. The magnetic microalgae production has been achieved by introducing magnetic nanoparticles functionalized with lipid-bilayer inside the cells through electroporation. Regarding high-throughput procedure for electroporation, a flow-cuvette, that fits to a standard electroporator, was used, which has been designed, constructed and tested in our laboratory.

A solution ($20x10^6$ ml) of microalgae cells from 3 different microalgae strains, pre-treated enzymatically (cellulase) or mechanically (glass bead vortexing) for the partial removal of the cell walls, have been mixed with magnetic nanoparticles (10 ng/ml) and pass continuously, with the use of a peristaltic pump, through the flow cuvette for electroporation. The electroporation parameters used were 1 pulse of 1 kv/cm, with the duration 13-17 msec for several times. With this method a high volume of a liquid culture (e.g., 100 ml) can be electroporated in a short period of time (e.g., 10 minutes).

The cell concentration and the electroporation time depend on the microalgae strain used. Subsequently, different carbon and amino acids sources were added in the recovery medium as key metabolites for enhancement of the recovery trends of the protoplasts after the cellulase treatment or glass beads agitation. The last step was the growth of selected magnetic cells on the magnetic side of a flask.

PP_077

DISSECTING THE FUNCTIONAL ROLE OF MICROBES IN BIOREACTORS PRODUCING BIOMETHANE USING GENOME CENTRIC METATRANSCRIPTOMICS

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There is an urgent need of a sustainable solution for renewable energy generation and waste resource recovery. Anaerobic Digestion (AD) of organic matter is an efficient technology for anyway, the intricate set of microbial species that by their activity and abundance drives the AD process is far from being completely understood. Additionally, the biologically mediated CO2 methanation through H2 addition has been proven an effective technology for energy production and storage. The study of AD through combination of -omic tools permit identification of the active metabolic pathways and understanding of interactions within microorganisms. The objective of this work is to identify connections among microbial activities and methanogenic performance of a biogas producing system using hydrogen for methanation of carbon-dioxide. Genome-centric metagenomic and metatranscriptomics were used to investigate the microbiome of hydrogen-fueled systems. Genome-centric metagenomics and metatranscriptomic analyses were applied to understand the dynamics in different AD systems and to elucidate the complex microbial interactions occurring. At present, the metabolic profiles of more than 1600 different species involved in AD have been determined; among them ~200 uncultivated metagenome-assembled-genomes are involved in H2 -assisted methanogenesis. To make optimal use of microbial community driven processes, multi -omics data have been integrated with mathematical modeling to resolve intracellular microbial activity. In particular, the role of different shuttle compounds (e.g. formate) on syntrophic associations was determined and the effects on CH4 production were estimated. The complex and integrated pattern of functions of microbial specialized consortium was clarified. This study identified the connections linking microbial activity to biotic and environmental factors and to the methanogenic performance of the reactors. It could be hypothesized that H2 utilizers can work synergistically, forming a specialized population within the community. the metabolic pathways present in the main species responsible for biogas upgrading were deciphered.

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PP_078

GENETIC AND FUNCTIONAL ANALYSIS OF THE ZYMOMONAS MOBILIS STRAIN CP4 PLASMIDS

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Zymomonas mobilis is a candidate organism for large scale bioethanol production due to its ability to ferment sugars more productively than yeasts. Strain CP4, isolated from Recife, Brazil, is closely related to the highly patented strain ZM4 (ATCC 31821) and has often been considered identical in bacterial depository descriptions, literature and patents, despite its distinct genomic features (10.1128/genomeA.00845-13).

Thorough restriction analysis as well as DNA sequencing of the CP4 plasmid genome revealed that CP4 has five almost same-sized plasmids, ranging from ~31 to ~37 kb (US DOE-JGI Program CSP_788284; results combining whole-genome sequencing and plasmid-only sample sequencing). The plasmids bear genes plausibly involved in multiple functions: basic metabolism – including an iron containing alcohol dehydrogenase, cell structure formation, membrane transport, phage particle assembly, transcriptional regulation, restriction-modification, acriflavin resistance, as well as housekeeping genes, i.e. replicases, active partitioning genes and post-segregational host-killing genes. However, approximately 30% of the genes remain of hypothetical function. Excluding ZM4 and comparing with the other sequenced Z. mobilis strains, the majority of the CP4 plasmid genes are strain-unique.

Nevertheless, notable resemblance in terms of gene content and synteny is observed with various discrete parts of the four, again same-sized and similar to CP4, plasmids of ZM4. Transcriptomic analysis of CP4 and of samples collected at mid-late exponential stage of growth (US DOE-JGI RNAseq Program CSP_52) has come to aid in terms of curating the CP4 plasmidome. In this work we present the results of this analysis and we furthermore compare the plasmidomes of strains CP4 and ZM4 and discuss their possible evolutionary relations.

PP_080

SECRETOME ANALYSIS OF TWO GREEK BASIDIOMYCETE WILD STRAINS GROWN ON CORN STOVER REVEALED TWO NOVEL LACCASES WITH SUPERIOR PROPERTIES.

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White-rot basidiomycetes are the only microorganisms with the ability to produce both hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) enzymes for degrading all the constituents of lignocellulosic biomass. Their rich enzymatic machinery makes them ideal candidates for the targeted discovery of novel enzymes with desirable properties. In the present work, two wild strains of Basidiomycetes fungi, Pleurotus citrinopileatus and Abortiporus biennis, from Greek habitats were grown in corn stover, a common agroindustrial residue, and the production of lignocellulose-acting enzymes was studied with standard biochemical methods, as well as secretome analyses. The results revealed the presence of all the necessary enzymatic activities for the complete breakdown of the lignocellulosic substrate, while the prominent role of the oxidative enzymes in the lignocellulolytic strategy of white rot fungi became apparent. Based on the enzymatic activity profiling of both fungi, A. biennis was selected for the isolation of novel laccase enzymes. Two novel laccases, AbiLac1 and AbiLac2, were isolated from the culture supernatant of this fungus with ion-exchange chromatographic methods. Biochemical characterization of both enzymes revealed that they are able to oxidize a wide variety of phenolic and non-phenolic substrates, and they showed typical properties of fungal laccases, such as slightly acidic pH optima and mesophilic temperature optima. AbiLac1 was found to be valuable in environmental pollutants biodegradation applications, as demonstrated by its ability to oxidize chlorophenols and tetracycline. Overall, the results of the present study demonstrate the prominent biotechnological potential of the unexplored enzymatic machinery of white-rot Basidiomycetes from Greek habitats.

PP_081

OVERVIEW AND BENCHMARKING OF AMPLICON-ORIENTED BIOINFORMATICS TOOLS, FOR PREDICTING FUNCTIONAL FEATURES OF MICROBIAL COMMUNITIES, AND THEIR COMPARISON WITH SHOTGUN METAGENOMIC DATA ANALYSIS

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Microbial ecology investigates the interactions of microorganisms with each other and with the environment. Traditionally, culture-dependent techniques were used to investigate and evaluate microbial diversity. However, after the advent of pyrosequencing, isolation and culture of microorganisms is no more a limiting step.

One of the commonly used culture-independent techniques is metagenomics sequencing that can be subdivided into a) targeted sequencing of a specific gene (e.g. 16S rRNA), i.e. metabarcoding and b) sequencing of random segments of all the microbial genomes of a sample, i.e. shotgun metagenomics. While there is a huge amount of 16S rRNA data in nucleotide sequence databases, shotgun metagenomics is the method that provides us with the functional repertoire of the microorganisms under study.

In recent years, several bioinformatics tools have been developed that can link 16S rRNA sequences with their predicted functional profile (e.g. iVikodak [1], FAPROTAX [2], Tax4Fun [3], PICRUSt [4]), thus aiming to enhance the information deriving from these sequences.

The aim of the present study was to review and benchmark bioinformatics tools designed to predict functional features of microbial communities based on the 16S rRNA gene in order to draw conclusions about their effectiveness and reliable use.

Sequence data from pristine and impacted aquatic environments were retrieved from MGnify, a widely known microbiome analysis resource. 16S rRNA sequences were extracted from the shotgun metagenomic datasets and were processed with tools designed to annotate functions. The predicted functional profiles for each environment, based on the 16S rRNA gene data, were compared with the actual functional profiles derived from the shotgun metagenome data, in order to draw conclusions on a) the reliability of bioinformatics tools and workflows that predict community functions, b) the functional prediction efficiency in different types of environments and c) the possibility of replacing shotgun metagenomics analysis with metabarcoding.

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PP_082

ENDOPHYTIC FUNGI OF CANNABIS SATIVA AND THEIR POTENTIAL BIOTRANSFORMATION PROPERTIES

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Fungal endophytes are well-established sources of biologically active natural compounds. However, the diversity of endophytic fungi inhabiting plant species is insufficiently studied. In the present study, we investigated the Cannabis sativa endophytic mycobiota, as well as their underexplored biotransformation potential regarding cannabinoids, with the aim to bio-synthesize novel compounds with better therapeutic indices.

To this objective, twenty-five endophytic fungi were isolated from leaves, stems and bracts of hemp "Carmagnola" variety cultivated in Evvoia.

The morphological characters of all strains were studied macroscopically and microscopically, and it was found that thirteen of them belong to the family Chaetomiaceae, two to the genus Aspergillus, one to Cladosporium, one to Aureobasidium, one to Beauveria, one to Arthrinium and one to the species Microascus trigonosporus, while for the rest the morphological identification was impossible due to the absence of reproductive structures.

Compared to the quite limited data available from literature, it appears that most of the strains belong to species that are new entries to cannabis' mycobiota, something that is expected to be verified after their molecular characterization.

Three strains of the family Chaetomiaceae were tested for cannabinoids production and were also used for cannabidiol (CBD) biotransformation experiments in liquid cultures. None of the strains produced CBD. The strain with the most promising chemical profile, belonging to the species Chaetomium globosum, was subjected to a scaled-up liquid fermentation process for the isolation of fungal biotransformation products. The compounds contained in the EtOAc extracts were purified via column chromatography and semipreparative RP-HPLC and characterized by 1 & 2D NMR and HRMS spectra.

A hydroxylated glycosidic derivative of CBD, which had not been previously described, and four new natural products belonging to the cannabielsoin type of cannabinoids were isolated. In addition, one known mono-hydroxylated, two di-hydroxylated and one O-glycoside were purified. Finally, the antibiotic chlochidinol was isolated, a fungal secondary metabolite with cytostatic properties considered characteristic for the genus. To conclude, it seems that with the aid of endophytes from C. sativa, cannabinoids can be transformed into a large inventory of novel compounds with potential pharmaceutical applications.

PP_083

BIODEGRADATION OF ENVIRONMENTAL POLLUTANTS: A BIOINFORMATICS APPROACH

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Bioremediation is the application of biological processes for the restoration of contaminated regions. Compared to other methods, it is a low-cost, sustainable and technologically simpler clean-up method for ecosystems[1]. The abundance and the presence of microorganisms in different and even extreme environmental conditions, render them a constant source of study and research, aiming at their recruitment for the biodegradation of contaminants. As a vast amount of biological data is now available in specialized databases, bioinformatics analysis can play a significant role in this field. Bioinformatics analysis can contribute to the identification of novel microorganisms, which biodegrade contaminants, or of participating proteins and enzymes, and the elucidation of the complex metabolic pathways involved in biodegradation[2]. In our study, we have collected data about the most widespread soil and water pollutants in the EU, namely PAHs (Polycyclic Aromatic Hydrocarbons), PBDEs (Polybrominated diphenyl ethers) and pesticides. Previously identified bacterial species with the potential to degrade the target pollutants, as well as the pathways and enzymes involved have been collected from bibliographical data and the KEGG and UM-BBD databases, and categorized according to phyla, types and enzyme families. We then focused our analysis on the C-P lyase and GOX enzymes which degrade glyphosate, a widely used pesticide. We collected the amino acid sequences from a broad range of microorganisms, and the conserved residues, identified via multiple alignment, were mapped onto the 3-D structures of the enzymes, using PyMOL. Novel insights on the function of these enzymes will be discussed. This approach can yield insights into more enzymes important for bioremediation.

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PP_084

ISOLATION, CHARACTERIZATION AND APPLICATION OF A MYCOSPHAERELLA TASSIANA FUNGAL ISOLATE FOR THE REMOVAL OF IMAZALIL FROM AGRO-INDUSTRIAL EFFLUENTS

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Imazalil (IMZ) is an imidazole fungicide, commonly used by fruit packaging plants for the control of fungal infestations during storage. Its application in dense solutions results in the production of large pesticide-contaminated wastewater volumes, which according to the European Commission need to be treated before environmental discharge. Biodepuration systems inoculated with specialized tailored-made pesticide-degrading microbial inocula are considered an efficient solution. However, nothing is known about the biodegradation of IMZ. Here, we report for the first time, the isolation of a microorganism able to degrade IMZ. A fungal strain capable of IMZ degradation was isolated via enrichment cultures from a soil that was receiving regular discharges of effluents from a fruit-packaging plant. Phylogenetic analysis classified the isolate in the Mycosphaerella tassiana species. The isolate was verified to be the sole degrader of IMZ via cultivation in the presence of antibacterial and antifungal agents. Inoculation of fruits commonly processed by fruit packaging plants negated any potential phytopathogenic activity by the fungal degrader. The degrading capacity and the growth of the fungal isolate were evaluated at increasing IMZ concentrations (0, 20, 50, 100 mg/L), in both selective (MSMN) and nutrient-rich (PDB) growth media. Fungal growth and degradation rates were reduced in a dose-dependent manner. This response indicates that the degradation of IMZ by the fungal isolate is most probably a detoxification mechanism rather than an energy-gain process. The isolate was able to tolerate and gradually degrade other fungicides, like fludioxonil, thiabendazole and iprodione, which are commonly used in fruit packaging plants and are expected to co-occur with IMZ in the industrial effluents. However, its growth was inhibited by ortho-phenyl phenol. The capacity of M. tassiana to remove IMZ (200 mg/L) from wastewater under practical conditions was evaluated in a benchtop immobilized cell bioreactor. The fungal strain established and dominated the fungal community in the bioreactor, as determined by amplicon sequencing analysis, and effectively removed IMZ (>98% removal). Our study provides first evidence for the biodegradation of IMZ by a microbial isolate and explores the potential use of the isolate for the treatment of pesticide-contaminated effluents.

PP_085

UPGRADE OF AGRO-INDUSTRIAL WASTES INTO PROTEINACEOUS ANIMAL FEED BY SOLID STATE FERMENTATION

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The intense industrialization of the agricultural sector, results in the production of huge volumes of wastes. Most of these wastes are consisted of a complex of polysaccharides justifying their lignocellulosic structure. Due to their physicochemical properties and their rich nutritional composition, can be characterized as raw materials facilitating thus microbial growth. Study herein concerns the application of solid state fermentation (SSF) bioprocess of agro-industrial wastes, in order to upgrade their nutritional composition, targeting to their exploitation as proteinaceous animal feed. The developed SSF procedure was initiated by the edible fungi Pleurotus ostreatus, a natural source of proteins, β-glucans and various metabolites, which are essential for animals' welfare. The fermentation outcomes, having Olive Mill Stone Waste (OMSW) as principal material, were mixed at various ratios with other lignocellulosic agro-industrial wastes such as, Pistachio Shell (PS), Lathyrus clymenum pericarp (LPs), Walnut Shell (WS), Industrial Hemp (IH), Oat Bran (OB) and Brewer's Spent Grain (BSG) with or without Urea's addition at different proportions. The most critical parameter is the substrates' proteins content after SSF, which is indicative of their conversion efficiency into high-added nutritional protein materials. At the end of the fermentation, proteins content was increased to all the examined substrates as compared to their initial content. Cellulose concentration was found to be augmented to the most of them whereas, lignin presence was decreased. β-Glucans concentration recorded a notably increment to all the studied substrates. Urea's supplementation to the mixtures of BSG with LPs, forms an intriguing case hence the incorporation of 1% w/w, revealed adverse effects by recording diminished proteins concentration and β-glucans content to all studied substrates at the end of the fermentation. Overall, this study highlights the potential of the fermentation outcomes, as supplements in animals' dietary which can be rationalized concerning their nutritional upgrade through SSF process. The application of SSF contributes to the circular economy and the reduction of environmental impact, hence agricultural wastes can be harnessed productively.

PP_086

COMPARISON OF HYDROCARBON-DEGRADING CONSORTIA FROM SURFACE AND DEEP WATERS OF THE EASTERN MEDITERRANEAN SEA: CHARACTERIZATION AND DEGRADATION POTENTIAL

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The Eastern Mediterranean Sea (EMS) has attracted great interest in recent years due to large oil and gas reservoirs that have been discovered in deep and ultra-deep waters. A potential oil spill accident, similar to that of Deepwater Horizon in the Gulf of Mexico, could have severe impact in the marine life and economy of the surrounding countries in Eastern Mediterranean basin. Here, the diversity and degradation capacity of hydrocarbon-degrading consortia enriched from surface (10m) and deep (1040m) waters of EMS, were studied in a series of experiments. Microcosms were set up in ONR7a medium at in situ temperatures, 25°C and 14°C for Surface and Deep sea consortia respectively with crude oil as the sole carbon source. The Deep sea consortium was additionally investigated at 25° C to allow the direct comparison of degradation rates to the Surface consortium. By the end of the experiment, ~50% of alkanes and ~15% of polycyclic aromatic hydrocarbons were degraded in all treatments. Approximately ~95% of the total biodegradation by the Deep consortium took place within 6 days regardless of temperature, whereas comparable levels of degradation were reached on day 12 by the Surface consortium. Both consortia were dominated by well-known hydrocarbon-degrading taxa. Temperature played a significant role in shaping the Deep sea consortia communities with Pseudomonas and Pseudoalteromonas dominating at 25°C and Alcanivorax at 14°C. Overall, the Deep sea consortium exhibited a higher efficiency for hydrocarbon degradation within the first week following contamination, which is critical in the case of oil spills and thus, further investigation is required to arrive at site-specific bioremediation technologies exploiting the characteristics of deep sea consortia.

PP_087

INITIAL REASSESSMENT OF THE DIVERSITY OF THE GENUS AGARICUS (AGARICACAE, BASIDIOMYCOTA) IN GREECE BY USING MORPHOANATOMIC AND PHYLOGENETIC APPROACHES

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The cosmopolitan genus Agaricus includes more than 500 edible or poisonous saprotrophic species. Although numerous studies were performed to elucidate the taxonomy and phylogenetic relationships of Agaricus spp., large gaps still exist in pertinent knowledge; these are mainly due to the large morphological variability of basidiomes and to the fact that large regions, including the East Mediterranean area, are still poorly investigated. Over 40 Agaricus spp. have been recorded to date in Greece; however, their occurrence is reported on the basis of morphological features only.

This research aims to inventory the diversity of the genus Agaricus in Greece through a large-scale sampling process and the re-examination of several fungarium specimens by DNA sequencing in conjunction with detailed morphoanatomical studies. The preliminary results revealed that the 55 Agaricus samples initially assessed correspond to at least 16 species grouped in 10 infrageneric sections: i.e., Hondenses, Bivelares, Xanthodermatei, Chitonioides, Bohusia, Agaricus, Subrutilescentes, Spissicaules, Minores and Arvenses. Among them, A. coniferarum and A. iesu-et-marthae were collected from Andros island, and are recorded for the first time in Greece.

In addition, the existence of Agaricus augustus, A. brunneolus (syn. A. porphyrizon) and A. gennadii from Andros, A. bitorquis and A. bresadolanus from Attica, A. comtulus from Corfu, A. cupressicola and A. litoralis from Chalkidiki, A. freirei from Mt. Parnassos and Ikaria island, A. langei and A. macrocarpus from Grevena, and A. parvitigrinus from Trikala and Ikaria island is confirmed for the first time on the basis of molecular evidence. Moreover, several collections might correspond to species new to science and their identity will be established through the examination of additional collections and the use of multi-gene phylogenetic analyses.

PP_088

DIVERSITY, PHYLOGENY AND CHEMICAL CHARACTERIZATION OF TUBER SPECIES FROM GREECE – ESTABLISHMENT OF FUNGAL INOCULA TO FOSTER TRUFFLE CULTIVATION ON NATIVE PLANTS

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The genus Tuber F.H. Wigg. (Ascomycota, Pezizales) is famous for the production of hypogeous ascomata ('truffles') with unique organoleptic properties. Despite the rather thorough documentation of the distribution of Tuber spp. in Europe, the Balkans and the greater East Mediterranean area are still poorly investigated. Moreover, although truffle products are of high commercial value, most of the traded articles are identified on the basis of morphological/empirical characters, and thus some of them appear in the market under false names.

Species-specific chemical profiles of truffles could provide significant information related to their identity, biochemical properties and content in bioactive compounds, and thus confer at maximizing their exploitation potential and at minimizing fraud or mislabeling incidents in marketed products. The assessment of the diversity and the distribution of Tuber species in Greece, the determination of the unique ascomata content and the optimization of truffle cultivation under local conditions are of paramount importance in both research and commercial context. Until recently, 16 Tuber species were reported from Greece; however, the existence of only four of them had been confirmed by DNA sequencing.

Our preliminary results involving the implementation of multidisciplinary approaches revealed the presence of more than 20 species. Various local truffle samples of high commercial importance are examined in relation to their chemical profiles (e.g., volatile organic compounds, elemental profile including rare earths content and FTIR spectra), and possible associations with their geographical origin will be determined. In addition, selected native tree species are inoculated with indigenous truffles to establish viable symbioses which would then be evaluated in respect to various properties of the plant-fungus system.

PP_090

EXPLOITING THE UNIQUE MICROBIAL DIVERSITY OF ETOLIKO LAGOON TO IMPROVE OUR UNDERSTANDING OF THE MICROBIAL COMMUNITY ASSEMBLY, EVOLUTION, AND GLOBAL CARBON CYCLE IN SEDIMENTS

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Etoliko lagoon is part of a complex and unique wetland in Western Greece. A single cell genomics study identified Etoliko Lagoon as one of eight unique environments worldwide with an extremely high representation of candidate phyla, exhibiting a unique and unexplored bacterial and archaeal diversity. In this study we aim to deploy a single cell genomics / metagenomics / metatranscriptomics approach, combined with a detailed 16S rRNA/ITS2 amplicon sequencing from anoxic sediment cores of the Etoliko lagoon. These advanced molecular tools will enable us to unravel the: (i) structure and microbial assembly from shallow to deep sediments, (ii) the ecophysiologies of anaerobic uncultured natural organic matter degrading microorganisms, (iii) the biogeochemical processes of the global carbon and sulfur cycle and (iv) viral diversity in sediments. Moreover, it will enable us to examine the hypothesis which states that due to extreme energy limitation in the deep of the lagoon, DNA repair potential is limited therefore leading to mutation accumulation. The project will be carried out in four work packages. The first is the sediment core sampling. These cores will be separated into vertical layers and each layer will be characterized phylogenetically. The second is the physicochemical analysis of the core samples. The third step is the metagenomic, single cell and metatranscriptomic analysis. Finally, the fourth step is the exploration of the microbial dark matter via culture-dependent techniques.

