ΜΙΚΡΟBIOΚΟΣΜΟΣ

Microbial Communities as growth engines for Greece

Book of Abstracts

KEYNOTE LECTURE

Feedback with soil microbes drives plant community composition in temperate ecosystems

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SHORT BIO

John Klironomos, Professor, Biology /BRAES Institute, Associate Dean of Research, Irving K. Barber School of Arts and Sciences, The University of British Columbia, Okanagan Campus, Canada. Prof. Klironomos is a fellow of the Royal Society of Canada. His research group "focuses on one group of mycorrhizal fungi (arbuscular mycorrhizal fungi – Phylum Glomeromycota), although they often include other fungi (mycorrhizal, saprobic, pathogenic), and other soil biota as well (e.g., bacteria, protists, fungi, invertebrates), using two approaches in studying mycorrhizal ecology: (a) comparative and manipulative field studies, and (b) controlled experiments under laboratory and greenhouse conditions". More information may be found at https://biodiversity.ubc.ca/people/faculty/john-klironomos.

O1. PLENARY LECTURE I

Plant disease susceptibility; on host genes and pathogen effectors controlling infection

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Susceptibility to infectious diseases caused by pathogens affects most plants in their natural habitat and leads to yield losses in agriculture. But plants are not helpless because their immune system can deal with the vast majority of attackers that our recognized through molecular patterns by specialized receptors. Nevertheless, adapted pathogens have evolved effectors to circumvent or avert host immunity making plants susceptible to these uninvited guests. In addition to the failure of the plant immune system, there are other host processes that contribute to plant disease susceptibility. In my presentation I will discuss the active role that the host plays in supporting disease. Mutation of susceptibility genes can render plants resistant to pathogen attack thereby providing a method for obtaining disease resistant crops. In my presentation I will provide some insight into the translation of our fundamental science from the Arabidopsis-downy mildew system to its use in breeding for resistance in vegetable crops.

O2.

Priming of the plant immune responses through epigenetic modifications triggered by a beneficial bacterium

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Numerous epigenetic modifications can be caused by biotic and abiotic factors but only some of them can be inherited to the next generation. The aim of this work was to study the epigenetic background of the inherited resistance of *A. thaliana* to *Verticillium dahliae* infection observed in the offspring of plants treated with the biocontrol agent *Paenibacillus alvei* K165. Transcriptomic analysis in the above ground tissue of K165 treated plants and their offspring revealed significant changes in the expression levels of genes in phenylpronanoid biosynthesis pathway which gives lignin as a final product. This was confirmed by the high lignin levels observed in K165 treated plants and their offspring. The whole genome acetylation levels of resistant plants were found higher than those of control plants using western blot analysis. Moreover, Chromatin Immunoprecipitation experiments have shown a correlation between the expression levels of *PDF1.2* and *CAD8* genes and the acetylation levels in their promoters. Finally Arabidopsis mutants in CAD genes lost the resistance caused by K165 as well as the inherited resistance. The results of this study demonstrate the importance of epigenetic modifications for the activation of plant immune responses by biocontrol agents. Priming the plants' immune system using a biocontrol agent can result in offspring with an innate epigenetically inherited resistance to a pathogen.

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) and the General secretariat for Research and Technology (GSRT).

O3.

Roles and interactions of the members of a bacterial consortium along the degradation of the recalcitrant fungicide thiabendazole revealed via multi-omic approach

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Recalcitrant to degradation compounds used in agriculture pose a challenge for environmental management. Thiabendazole (TBZ), a benzimidazole commonly used against postharvest fungal diseases and as anthelminthic in livestock farming, is highly persistent in soil (DT50> 1-2 years) without known microorganisms with potency to degrade it. Our group has recently enriched from soil a bacterial consortium able to rapidly degrade TBZ through cleavage of the benzimidazole ring. However, no pure TBZ-degrading isolate was obtained, despite numerous attempts, suggesting complex interactions between consortium members. We employed a multi-omic approach to elucidate the microbial interactions that maintain the consortium TBZ-degrading capacity complemented by stable isotope probing (SIP). Metagenomics resulted in 19 high quality metagenome assembled genomes (MAGs) with six being dominant. Alongside, SIP 16S-rRNA-gene-amplicon sequencing verified previous group findings of the key degrading role of a *Sphingomonas* strain comprising the most dominant metagenome bin. RNA sequencing of the consortium supplied with TBZ or succinate, as sole carbon sources, showed the enhanced expression in *Sphignomonas* of (i) a a carbazole dioxygenase locus having a probable role in the initial transformation step of TBZ (ii) interesting signaling, transport, secretion and conjugation associated genes. RNA data networking analysis suggested the interaction of *Sphingomonas* with a *Hydrogenophaga* strain and possible contribution of the latter to the overall cobalamin balance via upregulation of the complete *cob* locus during TBZ degradation. Proteomics supported the mRNA differential expression patterns with a more stable expression profile over time. On-going metabolomics and associated modeling are expected to elucidate the full metabolic pathway of TBZ.