PP_091

INACTIVATION OF ANTIBIOTIC RESISTANT BACTERIA IN WATER BY ADVANCED SOLAR APPLICATIONS: ASSESSMENT OF PROCESS VARIABLES EFFECTS & ELIMINATION OF ANTIBIOTIC RESISTANCE GENES

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The constantly growing demand for high hygiene standards has led to the exploration and the development of effective water disinfection techniques. However, the effective application of any disinfection procedure should include a special group of microorganisms, which is consisted of the antibiotic resistant bacteria (ARB). The prevalence of ARB and antibiotic resistance genes (ARGs) in the environment is a growing public health concern, as they have been regarded as emerging contaminants. On-going research focuses on the exploration of alternative methods with beneficial effects on public health, green economy and the environment. Advanced oxidation processes, like solar photocatalysis have emerged as a group of techniques with high oxidation and biocidal potential. The aim of the current study was to assess novel solar disinfection techniques, as eco-friendly procedures with the view to inactivate microorganisms of different resistance levels and to eliminate ARB & ARGs. The catalysts used were synthesized perovskites (ZnTiO3), which were activated under natural sunlight and/or upon application of persulfate. The tested microorganisms included representative Gram positive (Bacillus cereus) and Gram negative (Escherichia coli, Pseudomonas aeruginosa) bacterial strains.

Solar photocatalysis with 100mg/L of ZnTiO3 resulted in an almost 6 Log reduction of E.coli and P.aeruginosa but only after 150min of irradiation, while B.cereus population was decayed only by 3 Logs. The addition of 200mg/L persulfate improved disinfection efficiency as complete bacterial elimination occurred in shorter periods of time (30min). The antibiotic resistance profiles of the bacteria were recorded before and after treatment, so as to detect any changes upon disinfection. All the three bacteria showed variant profiles depending on the antibiotic tested each time. For example E.coli became more sensitive to sulfamethoxazole after treatment, while B.cereus' response remained almost the same. The ARGs that were tested provide resistance to β-lactams (ampC), sulfamethoxazole (sul2) and quinolones (qnrA). Although they were not detected in all samples, the general observation was that certain ARGs remained after disinfection and in some cases more gene-copies were measured.

Solar processes have an obvious head start in the quest of efficient treatment technologies, capable of eliminating ARB but certain concerns still remain about ARGs.

PP_092

EFFECTS OF APPLICATION OF SEWAGE SLUDGE TREATED WITH CLAY MINERALS AND BIOCHAR ON MICROBIAL AND CHEMICAL PROPERTIES OF TWO SOILS CULTIVATED WITH A GRASS AND A LEGUME

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Stabilized sewage sludge can be applied as a soil amendment. The effect of sewage sludge, treated with 0 and 15% bentonite, vermiculite, biochar and Ca(OH)2, on soil microorganisms and selected properties of an acid and an alkaline-calcareous soil was investigated. The treated-sludge after air-drying was added at 2% rate to the two soils, and equilibrated with periodic wetting and air-drying. Then, the treatments were planted with Lolium perenne L. (ryegrass) and Trifolium repens L. and tested against fertilized or non-treated soil, in pots. Soil samples of each treatment were analyzed for the rate of microbial respiration, microbial biomass N (MBN), metabolic quotient (MQ) and mycorrhizal colonization, pH, electrical conductivity (ECse) and available nutrients. After equilibration, in the acid soil, the MQ of the control was the highest and MBN the lowest, while among the other treatments there was no difference. This is likely related to moderate (8,5mg kg-1) NO3-N of the control and high NO3-N (15-68 mg kg-1) of the other treatments. In the alkaline soil, limed-sludge treatments had the highest MBN (12.5mg kg-1), higher than the control and treatments with vermiculite- or bentonite-sludge mixtures. For both soils, after ryegrass, there was no difference between treatments and the control for any of the microbiological parameters measured. In contrast, after trifolium in acid soil, MBN was higher with inorganic fertilizer (34mg kg-1), while in alkaline soil, higher MBN was observed with vermiculite-treated sludge (24mg kg-1). In all treatments of both soils, ECse increased (1.2-3.1 dS m-1), compared to the control (0.33 and 0.88 dS m-1 for the acid and alkaline soil, respectively), while available Zn increased up to eight times. Although addition of treated sewage sludge to soils is a soil disturbance, MQ was non different among treatments in both soils, which is an indication that soil microorganisms were not stressed by the increased amounts of Zn nor by the increased ECse. The application of treated sewage sludge with clay minerals, biochar, or Ca(OH)2 did not cause a negative reaction to soil microorganisms. In contrast, in some cases, it led to an increase of soil microbial biomass
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PP_093

DISSECTION OF MEDITERRANEAN FOREST SOIL LIGNOCELLULOSE-DEGRADING ACTIVITY

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Forests cover vast areas of the Earth's surface and are the most important terrestrial renewable resources of carbon-based material in the form of woody biomass. Complex soil microbial communities decompose woody material and, therefore, recycle carbon, providing valuable nutrients and sustaining life in forests. However, exploration of soil microbiomes is a challenging task because the majority of soil microorganisms and their precise functions in these habitats are largely unknown. In this work, we analyzed soil samples from a coniferous forest (mainland; Parnitha) and an angiosperm forest (island; Andros) of Greece. The microbial communities, determined by ITS and 16S metagenomics, revealed that the most abundant fungi in these soils were Basidiomycota (primarily Agaricales and to a lesser extent Russulales, Sebacinales, Gomphales, Geastrales, Hysterangiales and Trechisporales) and airborne Ascomycota (mainly on soil surface, drastically reduced in deeper soil samples). Bacteria were abundant, whereas Archaea were very rare. The most frequent belonged to Proteobacteria, Actinobacteria and Acidobacteria, while Verrucomicrobia, Planctomycetes and Bacteroidetes were less abundant. Furthermore, we performed targeted metagenomics and metatranscriptomics analyses focusing on Class II peroxidases, and we obtained a large number of manganese peroxidases (MnPs), versatile peroxidases (VPs) and lignin peroxodases (LiPs). Our investigation confirmed that the active taxa in lignocellulolysis of these forest soils were most frequent at late spring, and that they increased their presence with depth in both forest types. The obtained peroxidase-like nucleotide sequences were aligned and compared to known functional MnPs from Agaricomycetes. Their structures revealed extended polymorphisms and presence of unknown introns that possibly affect the expression of these enzymes, and numerous novel enzymes. In addition, the detection of several bacterial peroxidase-like uncovered an important synergistic role in lignocellulose biomass degradation. Our results underline the importance of targeted meta-omics analyses for the functional characterization of different soil microbial communities, in order to detect novel enzymes and indicate potential sequence targets for genetic modifications.

PP_094

PREDICTED OCCURRENCE OF ANTIBIOTIC RESISTANCE IN GROUNDWATER RESOURCES THROUGH IN SILICO ANALYSIS OF PUBLICLY AVAILABLE METAGENOMES.

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Despite the importance of groundwater reservoirs as freshwater resources, a gap regarding the global dynamics of antibiotic resistance in groundwater environments remains. To tackle this gap, we here retrieved publicly available groundwater metagenomes from sequencing read archive and analyzed their antibiotic resistance gene (ARG) and microbial community profiles. The resistome profiles showed dependence on location (PERMANOVA test, Euclidean Distance, R2 = 0.33, p = 1*10-6, n=4-30). Out of all ARGs, the genes aph(3'), aph(3''), sul1, sul2, blaOXA and blaTEM were present in more than 30% of the analyzed metagenomes and occurred in the highest relative abundances. Genes commonly occurring in high abundance in wastewater, such as sul1 and sul2, exhibited higher relative abundance in Saudi Arabia groundwater, compared to the groundwater from the rest of locations. In contrast, the β-lactam ARGs blaOXA and blaTEM showed a more uniform distribution over the different locations. Moreover, the bacterial community profile clustered per location (PERMANOVA test, Bray-Curtis distance, R2 = 0.24, p = 10-6, n=6-41) as well. However, bacterial community composition weakly correlated with the ARG profile based on Mantel test (r = 0.25, p= 0.001, n= 69). Furthermore the fungal/bacterial rRNA ratio positively correlated mainly with the β-lactam ARG blaTEM gene (Spearman Correlation Coefficient, r= 0.48, p=0.0030, n= 35). Consequently, fungal abundance contributed mainly to the dissemination of the β-lactam ARG blaTEM. This might explain its more uniform distribution across the global groundwater environments, in comparison with the rest of ARG. In conclusion, the resistome and the bacterial community composition of groundwater metagenomes clustered per location, with a limited fraction of known highly abundant ARGs occurring in more than 30% of analyzed metagenomes. Moreover the biotic interactions due to antibiotic production from microorganisms have a limited effect on the spread of natural/naturalized occurring ARGs, influencing mainly a few β-lactam ARGs.

PP_095

EPIBIOTIC MICROBIAL DIVERSITY OF MEDITERRANEAN LOGGERHEAD SEA TURTLES

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Loggerhead sea turtles carry diverse, often unique epibionts, and several species of barnacles, red algae or amphipods, known to inhabit only sea turtle shells. However, studies of epibiotic microbial communities of sea turtles are generally scarce and focused mainly on morphological analyses of sea turtle-associated metazoans and diatoms. On the other hand, sea turtle microbiome studies are largely limited to those exploring the endobiotic gut, oral and cloacal microbial communities. The current research aims to characterize both prokaryotic and eukaryotic microorganisms associated with the carapace and skin of 26 loggerhead sea turtles from Ionian and Adriatic Seas, sampled in different seasons during 2018 and 2019. To investigate the overall microbial diversity and composition, amplicon sequencing of the 16S and 18S rRNA genes was performed using the Illumina MiSeq Platform. Data were analyzed using the QIIME 2 pipeline, while SILVA and PR2 were used as reference databases for prokaryotes and eukaryotes, respectively. Most abundant detected prokaryotic phyla were Proteobacteria and Bacteroidota, while eukaryotic microbes were mostly represented by TSAR (Alveolata and Stramenopila). Our findings show that overall microbial communities differed among sampling locations (Adriatic vs. Ionian Sea) and sampling seasons, while prokaryotic communities differed significantly between sampled body parts (carapace vs. skin). This research is the first to describe prokaryotic and eukaryotic component of epibiotic microbiome of loggerhead sea turtles using amplicon sequencing. Additionally, this study supports the claim that sea turtle epibiotic microbiota is a reservoir of vast variety microbial species.

PP_096

CITIZEN SCIENCE AND RESEARCH ON MUSHROOM DIVERSITY: NEW REPORTS OF FIRST RECORDS IN GREECE CONFIRMED BY DNA SEQUENCING

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During the last 20 years mushroom citizen scientists have largely contributed to the knowledge of the diversity of macrofungi in Greece through intensive sampling, production of detailed taxonomic data, and publication of regional and national field guides. For promoting/enhancing the analysis of such material collected from various habitats in the country, the Mycological Research Group of the Agricultural University of Athens (MRG-AUA) and the Greek Mushroom Society (GMS) embarked on a fruitful collaboration that lasts more than four years. Hence, hundreds of specimens collected by GMS members were delivered to the MRG-AUA's facilities and have been subjected to DNA sequencing, followed by phylogenetic analyses and detailed morphoanatomical examination of selected samples. Such studies contributed significantly at expanding the knowledge on the distribution of fungal taxa in Greece, they helped to validate previous records and to accurately determine the species identity of several challenging specimens. Most importantly, they promoted a coherent model of collaboration between Universities and citizen scientists which is quite uncommon in Greece. The study of recent findings revealed the existence of eight species which are new records for the Greek mycobiota. Among them, the most interesting one is Cleistocybe carneogrisea, i.e., an extremely rare Mediterranean species recorded in Rhodes, which has been proposed by the IUCN for inclusion in the Global Red List of Fungi; it is known to date only from Spain and Morocco. Moreover, Hebeloma limbatum (a species so far reported from south Europe), the strictly Mediterranean Infundibulicybe alkaliviolascens and Melanoleuca cinereifolia were collected in Attica. In addition, Lepiota farinolens and Pholiotina mediterranea were recorded in Paros island, Leucoagaricus pilatianus was found in Lesvos island, while Saproamanita codinae was collected in Magnesia and Attica. All these species are also considered to be rare and were known predominantly or exclusively from west Mediterranean countries. Last, our preliminary phylogenetic analyses indicated that several other collections might represent undescribed (i.e., new to science) species, and further studies are in progress to assess their exact taxonomic status and phylogenetic relationships.

PP_097

FOLLOWING THE ROUTE OF VETERINARY ANTIBIOTICS TIAMULIN AND TILMICOSIN FROM LIVESTOCK FARMS TO AGRICULTURAL SOILS

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Veterinary antibiotics (VAs) upon their administration are not metabolized in the animal body and are mostly excreted in feces. Their use for soil nutrition or energy production may facilitate VA dispersal impacting environmental quality and human health. We studied the persistence of two VAs, tiamulin (TIA) and tilmicosin (TLM) along their route from pigs to feces and receiving environments. We asked the questions: (a) how different administration modes affect their excretion temporal patterns; (b) how efficient are, anaerobic digestion and ambient storage in removing VAs from feces and conversely how VAs affect the anaerobic digestion process; (c) how persistent are VAs in agricultural soils and (d) how VAs affect soil microbial processes. TLM was detected in feces at levels folds higher (4.27-749.6 μg g-1) compared to TIA (0.55-5.99 μg g-1), with both VAs peaking during the administration period followed by a gradual but incomplete dissipation during the withdrawal period. Administration through water resulted in delayed appearance or lower levels for TIA and TLM respectively, compared with feed administration. TIA and TLM (fortification levels 0.5, 5 and 50 μg g-1) dissipated gradually from during manure stockpiling (DT50 5.85-35.9 and 23.5-49.8 days respectively). Both VAs showed longer persistence during anaerobic digestion (DT90 > 365 days) and negatively affected biomethanation at levels > 5 μg g-1. In direct soil application, TLM was more persistent than TIA with soil fumigation extending their persistence, suggesting a major role of soil microbiota in the degradation of these compounds. Soil application of VAs through feces increased their persistence, probably due to increased sorption to the organic fecal matter and hence reduced bioavailability. Potential nitrification rates were suppressed by TIA after the second application and enhanced by TLM in the direct application and at the low pH soil, whereas little/no effect was observed during their application through manure and at the high pH soil. Our results suggest that the administration of TIA and TLM in pig farms and the subsequent use of feces in agricultural soils is expected to lead to dispersal of VA residues in soil with yet unexplored consequences for environmental contamination and human health.

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PP_098

DIVERSITY OF CULTIVATED MICROORGANISMS ISOLATED FROM TWO-PHASE OLIVE MILL WASTE AND EVALUATION OF THEIR ENZYME ACTIVITIES

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Olive oil production represents a major agro-industrial activity of great importance for the Mediterranean region. A continuously increasing number of olive mills are adopting the “two-phase” process for oil extraction, which results in the generation of large quantities of a sludge-like effluent (‘alperujo’). The composition of this by-product (and mainly of the organic compounds it contains) largely determines the abundancy, diversity and functionality of the indigenous microbiota. The objective of the present work was to obtain pure cultures of fungi and bacteria existing in alperujo by using various selective media and incubation temperatures, and to identify them through the use of colony characteristics and DNA sequencing (ITS and 16S rDNA, respectively). A total of 109 fungal and 103 bacterial cultures were obtained, and the outcome of the phylogenetic analyses revealed the existence of the following genera among Fungi: Aspergillus, Penicillium, Talaromyces, Pleurostoma, Phialophora, Beauveria, Sarocladium, Trichoderma, Cladosporium, Neocucurbitaria, Stagonosporopsis, Candida, Geotrichum, Fuscoporia, Phlebia, Russula, Peniophora and Mucor. As regards Bacteria, the main genera detected were Paenibacillus, Lysinibaillus, Niallia, Bacillus, Acinetobacter, Streptomyces, Brevibacterium, Janibacter, Ameyamaea, Gluconacetobacter, Roseomonas, Pseudocitrobacter and Pseudomonas. In addition, selected strains were evaluated in respect to their laccase, Mn-peroxidase, lignin peroxidase, chitinase, cellulase and hemicellulase activities. The respective results demonstrated that most fungi could grow on media with cellulose or lignin as sole carbon source; however, activities for laccases and peroxidases were detected for 10% and 14% of the isolates, respectively. On the other hand, ca. 30% of bacteria hydrolyzed chitin, 60% and 46% of them produced extracellular cellulases and hemicellulases, respectively, whereas peroxidases activities were rather low compared to fungi.

PP_099

3,4-PROTOCATECHUIC ACID DIOXYGENASE: A POINT MUTATION CHANGES THE GAME

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Despite the fact that the world is already heading to the end of the first quarter of the 21st century, a major problem still remains unsolved: the deterioration of the environment due to the population growth and the rapid industrialization. A vast amount of pollutants with carcinogenic, mutagenic or toxic properties, are being released each day in the environment. A major category of such pollutants consists of aromatic compounds which include Polycyclic Aromatic Hydrocarbons (PAHs), heterocycles and substituted phenols. Bioremediation is considered to be the most sustainable solution to the problem of these environmental pollutants due to its low cost, high efficiency, minimum byproducts and no secondary pollution. Despite the great variety of the aforementioned pollutants, their degradation occurs through a limited number of specific metabolic pathways to the formation of central intermediates consisting mainly of catechols and protocatechuates. Our previous studies have demonstrated that Pseudarthrobacter phenanthrenivorans Sphe3, isolated from a creosote contaminated site, metabolizes phenanthrene through protocatechuate (PCA) [1].

In this study, our goal is to broaden the specificity of protocatechuate 3,4-dioxygenase (3,4-PCD) of Sphe3 (a non-heme ferric ion intradiol dioxygenase, characterized by narrow substrate specificity) in order to identify other central intermediate metabolites. Computational studies combined with literature data, lead to a set of possibly functional enzyme variants. After docking experiments and molecular dynamics simulations conducted to 3, 4-PCD (PDB ID: 2PCD) with catechol, one specific amino acid residue (R133H) was altered in order for 3,4-PCD to functionally replace 1,2-catechol dioxygenase (1,2-CAD). In silico experiments were followed by site-directed mutagenesis via PCR. After cloning of the mutant gene in an overexpression plasmid vector and inducing heterologous expression of the recombined enzyme kinetic studies showed that the mutant 3,4-PCD successfully acquires the ability to break down catechol through 1,2-cleavage while maintaining its native ability to break down PCA as well. Further studies may allow the construction of a handful of enzymes able to recognize and catabolize a broader range of aromatic substrates.

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PP_100

CULTIVATION OF THE FRESHWATER COMPLEX MICROALGAE-CYANOBACTERIA AND INVESTIGATION OF ASSOCIATED INTERACTIONS

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Microalgae and cyanobacteria are photosynthetic microorganisms that inhabit both seawater and freshwater. They are considered as natural resources with multiple properties of high added value for natural ecosystems. Currently, there is growing interest in their potential use in several applications as in the nutraceutical and pharmaceutical fields, in the treatment of effluents, and the production of bioenergy. Interestingly, several functional interactions between cyanobacteria and/or algae and bacteria have been reported and these interactions are ubiquitous.

Within this framework, the aim of this study is to investigate the microbial diversity of freshwater ecosystems by isolation and characterization of microalgae, cyanobacteria and associated heterotrophic bacteria from different Tunisian eco-regions. Therefore, freshwater samples and geothermal algal mats were collected from water sources located in the North and South regions of Tunisia. The isolation of cyanobacteria and microalgae was performed using BG11 and BBM media respectively. Cultures were incubated at 25°C under 16:8 light/dark photoperiods for 30-40 days. In total, 198 cultures were obtained and subjected to morphological and molecular analysis. Molecular identification of cyanobacteria and heterotrophic bacteria was performed via the sequencing of the 16S rRNA gene, while identification of microalgae was based on the ITS and 18S rRNA gene markers. Microscopic observation of cyanobacterial cultures showed the dominance of filamentous genera like Phormidium and Leptolyngbya, while morphological characterization of microalgae demonstrated the presence of both unicellular and filamentous cells. Molecular identification of heterotrophic bacterial isolates showed their affiliation to Alphaproteobacteria, Gammaproteobacteria and Actinobacteria. Moreover, next generation amplicon sequencing was applied for an in-depth analysis of the bacterial community of these complex environmental samples.

Our results confirmed that cyanobacteria and microalgae provide microenvironments for heterotrophic bacterial growth. Hence, the assemblages between those microorganisms constitute a complex ecological superstructure. Therefore, understanding those interactions and the microbial biodiversity can provide important answers to the ecology of freshwater ecosystems.

PP_101

PLANT GROWTH PROMOTING MICROBES FROM ROOTS AND RHIZOSPHERES OF INDIGENOUS DRYLAND PLANTS TO BE USED IN QUARRY RESTORATION OPERATIONS IN A TYPICAL INDUSTRY-DEGRADED ECOSYSTEM OF MILOS (GREECE)

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Soil microorganisms intimately interact with each other, and with plants, providing essential ecosystem services that include biogeochemical, nutrient cycling, water management, and carbon sequestration. Quarrying operations, cause irreversible damage to the local environment creating vast degradation problems because of fertile soil depletion, vegetation removal, and alterations in the original topography. Restoration practitioners try to re-install plants and restore ecosystem functions, focusing on recreating specific plant communities based on historical, reference, or desirable output. However, routine restoration interventions often lack an integrated ecosystem approach and largely ignore the importance of soil microbial communities and plant-microbe interactions. Producing and utilizing soil microbial inoculants derived from roots and rhizosphere microbial communities from plants in nearby undisturbed sites may assist in meeting this challenge. The rooting zone, a prime source of microbial inoculants, serves as an ecological niche, where the plant-microbe interactome has developed a spectrum of associations that range along a broad continuum, from strong mutualism to parasitism based on adaptation, co-adaptation and antagonistic processes. In this work, bacteria and fungi were isolated from the rhizospheres and roots of indigenous dryland plants from undisturbed ecosystems located on the island of Milos (Greece). Moreover, rhizospheric soil and small parts of roots of the plants of interest were used in trap cultures with different trap plants in pots, to isolate arbuscular mycorrhizal fungi (AMF). Bacteria were tested in vitro for the biosynthesis of auxins, phosphate solubilization, and siderophore production. Bacteria and fungi are now molecularly identified and a trait-based selection approach will also be applied to potential plant growth-promoting fungi in parallel to the application of taxonomy-based pre-selection criteria. In the next phase, bacteria and fungi with plant growth-promoting traits, combined with propagules of AMF isolates will be used as inoculants to enhance the survival, growth and fitness of these native plants in restoration applications. Synthetic communities and crude inocula will also be tested, shedding light on the role of local microbial isolates plant-microbe interactions and community effects as drivers in the restoration and maintenance of aboveground vegetation, soil functions and services in a typical dryland ecosystem that has been degraded due to quarrying activities.

PP_102

ASSESSING THE TOXICITY OF PESTICIDES ON NATURAL SOIL AND PLANT ASSEMBLAGES OF AMF

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Pesticide usage is regulated by the international standardized methods, to access their toxicological effects on the environment. However, how these compounds affect the off-target soil microorganisms, remains elusive, due to the no-well-defined regulatory-tiered scheme for assessing the soil microbial toxicity of pesticides. In reference to the European Food Safety Authority call, Arbuscular mycorrhizal fungi (AMF) have been considered as ideal soil microbial indicators of pesticides toxicity, where the basis for that was its ecological role in the ecosystems (1). In addition, to date most studies have investigated the toxicity of pesticides on soil microorganisms at the lab- and field-scale in a nonstandardized way (2) and potential recovery is overlooked (3). In the current research work, advanced experimental lab and field tests will be developed and implemented for that purpose.

Potato is used as a host plant as it can be colonized by a wide diversity of AMF taxa. To have more realistic scenarios, 3 types of soils which vary in their physicochemical characteristics and are from 3 geographical zones of the EU are used and are exposed to high pesticide dose rates. It is expected to monitor the pesticide's dissipation for identifying the correlations between exposure and effects, and the pesticide's possible effects on the host plant, e.g. productivity, ionome. Possible effects of the pesticides on natural AMF assemblages, e.g. function, diversity, colonization level are considered as main toxicity endpoints. To have a holistic view of pesticides toxicity on the main functional microbial group, the pesticide's toxicity mechanisms are going to be identified. This work is a big step in the establishment of guidelines on how to assess the toxicity of pesticides on AMF at lab and field scales.

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PP_103

DECIPHERING THE ROLE OF A NOVEL DIOXYGENASE OF THE VOC FAMILY FROM PSEUDARTHROBACTER PHENANTHRENIVORANS SPHE3

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Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous organic pollutants present equally in aquatic and terrestrial ecosystems with strong genotoxic, mutagenic and carcinogenic properties. Biodegradation is the most cost-effective and environmentally sustainable alternative to tackle the PAH pollution challenge. Pseudarthrobacter phenanthrenivorans Sphe3 is a Gram(+) bacterium of the phylum Actinobacteria with the ability to degrade phenanthrene at concentrations up to 400 mg/L [1]. Sphe3 has also been shown to contain two plasmids, pASPHE301 and pASPHE302. A derivative strain cured of the largest plasmid, pASPHE301 (Sphe3c), was unable to grow on phenol or gallate as the sole source of carbon and energy. Mono- and di-hydroxybenzoates are key intermediates in the aerobic metabolism of aromatic compounds further catabolized by strain-specific pathways. A thorough in silico analysis of pASPHE301 plasmid led to the identification of a number of genes whose absence could be responsible for Sphe3c abolishing the ability to catabolize these compounds. A putative orf annotated as catechol 2,3-dioxygenase was selected for further investigation as this enzyme could be involved in phenol degradation. Subsequently the gene was heterologously expressed in E. coli BL21 (DE3) cells but no such activity was observed. Blast analysis suggested that this protein is a member of a new VOC (Vicinal Oxygen Chelate) family protein. Attempting to characterize the catalytic activity of the protein, the enzymatic reaction UV-Vis spectra with several mono-, di- or trihydroxylated aromatic compounds as substrates was investigated. Interestingly, of the aromatics tested protocatechuate, gallate, pyrogallol, gentisate and hydroquinone exhibited shifts in the spectrum, leading to the conclusion that it is an enzyme that requires at least two hydroxyl groups in either ortho- or para-position.
Further kinetic studies are expected to shed more light in the role of this novel dioxygenase from P. phenanthrenivorans Sphe3.

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PP_104

CULTIVATION OF PLEUROTUS ERYNGII MUSHROOM ON ENRICHED AGRICULTURAL RESIDUES

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The increment of agro-industrial residues per year is associated with many environmental problems because of their burning or improper disposal. The re-utilization of lignocellulosic wastes is very important for the environmental balance, as well for economic reasons. Mushrooms and particular those of Pleurotus spp. possess an enzymatic system that enables them to degrade and flourish on these wastes. The use of substrates enriched with additives are usually more beneficial than the homogenous ones, considering mushrooms' cultivation (duration, yield, quality). Therefore, the influence of three enriched substrates (wheat straw-WS, barley and oat straw - BOS and rice bark - RB supplemented with 2 and 5% w/w of sunflower oil and yeast extract) in respect to non-supplemented ones (control) was examined in the vegetative phase of P. eryngii mushroom AMRL 173-6. Evaluation included mycelium growth rate, biomass production (estimated as glucosamine content) and laccase accumulation. Colonization rate measurements demonstrated that enriched substrates enhanced growth rate at WS (except for 2% w/w sunflower oil) and BOS (except for 2% w/w yeast extract) with values ranging between 5.75-8.27 mm/day, whereas at RB the additives acted as inhibitors. Contrariwise, the supplements enhanced biomass production on RB with the highest values being detected for sunflower oil 5% (218.98 mg/g d.w.) and yeast extract 2% w/w (196.11 mg/g d.w.). Yeast extract additive in WS was unfavourable for biomass production, while all examined supplements except for sunflower oil 2% w/w, promoted high biomass production on BOS substrate (225.64-264.60 mg/g d.w.). Finally, yeast extract enhanced laccase accumulation for WS and BOS, opposed to sunflower oil which was an unfavourable additive. Exceptionally, at BOS substrate laccase accumulation was almost two-folded with the addition of 2 and 5% w/w yeast extract (2974.88 and 3184.43 U/g d.w. respectively), compared to the control substrate (1746.00 U/g d.w.). On the other hand, at RB substrate the supplements addition had negative effect on laccase synthesis. It seems therefore that colonization process parameters were affected by the substrate synthesis as well the additive used, mostly in a positive way and therefore evaluation of these supplements should continue on the stage of mushroom fructification and cropping.

PP_105

EFFECT OF SMALL ORGANIC ACIDS ON BACTERIAL CELLULOSE YIELD AND MECHANICAL PROPERTIES

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Bacterial cellulose (BC) is an ultrafine, nanofibrillar material with an exclusive combination of properties spanning high crystallinity, high surface area, high flexibility, tensile strength and high water-holding capacity. However, the high cost of fermentation media has limited the industrial production of BC. Complex and expensive Hestrin–Schramm medium (HS) is commonly used in the cultivation of BC and contains: 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). 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).

The aim of this work was to investigate if small organic acids could be used as a substitute for glucose in HS medium and if their presence may affect mechanical properties of the obtained material. Commercial and post-consumer terephtalic acid (TPA), benzoic acid and glutaric acid were tested in different glucose/acid ratios (80/20, 50/50, 30/70, 0/100). BC producing strain Komagataeibacter medellinensis sp. ID13488 was incubated in modified HS media for 2 weeks in static conditions (5% inoculum (v/v), 25℃, pH 5.5). Bacterial cellulose films were weighed and their mechanical and thermal properties were examined (tensile strength, elastic modulus, strain-at-break, degree of crystallinity, glass transition temperature).

It was shown that benzoic and glutaric acid have an inhibitory effect on BC production in all tested ratios, whereas in the presence of TPA, grown BC films weighed more than films obtained using the positive control (regular HS medium) under the same conditions.

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PP_106

VERTICAL AND TEMPORAL VARIATIONS OF BACTERIAL COMMUNITY COMPOSITION IN SHALLOW SURFACE SEDIMENT OF EUTROPHIC LAKE PAMVOTIS IN NW GREECE

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Within the lacustrine ecosystem, sediment accumulates sinking organic matter and provides distinct microniches for the development of a diverse microbial community with a key part in biogeochemical nutrient cycling. The present pilot-scale study investigated vertical and temporal variations of the sediment bacterial community composition in a shallow coastal site of eutrophic Lake Pamvotis (NW Greece) across three, 1 cm thick sediment horizons within the first 5 cm below the lake floor (cmblf) during the transition to the cold period of the year (Oct–Dec 2018), through monthly samplings. Bacterial community analysis was based on Illumina sequencing of the V3–V4 hypervariable regions of the 16S rRNA gene. Meanwhile, the concentration of chlorophyll α and pheophytin in the sediment was measured using extraction in 90% acetone and spectrophotometry, and organic matter content in the sediment was calculated using the Loss on Ignition (LOI) method. Operational taxonomic unit (OTU) richness ranged between 3565 and 4835 in different samples, and bacterial community composition was dominated by members of Proteobacteria, Bacteroidetes, and Chloroflexi. Overall, a core community of ~1000 OTUs associated to the above-mentioned phyla persisted across months and increasing sediment depth. The observed diversity was mainly made up of rare (< 1% of relative abundance) OTUs (~96% of richness per sample), which tended to remain rare over the course of the sampling period. Most genera associated with the shared abundant (≥ 1% of relative abundance) OTUs of the examined samples are commonly isolated from polluted aquatic systems or wastewater treatment facilities. Additionally, several abundant OTUs correlated significantly with pigment or organic matter concentration, and community composition appeared to begin shifting along the transition to winter. The present study hinted at the potential of specific bacterial taxa (e.g., Anaerolineae, Bellilinea) to serve as monitors of organic pollutants (e.g., polycyclic aromatic hydrocarbons) as well as the need to elucidate the role of the rare biosphere in ecosystem functioning. Furthermore, insights may have also been provided regarding the sediment microbial community of other lakes with common characteristics (e.g trophic state, human impact level, location), that have not been studied in a similar way.

PP_108

SEARCHING FOR NOVEL MICROORGANISMS AND ENZYMES FOR POLYURETHANE DEGRADATION

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With global plastic production over 350 million tons annually plastic waste has become a global environmental and health problem. Polyurethanes (PU) make up 7.7% of the global production, they can be found in mattresses, car seats, they are an integral part of thermal insulation of buildings and account for more than 60% of plastic foams. Because of their specific crosslinked 3D structure and the number of different monomeric units that can be incorporated, they are almost impossible to recycle by standard mechanical and chemical methods. Therefore, biotechnological degradation and recycling of PU is in research focus. Numerous microorganisms capable of degrading specific PUs have been identified however, a need for more efficient biocatalysts still remains [1]. In this work, we aimed to identify novel plastic degrading biocatalysts by screening novel microorganisms and employing a set of specific PU model substrates with different levels of complexity.

By structural analysis of common PU materials, we selected and synthesized eight PU model substrates of which 6 were completely new compounds. Model substrates were synthesized by reacting hydroxyalkyl esters with phenyl isocyanate/toluene diisocyanate followed by hydrogenolysis. These compounds were used as substrates for microbial growth and in biocatalytic reactions to increase specificity of screening for novel PU degraders.

A total of 251 microorganisms were isolated from contaminated and uncontaminated sites using four different growth media, in order to promote the diversity of isolated microorganisms. All of the isolated strains were screened for their potential to degrade PU. The screening was carried out on Mineral Salt Medium (MSM) agar plates containing Impranil DLN-SD, DL 2077 and PU powder as the sole carbon source. Strains able to produce clearing halos on such plates were selected for further experiments including biotransformation reactions with PU model substrates in a whole-cell biocatalytic system. Twenty-three strains showed high PU degrading potential. The most potent three strains were selected for whole genome sequencing and identification of potential PU degrading enzymes.

1. Nikolaivits, E., et al., Progressing plastics circularity: A review of mechano-biocatalytic approaches for waste plastic (re) valorization. Frontiers in Bioengineering and Biotechnology, 2021. 9: p. 535.

PP_109

ASSESSING THE TOXICITY OF BIOPESTICIDES ON SOIL MICROBIOTA USING A MODIFICATION OF THE OECD 216 TEST

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Synthetic pesticides are widely used in agricultural systems to protect crops against diseases and pests. Despite their undeniable importance for crop production, studies indicate that the indiscriminate use of synthetic pesticides threatens the environment and human health. As an alternative, biopesticides (chemicals of biological origin), including many natural products of plants and microbes, are gaining the attention of the general public and the pesticide market. In general, biopesticides are considered low-risk compounds and environmentally safe even though evidence supporting this idea are still lacking. Given the increase in the numbers of biopesticides in the agricultural market and the scarcity of literature regarding these products, studies on the effects of biopesticides on the soil microbial community and nutrient cycling become necessary. Hence, to fulfill this gap of knowledge, we conducted a microcosm experiment to assess the effects of biopesticides, after a single exposure, on soil microbiota and nitrogen mineralization process. Briefly, we set up a modified OECD 216 assay using two different soils, one from France (silt clay loam) and another from Germany (sandy loam). Six biopesticides including azadirachtin, spinosad, aliphatic phenol, dihydrochalcone, isoflavone, pyrethrins, plus a synthetic pesticide (3.5 DCA - used as a negative chemical control known for its toxicity against soil microbiota) are being tested. Each biopesticide was applied in the recommended dose and 10x the recommended dose. Samples were collected at 0, 7, 14, and 28 days of incubation. The effects of biopesticides on the soil microbiota will primarily focus on nitrification and the ammonia-oxidation process by measuring potential nitrification (PNT), ammonium (NH_4^+), nitrate (NO_3^-) levels, and the abundance of relevant ammonia-oxidizing microbial groups (archaea, bacteria, Comammox) via q-PCR. Effects on the soil microbial diversity (bacteria, archaea, fungi and ammonia-oxidizers) will also be determined via amplicon sequencing. This research has the potential to provide a new holistic view to the field of Soil Ecotoxicology and generate benchmarking knowledge to comprehensively assess the potential toxicity of biopesticides to soil microbiota and their impact on nitrogen transformation process.

PP_110

ENHANCED BIODEGRADATION IN SOIL OF THE VETERINARY ANTIBIOTIC TIAMULIN: ISOLATION OF TIAMULIN-DEGRADING BACTERIA

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Tiamulin (TIA) is a veterinary antibiotic heavily used in livestock farms to support animal growth and to treat enteric and respiratory infections of pigs and poultry. Upon its administration most of TIA (>90%) is excreted intact in urine and feces which are subsequently used as manures in agricultural soils. This practice results in the environmental dispersion of TIA residues with serious implications for environmental protection and human health. Beyond environmental and health risks, the regular exposure of agricultural soils to veterinary antibiotics might also lead to adaptation of the soil microbiota to their growth-linked biodegradation. This phenomenon has been reported for certain groups of antibiotics like sulfonamides and soil bacteria able to actively degrade these compounds have been isolated. Our study aimed to isolate the first TIA-degrading bacteria and evaluate their potential for future implementation in bioremediation of manures before their land spread. Enhanced biodegradation of TIA was observed in a soil collected in Rodia village, Thessaly, Greece, after repeated applications of the antibiotic. Thus, liquid enrichment cultures in which TIA was the sole source of C or C/N were set up for the isolation of TIA-degrading bacteria. After 6 enrichment cycles TIA was completely depleted within 3 days. Plating in selective media with TIA constitutes the sole C/N source, colony selection and degradation assays is currently on going aiming to isolate in pure culture the microorganisms responsible for the observed TIA biodegradation.

PP_111

THE ECTOMYCORRHIZAL GENUS XEROCOMELLUS (BOLETACEAE, BASIDIOMYCOTA) IN GREECE – INTERESTING FINDINGS INCLUDING FIRST NATIONAL RECORDS AND ONE SPECIES NEW TO SCIENCE

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The boletoid genus Xerocomellus Šutara was segregated from Xerocomus Quél. relatively recently by combining molecular and morphological data, such as the pileipellis structure and the fine structure of the spores' surface. The genus Xerocomellus includes several species that exhibit a more or less distinct Mediterranean distribution and grow in association with Quercus spp. in xerothermic habitats. Surprisingly, no published information is available about the diversity of this genus; hence, very little is known about the occurrence of Xerocomellus species in Greece. A long-term investigation on this genus has been performed during the last 15 years which included an extensive sampling in Quercus ilex and Q. coccifera stands of Aegean islands (e.g., Andros, Naxos, Ikaria, Crete), and the study of fungarium specimens from various regions of Greece as well as from other European countries, through the use of high resolution optical and scanning electron microscopy combined with multi-gene phylogenetic analyses (ITS, LSU and RPB2 markers). The results led to the reassessment of several old herbarium specimens, and the existence of the following species was assessed: X. redeuilhii from Andros and Ikaria as well as from Mt. Parnitha, X. sarnarii from Naxos and Ikaria (both species are first national records), X. cisalpinus from Andros, Ikaria and Crete (previously reported only once from the mainland), and X. chrysenteron and X. porosporus (recorded so far from continental Greece). In addition, three collections from Naxos represent a phylogenetically and morphologically distinct species which is new to science. Interestingly, our phylogenetic analyses demonstrated that specimens labeled as X. marekii – including the holotype of this species – were conspecific with X. porosporus.

PP_112

ASSESSING THE EFFECTS OF MICROPLASTICS ON THE SOIL MICROBIOTA: THE PROJECT MINAGRIS

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Awareness of the plastic pollution in our environment, including agricultural soils, is increasing. Micro- and nano-plastics (MNP) that are derived from different plastic types are now considered emerging pollutants of global importance. They are small enough to be ingested by a wide range of organisms and at nano-scale, they can cross biological barriers and enter plants systems. The emerging threat of MNP to terrestrial environments has prompted first studies which confirmed the high potential of MP to accumulate in soils and cause changes in physico-chemical soil properties, thereby also altering soil functions. These effects could be magnified when MP occur in combination with other soil pollutants such as pesticides and veterinary drugs or plastic additives.

It is therefore crucial to reach a better understanding of any potential harmful impacts of MNP to soil biodiversity, soil functions and related ecosystem services. An EU H2020 Research Project on Micro and Nano-plastics in Agricultural Soils (MINAGRIS) has been launched in September 2021 to tackle these challenges. The overall aim of MINAGRIS is to contribute to healthy soils in Europe by providing a deeper understanding and tools to assess the impact of MP and NP in agricultural soils on biodiversity, plant productivity and ecosystem services and their disaggregation fate in the environment. Based on 11 Case Study Sites across Europe, MINAGRIS will also provide recommendations for sustainable use of plastic in agriculture at the farm and field levels for ensuring safe and economically viable food systems in Europe.

One of the main research focusses will be to determine the effects of MNP (alone or in combination with other stressors) on the function and diversity of the soil microbial community. This will be determined in a series of lab, pot and field experiments with a range of MPs of different chemistry targeting specific functional microbial groups but also the overall soil microbial diversity using advanced molecular tools. Interactions of the soil microbiota with the plastics on the plastisphere, the interface between plastics and soil surfaces, will be also addressed using metagenomic approaches. Further details and first results will be presented in the MIKROBIOKOSMOS conference

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PP_113

CAN PGP BACTERIA HELP PLANTS DEAL WITH FLOODS CAUSED BY THE CLIMATE CHANGE?

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Extreme climate events will increasingly disrupt vulnerable agricultural productivity, threatening food security, quality, and price stability. There is an urgent need for development of novel approaches for adaptation of crops to a quickly changing climate. Researchers have just started to focus on soil microbiota. Bacteria which colonize plant root and provide benefits (through variety of mechanisms) for plant growth and biocontrol so-called PGPB (plant-growth-promoting-bacteria) are of great importance. Within project entitled "Potential of the rhizosphere microbiome in the adaptation of agriculture to climate change (PERSPIRE)", funded by the EU Regional Development Fund, we focused our research on the effects of flood (water retention following heavy rain), as a consequence of climate change, on the plant holobiont i.e. on this positive interaction that exist between soil microorganisms and its host plant. We settled our experiment in the controlled conditions (16 hours day/8 hours night; 25 ˚C per day/20 ˚C per night; 60-70% relative humidity), with the cabbage (Brassica oleracea var. capitata f. alba) used as a model plant. The experiment lasted, from seeding to the full end, for 57 days. Plants (triplicate trials) were subjected to either one (72 h duration) or two short-term flooding events (72 h duration, 10 days recovery between floods) at different stages of development. At different time points (day 0, after flooding and after recovery period) whole soil was removed from the pots, thoroughly mixed and subsamples were taken for PGPB isolation and analysis. PGPB were isolated by inoculation onto non-selective nutrient agar and incubation at 30 ˚C (3 Days). From each of the sample points cca. 20 morphologically different bacterial colonies were selected, purified and screened for different PGP characteristics by using plating culturable methods on various media. All together 140 isolates were tested for the ability to: produce indole acetic acid (IAA), asymbiotic N2 fixation, solubilisation of phosphate, production of siderophores and the synthesis of enzymes. We believe that, full understanding of the effects of climate change on PGPB, could represent first step forward toward potential using of PGPB isolate as bio inoculums for crops affected by flooding conditions.

PP_115

IMMOBILIZATION OF AEROBIC BACTERIA ON VARIOUS CARRIERS FOR OIL BIODESULFURIZATION

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Oil biodesulfurization (BDS) requires large amounts of biomass and sometimes separation of the cells from the oil is difficult. The 4S pathway provides a non-destructive oxidative process used by the cells to obtain the sulfur required for their growth; it involves the transformation of Dibenzothiophene (DBT, the model compound for sulfur heterocycles present in the oil), into 2-Hydroxybiphenyl (2HBP), and sulfite. More specifically for BDS, several separation schemes can be evaluated, including settling tanks, hydrocyclones, and centrifuges. However, these procedures are time-consuming and costly. To overcome these problems, immobilized whole cells can be used in the BDS process. Immobilization of microbes to carriers contributes to better catalytic efficacy and easy separation of products, enabling reuse of degradation agents. Towards this, the possibility of severe contamination is reduced, and the efficient recovery of the product is enabled. Thus, the more adequate immobilized particles that bacteria can be attached to will be examined. The immobilized particles need to sustain the activity of the BDS over time. Therefore, to increase the BDS process, three carriers (biochars) were examined along with two bacteria species, Serratia sp. and Burkholderia sp., which were isolated from relevant environmental samples. The experiments took place in a 200 ml flask (80 ml working volume), and initially, 10% carrier was used. The aerobic BDS consortium was inoculated, and the DBT reduction over time was monitored through GC-FID. The results are presented and discussed.

PP_116

POSITIVE FEEDBACKS OF WHOLE RHIZOSPHERIC MICROBIAL COMMUNITY INOCULATION ON PLANT PERFORMANCE AND FITNESS OF THREE LEGUME SHRUBS GROWN ON BARREN QUARRY DEPOSITS.

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In this work, we investigated the effects of inoculation with the whole rhizospheric microbial communities on the early plant growth and on the establishment of beneficial symbiosis with rhizobia and arbuscular mycorrhizal fungi (AMF) for three pioneer legume shrubs, indigenous to the island of Milos, Medicago arborea, Anthyllis hermanniae and Calicotome villosa. Seeds of each plant species were sowed in pots filled either with barren quarry deposits material derived from a bentonite quarrying operation at Milos Island – Greece (inorganic growth medium) or with a typical propagation substrate, high in organic matter (organic growth medium). Following half of the pots of each plant species was inoculated with 10ml of a soil slurry (crude inoculum - 1:10 v/w soil to water) made from the rhizospheric soil of each plant species collected from natural plant formations. The rest 50% of the pots were not inoculated and served as controls. Inoculations with each plant's crude inoculum were applied twice, at sowing and one week after sowing. Following a six-moth growth period the plants were destructively sampled and the above and below ground biomass, the number and weight of root nodules, the N2 fixation rate and the extend of AMF root colonization were measured. Overall, for all three plants grown in the inorganic medium, inoculation with their indigenous rhizospheric microbial community enhanced plant growth, both belowground and aboveground and symbiotic associations were favored, leading to more and heavier nodules, higher N2 fixation rate and increased root colonization by AMF. On the other hand, plants grown on the organic medium did not respond uniformly to inoculation, and each plant taxon performed differently. The increase in AMF root colonization, which was observed for all inoculated plants in the organic growth medium may be related to this inconsistent performance, as AMF may enhance plant nutrition, but at the same time overexploit plant-C resources. In conclusion, inoculation of plants with the whole rhizospheric microbial community they maintain under natural conditions reinforces plant fitness in infertile and semi-sterile environments as baren quarry deposits, a strategy that may prove to be important for revegetation and restoration of soil functions.

PP_117

DEPTH-DRIVEN ALTERATIONS IN VIRAL LYTIC COMMUNITY COMPOSITION AND PRODUCTION RATES IN OFFSHORE OLIGOTROPHIC WATERS

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Viruses are a fundamental component of the marine ecosystem, influencing global biogeochemical cycling, microbial plankton mortality and community evolution. During an expedition in the Western Levantine Sea, water was collected by Niskin deployment at four locations and four depths (5, 50, 75, 1000 m) to study viral abundance, replication cycles and community composition in this offshore, ultra-oligotrophic and largely-unexplored environment. Abundances were estimated using flow cytometry, lysis over lysogeny using the viral reduction approach and taxonomy using whole-metagenome sequencing at Illumina HiSeq platform. Physical characteristics, nutrient, oxygen and chlorophyll concentrations in the seawater were also assessed with conventional methods to find potential driving forces of viral communities. Viral abundances did not differ within the surface layers, but significant differences were seen between surface and 1000 m samples (one-way ANOVA, $p<0.05$); abundances were significantly lower at 1000 m and the resulting virus-to-bacteria ratio (VBR) was significantly higher at 1000 m than at the other depths (post hoc Tukey tests, $p<0.05$). Lytic viral production did not differ between the sampling depths and was estimated between 4x105 and 3x106 viral particles mL-1 h-1. Viral community composition differed with depth at the taxonomic levels of family (PERMANOVA, Pseudo-$F_{3,10}=6.23$, $p<0.01$) and genera (PERMANOVA, Pseudo-$F_{3,10}=3.59$, $p<0.01$). Communities at 50 and 1000 m showed the most discrete patterns. Within the assigned contigs, the dominant families were Podoviridae (29±3%) and Siphoviridae (23±3%). Significantly lower contribution of Podoviridae and Siphoviridae was seen at 1000 m. Using the distance-based Redundancy Analysis test of the distance-based linear model fitted to the set of the abiotic and biotic variables, we found that the viral community composition at the family level was best explained by dissolved oxygen concentration and bacterial production, while the concentrations of chlorophyll and total nitrogen, and salinity increased the explained variability (adjusted $R2=0.75$, stepwise type of selection, $p<0.05$). A list of 143 auxiliary metabolic genes was counted in the viral metagenomes and then associated with a metabolic pathway. AMG content differed with depth (PERMANOVA, Pseudo-$F_{3,10}=4.32$, $p<0.01$); samples at 1000 m were the most discrete ones, with AMGs involved in “amino acid metabolism” and “carbohydrate metabolism” showing peculiar percentage contribution.

PP_118

SHOTGUN METAGENOMICS ASSESSMENT OF THE RESISTOME, MOBILOME, PATHOGEN DYNAMICS AND THEIR ECOLOGICAL CONTROL MODES IN FULL-SCALE URBAN WASTEWATER TREATMENT PLANTS.

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Antibiotic resistance is a global issue of utmost significance. Urban wastewater treatment plants (UWTPs) are considered evolutionary hotspots for multi-drug resistance of bacteria with known pathogenic potential. The conventional activated sludge (CAS) process, widely used worldwide for the biological treatment of urban wastewater, was shown to remove the majority of the bacterial load. It is known however, that pathogenic microorganisms and antibiotic resistance genes (ARGs) remain in final treated effluents, consequently discharged into receiving environments. Membrane bioreactors (MBR) are good alternatives to CAS systems in reducing the load of ARGs and pathogens in treated effluents. To this end, the overall performance of two full-scale UWTPs, an MBR and a CAS system was assessed, while shotgun metagenomics was employed to determine the fate of putative pathogenic bacteria and resistance determinants. Their co-occurrence patterns with biocide resistance genes (BRGs), mobile genetic elements (MGEs) and bacterial-predatory microorganisms were also explored to define microbial interactions that facilitate dispersal of ARGs and/or control putative pathogenic bacteria in UWTPs. The two examined UWTPs showed a significant difference ($p<0.05$) in BOD, COD and TSS (97.6-99% reduction) between their influent and effluent concentrations, while there was a variation in TP values with a reduction of 94% after CAS and 61% after MBR treatment. A higher decline in the abundance of pathogen-containing taxa and ARGs was observed in the MBR effluents compared to that of CAS. MBR treatment favored the abundance of ARGs associated with triclosan or with naturally occurring antimicrobials like streptothricin, among those detected. In contrast, CAS effluents contained a diverse set of ARGs associated with antibiotics of clinical importance. Correlations between putative pathogenic bacteria, ARG/BRGs/MGEs and bacterial-predatory microorganisms showed that: (i) opportunistic pathogens like Clostridia and Nocardia may acquire resistance traits against first-line treatments and (ii) that bacteriophages play an important role in controlling bacterial populations in such systems and thus, may be considered as a natural mechanism for pathogen-specific removal in UWTPs. Overall, these findings reinforce the capacity of MBR systems of retaining pathogenic loads, hence reducing the potential health risks associated with the reuse of treated wastewaters or their disposal in aquatic environments.

PP_119

MONITORING THE ACTIVITY OF AN INNOVATIVE SURFACTANT BASED ON TIO2 NANOPARTICLES AGAINST FOODBORNE PATHOGENS IN LABORATORY SCALE AND IN FOOD INDUSTRY

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Biofilms have been associated with several problems in the food industry as the conditions in food processing environment may support biofilm formation. Biofilms may pose a serious health hazard because upon subsequent de-attachment, that constitutes a significant source of food contamination and leading to foodborne disease outbreaks. To this respect, emphasis should be given on the removal of microorganisms from food industry surfaces by the use of disinfectants. In this study, the disinfecting and antibiofilm effectiveness against foodborne pathogens was checked in two food industries, as well as on surfaces and materials used in food industry. More specifically, the potential antimicrobial effect of the developed product against growth and survival of Salmonella, Listeria monocytogenes and Escherichia coli was monitored. For the pilot application, swabbing was performed before and after application of the disinfectant for 10 (pilot 1) and 5 (pilot 2) minutes, and the presence of Salmonella, Listeria monocytogenes and Escherichia coli was monitored by microbial enumeration. In addition, the pathogens were left to form biofilm in mono- or co-cultures on stainless steel (SS) coupons immerged in TSB at 20°C for 6 days. After biofilm formation, the SS coupons were covered by the disinfectant with different concentrations of TiO_2 (5, 10, 20 and 50% w/v) and exposed to UV for 2h for each side, separately. Biofilm population was enumerated by bead vortexing-plate counting method. According to the obtained results, 10 minutes of application in food industry, found to be effective for the reduction of Listeria and E. coli while Salmonella was not detected in both industries. However, the reduction of Salmonella Enteritidis, L. monocytogenes and E. coli population of mature biofilms was less than 0.5 log in most cases. In conclusion, the use of the surfactant based on TiO_2 nanoparticles as an alternative way of cleaning contaminated surfaces presents an intriguing case that may provide a powerful solution regarding surface disinfection within the food processing environments.

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PP_120

IMMUNOMODULATING ACTIVITY OF PLEUROTUS ERYNGII FOLLOWING ITS IN VITRO FERMENTATION BY HUMAN FECAL MICROBIOTA

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Introduction: Edible mushrooms have been used for centuries in traditional medicine as enhancers of longevity and well-being. Current research has identified many of their health-promoting properties, ranging from antioxidant, antimicrobial and anticancer activities to immune enhancement and prebiotic action. These beneficial effects have been attributed to a plethora of biomolecules that are found in these mushrooms, especially polysaccharides, with beta-glucans being in the spotlight lately, due to their possible prebiotic activity on gut micro-organisms.

Methods: In the present study, we investigated the immunomodulatory properties of Pleurotus eryngii, an edible mushroom rich in beta-glucans, selected due to its strong lactogenic effect and anti-genotoxic properties. Lyophilized P. eryngii underwent in vitro static batch fermentation for 24 hours in the presence of human fecal microbiota from 5 healthy elderly volunteers (aged >60 years old). Consequently, the fermentation supernatants (FSs) were administered at a concentration of 1% v/v in U937-derived human macrophages for 6 and 24 hours. Gene expression of pro- and anti-inflammatory cytokines of interest (IL-1β, IL-1Ra, IL-8, IL-10 and TNFα) was assessed via real-time PCR. The corresponding protein levels released in the cell culture supernatant were assessed via immunoassays. Additionally, the effect of FSs corresponding to selected volunteers on their PBMCs was investigated through CyTOF analysis.

Results: The presence of P.eryngii in the fermentation process led to altered immune response, as indicated by the altered gene expression and protein levels of pro- (TNFα, IL1β, IL-8) and anti-inflammatory cytokines (IL10, IL1Rα) in human macrophages. This finding was consistent for all volunteers. CyTOF analysis performed in selected volunteers' PBMCs treated with FSs under the same conditions revealed that the products of the in vitro fermentation in the presence of P.eryngii affected the subpopulations distribution within monocytes in PBMCs.

Conclusion: Overall, the products of the in vitro fermentation of P.eryngii by human fecal inocula clearly affect the cytokine expression and alter the monocytes' distribution in PBMCs of healthy elderly volunteers, suggesting a potent immunomodulatory action for this edible mushroom. Further investigation is currently ongoing to underpin the molecular mechanisms and correlate these findings with fermentation metabolites and fecal microbiota composition.

PP_121

IN VIVO GENOPROTECTIVE PROPERTIES OF PLEUROTUS ERYNGII HOT WATER EXTRACT

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The exploitation of natural bioactive ingredients is a current trend in cancer and other multifactorial diseases prevention. Edible mushrooms are known for their beneficial biological properties, and many studies indicate that at least some of these activities are due to their prebiotic effect which alters the gut microbiome and consequently, affect the immune-responses and various metabolic pathways.

In the present study, the anti-genotoxic effect of Pleurotus eryngii, an edible mushroom from Greek habitats of the Greek flora, was investigated in vivo. Young (8-9 weeks old) and aged (17-18 months old) CD-1 mice were treated orally (gavage), once daily, with mushroom's hot-water extract at different dosing regimen for 14 days. At the end of the treatment period, the genotoxic agent cyclophosphamide was administered intraperitoneally. The genoprotective properties of the mushroom extract were assessed in whole blood cells and bone marrow cells, against the damage caused by cyclophosphamide, by the micronucleus assay. Furthermore, the ability of the extract to regulate the antioxidant and inflammation mechanisms of the cell were studied by quantifying the mRNA expression levels of Nrf2 and Nfkβ genes in gut and liver tissues, using qRT-PCR.

According to our results, the . eryngii extract exerts significant genoprotective activity mainly in the bone marrow cells of both young and aged animals. After the administration of the mushroom extract, NrF2 expression levels were increased in liver whereas, Nfkβ levels were increased in gut. These results are indicative of alterations in antioxidant defense, inflammation and gut homeostasis signaling pathways. However, further studies are needed in order to elucidate the P. eryngii health promoting properties and its beneficial for the organism mode of action.

PP_122

EFFECT OF DAILY CONSUMPTION OF A NOVEL BISCUIT ENRICHED WITH PLEUROTUS ERYNGII MUSHROOM POWDER ON INTESTINAL HEALTH-RELATED PARAMETERS: A CROSS-OVER, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL STUDY

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The purpose of the present ongoing study is to assess the effect of the daily consumption of a novel biscuit (enriched with P. eryngii mushrooms rich in β-glucans) on gut health related parameters of healthy subjects over 60 years old.

Apparently healthy participants of both sexes, between 60-80 years old, who met the eligibility criteria, provided us with biological samples and were randomly assigned in a double-blind manner to one of the intervention groups, i.e., daily consumption of the novel biscuit (biscuit enriched with mushroom powder containing 3g of β-glucans) or daily consumption of the placebo biscuit for 3 months. After a washout period of 2 months, the subjects consumed the novel biscuit or the placebo biscuit in a cross-over design for further 3 months. Throughout the interventional period subjects' dietary intake, anthropometrical data, biochemical markers and food's tolerance markers (i.e., daily stool frequency, 7-days recorded gastrointestinal symptoms focusing on abdominal pain, distension, flatulence and borborygmi) were monitored with the use of validated questionnaires. Gut microbiota parameters analysis (qPCR-based quantification of selected bacteria) was also performed. Differences in the end compared to the baseline of each trimester were analyzed and a p-value of <0.05 was considered statistically significant.

Preliminary analysis on data from 13 participants pointed out that there were no significant differences on anthropometrical or biochemical measurements and food's tolerance markers, with no recorded side effects between placebo and mushroom-enriched groups. Baseline levels of quantified microbes were comparable between groups, independently of the type of biscuit consumption. Interestingly, when taking into consideration the pre-existing gastrointestinal healthy background, our analysis revealed a lactogenic effect by displaying a significant relative increase (%) of the Lactobacillus group levels only in the group who consumed the enriched biscuit.

Our preliminary data highlighted the safe use of a novel biscuit enriched with P. eryngii mushrooms and indicated some positive effects on selected microbial populations. Further analysis could untangle its prebiotic potential and reveal future exploitation prospects.

PP_123

THE EFFECT OF VINTAGE AND GRAPE CULTIVAR ON WINE YEAST COMMUNITY COMPOSITION

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The yeast community associated with grapes is an essential part of wine production with significant effects on product quality. Previous studies have unravelled various environmental drivers of grape fungal community structure, but we have not as yet a clear picture about the vine variety or the vintage effect. In the present study, we analysed the wine yeast populations associated with four different grape varieties from the Greek national collection vineyard, growing under the same micro-environmental conditions, with the aim to evaluate the effect of varietal factor on yeast community assembly. The vintage effect was also considered by sampling grapes for two consecutive years. Fourteen yeast species were recovered and genotyped by molecular fingerprinting techniques. A relatively stable yeast community structure was detected in different vintages, with Hanseniaspora guilliermondii being the core species of the vineyard under study. Importantly, it was shown that different grape cultivars were associated with distinct yeast populations. Strains within each species showed a relatively high genetic similarity to each other with several genotypes persisting in different vintages. This study shows that the varietal factor is an important driver of vineyard-associated wine yeast community structure, while the community remains rather stable in different vintages.

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PP_124

BIODIVERSITY OF THE WINE YEAST TORULASPORA DELBRUECKII POPULATIONS ACROSS GREEK VINEYARDS

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Non-Saccharomyces yeasts are an important regulator of the vine-wine system function. It also provides an important reservoir of promising enological phenotypes for the wine industry. In view of the upcoming climate change and its detrimental effects in winemaking and wine quality, the isolation and characterization of special wine yeast strains has become a great challenge. Torulaspora delbrueckii is a non-Saccharomyces yeast that is commonly employed in winemaking. The aim of this study was 1) to estimate the genetic diversity of T. delbrueckii across Greek vineyards and 2) to evaluate the winemaking potential of various strains. T. delbrueckii isolates were recovered from spontaneously fermented grape musts originating from 3 different wine-growing regions. Species identification was accomplished by analysis of the 5.8S-ITS rDNA region. Strain differentiation was investigated by different molecular fingerprinting methods (TRtRNA-PCR, RAPDs, and SSRs). SSRs showed the highest discrimination power. Interestingly, distinct strains were associated with their vineyards of origin, except for two strains that showed broad dispersal. Different strains showed diverse fermentation profiles. Present results show that Greek vineyards harbor several promising T. delbrueckii genotypes for the selection of special phenotypes for the wine industry.

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PP_125

DEVELOPMENT OF FOOD PRODUCTS ENRICHED WITH PROBIOTIC CULTURES EMBEDDED IN PREBIOTIC MATRICES

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Probiotic microorganisms are widely known for their beneficial effects on human and animal health and well-being. Various bacterial genera may be used as probiotics between them, Lactic Acid Bacteria (LAB) are recommended as a high-value source of probiotic microorganisms. The species of the genus Lactobacillus appear to have significant importance for novel probiotic properties in this group, originated both from the gastrointestinal tract (GIT) and from dairy products.

The laboratory of Microbiology of National and Kapodestrian University of Athens participates in ProbiYo Project, entitled "Development of food products enriched with probiotic cultures embedded in prebiotic matrices". The aim of the project is to promote partnership in R&D activities involving Greek and Chinese research institutions and companies which manufacture and distribute food products, with an emphasis on the development and application of innovative ingredient technologies for the production of novel functional dairy products, including potential cancer and gut barrier function preventative activity.

Our work is to provide the partners of the project with freeze-dried novel probiotic strains, derived from dairy products and the gastrointestinal tract. From Greek Protected Designation of Origin (PDO) fermented products, such as Feta cheese, Gruyere cheese, Kasseri cheese, and Goat yogurt, 240 isolates were derived. Furthermore, 92 isolates were recovered from fecal specimens of ten healthy, breast-fed, full-term infants, sampled at the age range of four to ninety days. All isolates were characterized by 16S rDNA gene sequencing and were examined for their probiotic potential. Five specific probiotic assays were performed according to in vitro assays suggested by the Food and Agriculture Organization/World Health Organization to each one of them; namely adhesive properties, antimicrobial properties toward pathogenic bacteria, antibiotic susceptibility, tolerance to low pH, and bile salts. 13 Lactobacillus sp. isolates exhibited satisfactory probiotic properties and their impact on carcinogenesis will be evaluated in In vitro systems of breast and colon cancer cell lines.

PP_126

EFFECT OF HIGH PRESSURE COMBINED WITH TEMPERATURE ON THE SHELF LIFE OF ORANGE JUICE CONTAMINATED WITH ALICYCLOBACILLUS ACIDOTERRESTRIS SPORES

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Nowadays consumers demand healthier and minimal-processed products, therefore juices with the above standards have an important place in human diet. One of the main problems in fruit juice industry is the presence of Alicyclobacillus acidoterrestris spores. Although this microorganism is not pathogenic, it has the ability to form guaiacol that can cause spoilage, which is difficult to be detected before consumption of the juice, due to the lack of acid or gas production. High pressure processing (HPP) combined with mild temperatures has been indicated to have high potential in guaranteeing microbiological safety with minimal effects on the sensory and nutritional characteristics of the juice. The objective of this work was to study the effect of HPP against two different strains of A. acidoterrestris a wild type strain - (strain A) and a reference strain (DSMZ 2498). For this purpose, spores were inoculated in pasteurized orange juice (pH 3.7, 11.45 °Brix) and treated with HHP of 600 MPa with combination of heat at 60 ℃ for 5 and 10 min. The spore population was evaluated before, after the treatment and during storage at 4, 12 and 25 ℃. It was shown that the spores population reduced from 3-3,5 log after the HHP treatment and remained stable during storage, taking in consideration small fluctuations. The results showed that HHP may enhance the shelf life of fresh juice when contaminated with A. acidoterrestris.

PP_127

RAPID SPOILAGE ASSESSMENT OF CHICKEN BURGERS AT ISOTHERMAL AND DYNAMIC TEMPERATURE CONDITIONS BY FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

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In the present study, FT-IR spectroscopy was evaluated as a rapid method to predict the spoilage of chicken burgers. Burgers (100g each) of two batches were stored aerobically at 4, 8, 12 and 16°C (isothermal conditions) and at 4-8-12°C/8h (dynamic conditions). The dynamics of total viable counts (TVC), pseudomonads, Brochothrix thermosphacta, lactic acid bacteria (LAB), Enterobacteriaceae and yeasts were determined by microbiological analyses (duplicates) and FT-IR spectra were collected (triplicates) at regular time intervals.

The TVC (initial 6.02±0.1 logCFU/g) progressively increased exceeding 9 logCFU/g by the end of storage at both isothermal and dynamic conditions. Pseudomonads dynamics were in accordance with TVC, suggesting them as the dominant group, followed by Brochothrix thermosphacta and LAB which reached high populations by the end of all storage conditions. In an attempt to predict the spoilage of the product, the microbiological counts were correlated with spectral data (1800-900 cm-1) and partial least squares regression (PLS-R) were developed.

The models were calibrated and externally validated with data collected from isothermal and dynamic conditions, respectively, for the main microbial groups associated with the spoilage. The obtained values of Bf factor were close to unity, indicating no systematic over/under-prediction of the models and specifically were 0.996 for TVC, 1.034 for pseudomonads, 1.038 for Brochothrix thermosphacta and 1.929 for LAB. Except for the case of LAB, good Af values were also obtained, showing that predictions are close to observations and ranged from 1.052 for TVC, to 1.084 for pseudomonads and 1.08 for Brochothrix thermosphacta. The RMSE and R2 values were higher than 0.80, while also the %PE values were always found higher than 70% for all microbial groups, except for the case of LAB.

The developed models gave an overall good performance, with TVC and pseudomonads models giving better predictions. In conclusion, the use of FT-IR spectroscopy may serve as a rapid and reliable tool to estimate the spoilage of the product.

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OCCURRENCE OF THE MOST COMMON MYCOTOXINS FOUND IN CEREALS AND CEREAL-BASED FOOD PRODUCTS: A REVIEW STUDY

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The global production of cereals exceeds 2,667.64 billion metric tons (BMT) (agricultural year 2020-21) to cover the ever-increasing requirements of human diet and livestock feed. According to Food and Agriculture Organization (2020), one of the main reasons that contribute to the excessive cereal grains waste (~30% of the annual worldwide production) is the contamination of agricultural products with mycotoxins. Mycotoxins can be produced at all stages of the food and feed supply chain. Their occurrence is mainly affected by the environmental conditions prevailing during the harvest and storage period of cereals. The present review is based on international published research articles, the majority of which focus on maize, which is the most highly produced cereal in the world (≈1,125 BMT/2020-21), followed by wheat and rice with annual productions of 775.8 and 505 BMT, respectively. Most surveys show mycotoxins' prevalence in almost 25% of the global commodities. Although the studies indicate that more than one mycotoxin may occur in cereals grains and their derived products (flours, starches, breakfast cereals, infant formulas), it was observed that the most frequent mycotoxins are Aflatoxins (66%), followed by Ochratoxin A (48%), Fumonisins (38%), Zearalenone (33%) and Deoxynivalenol (30%). Yet, the vast majority of contaminated food samples don't exceed the established maximum permitted limits. Various chemical and biochemical methods have been developed to detect and quantify mycotoxins in food and animal feed. The most widely used are chromatographic techniques such as High-Performance Liquid Chromatography since they provide accurate and reliable results simultaneously with significantly low limits of detection and quantification. Recent approaches introduce rapid, non-invasive analytical methods such as Fourier Transform Infrared Spectroscopy to monitor the presence of mycotoxins in cereals. In this direction, more efforts are needed to apply new, faster, economical and less time-consuming analytical methods that will improve significantly the mycotoxins monitoring and thus increase food safety, food security and reduce food waste.

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PP_129

VOLATILOME OF OCHRATOXIGENIC FUNGI INTERACTED WITH SACCHAROMYCES CEREVISIAE

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Foods including cereals, coffee beans, grapes, dried vine fruits, and wine are all susceptible to fungal spoilage, which is the leading cause of quality degradation through the production of a variety of metabolites including toxins. Among them, Aspergillus carbonarius and Penicillium verrucosum are two of the most common ochratoxin A (OTA) producing fungi. The purpose of this work was to describe the volatilome of the above fungi grown in vitro on CYA (Czapek Yeast Agar) medium with/without the presence of the yeast Saccharomyces cerevisiae. To this end, headspace vials (20 mL total volume) were used to grow the fungi as mono-culture and in co-culture with Saccharomyces cerevisiae. The analytical method of solid phase microextraction GC-MS was employed for the identification of the volatile organic compounds profile (VOCs) during a 7-day incubation period at 25 °C. Seventy-one (71) different volatile compounds were identified for A. carbonarius and eighty-six (86) for Penicillium verrucosum until day 7. Alcohol and ester metabolites were detected in highest concentrations for A. carbonarius, whereas the level of ketones remained unchanged during the incubation period. Some characteristic sisquiterpenes such as a-cadrene and α-Guaiene were also detected. On the other side, most sisquiterpenes of Penicillium verrucosum were detected at day 5, such as α-Terpinene, Cabrene A, b-Farnesene (E). Alcohols, esters, ketones, acids, alkanes, heterocyclic aromatic organic compounds, and benzene compounds were also detected for P. verrucosum. The inhibition of fungal growth could be perceived by comparing VOCs profile of fungi during mono and co-culture with S. cerevisiae. The accumulation of compounds associated with yeast growth, especially the ethyl ester group, could be a possible indication of the inhibiting action of S. cerevisiae against fungal growth.

PP_130

USE OF FOURIER-TRANSFORM INFRARED SPECTROSCOPY IN TANDEM WITH MACHINE LEARNING FOR THE ESTIMATION OF MICROBIOLOGICAL QUALITY OF CHICKEN LIVER IN THE EVENT OF SALMONELLA CROSS-CONTAMINATION

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Chicken liver has a limited shelf-life, while it is generally recognized as a vehicle for the transmission of foodborne pathogens, such as Salmonella. This study evaluated the ability of Fourier-transform infrared spectroscopy (FTIR) to assess chicken liver microbial spoilage under conditions simulating potential Salmonella cross-contamination, through the development of a spectral analysis and prediction building workflow. Chicken liver samples, non-inoculated and inoculated with Salmonella (four strain-cocktail), were stored under isothermal (0, 4, and 8°C) and dynamic temperature conditions. At regular time intervals samples (n=4, two batches) were retrieved and were subjected to 1) microbiological analysis for the enumeration of selected microbial groups and 2) FTIR measurements (n=12). To overcome the high dimensionality of spectral data a feature selection step based on extra-trees algorithm was initially introduced. Then, a support vector machine (SVM) regression analysis with radial basis function (RBF) kernel was applied for model development and validation. The dataset from both non-inoculated and inoculated with Salmonella samples (to incorporate in the model the biochemical fingerprint of the pathogen potentially present in the processing line) was randomly partitioned over 50 iterations into a training and test dataset (70% and 30% of the samples, respectively) for model development and external validation. The performance of the models was evaluated by calculating the root-mean-square error (RMSE), the correlation coefficient (R2), the bias (Bf) and accuracy (Af) factors, and the accuracy of prediction (A%). The developed models achieved a reasonably good prediction accuracy between the measured via the microbiological analysis and the estimated microbial populations (ranging from 71.76% to 84.33%, depending on the microbial group). The calculated R2 and RMSE values ranged from 0.737 to 0.797 and 0.696 to 0.949 log CFU/g, respectively, for the different microbial groups indicating a satisfactory relationship between spectra and the specific spoilage microorganisms studied. Results of this study demonstrate the considerable potential of FTIR spectroscopy in tandem with the proposed machine learning pipeline to satisfactorily describe chicken liver spoilage in case of Salmonella cross-contamination.

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PP_131

IDENTIFICATION OF AEGINA'S PISTACHIOS YEAST FLORA AND INVESTIGATION OF INTERACTION WITH MYCOTOXIN PRODUCING FUNGI ASPERGILLUS FLAVUS AND ASPERGILLUS CARBONARIUS.

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Pistachios of Aegina Island in Greece are a type of nuts that can be considered as one of the healthiest and delicious snacks due to their high content of unsaturated fats, minerals, and antioxidant polyphenols. Pistachios have been recognized as a PDO product since 1994. They are mainly affected by fungi, specifically Aspergillus spp. so it is important to employ an antifungal treatment based on biological control without the use of chemicals. Based on this goal, the antifungal activity of indigenous yeasts of pistachios was studied on the growth of mycotoxin producers Aspergillus flavus and Aspergillus carbonarius. Fungal growth was assayed in vitro in the presence and absence of the yeasts on Malt Extract Agar. The plates were incubated at 25°C for 14 days. The majority of the indigenous yeasts inhibited the growth of the two fungi. Specifically, the growth of A. carbonarius was inhibited to a greater extent compared to A. flavus. Subsequently, a total of 49 yeast isolates were identified at species level using sequencing analysis of the D1/D2 domain of 26S rRNA gene with pair primers NL1-NL4. The isolated strains were grouped to 5 species and identified as Aureobasidium pullulans, Rhodotorula spp., Vishniacozyma carnescens, Cryptococcus magnus and Kwoella dendrophila. The results of this study suggest that the indigenous yeast flora of pistachios is potentially effective as biological control agent to A. flavus and A. carbonarius.

PP_132

LUMINOUS PHOTOBACTERIUM SPP. IN CHICKEN MEAT SPOILAGE: GROWTH PATTERNS AND GENOTYPIC DIVERSITY

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Microbial chicken meat spoilage has been traditionally attributed to genera Pseudomonas, Brochothrix thermosphacta, Enterobacteriaceae and lactic acid bacteria (LAB). Yet, recent metagenomic analyses on spoiled chicken meat revealed the presence of genus Photobacterium at significantly high abundances. This study was conducted to a) assess the presence and growth patterns of luminous Photobacterium spp. on refrigerated chicken breast fillets and b) explore and describe their genotypic biodiversity. Chicken breast fillets (2 samples/batch, 3 batches) were stored under aerobic conditions at 4°C. Samples were analyzed microbiologically for the estimation of total mesophilic bacteria, Pseudomonas spp., Brochothrix thermosphacta, LAB, Enterobacteriaceae and presumptive Photobacterium. For the characterization of presumptive Photobacterium, a total of 91 luminous isolates were recovered from Marine Broth (MB) agar medium at the beginning (time of first appearance), middle and end stages of storage (shelf life, 144h). Bacterial identification at the genus/species level was performed by sequencing of 16S rRNA and gyrB gene. Different DNA fingerprinting techniques (RAPD and rep-PCR) were applied for differentiation of presumptive Photobacterium isolates. The microbial community of fillets was largely dominated by Pseudomonas (ca. 8.2 logCFU/g) followed by B. thermosphacta (ca. 7.5 logCFU/g), while levels of LAB and Enterobacteriaceae remained at lower levels (ca. 5.4 and 4.8 logCFU/g, respectively). Luminous bacteria were scarce at the beginning of storage (detected in one out of three batches at ca. 2.0 logCFU/g). However, they were encountered at relatively high populations, ranging from 5.3 to 7.0 logCFU/g, at later stages of storage in chicken breast samples from two batches, while they were below the detection limit in samples of the third batch. In the latter case, luminous bacteria were sporadically encountered throughout storage at populations of up to 3.9 logCFU/g. Genetic fingerprinting revealed numerous highly diverse DNA fingerprints of luminous bacterial isolates. Sequencing analysis assigned these isolates to Photobacterium genus. Results of the present study highlight the potent role of Photobacterium spp. to chicken meat spoilage and reveal the significant genetic biodiversity of Photobacterium isolates.

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THE POSITIVE EFFECT OF CORN OIL ON NUTRITIONAL ATTRIBUTES OF PLEUROTUS MUSHROOMS

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The effect of corn oil, used as additive into wheat straw (WS) and barley and oat (BOS) cultivation substrates, on the nutritional properties of Pleurotus mushrooms is presented. P. ostreatus (AMRL 150) and P. eryngii (AMRL 173-6) after cultivation in BOS and WS substrates enriched with commercial corn oil (2 and 5% w/w)were dried at 45°C/2days and analyzed for their total lipid, protein and intra-cellular polysaccharidecontent in respect to control samples (no addition of oil). Results supported the positive effect of corn oil on all parameters tested. Total lipid content values of both P.ostreatus and P. eryngii were the highest at BOS substrate and 5% oil addition,whereas theypresented a two-fold increaseat WS and a three-fold at BOS regardless the oil concentration used compared to the control (5.23 and 6.87% w/w in contrast to 2.32 and 3.23% w/w).Total protein values of P. ostreatus have been significantly increased from 10.9% to 16.7 (2% corn oil) and 18.4% w/w (5% corn oil) at WS and from 15.9 to 20.5% w/w (5% corn oil) at BOS, while for P. eryngiithe protein content was significantly lower (about 10% w/w) for both substrates without or with the presence of oil.Regardingpolysaccharide concentration, it was also positively affected by the presence of oils (particularly at 5%) supplemented to WS at both P. ostreatus and P. eryngii strains (60.78 compared to 52.22% w/w and 54.06 compared to 37.88%w/w respectively) and so did P. ostreatus at WS (51.16 to 37.78% w/w). At P. eryngii mushrooms, polysaccharides presented high values (52% w/w) that remained almost stable at all treatments of BOS. Mushrooms' polysaccharides, proteins and fatty acids have shown multiple benefits on human healthused as food and by constituting ingredients for formulation of functional foods and nutraceuticals, so their enhancement through mild alteration of substrate synthesis and cultivation technique is an easy and promising way. The use of BOS substrate instead of WS in commercial cultivation of mushrooms is positive for financial and environmentalreasons too.

PP_134

YEAST DIVERSITY OF FERMENTED GREEK TABLE OLIVES BY METAGENOMIC ANALYSIS INDICATES OLIVE VARIETY AND DESIGNATION OF GEOGRAPHICAL ORIGIN

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Next generation sequencing (NGS) technology was applied to assess the yeast diversity of fermented table olives of two Greek varieties collected from different regions. The main inquiry was whether the metagenomic analysis of yeast communities is capable of providing information regarding the olive variety and designation of geographical origin. A total of 34 olive samples of cv. Halkidiki were collected from Kavala and Halkidiki region and cv. Konservolia, from Magnesia and Fthiotida region from two seasons. DNA was directly extracted from the olives' surface and subjected to NGS for the identification of yeast communities. Pichiaceae was the most abundant detected family in most of the cases. In brief, the family Pichiaceae was detected in higher percentage in Halkidiki olives regardless the region of origin or season. However, differences were observed at species level, where Pichia manshurica was the most abundant species in 3 (out of 6) and 6 (out of 9) samples from Kavala and Halkidiki region, respectively. On the other hand, Brettanomyces custersianus was the most abundant species in the rest samples from Kavala, and Pichia membranifaciens in 4 samples collected the first (2 samples) and second (2) season. In one sample of Halkidiki olives, Schwanniomyces etchelsii of Debaryomycetaceae family was the most abundant species. In the case of Konservolia olives, Phaffomycetaceae was the most abundant family in 4 samples from the Magnesia region. Pichiaceae dominated the yeasts microbiota in 2 samples from Magnesia and from Fthiotida. At species level, Wickerhamomyces anomalus was detected in 4 samples and Pichia membranifaciens in 4 samples from Magnesia. In olives collected the first season from Fthiotida, different species i.e Pichia manshurica (3 samples), Pichia membranifaciens (2), Brettanomyces custersianus (3) και Aureobasidium pullulans (1) dominated the yeast community. On the other hand, Candida boidinii was the most abundant species detected in Konservolia olives of second period. In conclusion, the results obtained reveal the complex structure of the microbiota in olive fermentations and the microbial key taxa that may be linked to specific geographic areas.

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PP_135

MICROBIAL DYNAMICS DURING FERMENTATION OF BLACK OLIVES CV. KONSERVOLIA FROM DIFFERENT ALTITUDES OF FTHIOTIDA REGION EVALUATED BY CULTURE-DEPENDENT AND -INDEPENDENT METHODS.

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The present study aims to assess the microbial ecology of fermentation of olives from different altitudes of a geographical region. The spontaneous fermentation process of black olives cv. Konservolia from different altitudes (mountainous and lowland) of Fthiotida region was monitored for 130 days via microbiological and physicochemical analyses. Moreover, the microbiota of fresh and fermented olives of both altitudes was determined by next generation sequencing (NGS). Microbiological analysis, reveal that lactic acid bacteria dominated the fermentation process, with levels of ca 6.5 logCFU/g, while yeasts population started from 2.5 log CFU/g, and reached ca 4.8 logCFU/g at the end of the fermentation in both cases. The population of Enterobacteriaceae decreased gradually reaching the enumeration detection limit (1 log CFU/g) in 20 days, while the pH in the brine progressively decreased reaching ca 4.2 at the end of the process. The NGS analysis of yeasts revealed Sporidiobolaceae and Saccotheciaceae as the dominant families on fresh mountainous olives with Aureobasidium pullulans, Rhodotorula sp., Zygowilliopsis californica and Cryptococcus sp. being the most abundant species. Alternaria alternata of Pleosporaceae family was the most abundant species on fresh lowland olives. Pichiaceae was the dominant family on fermented olives from both regions with Candida boidinii being the most abundant species. Moreover, the Aspergillaceae family and Penicillium roqueforti was found in great abundance on fermented olives from the mountainous region.
The results indicate that the altitude of the geographical region as well as the fermentation process affected the yeast diversity. Indeed the diversity was higher on fresh olives growing in mountainous area than those from the lowland area. Furthermore a higher diversity observed in yeast population of fresh olives compared to fermented ones.

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IMMOBILIZED PROBIOTICS AS EFFECTIVE DELIVERY VEHICLES ENSURING CELL SURVIVAL UNDER SIMULATED GASTROINTESTINAL TRANSIT.

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Over the past decades, an upsurge of interest in developing novel functional foods containing pro- and prebiotic constituents, such as lactic acid bacteria (LAB) and dietary fibers is witnessed. Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”. According to the International Probiotics Association (IPA) Europe, probiotic products need to contain an adequate amount of live bacteria in order to achieve a daily intake of at least 109 viable cells, able to survive the harsh degradative conditions of the upper gastrointestinal tract and induce the health benefits. Nevertheless, this requirement is not always fulfilled. Previous studies have documented maintenance of high cell viability of immobilized cells. In this vein, the effect of immobilization on cell survival of a new presumptive probiotic Lacticaseibacillus rhamnosus strain isolated from Greek olives, with potential antidiabetic capabilities, was evaluated under simulated digestion and compared to free cells. Notably, both wet and freeze-dried immobilized cultures on cereals and fruit pieces retained high cell loads at levels significantly higher compared to free cells after simulated gastrointestinal (GI) transit. In particular, immobilized cells on oat and wheat flakes were encountered > 6 logcfu/g, while the corresponding values for immobilized cells on apple and banana pieces were significantly lower (> 4 logcfu/g) after simulated digestion. On the contrary, the levels of free cells were drastically reduced down to 3.51±0.12 logcfu/mL In conclusion, the results clearly suggested the potential use of immobilized cultures as suitable delivery vehicles for beneficial microorganisms that ensure cell viability during GI transit.

PP_137

THE EFFECT OF SWEETENERS ADDITION ON LISTERIA MONOCYTOGENES AND SALMONELLA ENTERITIDIS GROWTH DYNAMICS

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The increasing prevalence of obesity and various metabolic diseases worldwide, has been partially attributed to the excessive consumption of sugar. Sweeteners have become a popular sugar substitute, allowing a variety of food products to retain their palatability with parallel reduction of the caloric content. However, it has been reported that sugar replacement may affect the microbial food safety. The aim of this study was to assess the potential effect of sweeteners addition on the planktonic growth behavior of Listeria monocytogenes and Salmonella enterica serovar Enteritidis. Six, commonly used in food industry, sweeteners were tested. The bacterial strains were inoculated with a cell population of about 102 CFU/mL in nutrient broth supplemented with i) the selected sweeteners and ii) glucose (control). Different sweeteners' concentrations were evaluated based on their sweetness equivalence as compared to 2.5 and 5% w/v glucose. Bacterial growth was monitored by means of absorbance detection times, and was quantitatively described, through the estimation of the following kinetic parameters: i) the detection time (Tdet) needed for optical density changes to occur in the growth medium, ii) the maximum slope (rate) of optical density changes (MSrODC) and the area (Area) under the optical density/time curve. Statistical analysis (ANOVA) was performed to determine any significant differences between the abovementioned treatments. With regard to the lower concentration, L. monocytogenes had a significantly better growth in broth with glucose in terms of Area and MSrODC. However, Tdet was significantly higher in glucose compared to sweeteners. Similar were the results for the higher concentration, although the Area parameter showed no statistically significant differences between glucose and the majority of sweeteners. On the other hand, Salmonella Enteritidis had significantly higher growth in broth with sweeteners in terms of Area and Tdet for both concentrations. In the case of MSrODC, the values were similar or slightly higher in glucose compared to sweeteners. To conclude, the effect of sweeteners addition should be studied thoroughly to ensure optimal food safety.

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MONITORING GROWTH AND BACTERIOCIN GENE TRANSCRIPTION OF STARTER AND BIOPROTECTIVE ADJUNCT LAB STRAIN MIXTURES IN MODEL MILK FERMENTATIONS

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Lactic Acid Bacteria (LAB) predominate and contribute in forming the desirable aroma and flavor of fermented dairy foods. Certain LAB species have a long safe use as industrial dairy starters, while numerous LAB strains produce natural antimicrobial compounds, including bacteriocins, active against food pathogens. However, the inclusion of bacteriocin-producing (Bac+) LAB in (dairy) starter cultures remains a challenge because they may retard growth of the starter strains or, vice versa, the starter/s may suppress growth and activity of the Bac+ strains. Hence, proper enumeration of LAB species and monitoring of beneficial (bacteriocin) genes' expression is crucial in studying their role and dynamics in fermented milk products.

This study aimed to monitor the growth of mixed LAB strain populations during model milk fermentations by real-time quantitative PCR (qPCR) and to assess the expression levels of bacteriocin genes by real-time reverse transcription PCR (RT-qPCR).

LAB cocultures were generated by inoculating sterilized raw milk (SRM) with Streptococcus thermophilus ST1 and Lactococcus lactis subsp. cremoris M78 as the basic starter composite (treatment A1). Enterococcus faecium KE82, E. faecium GL31 and Lactobacillus plantarum H25 were added in A1 to form treatments A2, A3 and A4, respectively. Specific genes, lacZ, nisin, entB, entA and recA, were selected for the respective detection of ST1, M78, KE82, GL31 and H25 by qPCR, and RT-qPCR allowed the quantification of the bacteriocin genes transcripts in order to study their expression levels in the cocultures at preset time-points. Nisin (nisA) synthesized by M78, and enterocins B (entB) and A (entA) synthesized by KE82 and GL31, respectively, were quantified. The expression of the selected genes was evaluated in relation to time and population, as well as compared to each other.

Streptococcus thermophilus ST1 prevailed in all treatments. M78 grew similarly with KE82 and GL31 in A2 and A3; H25 showed delayed growth in A4. Nisin expression varied between treatments; in specific, nisA and entB and entA expression was interrelated indicating an antagonistic activity, with KE82 in A2 being more antagonistic than GL31 in A3. Changes in all bacteriocins expression levels were population-independent and most likely related to changes in mRNA transcript levels.

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RESISTANT STARCH DRIVES GUT MICROBIOME CHANGES AND AMELIORATES NAFLD PROGRESSION: A RANDOMIZED, DOUBLE-BLINDED CLINICAL STUDY.

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Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of a combination of metabolic dysfunctions mainly characterized by insulin resistance, dyslipidemia, impaired glucose tolerance and cardiometabolic syndrome. The progression of NAFLD is still poorly understood, but we know that the gut microbiome plays a key role in the pathogenesis of NAFLD and contributes to the development of the disease.

Previous studies have shown that resistant starch is a promising diet for the prevention or treatment of obesity and its related diseases, as it can reduce fat accumulation, enhance insulin sensitivity, regulate blood glucose levels and lipid metabolism. To investigate the effect of resistant starch in the gut microbiome in NAFLD subjects, in this intervention study we performed metagenomic and targeted metabolomic analysis of fecal samples from 100 NAFLD subjects in week 0 and 16 in a control (CS) and an intervention (RS) group, and we integrated our findings with clinical and biochemical data.

We observed that only individuals who received the RS intervention had significantly reduced their intra-hepatic triglyceride content (IHTC) as well as reduced levels of liver enzymes and had altered lipid profiles. Alpha and beta diversity on the species level, were significantly different between CS and RS in week 16, suggesting that resistant starch induces changes in the microbiome composition. We performed statistical analysis between RS in week 0 and 16, and between CS and RS in week 16 and we found 31 significantly changing species and 17 metabolites. In particular, the levels of Branched-Chain amino acids (BCAAs) were all reduced in the RS groups. Finally, these findings were significantly correlated with the improvement of crucial clinical measurements for NAFLD.

Here, we revealed that resistant starch diet prevents the progression of NAFLD by altering the microbiome and metabolome composition. After statistical and correlation analysis, we found key species that have an important role in the progression of NAFLD and are potential therapeutic targets.

Keyword 1: gut microbiota * Keyword 2: metagenomics * Keyword 3: diet intervention

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BIOFILM REMOVAL ACTIVITY OF WILD TYPE LACTIC ACID BACTERIA

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The rising anti-microbial resistance of pathogens consists a worldwide risk to human health and thus efficient antimicrobial alternatives are required urgently. Bacterial biofilms, formed by the polymeric metabolites secreted by microbes, are one of the main resistance mechanisms that bacteria utilize to survive against various stresses, including antibiotics, disinfectants, and host defenses. The use of Lactic Acid Bacteria (LAB) to combat bacterial biofims is a rapidly growing trend. It is documented that LAB produce several active metabolites, such as organic acids, bacteriocins, hydrogen peroxides, exopolysaccharides, and biosurfactants that eliminate biofilm formation. Most of these metabolites are secreted during cultivation in a broth medium following proliferation of bacteria cells, known as a supernatant. Hence, the aim of the present study was to assess biofilm removal potential of cell-free supernatant (CFS) of 8 LAB strains belonging to Lacticaseibacillus rhamnosus, Lacticaseibacillus paracasei, Lactiplantibacillus pentosus, and Leuconostoc mesenteroides species, isolated from traditional Greek foods, formed by foodborne pathogens (Salmonella Enteritidis, Salmonella Typhimurium, Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus). Biofilm removal activity of LAB was assessed on two-days mature biofilm using microtiter plate-based procedure. The results showed that addition of either non-neutralized or neutralized CFS removed biofilms in a concentration-dependent manner. Biofilm removal activity of the non-neutralized CFS was significantly higher than neutralized, as expected. Notably, the non-neutralized CFS of a Lacticaseibacillus rhamnosus strain exhibited the highest removal activity against all pathogens. In conclusion, application of LAB CFSs seems to have a high industrial potential as agents to control biofilm formation of foodborne pathogens.

PP_141

DEVELOPING FUNCTIONAL FOOD INGREDIENTS CONTAINING IMMOBILIZED KEFIR CULTURE ON DIETARY FIBERS SUPPORTS

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Kefir, a traditional milk product, originated in Caucasus, with a slight acidic taste, natural carbonation and a pleasant aroma, fermented by a mixed symbiotic culture is associated with several health-promoting effects. To induce the health effects, functional products should contain an adequate amount of viable beneficial bacteria (>10^7cfu/g of product, according to IPA Europe recommendations), able to survive the acidic conditions of the upper gastro-intestinal tract and proliferate in the gut, a requirement that constitutes a real bottleneck for the food industry. Since the use of wet cultures is incompatible with the commercial and industrial needs, a preference to dried cultures is witnessed due to multiple technological advantages. However, the drying and the food production process, along with long-term storage, are usually related to important losses in cell counts. Having been documented that cell immobilization offers multiple technological advantages, including maintenance of microbial viability, immobilization of kefir culture on natural supports, rich in dietary fibers, such as oat and what flakes, as well as freeze-drying of the immobilized cultures was of interest in the present study. For the optimization of the immobilization process, factors, such as the incubation time of cells/food matrix, the proportion of cells/food matrix and the use of cryoprotectants during freeze-drying were studied, aiming at the highest levels of immobilized cells, and considering cost effectiveness and maximum productivity. In oat flakes, the levels of immobilized lactobacilli, lactococci and yeasts/molds ranged 7.54-8.15, 7.58-8.05 and 5.87-7.56 logcfu/g, respectively, whereas the corresponding values in wheat flakes ranged 7.02-8.03, 7.73-8.02 and 6.40-7.22 logcfu/g. After freeze drying, the highest levels were recorded when milk was used as a cryoprotectant and cell loads of lactobacilli, lactococci and yeasts/molds in oat were 7.82, 7.38 and 4.59-6.89 logcfu/g, respectively, whereas 7.42, 6.51 and 4.23-6.67 logcfu/g were noted in wheat flakes. In conclusion, a simple, easy, and cost effective process for developing functional ingredients containing immobilized kefir culture on dietary fibers, suitable for multiple industrial applications, is presented.

PP_142

RAPID MICROBIOLOGICAL QUALITY ASSESSMENT OF EDIBLE SEAWEED ALARIA ESCULENTA VIA FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FT-IR)

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Alaria esculenta is one of the most commonly cultivated edible seaweed in western countries. Given that seaweeds rapidly deteriorate and that the seaweed aquaculture sector expands, the importance of implementing rapid, real-time techniques for quality assessment becomes more apparent.

Alaria esculenta samples originated from Scotland (two harvest years, n=130) and Ireland (three harvest years, n=200), were stored under various temperature conditions for specific time intervals. Microbiological analysis was performed on the day of their arrival at the lab and at specific days of storage, assessing the Total Viable Counts (TVC) values, while in parallel, FT-IR spectroscopy analysis was conducted. The collected data were used for the development of predictive models concerning microbial populations estimation. In brief, the processing pipeline consists of feature selection, i.e., specific informative wavenumbers on the basis of random forests (RFs) regression ensemble selection, followed by partial least squares (PLS) regression coupled with an automated selection of a number of components for reducing the dimensionality. The datasets were split into training and test set, while R-square and Root Mean Square Error (RMSE) were used as metrics for the evaluation of models' performance. Three different models were developed, one for each geographical area and one for their combination.

The microbial counts presented high variability among harvest years and geographical origins, being in the range of 2.0 to 10.0 log CFU/g. The results of the models developed both for the Alaria from Scotland and from Ireland indicated a good prediction performance on the external test dataset, although exhibiting rather high RMSE values (1.84 and 1.50, respectively). The model developed with all data combined (both Irish and Scottish origin), resulted also in satisfactory prediction performance, exhibiting enhanced robustness as being origin unaware towards microbiological population prediction (R2=0.75, RMSE=1.77).

FTIR spectroscopy in tandem with machine learning was found to be promising for microbiological quality assessment of seaweeds and would enable the seaweed industry to make fast, real-time, and reliable decisions.

PP_143

ALICYCLOBACILLUS SPP. SPOILAGE POTENTIAL IN PLANT-BASED DAIRY BEVERAGES MIXED WITH FRUIT JUICES DURING STORAGE

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The scope of the present study was to assess the spoilage potential of different Alicyclobacilli in commercial pasteurized (ambient-stable) plant-based dairy beverages mixed with fruit juices at different inoculation levels and storage temperatures.

Different products (Coconut & Berry–CB; Almond, Mango, & Passionfruit–MP; Oat, Strawberry, & Banana–OS) were inoculated with 10 or 2 x 103 spores/mL of either Alicyclobacillus acidoterrestris or A. fastidiosus or A. acidocaldarius strain composites, while non–inoculated samples served as controls. Samples inoculated with A. acidoterrestris and A. fastidiosus were stored at 30°C and 45°C, while in case of A. acidocaldarius storage took place at 50°C for 240 days. Gas composition, Alicyclobacillus spp. populations, total viable counts, pH, aw, color, and guaiacol off-taste were monitored.

CB and MP supported growth of A. acidoterrestris and A. fastidiosus, reaching populations of 4.0–5.0 log CFU/mL. In OS, populations of the latter Alicyclobacilli remained close to the initial inoculation levels during storage at 30°C, while at 45°C, the populations declined <1 CFU/mL. A. acidocaldarius growth was supported only in CB samples, reaching ca. 3.0 log CFU/mL at 50°C, regardless of initial inoculum size. Total color change was increased during storage; however, the instrumentally recorded color changes were not macroscopically visible. Spoilage in terms of guaiacol off-taste, was identified only in CB and MP samples inoculated with A. acidoterrestris after 60 days at 30°C and 45°C.

Considering that these products become increasingly popular and the scarcity of existing literature related to their spoilage by Alicyclobacillus spp., the contribution of the findings and data of present study are critical for assessing the significance of Alicyclobacilli as a potential spoilage hazard in these products and thus, to assist in the design and implementation of effective mitigation strategies by the beverage industry.

PP_144

COMPARATIVE EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF HYPERICUM SPECIES BASED ON THEIR PHYTOCHEMICAL PROFILE

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Species of the genus Hypericum (Hypericaceae) have been used worldwide for centuries in traditional medicine for their curative properties. Hypericum perforatum (St. John's Wort) is the most studied species as antidepressant, antiseptic, anti-inflammatory, expectorant and tonic for the immune system. It has also been studied for its antibiotic, antifungal, antiviral and anticancer properties, which have been related to its chemical composition, specifically its phenolic compounds content. In the current study, hydromethanolic extracts of four Hypericum species (H. perforatum, H. cycladicum, H. rumeliacum ssp. apollinis, H. perfoliatum) were investigated for their in vitro antibacterial activity against four Gram positive (Staphylococcus epidermidis, S. aureus, Listeria monocytogenes, Streptococcus mutans) and two Gram negative (Salmonella enterica ser. Typhimurium, Escherichia coli O157:H7) bacterial species using two methods, i.e. agar well diffusion and broth microdilution assay with resazurin (as metabolic activity indicator). The chemical composition of the extracts was determined by liquid chromatography, and their total phenolic content was evaluated by the Folin – Ciocalteu method. Both used methods revealed the effective antibacterial activity of all the extracts against all the Gram positive bacteria, with L. monocytogenes being the most susceptible (MIC 0.02 – 0.39 mg/mL) and S. mutans being the most resistant (MIC 0.78 – 1.56 mg/mL) species. On the contrary, the Gram negative E. coli required around more than 100-fold concentration (50 mg/mL) for the same effect, whereas no antibacterial activity was observed against S. Typhimurium. Hypericum perforatum extract exhibited the most potent antibacterial activity (MIC 0.02 - 0.78 mg/mL), while the extract of H. rumeliacum had the weakest antibacterial activity (MIC 0.39 - 1.56 mg/mL). As expected, the chemical composition of each extract was found to influence its antibacterial action. Our results enforce current knowledge on the antibacterial action of the extracts of Hypericum species native in the Mediterranean region.

PP_145

A NEW LEUCONOSTOC SP. ISOLATED FROM DEBINA GRAPE MUST PRESENTING ANTIMICROBIAL ACTIVITY AGAINST BOTH GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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Lactic acid bacteria (LAB) are traditionally used as starter cultures at fermentation processes and are part of the indigenous human gut microbiota. Due to the production of ribosomally synthesized antibacterial substances termed bacteriocins, LAB can act as bioprotective and/ or probiotic strains.
In the present study, a Leuconostoc sp. strain amSF03 was isolated from the mid-phase of a spontaneous fermentation of Debina grape must, based on its in vitro antibacterial activity against the indicator strains E. coli ATCC 25922 and Bacillus subtilis. The strain was identified as Leuconostoc suionicum based on its 16S rRNA gene sequence analysis. To investigate safety aspects of strain amSF03, detection of genes encoding virulence factors was performed by PCR and antibiotic susceptibility was determined by disk diffusion and broth dilution methods. Isolate amSF03 presented resistance against kanamycin and vancomycin.
Strain amSF03 was tested for antimicrobial activity against 19 indicator bacterial strains using agar overlay method. Inhibitory activity was detected against 12 of them, including both Gram-positive and Gram-negative bacteria, with the strongest antimicrobial activity being against E. coli ATCC 25922 and B.subtilis. Maximum extracellular antimicrobial activity by the isolate was recorded in MRS medium (pH 6,5) at 30°C and it showed strong correlation with bacterial growth. Antibacterial activity was found at early exponential phase and reached its peak at the early stationary phase which remained unchanged up to late stationary phase.
Treatment of the culture supernatant with proteinase K eliminated antimicrobial compound's inhibitory activity, which proved that it is a proteinaceous molecule. Also, it remained active at pH range 4-10 and stable to 121°C for 20 min (autoclave). Cell-free supernatant of Leuconostoc suionicum amSF03 was subjected to ammonium sulfate precipitation in different saturations and pH conditions and it was shown that in optimal conditions (80% saturation, pH 6.5) the precipitants retained their activity against the two selected indicator strains (E. coli, B. subtilis) even after 3 months.
Because of its stability and bactericidal activity against both Gram-positive and Gram-negative bacteria, this antimicrobial compound could potentially be used as a promising bio-preservative in food and beverages industry.

PP_146

INVESTIGATION OF PROTECTIVE ROLE OF PROBIOTIC LACTOBACILLUS SP. STRAINS OF HUMAN ORIGIN ON EPITHELIAL CELLS AGAINST THE FOODBORNE PATHOGEN LISTERIA MONOCYTOGENES

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In the probiotic field of research special attention is given to strains that contribute to host protection against invading pathogens in the gastrointestinal tract. In the present study, we aimed to examine the potential protective role of Lactobacillus sp. strains isolated from newborn infants against the foodborne pathogen L. monocytogenes on human intestinal epithelial cells.

Inhibition assays were performed using L. monocytogenes EGDe strain and ten Lactobacillus strains belonging to species L. acidophilus, L. rhamnosus, L. delbrueckii, L. gasseri, L. crispatus, L. paracasei, and L. plantarum, isolated from infant feces and tested for their probiotic properties. Caco-2 monolayers in 24-well plates were infected with a single bacterial strain for individual assays or with a combination of L. monocytogenes and Lactobacillus for inhibition assays (~10^6 CFU/ml). Protective activity was estimated under three conditions: (i) competitors were added at the same time, (ii) Lactobacillus strain was added 1 h before L. monocytogenes, and (iii) L. monocytogenes was added 1 h before the Lactobacillus strain. Plates were incubated at 37 °C in a 5% (vol/vol) CO_2 atmosphere for 30 min. Bacterial cell populations were enumerated on selective media.

All Lactobacillus strains were able to adhere to Caco-2 monolayers within 30 min, with L. paracasei exhibiting the highest adhesive properties ($P < 0.05$). When competitors infected Caco-2 cells simultaneously, five Lactobacillus strains were able to inhibit L. monocytogenes adhesion, resulting in lower adhered pathogen population in dual compared to single cultures ($P < 0.05$). L. acidophilus and L. rhamnosus strains were able to exclude or displace pathogen adhesion ($P < 0.05$) when they were added 1 h before or after L. monocytogenes, respectively. The protective role was stronger when competitive strains were added simultaneously rather than apart. The invasion efficiency of L. monocytogenes was reduced In presence of Lactobacillus strains and the inhibition of invasion varied depending on the treatment and the Lactobacillus strain.

These results are indicative of Lactobacillus sp. ability to inhibit L. monocytogenes adhesion and invasion on epithelial cells.

PP_147

METSCHNIKOWIA PULCHERRIMA MAY AFFECT GROWTH DYNAMICS AND METABOLIC PROFILE OF OTHER WINE YEAST SPECIES BY A CELL-CONTACT MEDIATED MECHANISM

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Research on wine yeast interactions could provide deep insight on the microbial diversity and function of wine ecosystem thereby allowing for successful implementations of enological practices. Metschnikowia pulcherrima (MP) is a major yeast species on grapes and in fresh grape must. In preliminary experiments it has been shown to exert antagonistic effect on important wine yeast populations.

This study investigated the impact of MP on growth and metabolic response of different wine yeast species and evaluated whether the observed yeast interactions are dependent on cell-contact.

The growth of seven key wine yeast species (Saccharomyces cerevisiae (SC), Torulaspora delbrueckii (TD), Lachancea thermotolerans (LT), Hanseniaspora opuntiae (HO), Hanseniaspora guillermodnii (HG), Hanseniaspora uvarum (HU) and Starmerella bacillaris (SB) was determined in single and mixed cultures with MP with or without cell-contact. At the end of incubation the concentration of different metabolites was determined.

MP showed a competitive growth advantage when co-cultured with other yeast species. All but SB showed significant differences in growth dynamics between single and mixed cultures. Differences were manifested mainly as lower final populations and earlier death for yeasts in co-cultures. Cell contact significantly enhanced growth inhibition of yeasts by MP. Growth competition was contact- dependent for all yeast species except for LT.

The comparison of metabolic profiles between single and mixed cultures revealed complex patterns. For instance, the concentration of acetaldehyde was diminished in mixed MP/Hanseniaspora cultures compared to the respective monocultures while it was increased in MP/SB co-culture compared to the single cultures. The concentration of ammonia was always significantly lower in single cultures compared to mixed cultures. Interestingly, the absence of cell contact in co-cultures led to the highest observed concentrations of ammonia.

Present results suggest that the competitive advantage of MP over other wine yeast species is cell-contact related exerting a species-dependent effect on their growth and metabolic response.

PP_148

ORGANOLEPTICALLY AND DIETARY IMPROVED DEBINA WINES

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Traditionally, Debina wines, as well as nearly every other kind of wine, were produced employing exclusively commercial strains of Saccharomyces cerevisiae (S. cerevisiae). A "second generation" of Debina wines was produced using various combinations of S. cerevisiae and non-Saccharomyces indigenous yeast isolates (Parapouli et al., 2010), an effort aiming the improvement of wine's organoleptic characteristics. Due to consumers' and therefore producers' demands as well, production of wines took recently new directions towards lower alcohol and sulfites content, without compromising their organoleptic values. To comply with these demands in respect to Debina wine production, we implemented a dual approach for its production consisting of the use of various combinations of S. cerevisiae and non-Saccharomyces indigenous yeast isolates combined with the addition of various amounts of aronia berry juice to the Debina must. Aronia melanocarpa produces berries with a high content of antioxidants (Jurikova et al., 2017). The yeast combination can lead to wines with lower alcohol content, while the aronia juice functions both as an antioxidant and antimicrobial compound, thus allowing the reduction of the sulfites' content in the produced Debina wine. Our efforts resulted in the production of wines with lower alcohol levels ranging from 4 to 8 %, while concomitantly the sulfites' content was reduced approximately three times. Aroma compounds of the wine products were analyzed by solid-phase microextraction with gas chromatography-mass spectrometry (SPME-GC/MS). The odorants are associated with "fruity" and "flowery" odor descriptors.

Jurikova T., Mlcek J., Skrovankova S., Sumczynski D., Sochor J., Hlavacova I., Snopek L., Orsavova J. Molecules 2017, 22, 944. https://doi.org/10.3390/molecules22060944

Parapouli M., Hatziloukas E., Drainas C., Perysinakis A. J Ind Microbiol Biotechnol 2010, 37:85. DOI 10.1007/s10295-009-0651-7

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PP_149

PRODUCTION OF WINES USING VARIOUS COMBINATIONS OF INDIGENOUS YEASTS.

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Consumers' demands for novel wine products, such as wines with lower alcohol content, or with improved organoleptic characteristics, led among others, to the search for: i) new, mostly indigenous, yeast isolates and ii) mixed inocula combinations consisting of different yeast genera/species. Such combinations of diverse Saccharomyces cerevisiae and so-called non-Saccharomyces strains are nowadays a widespread practice.

To this end, an extended sampling was undertaken, to isolate yeasts indigenous to cultivated vines from Zitsa (Epirus, Greece). A large number of yeasts was purified and partially characterized at the molecular level, through sequencing of their ITS1- 5,8rDNA-ITS2 regions. Among the isolated samples were included yeast species of known value in respect to vinification (indicative reference: Parapouli et al. 2020), such as, Hanseniaspora uvarum, Hanseniaspora vineae, Pichia terricola, Metschnikowia pulcherrima, Saccharomyces cerevisiae and others.

Several combinations of the above isolates were used as inocula to ferment Debina must and the most promising combinations, although final evaluation still stands out, were Pichia terricola - Saccharomyces cerevisiae and Metschnikowia pulcherrima - Pichia terricola - Saccharomyces cerevisiae.

Parapouli M, Vasileiadis A, Afendra A-S, Hatziloukas E. (2020). AIMS Microbiology, 6(1): 1–31. DOI:10.3934/microbiol.2020001

Keywords Mixed inocula must fermentation, Saccahomyces cerevisiae, nonSaccharomyces yeasts

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PP_150

EVALUATION OF LOCAL BASIDIOMYCETES FOR THE DEGRADATION AND EXPLOITATION OF WOOD INDUSTRY BYPRODUCTS

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Wood industries accumulate byproducts that usually remain unexploited and are considered as putative pollutants. We have investigated indigenous basidiomycetes for their potential to degrade byproducts of the wood industry by exploiting their cellulolytic and ligninolytic properties. Apart from their contribution to the bioremediation, the produced edible fungi could serve as a food source, while non-edible fungal biomass, as a source for the production of substances of high additive value, such as non-digestible oligosaccharides.

Basidiomycetes grown on decaying wood were collected from local forest habitats under partially aseptic conditions and inoculated in a variety of solid growth media. After the establishment of the most appropriate cleaning / sterilization method and growth conditions, the fungi were subjected to several rounds of subculturing to obtain pure cultures. Morphological and partial molecular characterization (sequencing of the ITS1-5,8S rRNA-ITS2 region), revealed the isolation of members of several genera including Trametes, Hypholoma, Lycoperdon, Auricularia and Cistella. Selected fungal isolates were successfully grown on various wood substrates, along with the commercially available basidiomycete Pleurotus ostreatus. To investigate the potential of exploiting the cellulolytic and ligninolytic properties in a first approximation, the expression of certain cellulase and lignin peroxidase genes from Pleurotus ostreatus was studied using qRT-PCR at various growth stages. It was shown that the transcriptional level of vp3 and mnp3 genes was increased in relation to the growth stage of the basidiomycetes implying that their expression is upregulated during fungal growth on wood substrates.

Abdel-Hamid AM, Solbiati JO, Cann IKO (2013). Advances in Applied Microbiology 82: 1-28.

We acknowledge support of this work by the project "Research Infrastructure on Food Bioprocessing Development and Innovation Exploitation – Food Innovation RI" (MIS 5027222), which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

PP_151

ENCAPSULATION OF OREGANO (ORIGANUM VULGARE L.) ESSENTIAL OIL INTO B-CYCLODEXTRIN AND LIPIDIC DISPERSIONS: CHARACTERIZATION AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY AGAINST LISTERIA MONOCYTOGENES IN BROTH AND MODEL FOOD

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The hydrophobicity, strong flavor and high volatility of essential oils impede their commercial application in foods. As such, we aimed to study the encapsulation of Oregano essential oil (OEO) in β-cyclodextrin (β-CD) and lipidic dispersions as means to enhance dispersion and controlled release of EO and evaluate their effect on the survival of Listeria monocytogenes in Tryptic Soy Broth (TSB) and cheese broth.

The encapsulation of OEO in β-CD was performed with the co-precipitation method, whereas for the incorporation of OEO into lipidic dispersion the thin-film hydration method was applied. Size distribution (PDI) and zeta-potential (ζ) of the formed inclusion complexes (IC) were assessed by Dynamic Light Scattering (DLS). UV-Vis spectroscopy was used for the determination of the encapsulation efficiency (EE%) and for assessing the release rate of the formed IC. The effect of ICs and free OEO at MIC, ½ MIC and 2xMIC against L. monocytogenes (3-strain cocktail: 106 CFU/ml), was determined in TSB (pH 7.0, 4.3) and cheese broth prepared from 'katiki' (pH 4.3) at 7oC.

The complexes presented nanoscale size (<1000 nm), 127.24 ±25.83 nm with an intensity of >90% for OEO-β-CD and 106.84 ±42.81 and 948.05 ±409.95 nm with intensity of 47% and 51.9%, respectively for OEO-lipidic dispersion. The size dispersion was 0.433 ±0.064 nm for OEO-β-CD and 0.872 ± 0.070 nm for OEO-lipidic dispersion. An acceptable stability was observed (>20 mV) with ζ-potential -23.57 ±6.76 mV for OEO-β-CD and -49.5 ± 6.18 mV for lipidic dispersion. EE% was 80.3% ± 4.0 and 87.8 ± 3.7 for OEO-β-CD and OEO-lipidic dispersion, respectively. The OEO release from both complexes occurred continuously for 18 days. The dose-dependent inhibitory effect of encapsulated OEO against L. monocytogenes was higher in TSB than in cheese-broth and increased at lower pH. Lipidic dispersion was more effective than OEO-β-CD at both pH values. The antimicrobial effect of encapsulated OEO was not as rapid as the effect of free EO, confirming the slow release of EO due to encapsulation. The results are indicative for the application of encapsulated OEO to control of L. monocytogenes in foods.

PP_152

DITECT – DIGITAL TECHNOLOGIES AS AN ENABLER FOR A CONTINUOUS TRANSFORMATION OF FOOD SAFETY MANAGEMENT SYSTEMS (EU-CHINA PROJECT 861915)

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The DiTECT* project has the following specific objectives: (i) To explore the needs, preferences, and acceptance criteria of various food-chain user groups; (ii) To implement product-specific food safety models for international food chains involving multiple Food Business Operators (FBOs) in the EU and in China; (iii) To bring together different food value-chain actors and regulatory authorities across the EU and China; (iv) To design and develop the open DiTECT technology hub, referred to as "intelligent" Food Safety Management System (iFSMS); (v) To develop the novel food-chain-level DiTECT framework; (vi) To render the DiTECT offerings efficient, effective and attractive for commercial food actors, while infusing trust by guaranteeing lower risk of food hazards, therefore, pushing towards the higher sustainability of market operations; (vii) To demonstrate and validate the efficiency of the DiTECT approach through product-specific food safety use-cases; (viii) To scale up, disseminate and communicate the developed tools and project findings, in order to contribute to the EU and China world market vision.

In order to achieve these objectives, eight integrated WorkPackages (WPs) and four pilots have been designed (WP2), in which selected hazards/factors will be studied across the food chain, using rapid, non-invasive sensors for hazard detection and monitoring (WP3). WP4 will deal with the storage and management of the data derived from WP2, WP3 and WP7. Risk assessment on selected hazards/factors will be studied in WP5, while the validation of the iFSMS, which will be developed in WP6 (based on WP2 and WP3), will be performed in WP7. In particular, the web-based iFSMS will be made available to final users, by feeding the realization of ICT solutions and chain management in WP7. WP1 & WP8 are dedicated to project management and communication, dissemination and exploitation.

The successful implementation of DiTECT is expected to significantly reduce biological/chemical hazards and environmental contaminants, through means of early detection before they make their way to final products. Moreover, the collaboration between the EU-China food businesses and research partners will result in enhancing consumers' confidence in the safety of food traded between the two regions, throughout the farm-to-fork continuum.

DiTECT is an HORIZON 2020 EU/CHINA Project (Contract N. 861915)

PP_153

OLIVE MILL WASTEWATER AS A RESOURCE OF BIOLOGICALLY ACTIVE PHENOLS FOR FOOD AND BEVERAGES

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In a circular economy perspective, the reuse of agro-food by-products represents a strategy for the recovery and production of high added value products. The olive mill wastewater (OMWW), a liquid effluent from the olive oil industry, is a source of bioactive compounds, such as hydroxytyrosol and tyrosol.
In the present study, OMWW were subjected, at industrial scale, to a concentration process through a tangential filtration membrane technique. Three retentates and three permeates were obtained: ultrafiltration retentate (sample R1); osmosis retentate I (sample R2); osmosis retentate II (sample R3); permeate of R1 (sample P1), permeate of R2 (sample P2) and permeate of R3 (sample P3). Retentate and permeate samples were subjected to microbiological and chemical analyses and P2 and P3 samples were subjected to analyses according to the UE Regulation 2020/741 of 25 May 2020. In addition, the antioxidant and antimicrobial properties were evaluated by DPPH and disc diffusion assay, respectively. Samples were tested against 9 pathogenic target strains, such as Listeria monocytogenes, Escherichia coli, Candida albicans, Staphylococcus aureus, Pseudomonas aeuroginosa, Salmonella typhimurium, Bacillus subtilis, Clostridium sporogenes, Enterococcus faecalis and against 4 probiotic target strains, such as Lacticaseibacillus rhamnosus, Lactiplantibacillus paracasei, Propionibacterium freudenreichii, and Bifidobacterium animalis subsp. paracasei.
The R2 and R3 samples, obtained by osmosis filtration, showed the highest phenolic contents, with 7204 and 6728 mg/L of hydroxytyrosol, and 6045 and 933 mg/L of tyrosol, respectively. In addition, the samples exhibited the highest antioxidant activity with an IC50 value of 41.17 and 50.95 µl respectively. The R2 and R3 concentrates showed inhibitory activity against E. coli and P. aeuroginosa, while only the sample R2 showed antimicrobial activity against B. subtilis, Cl. sporogenes and E. faecalis. It is interesting to highlight that no inhibitory activity was observed against all the considered probiotic target strains.
Finally, results of microbiological analyses revealed that permeate samples (P2 and P3) met the microbiological criteria set by Regulation (EU) 2020/741 on minimum requirements for water reuse, being suitable for crop irrigation or farm recirculation.
Therefore, OMWW may represent an alternative resource of biologically active phenols suitable to fortify food and/or beverages with antioxidant and antimicrobial activities.

PP_155

MONITORING SUB-LETHAL INJURY OF LISTERIA MONOCYTOGENES AT SINGLE CELL VERSUS POPULATION LEVEL DURING FRANKFURTERS REHEATING

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Listeria monocytogenes is an ubiquitous foodborne hazard that may contaminate the surface of ready-to-eat cooked meat products, like frankfurters. As such, the majority of manufacturing companies apply a post-package mild heat treatment by immersion in water or recommend reheating prior to consumption. However, such a treatment may be sub-lethal for well-embedded cells in niches on or below the product surface.

The objectives of the present study were to evaluate the sub-lethal thermal injury of L. monocytogenes on frankfurters surface at single cell versus population level, at temperatures that may occur on product surface during post-package pasteurization of frankfurters and comparatively estimate the distribution of pathogens' culturable, injured and dead cells during exposure to heat stress, using culture-based methods and fluorescent microscopy.

Frankfurters were inoculated (8.0 log CFU/cm2) of L. monocytogenes strain EGDE-e. Reheating of frankfurters was simulated in a water bath at 61°C (60 min) and 64°C (20 min). Temperature changes were monitored by placing a K-type thermocouple on the surface of frankfurters prior to vacuum packaging. Determination of injured sub-populations was performed by subtracting the number of colonies on Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) supplemented with 5% NaCl from those on TSAYE with 0.5% NaCl. Sub-lethally injured cells assessed by plating were also compared to those assessed by fluorescent microscopy, coupled with CFDA and PI to detect metabolically active and dead cells.
L. monocytogenes showed detectable logarithmic reduction and induction of injury during heat treatment at 61oC and 64oC. The highest sub-lethal injury (1.0–1.5 log CFU/cm2) was recorded after 2 and 4 min, or 6 min exposure at 64oC and 61oC, respectively. This was confirmed microscopically, at single cell level. After exposure to 61oC for 60 min, the whole population was below the detection limit (1.0 log CFU/ml); however, a considerable number of single cells appeared as CFDA+/PI+, suggesting the induction of sub-lethal injury and the underestimation of pathogen viability, when relying solely on culture-based enumeration.

Evaluation of in situ L. monocytogenes sub-lethal injury could be crucial for food microbiology, offering insights on the risks associated with overestimation of a process lethality.

PP_157

PREVALENCE OF SALMONELLA SPP., CAMPYLOBACTER SPP., AND LISTERIA MONOCYTOGENES IN RAW CHICKEN MEAT AND PRODUCTS FROM RETAIL STORES IN MYRINA, LEMNOS (GREECE)

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In this work, raw chicken meat and products sold in retail stores of Myrina were microbiologically analyzed for the prevalence of three important foodborne pathogenic bacteria, i.e., Salmonella spp., Campylobacter spp., and Listeria monocytogenes. In parallel, the total aerobic microflora, staphylococci, coliforms, and Escherichia coli were enumerated in each sample as hygiene indicator organisms. To do this, 60 samples of raw chicken meat and products (including whole carcasses, breasts, legs, wings, necks, drumsticks, and minced products) were collected the period from July to October 2021 from retail stores distributed in the town. To isolate and identify the pathogens, respective ISO protocols were strictly followed, while all other microbiological analyses were done by applying classical microbiological procedures. Salmonella spp. were recovered from 9 samples (15.0%), Campylobacter spp. from 52 samples (86.7%) while Listeria monocytogenes was present in 32 samples (53.3%). Total aerobic microflora surpassed 106 CFU/g in 16 samples (26.7%) and 5 samples (8.33%) contained coliforms above 105 CFU/g. E. coli population was above 102 CFU/g in 7 samples (11.7%), whereas staphylococci surpassed 102 CFU/g in only one sample. In sum, this study unravels the hygienic status of raw chicken meat and products sold in retail stores in an island Greek town (north-eastern Greece) and in parallel highlights the potential risk for public health in cases where good hygiene practices are not followed at later stages of the market (e.g., cross-contamination in the kitchen of other ready-to-eat products such as salads, and/or consumption of undercooked such chicken products). Future research will investigate drug resistance of the isolated pathogens.

Keywords: foodborne pathogens; raw chicken meat; food retail outlets; hygiene indicators; food safety; microbial risk assessment

PP_158

ONTHEFLY2.0: A TEXT-MINING WEB APPLICATION FOR AUTOMATED BIOMEDICAL ENTITY RECOGNITION, DOCUMENT ANNOTATION, INTERACTION NETWORK AND FUNCTIONAL ENRICHMENT ANALYSIS

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Extracting and processing information from documents is of great importance as lots of experimental results and findings are stored in local files. Therefore, extracting and analyzing biomedical terms from such files in an automated way is absolutely necessary. In this work we present OnTheFly2.0, a user-friendly application for extracting biomedical entities from documents in various file formats, including PDF documents, Office documents, plain text files and images (1). OnTheFly2.0 can generate informative summaries in popup windows containing knowledge related to the identified terms along with links to various databases. It uses the EXTRACT tagging service (2) to perform Named Entity Recognition for genes and proteins, chemical compounds, organisms, tissues, environments, diseases, phenotypes and ontology terms. Multiple files can be analyzed and combined to produce analysis datasets, which can then be explored through functional enrichment analysis or be statistically associated with diseases and PubMed entries. In addition, protein-protein and protein-chemical interaction networks can be generated by retrieving interactions from the STRING (3) and STITCH (4) databases. To demonstrate OnTheFly2.0's capacity for knowledge discovery, we interrogate published meta-analyses of clinical biomarkers of severe COVID-19 and uncover inflammatory and senescence pathways that impact disease pathogenesis. OnTheFly2.0 is publicly available through http://bib.fleming.gr:3838/OnTheFly/ or http://onthefly.pavlopouloslab.info/ as a web service, and https://github.com/PavlopoulosLab/OnTheFly as a downloadable package.

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PP_159

PREVALENCE AND CONCENTRATION OF ESCHERICHIA COLI IN BEEF: DEVELOPMENT OF A DATABASE FOR EUROPEAN UNION FOOD CHAIN

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Escherichia coli is a major concern for food safety with a lot of infections linked with beef and beef products. A lot of research efforts are dedicated to the detection and quantification of the hazard along the continuum from farm to fork. Nevertheless, this information is not translated into easily accessible data in a well- organized manner which are of high relevance for risk assessment and, generally, risk analysis. The objective of the present study was the collection of prevalence and concentration data (since 1991) of E.coli in beef and various beef products at different stages in the EU food chain and the construction of a database providing quantitative data along with information as food product type, food chain stage, packaging status etc. A systematic review was conducted on the Scopus electronic database. The review considered articles published between 1991-2021. Papers were deemed eligible for inclusion based on the microorganism (E.coli), the geographical location (countries of the European Economic Area) and the type of animal (cattle/cows) or product (beef) sampled. Prisma statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) was employed for reporting the screening process. 919 papers were retrieved from Scopus and after the entirety of the screening process, 222 were selected for data extraction. A database was developed and formatted according to the sampling site/stage, the type of product, the method of analysis and the prevalence and concentration. In addition, various supplementary information for each study was also recorded to improve the flexibility of the database. Meta - analysis will be performed and pooled data will be included. The compilation of the data and the construction of the database will constitute a valuable tool for the development of risk assessment models. The database offers ease of access to well organized and harmonized data and enable the study of various scenarios along the farm to fork continuum.

PP_160

FTIR SPECTROSCOPY AS A PROCESS ANALYTICAL TECHNOLOGY TOOL FOR CHARACTERIZATION AND PREDICTION OF PERFORMANCE AND QUALITY PROPERTIES IN CULTIVATED MUSHROOMS

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Nowadays, there is an increasing number of Fourier transform infrared (FTIR) spectroscopy applications related with characterization and quality assessment of food products (e.g. milk, vegetables, wine). However, the use of FTIR, as a process analytical technology (PAT) tool in mushroom science and technology, is still very limited. So far, FTIR has been exploited for the discrimination/authentication of Amanita, Boletus, Leccinum, Lignosus and Wolfiporia fruitbodies or sclerotia on the basis of their geographic origin, for separation between wild and cultivated basidiomes of Ganoderma spp., and to identify different types of mushroom glucans. Furthermore, FTIR combined with multivariate analysis have been applied to assess the post-harvest physical damage and aging of Agaricus bisporus mushrooms, and to determine the storage stability of Ganoderma lucidum water extracts. During the last years, our research team has exploited the large fungal culture collection it maintains, in order to implement FTIR approaches in the taxonomy of basidiomycetes as well as in mushroom cultivation and product quality. Towards this end, diffuse reflectance absorbance FTIR (DRIFT) were successfully implemented to identify strains of the same species and to discriminate 16 taxa of the genus Pleurotus, thus providing a fast, reliable, and cost-efficient solution for identifying pure cultures to species. In addition, the same technique was applied for the spectroscopic characterization of substrates commonly used in the cultivation of Cyclocybe cylindracea, and a FTIR-model was developed to predict mushrooms biological efficiency by advanced chemometrics. Furthermore, attenuated total reflectance FTIR (ATR-FTIR) was used to characterize Pleurotus fruitbodies produced on various substrates, while models predicting the content of mushrooms in health-promoting bioactive compounds (e.g. β-glucans, ergosterol, phenolic compounds, amino acids) were developed, achieving high performance (R^2 higher than 0.8) and low error (in most of the cases lower than 10%).

PP_161

ANALYSIS OF THE LACTIC ACID BACTERIA COMMUNITY OF THE GREEK FETA AND KEFALOGRAVIERA CHEESES USING THE BIOLOG MICROBIAL IDENTIFICATION SYSTEM

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Introduction: A key part of the microbial community of dairy fermented products is the lactic acid bacteria (LAB). Several fermented dairy products have been investigated, studying the microbial dynamics during the fermentation and ripening processes. The main role of LAB is related with their positive impact on the quality and organoleptic characteristics of the final products as well as on their safety, inhibiting the growth of undesired foodborne pathogens.
Purpose: The exploration of the diversity of LAB found in the Feta and Kefalograviera cheeses during their ripening as well as the rapid identification of the bacterial isolates.
Methods: Feta and Kefalograviera samples without the addition of commercial starters were collected at two stages of ripening (early and late). The samples were microbiologically analyzed using the following solid media (agar): MRS (a general medium for cultivation of LAB), Lactic Bacteria Differential (for differentiation of homofermentative and heterofermentative LAB) and HiCrome Nickels and Leesment Medium (for citrate-fermenting LAB). Microbial strains were identified using the Biolog MicroPlates method.
Results: In Feta and Kefalograviera, the main LAB genera identified were Lactobacillus, Lactococcus, Pediococcus, Enterococcus and Leuconostoc. Microbial diversity in Kefalograviera was lower with Lactococcus dominating microbiota. Phenotypic profiling revealed metabolic diversity in carbon source usage within species.
Significance: Rapid identification of LAB community of ripened Feta and Kefalograviera cheeses by sole-carbon source utilization.

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PP_162

ASSESSMENT OF THE MICROBIOLOGICAL QUALITY OF BEEF USING SPECTRAL DATA

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The limitations of traditional microbiological analysis (e.g., retrospective results) could be overcome with the development of spectroscopic-based sensors coupled with multivariate data analysis for the assessment of the microbiological quality of foods. Sensors based on spectroscopy have been extensively studied due to their properties, such as speed, low cost and non-destructive character. The aim of this work was to assess the microbiological quality of minced meat using Fourier transform infrared (FT-IR) spectroscopy and multispectral imaging (MSI). Minced beef (three batches) was purchased in three independent time periods. Patties of ca. 100 g each were prepared and stored aerobically under isothermal conditions (0, 4, 8, and 12 °C). The analysis was performed on duplicate samples for a maximum of 9 days. The total mesophilic microbial populations were determined with conventional plate counting, while in parallel FTIR measurements and MSI spectral data were acquired. In total 132 samples were analyzed and stratified sampling was applied so as 70% and 30% of the dataset was used for training and external validation of the model, respectively. The collected FTIR and MSI data were subjected to pre-processing, including Savitzky-Golay (smoothing, 1st and 2nd order derivatives) and/or standard normal variate (SNV) transformation. Partial least squares regression (PLS-R) was applied on spectral/imaging data and microbial counts, with the former constituting the input and the latter the output variables in the PLS-R models. The values of root mean squared error (RMSE, log CFU/g) for the prediction of the test (external validation) dataset for the FT-IR and MSI models were 0.99 and 0.91, respectively, while the corresponding values of the coefficient of determination (R^2) were 0.769 and 0.805, respectively. FT-IR and MSI spectroscopy showed considerable potential for the quantitative monitoring of the microbiological spoilage of minced meat.

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PP_163

INVESTIGATION OF THE ENDOMETRIAL MICROBIOME OF WOMEN UNDERGOING IN-VITRO FERTILIZATION

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PURPOSE: The microbiome is involved in maintaining health in the human body, therefore a change in its consistency could lead to pathological conditions. It has been suggested that the microbiome of the female reproductive system is involved in female infertility and that it may play a key role in the success of assisted reproduction. This study sought to test the composition of the endometrial microbiome in infertile patients undergoing in vitro fertilization.

MATERIALS-METHODS: Endometrial samples from a group of 17 women were examined with next generation sequencing technology, to investigate the composition of the endometrial microbiome. Nine hypervariable regions of the 16S rRNA gene (V2-4, 6-9) 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 and featured as Krona, Alpha and Beta diversity charts. Greengenes and Thermo Fisher's MicroSEQ 16S Reference Library were selected as the refence databases.

RESULTS: When bacterial communities from endometrial fluid samples were interrogated, different bacteria were detected among the samples. The bacterial profiles of the endometrial samples were dominated mostly by Burkholderiaceae (15 of the 17 samples) with an abundance varying from 1.4% to 82%. Lactobacillaceae were detected on 13 of the 17 samples at lower rates (1.5% to 59%). Pseudomonadaceae were the dominant population in 3 samples, ranging from 83% to 92%, whilst some samples presented a unique microbial profile.

DISCUSSION/CONCLUSIONS: Our results demonstrate that there is considerable variability in the composition of the microbiome amongst endometrial samples. Limitations in the investigation of the endometrial microbiome include the limited biomass, sample contamination, artifacts, various sampling, extraction and amplification techniques and different databases for determining the microbial composition. The course of the pregnancy of the women involved in the study will be monitored to clarify the role of the endometrial microbiome in the success of in-vitro fertilization.

Key words: Endometrial microbiome, in-vitro fertilization, Next Generation Sequencing, 16S rRNA, Ion Torrent

PP_164

QUORUM SENSING IN NON-PATHOGENIC BACTERIA

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Quorum sensing is a form of communication in microorganisms that allows coordinated regulation of gene expression in accordance with cell density. It was first discovered in the marine bacterium Vibrio fischeri, where the cognate quorum sensing (QS) system controls bioluminescence. The QS system of V. fischeri has offered a paradigm in bacteria in general and is well characterized to a molecular level. In V. fischeri, LuxI is the synthase of a signaling molecule, the acyl homoserine lactone (AHL) N-3-oxohexanoyl-L-homoserine lactone. The AHL diffuses to the external environment and at threshold concentrations that reflect certain cell densities, binds to and activates the transcription factor LuxR. The activated LuxR positively regulates the transcription of lux genes, encoding the luciferase which is necessary for bioluminescence, as well as the luxI and luxR genes, thus creating a positive feedback loop. Bioluminescence is essential for the mutualistic symbiosis of V. fischeri with the marine organisms it populates, such as the squid Euprymna scolopes. Since their discovery, QS systems have been intensively investigated in bacterial pathogens and this owes to the fact that they elicit responses harmful to the host; e.g., cytolytic or cytotoxic behaviors, formation of biofilms impermeable to treatment, and antimicrobial resistance. In this review, we examine the diversity and roles of quorum sensing systems in non-pathogenic bacteria overall: in bacteria important for human, animal and plant well-being because of their commensal or directly advantageous roles in health and disease, bacteria contributing to agriculture and ecosystem viability, to bioremediation and pest control, as well as those playing major role in food and drink production, industrial or traditional.

By focusing on certain paradigms, we will examine the different phenotypes regulated by QS in such non-pathogens and illuminate the intricate molecular networks by which they coordinate their behaviors. From the time the V. fischeri AHL-dependent QS system was first recognized, to date, a vast lexicon of signaling molecules has been discovered. QS is now known to act in simple or interwoven manners and to affect intra- or interspecies communication in the microbiota of different environments, as testified by recent community and metagenomics studies.

PP_165

EVALUATION OF NA-ALGINATE EDIBLE FILMS AS VEHICLES FOR DELIVERING PROBIOTIC BACTERIA TO SLICED CHEESE PRETREATED WITH HIGH PRESSURE PROCESSING

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The aim of the current study was to assess the efficacy of Na-alginate edible films as vehicles for delivering probiotic bacteria to sliced cheeses with or without high pressure processing (HPP). A three-strain cocktail of probiotic bacteria (Lactococcus lactis-T4, Leuconostoc mesenteroides-T25 and Lactobacillus paracasei-T26, previously isolated from Greek traditional cheeses), was incorporated in Na-alginate forming solution in a final population of 9 log CFU/mL. Cheese slices (without or with HPP treatment at 500 MPa for 2 min) were packaged in contact with the probiotic films and consequently packed under vacuum and stored at 4 °C until spoilage. Cheese slices without the addition of films with or without HPP treatment were used as controls. At all cases, microbiological, pH and sensory analyses were performed, while the presence and the relative abundance of each probiotic strain during storage was evaluated using Random Amplified Polymorphic DNA-PCR (RAPD-PCR). Results showed that in cheese slices without HPP treatment, the initial microbiota consisted mostly of LAB and lactic cocci (>7.5 log CFU/g), while HPP treatment caused a reduction in the initial microbial population of approximately 1-1.5 log CFU/g. Regarding the samples supplemented with the probiotic films, population of LAB and lactic cocci were maintained at levels of >6.8 log CFU/g during shelf-life, regardless the HPP treatment. Sensory evaluation revealed that the probiotic samples without HPP had a slightly more acidic taste compared to the control, whereas the HPP probiotic cheese samples exhibited the best organoleptic characteristics. RAPD-PCR confirmed that all the recovered strains were assigned to the three probiotic strains that have been incorporated in the films and survival of the strains depended on the different storage time. Overall, the results of the study are promising since probiotic bacteria were successfully delivered in the products by the edible films regardless of the HPP treatment.

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PP_167

RAPID ASSESSMENT OF QUALITY IN MARINATED CHICKEN SOUVLAKI VIA DATA FUSION OF FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FT-IR), MULTISPECTRAL IMAGING (MSI) AND ELECTRONIC NOSE (E-NOSE)

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Fourier Transform Infrared Spectroscopy (FT-IR), Multispectral Imaging (MSI) and electronic nose (e- nose) are three rapid, noninvasive methods that have been frequently applied in quality assessment of meat and poultry products.
The aim of this study was to investigate the efficacy of these methods, individually and in combination (mid-level fusion) for the assessment of the microbiological spoilage of chicken marinated souvlaki via Partial Least Squares Regression (PLS-R).
Chicken marinated souvlaki (n=116) samples were stored aerobically at three isothermal conditions, namely 0, 5, and 10 °C (two independent experiments) and a dynamic temperature profile (12 h at 0 °C, 8 h at 5 °C and 4 h at 10 °C). Samples were analyzed microbiologically for the determination of Total Viable Counts (TVC) while in parallel FT-IR, MSI and e-nose analyses were undertaken. PLS-R models were developed for the estimation of TVC for each sensor separately. Moreover, mid-level data fusion (First step: Principal Component Analysis, PCA; Second step: PLS-R) was performed for the evaluation of these three methods unitedly for TVCs assessment.
PLS-R model for FT-IR spectral data predicted efficiently TVC with Root Mean Squared error (RMSE) and r (correlation coefficient) values of 1.025 log CFU/g and 0.783, respectively, during model prediction. For MSI data, RMSE and r values were estimated at 0.763 log CFU/g and 0.878, respectively. For the PLS-R model developed on e-nose data, RMSE and r values were calculated at 1.921 log CFU/g and 0.249, respectively. Data fusion based on FT-IR, MSI and e-nose responses provided a PLS-R model that could estimate TVC with RMSE and r values of 1.367 log CFU/g and 0.722, respectively.
PLS-R models developed on FT-IR and MSI data could satisfactorily determine TVC loads on chicken marinated souvlaki, together with the data fusion approach based on the 3-sensor model. These findings could be implemented by poultry industries for the rapid assessment of product quality and hence could result in food loss reduction.

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PP_168

A CHEMICAL NAMED ENTITY RECOGNITION AND ASSOCIATION RULE MINING APPROACH TO FACILITATE THE STUDY OF METABOLIC PROCESSES IN HYPERTHERMOPHILIC MICROORGANISMS.

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The study of the metabolism of hyperthermophilic microorganisms is of industrial importance since these organisms' enzymes catalyze reactions at elevated temperatures.

Various hyperthermophiles of archaea and bacteria are established as laboratory models; their metabolic pathways are genetically engineered aiming at optimal production of fuels and chemicals at elevated temperatures in industrial scale.

This project aims to ease the literature study of the biological processes (and related metabolites) of microorganisms of interest. To this end mentions of chemical entities (metabolites, substrates, enzyme cofactors, etc.) in pertinent abstracts are identified and then co-occurrence associations between microorganisms and chemical entities, and between chemical entities are retrieved. In this project a chemical Named Entity Recogniser (NER), built upon the spaCy (https://spacy.io/) library and trained on the CHEMDNER corpus(1), was developed. A set of 9 hyperthermophile species (Thermococcus kodakarensis, Pyrococcus furiosus, Metallosphaera sedula, Thermotoga maritima, Caldicellulosiruptor bescii, Sulfolobus solfataricus, Thermus thermophilus, Thermoanaerobacter mathranii, Caldicellulosiruptor hydrothermalis) was used to demonstrate the chemical NER's applicability using literature (i.e PubMed abstracts) retrieved via the ORGANISMS web application (http://organisms.jensenlab.org). The chemical entities extracted from the aforementioned literature were further analysed for the appearance of frequent patterns and the co-occurrences among them by generating association rules between frequent itemsets (association rule mining).

The association of carbohydrates to C. bescii and of copper and T .thermophilus to heme emerged from this study. Manual curation of the literature showed that indeed Carbohydrate metabolism has been extensively studied in C. bescii for the production of ethanol. Similarly, extensive studies of T. thermophilus report that the heme–copper oxygen reductases are able to catalyze the reduction of nitric oxide to nitrous oxide under reducing anaerobic conditions (Figure 1, rules: R3, R4, R17, R18).

Beyond the hyperthermophilic microorganisms study, the presented method could be applied to any microorganism specific abstract collection. The chemical NER facilitates the identification of chemical entities in text. Furthermore, association rule mining provides co-occurrence associations between microorganisms and chemical entities and between chemical entities in abstracts.

Citations:: 1. Krallinger M, Rabal O, Leitner F, et al. The CHEMDNER corpus of chemicals and drugs and its annotation principles. J Cheminformatics. 2015;7(S1):S2. doi:10.1186/1758-2946-7-S1-S2

PP_169

FOOD SPOILAGE: MOVING TOWARDS A RISK-BASED FOOD QUALITY MANAGEMENT SYSTEM

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Over the last decade, food spoilage has become a major issue for both high- and low-income countries. According to Food and Agriculture Organization (FAO, 2011), nearly 1.3 billion tons of food per year is lost or wasted globally due to spoilage. Poor transportation and storage conditions as well as inadequate refrigeration cause a colossal amount of waste of both natural and human recourses that are used during food production, while consumers demand of high-quality food products is growing. Consequently, the development of a new, efficient and holistic quality and safety management system is necessary. Quantitative Microbiological Risk Assessment (QMRA) has been extensively used around the world within the context of food safety with the managers and food business operators adopting this modern approach. In comparison to the classical approaches, QMRA is notably different and allows for a rapid identification of safety and quality problems as well as investigation of suitable mitigation strategies to properly deal with them.

By developing probabilistic QMRA models for food spoilage, different scenarios regarding the characteristics and the handling of products, for example expiration date and environmental conditions, could be simulated for the estimation of their impact on risk of spoilage.

Moving towards a risk-based food quality management, the aim of this study is to describe in detail the methodology of the application of QMRA structure for assessing the risk of spoilage in perishable foods and further provide a basis to support the FBOs decision in selecting a more cost-effective expiration date while reducing the food waste.

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH- CREATE- INNOVATE (project code: T1EDK-04344).

PP_171

CHARACTERISING THE VAGINAL MICROBIOME OF GREEK WOMEN USING 16S RRNA SEQUENCING

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Introduction: The human microbiota is made up of 10-100 trillion symbiotic microbial cells present in varying amounts and compositions throughout the body. A wealth of evidence points to their critical role in human physiology and pathology. The normal vaginal microbiome is important in preventing genital and urinary tract infections, while perturbations in its composition have been associated with various pathological conditions including infertility or poor assisted reproduction outcomes. However, evidence suggests that the normal human microbiome may vary across populations.

Aim: Our study aimed to characterise the vaginal microbiome of healthy Greek women in comparison to other populations.

Methods: We employed next generation sequencing to sequence the V2-V4, V6-V9 regions of the 16S rRNA genes of the vaginal bacteria in 17 Greek women. The samples were processed using the Ion 16S Metagenomics kit and the data were analysed bioinformatically using the Ion Reporter software and the MicrobiomeAnalyst tool.

Results: In our population, four phyla (76% samples Firmicutes-dominant), 19 genera (76% samples Lactobacillus-dominant (LD)), and 51 species (29% samples L. iners dominant) were detected. Other dominant species in the Greek population included L. gasseri and L. jensenii (each dominant in 18% samples) and L. crispatus and L. delbrueckii (each dominant in 6% samples). Of note, 23% of the tested population displayed dominance of anaerobic bacteria, including Atopobium vaginae, Bifidobacterium dentium, or Prevotella amnii.

Discussion: Lactobacillus is reported as the most frequently dominant genus in the Caucasian (90% women), Asian (70-85% women), African (50-70% women) and Hispanic (50-70% women) populations, albeit with a different frequency. Among the LD Caucasian population 30-50% are dominated by L. crispatus, 15-30% by L. iners, and <10% by L. gasseri or L. jensenii. In contrast, L. iners is dominant in 30-50% of Asian, African and Hispanic women, while L. crispatus is dominant in only 15-30% of Asian and African, and <10% of Hispanic women. Interestingly, healthy Greek women deviate from other populations in terms of dominant vaginal species. If confirmed in a larger cohort, a unique Greek vaginal microbiome baseline should apply in future microbiome-phenome correlation studies, and microbiome-based predictive, diagnostic or prognostic tools

PP_172

MITOCHONDRIAL GENOME DIVERSITY OF THE GENUS MALASSEZIA AND ITS POTENTIAL FOR SPECIES IDENTIFICATION

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Malassezia is a unique yeast genus belonging to Malasseziomycetes, Ustilaginomycotina, Basidiomycota that currently comprises of 18 species, primarily known as human and animal skin inhabitors. The genus is the most dominant component of the healthy warm-blooded animal skin mycobiome, but also involved in various skin disorders like seborrheic dermatitis/dandruff and pityriasis versicolor. Moreover, some species, i.e. M. furfur, M. pachydermatis and M. sympodialis, are associated with bloodstream infections in immunocompromised patients and neonates specifically. The yeasts of this genus are lipid dependent, and some clinically relevant species are particularly fastidious, making them difficult to culture and diagnose with traditional tools. Recent advances in Malassezia genomics and genetics have focused on the nuclear genome. This work aimed to unveil the mitochondrial (mt) genetic heterogenicity of genus Malassezia. In detail, a comparative mitochondrial genome analysis of the genus revealed a high variability of Malassezia spp mt genomes. Genome size varied from approximately 28 kb to 60 kb and size variability was attributed into different intron abundance and variable intergenic regions. Introns were in several mt genes with cox1 as their main host. Genes of Homing endonucleases (HEGs) were located within introns and were divided to LAGLIDADG and GIY-YIG types with the former as the main HEG contributor. Intergenic regions were highly variable even within mt genomes of the same species. Human pathogens M. furfur, M. pachydermatis, M. restricta, M. symbodialis and M. obtusa were representatives of this diversity presenting an additional repeat region which may also carry trn genes. Moreover, several conserved blocks of genes were defined after the comparative analyses of the genomes of genus Malassezia. Syntenic diversity, the multicopy nature of fungal mt genomes, intron abundance, and variability of the intergenic regions make these genomes suitable targets for the development of sensitive tools for detection and identification purposes.

PP_173

THE IMPACT OF OXYGEN AVAILABILITY, STRUCTURE AND NUTRIENTS OF DIFFERENT SUBSTRATES ON INTER-STRAIN INTERACTIONS AND ACID RESISTANCE OF L. MONOCYTOGENES STRAINS

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Growth and interactions of different Listeria monocytogenes strains, present simultaneously in the same food product, are affected by the matrix. The study evaluated the effect of co-culture in/on different substrates, under different levels of oxygen availability, on growth, inter-strain interactions and acid resistance of L. monocytogenes,

Antibiotic-resistant (for selective enumeration) L. monocytogenes strains of serotypes 4b(C5,ScottA) and 1/2b(PL25), were inoculated (2-3 log CFU/mL or cm^2) in single or two-strain cultures (1:1 strain-ratio), in/on TSA-YE(Tryptic Soy Broth supplemented with Yeast Extract and 0.6% or 1.2% agar), in/on Ricotta and Camembert-based media (1 part dairy product and 2 parts ¼Ringer's solution supplemented with 0.6% or 1.4% agar) and in/on Ricotta and Camembert and stored at 7°C(n=3x2). Aerobic conditions were achieved with surface inoculation, while incorporation into the cheese mass or pour plated media, corresponded to hypoxic conditions. Survival of middle exponential and early stationary bacterial cells, co-cultured in/on dairy products, was evaluated after 10, 20, 40, 60 and 120 min in SGF(pH 2.0;37°C)(n=3x2).

Inter-strain interactions, as manifested by difference in the final population of singly and co-cultured strains, seemed to be affected by the characteristics of the substrate, with C5 and PL25 suppressing ScottA. The presence of C5 inhibit ScottA by 2.7 and 2.3 log units in Ricotta and Camembert, respectively, while the inhibition of ScottA mitigated in/on TSA-YE and the dairy-based substrates. The impact of PL25 on final population of ScottA was less pronounced during co-culture in/on the different studied substrates and did not exceed the 1 log unit. Under hypoxic conditions, the inhibition of ScottA by both C5 and PL25 was more pronounced in/on Ricotta-based than in/on Camembert-based media. The results indicate that substrate nutrients may manipulate the inter-strain interactions, however the impact of structure and oxygen availability was substrate and combination of strains-dependent. Habituation of the pathogen on Camembert resulted in acid susceptibility against subsequent exposure in SGF. Regarding Ricotta, ScottA, displayed increased acid resistance compared to C5 and PL25, even though it was outcompeted during co-culture.

Investigating inter-strain interactions during co-culture in/on different substrates could assist in explaining the mismatch between clinical and food samples, during outbreak investigations.

PP_174

THE NULL OUTLIER CLOCK TEST (NOCTES): A NEW MOLECULAR CLOCK TEST AND ITS APPLICATION IN PROKARYOTES

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Since the proposal of a Neutral Theory of Molecular Evolution by Motoo Kimura in 1968, the assumption of a 'strict' molecular clock that relies on constant mutation rates has greatly aided studies of molecular evolution. However, we know that gene, lineage, and residual effects can cause rate heterogeneity across biological entities and as more genomic data are being accumulated, this phenomenon is becoming more and more evident. As a result, it is crucial that this rate variation be taken into account and the null hypothesis of a molecular clock be tested, before applying molecular dating analyses or inferring divergence events. Here we present the Null Outlier Clock TESt (NOCTES), a non-parametric branch-length-based molecular clock test. NOCTES takes as input a set of rooted phylogenies and calculates the root-to-tip distances for each tree plus the variance of those distances. For the set of all variances, NOCTES performs a Tukey's fences test to check whether 0, which corresponds to an ultrametric tree, is an outlier value. If so, the molecular clock assumption can be rejected, either softly at k=1.5 or hard at k=3 interquartile ranges. By measuring the ultrametric value distance in interquartile ranges, NOCTES acts as a semi-quantitative metric of molecular clock violation. We validate NOCTES by applying it to all prokaryotic taxonomic units (at genus or higher level) in the GTDB normalized taxonomy system to test the strict clock hypothesis across prokaryotes.

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MITOCHONDRIAL TRANSCRIPTION OF ENTOMOPATHOGENIC FUNGI REVEALS EVOLUTIONARY ASPECTS OF MITOGENOMES

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Entomopathogenic fungi and more specifically genera Beauveria and Metarhizium have been exploited for the biological control of pests. Genome analyses are important to understand better their mode of action and thus, improve their efficacy against their hosts. Until now, the sequences of their mitochondrial genomes were studied, but not at the level of transcription. Except of yeasts and Neurospora crassa, for which everything related to mt gene transcription is well described, in Pezizomycotina, related information is extremely scarce. Therefore, in this work, mt transcription and key enzymes of this function were studied. RT-PCR experiments and Northern hybridizations reveal the transcriptional map of the mt genomes of B. bassiana and M. brunneum species. The mt genes are transcribed in six main transcripts and undergo post-transcriptional modifications to create single gene transcripts. In both cases the six transciption units determined were: rnl (rps3), trn(TEMMLAFKLQHM)-nad2-nad3-atp9, cox2-trnR-nad4L-nad5-cob-trnC, cox1-trnR, nad1-nad4-atp8-atp6, rns-trn(NSDY)-cox3-trnG-nad6-trn(WSILP). Promoters were determined in both mt genomes with a comparative in silico analysis, including all known information from other fungal mt genomes. The promoter consensus sequence is 5'-ATAGTTATTAT-3' and is in accordance with the definition of the polycistronic transcripts determined with the experiments described above. Moreover, 5'-RACE experiments in the case of premature polycistronic transcript nad1-nad4-atp8-atp6, revealed the 5' end of the RNA transcript immediately after the in silico determined promoter, as also known in yeast species. Since several conserved elements were retrieved from these analyses compared to the already known data from yeasts and N. crassa, the phylogenetic analyses of mt RNA polymerase (Rpo41) and its transcriptional factor (Mtf1) were performed in order to define their evolution within the Kingdom of Fungi. As expected, it was found that fungal Rpo41 originate from the respective polymerase of T7/T3 phages, while the ancestor of Mtf1 is of alpha-bacterial origin. Therefore, this study presents insights about the fidelity of the mt single-subunit phage-like RNA polymerase during transcription, and the correct identification of mt promoters from Rpo41 requires an ortholog to bacterial sigma factor, i.e. Mtf1.

PP_176

DEPARTMENT OF DAIRY RESEARCH OF HELLENIC AGRICULTURAL ORGANIZATION "DIMITRA" - ESTABLISHMENT AND OPERATION OF A CENTER OF INNOVATION AND SPECIALIZATION IN AGRI-FOOD – MILKQUALITY

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The Innovation and Specialization Center "MilkQuality" will be created as a certified Research Infrastructure (R&D) with modern equipment, which will aim to upgrade the quality and added value of fresh milk and cheese products. In addition, the infrastructure will be able to support other sectors, such as meat. "MilkQuality" will create an ecosystem of research, knowledge, and entrepreneurship, which will contribute to the creation of a healthy and friendly, in the livestock and industrial sector, a problem-solving system, based on modern technologies and scientific knowledge.

"MilkQuality" policy includes:

1. Provision of services and product development in Agri-Food.
2. Training of young researchers in the new technologies.
3. Training and consulting provided to companies and organizations.
4. Evaluation and improvement of methods and techniques.
5. Operation at national and international level.

The R&D Center will have equipment to perform microbiological, physicochemical, and molecular analyses on products of animal origin. Initially, "MilkQuality" will focus on the following areas:

1. Tackling quality problems.
2. Labelling (composition and physicochemical parameters).
3. Quality control and hygiene.
4. Determination of the shelf-life and safety.
5. Support in the development of new products through the determination of critical parameters (microbiological-physicochemical parameters and shelf-life).
6. Ensuring the authenticity of PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and GTSP (Guaranteed Traditional Specific Product) products.
7. Improving the competitiveness of livestock farms by reducing losses, veterinary costs, breeding costs and improving production (Precision Dairy Farming).

Responsible for the coordination of the project is Dr. Mataragas Marios, Principal Investigator in Molecular Dairy Microbiology of the Hellenic Agricultural Organization "DIMITRA" / Institute of Technology of Agricultural Products / Department of Dairy Research.

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PP_177

THE NAGOYA PROTOCOL AND COLLECTIONS OF MICROORGANISMS: CURRENT SITUATION OF THE GREEK COLLECTIONS OF MICROORGANISMS, IMPLEMENTATION ISSUES AND DEVELOPMENT PROSPECTS.

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The subject of the paper are microorganisms as genetic resources, in the frame of the Convention on Biological Diversity (CBD), the Nagoya protocol (NP) and the European Regulation 511/2014. CBD consists of three main pillars. The first one concerns the conservation of the biological diversity as a public good, the second one the sustainable use of its components and the third the fair and equitable benefit sharing arising from the utilization of the genetic resources and the associated traditional knowledge. The third pillar of CBD is the most specialized and seeks the fair access and benefit from the use of genetic resources, as a national heritage. The Nagoya Protocol and the European Regulation 511/2014 aim to introduce an international and a European framework, respectively, which will enable the effective implementation of the third pillar of CBD. The Protocol has been ratified by 129 countries, including the Greek ratification, in June 2019. Genetic resources are extremely important for the human kind. Especially microorganisms, which are significant genetic resources, have played a great role throughout human history, too. Their properties are subjects of research and development in various scientific fields, including medicine, agriculture, ecosystem decontamination, biotechnology, bioenergy, etc. They are also used for commercial purposes and cover a wide range of product applications (pharmaceuticals and, biofuels, cosmetics, food, beverages, vaccines, etc.). Collections that maintain microorganisms outside their natural ecosystems (ex situ collections), play a vital role in their study, reproduction and development of new products. The owners of the Greek collections answered various questions, which were processed by statistical methods and many interesting conclusions emerged. The level of knowledge of the Greek owners of collections of microorganisms for the CBD, and the NP is quite high, in contrast with the EU Regulation, which is low. Moreover, there is a lack of familiarity regarding the practical issues of the Protocol, due to their low involvement with their commercial use. There is also deficient knowledge on national research legislation, concerning licensing on genetic resources. The owners would think positive about collaborating with the Voluntary Register of Collections, which the EU Regulation 511/2014 suggests.

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GROWTH, PRODUCTIVITY, AMINO- AND FATTY ACIDS COMPOSITION OF AUXENOCHLORELLA PROTOTHECOIDES GROWN ON BIODIESEL-DERIVED GLYCEROL IN A CONTINUOUS CULTURE UNDER HETEROTROPHIC AND MIXOTROPHIC CONDITIONS

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With the aim to study the growth and metabolic production of the microalga Auxenochlorella protothecoides utilizing biodiesel-derived glycerol as carbon source under heterotrophic and mixotrophic conditions, continuous cultures were performed at different dilution rates (D) and initial glycerol concentrations. Under heterotrophic conditions and 30 g/l glycerol concentration, steady-state yield coefficient on glycerol (Yx/s) and biomass productivity (Px) were estimated within the range of D from 0.064 to 0.11 1/h. Yx/s was higher at lower D (0.027 - 0.040 1/h), while it started to decrease at D>0.040 1/h. The highest values of Px were also obtained at the lower D with the maximum Px (0.274 g/lh) being recorded at D=0.047 1/h. Lipids production was favored at the lower D, while as D increased a metabolic shift occurred favoring the flow to proteins production. Increased initial glycerol concentration (50 g/l) at higher D was not adequately catabolized and seemed to act as a growth restrictive factor. Mixotrophic growth was performed at 30 g/l glycerol within a range of D from 0.27 to 0.107 1/h. At the steady states, Yx/s and Px were kept almost constant at D between 0.027 and 0.08 1/h, but were drastically reduced at D>0.08 1/h. The Px values were significantly lower than the corresponding ones under heterotrophic conditions at the same initial glycerol concentration for all D, while the concentration of residual glycerol in the medium was generally increased, indicating possible inhibition of glycerol catabolism in the presence of light. Between the two metabolic modes heterotrophy was found to favor biomass and metabolites (total protein and lipids) production, but did not affect the amino acids and the fatty acids profiles. Oleic acid was found to be the most abundant fatty acid detected in a percentage up to 67% of the lipid content, observation with great importance given that the methyl esters of monounsaturated fatty acids are considered to be better feedstock for biodiesel production than polyunsaturated ones. The algal biomass contained many essential and non-essential amino acids especially glutamic acid, aspartic acid, arginine, and alanine, while the profiles showed no significant differences with increasing D.

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IDENTIFICATION OF AUTOCHTHONOUS BACTERIAL MICROFLORA OF GREEK CHEESE PRODUCTS AND SHEEP MILK THROUGH CLASSICAL AND MOLECULAR APPROACHES

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The complex ecosystem of the dairy products is constituted by a variety of microorganisms with the lactic acid bacteria (LAB) as the dominant microbiota. To understand the individual contribution of the bacteria, autochthonous or added, to the physicochemical and organoleptic characteristics of the products, their identification is fundamental.

The aim of the present study was to identify, at species level, the autochthonous bacterial microbiota of four cheese products (Kefalograviera and Feta cheese in the beginning and the end of the ripening) and five sheep milks from different milk producers through classical and molecular methods.

A total of 189 LAB isolates were examined according to the Bergey's Manual of Systematic Bacteriology. After microscope observation of morphological characteristics, the isolates were assessed by Gram stain. Subsequently, the isolates were evaluated for their ability to grow at 4, 10, 15, 35, 37 και 45°C as well as in presence of NaCl 4 and 6.5% (w/v), produce CO_2 from glucose and ferment carbohydrates (lactose, D-mannitol, sucrose, maltose, D-galactose, D-glucose, sorbitol, raffinose, D-ribose, cellobiose, a-trehalose, D-xylose, melibiose and L-arabinose). For the molecular approach, after the extraction of DNA, PCR-RARD, using M13 as primer, was carried out for clustering of isolates. Representative bacterial isolates of each cluster (40 in total) were selected for sequencing of their V1–V3 region of 16S-rRNA gene. The isolates belonging to the Lactiplantibacillus plantarum group were subjected to species-specific PCR.

The results of the classical and molecular methods were in complete agreement. A total of 46 isolates were identified as Enterococcus faecium, 32 as Weissella paramesenteroides, 29 as Lcb. plantarum, 29 as Levilactobacillus brevis, 29 as Pediococcus pentosaceus, 7 as Lactococcus lactis, 6 as Leuconostoc mesenteroides, 6 as Latilactobacillus curvatus, 3 as Enterococcus faecalis and 2 as Lactiplantibacillus pentosus. In the case of Kefalograviera cheese, the dominant species was E. faecium, while in Feta cheese was Lcb. plantarum.

The present research confirms the diversity at species level of the milk and cheese microsystem since the 189 isolates were classified to 10 different species.

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INVESTIGATION OF MOLECULAR LINKS BETWEEN ENVIRONMENTAL SENSING, DEVELOPMENT, AND VIRULENCE IN PATHOGENIC FUNGI

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Environmental sensing is crucial for the development and the adaptation of all organisms. In eukaryotes the TOR kinase is the main cellular hub that links environmental sensing with growth and proliferation. Filamentous fungi can orientate their hyphae towards environmental cues and studies in Fusarium oxysporum have shown that this chemotropic response is mediated by the pheromone receptor Ste2 and the MAP kinase signaling cascades (i.e., Fus3 and Slt2). The TOR signaling pathway in fungi has important roles in the regulation of nutrient sensing, hyphal elongation, and branching, but the molecular details are still elusive.

Here, we investigated the molecular machinery that is involved in environmental sensing and chemotropic response in the plant pathogen Verticillium dahliae. We optimized a quantitative chemosensing assay and tracked the chemotropic response of the fungus against a wide variety of signals. The MAP kinase Fus3, was indispensable for the chemotropic growth against nutrients, while Slt2 mediated the response against the plant host.

Deletion of the pheromone receptor Ste2 impaired chemotropism in the same way as the slt2 deletion mutant did. Interestingly, artificial infections of eggplant seedlings revealed that only the Fus3 kinase had a pivotal role in pathogenicity.

To examine the involvement of TOR signaling pathway we functionally characterized the Rag GTPase Gtr1, which is part of the GTR1/2 complex that activates TOR kinase in response to amino acids in yeast and mammals, and the TSC2, which is part of the TSC1/2 complex that inhibits TOR activation and promotes autophagy upon stress and starvation in mammals.

We report that both deletion mutants showed impaired chemotropism against plant-derived signals and attenuated virulence, while Δgtr1 was also unable to sense different kind of nutrients. During environmental sensing we detected a fine-tuned and time-dependent pattern of phosphorylation of both MAPKs, that was drastically altered upon deletion of both gtr1 and tsc2, implying a potential crosstalk between these pathways.

Collectively, our results demonstrate the conserved role of MAPKs in the regulation of chemotropic response and indicate a crucial role for components of the TOR signaling in this process through its putative interactions with the MAPKs.