Acknowledgements

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http://emigrate.bio.uth.gr / https://www.omic-engine.com

O4.

Characterization, Identification and Physiological Studies of a Pigment-producing Tentative Pseudomonas spp. with Antifungal Properties

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A Gram negative, psychrotrophic, alcalophilic bacterium of very small size (<0,45μm) with antifungal properties was isolated randomly in a lab refrigerator and identified by phylogenetic analysis using the 16sRNA, gyrB and rPOD nucleotide sequences. Its physiology, morphology, basic biochemical properties and growth conditions were studied, in addition to its antifungal activity.

This rod-shaped bacterium, which produces a melanin-type pigment, was found to be a Pseudomonas species, which so far cannot be fully identified with any of the known species of the genus. It is catalase and oxidase positive, urease negative, exhibits motility, lipase activity and can utilize citric acid.

The production of the pigment is dependent on environmental conditions and medium composition and appears as a reversible feature of the bacterium (thus probably controlled by plasmids), which is also fading gradually, along with the antifungal activity, after long preservation of the culture under refrigeration. Fresh cultures, especially under co-cultivation with fungi, can resume and exhibit high antifungal properties. The bacterium has a wide pH range for growth, exhibiting optimal growth at pH 9, is relatively heat resistant up to at least 60°C, and can rapidly outgrow many different fungi (by slowing down, or completely pausing fungal growth), even in fungal selective media, at a pH of ≥4. Its antifungal properties are dependent on the presence of viable cells, and are not due to any extracellular metabolite (no antimicrobial activity was found in cell culture filtrates). The bacterium showed significant inhibition of growth of *Penicillium expansum*, *Aspergillus flavus*, *Rhizoctonia solani* and *Rhodotorula mucilaginosa* in synthetic media, based on well diffusion assay on agar media, and on the measurement of target cell population in liquid cultures (assessed as cfu/ml, or spectrophotometrically as O.D. - Optical Density). However, it had no antibacterial activity against several bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*).

The antifungal properties of this bacterium can be of great biotechnological interest in food preservation, plant bioprotection and therapeutics and set the basis of future research on the biosynthesis of the melanin-type pigment and the mechanisms for the expression of antifungal activity.

Keywords: *Pseudomonas*, melanin-type pigment, antifungal, molecular identification, biochemical properties

O5.

Wastewater irrigation is affecting the prevalence of antibiotic resistance genes in surface soil and groundwater.

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Wastewater reuse is a necessary practice due to freshwater depletion, however the impact of wastewater irrigation on the prevalence of antibiotic resistance genes (ARGs) in soil and groundwater environments, is not yet completely elucidated. We hypothesized that wastewater irrigation is increasing the relative abundance of ARGs in the topsoil and the groundwater. To test our hypotheses we sampled an agricultural area that is irrigated with treated wastewater. We performed three different sampling campaigns. The sampling of the groundwater took place in the central groundwater station (10 m depth) of the agricultural area and started in April 2017 until December 2018. For the soil samples the campaign lasted from October 2017 to December 2018 in a selected field of the agricultural area. During those sampling campaigns the fields were irrigated with different type of irrigation, depending on the crops' needs in nutrients (rainfall, outflow & outflow mixed with digested sludge). In addition to gain further insight on the ARGs dynamics in the groundwater we performed an extra campaign on the groundwater of the selected field (10 m depth), before the start of irrigation in June 2018 and following the irrigation in July & September (2018). Using quantitative PCR, we analyzed five ARGs (*bla*TEM*, bla*CTX-M-32*, bla*OXA-58*, tetM*, *qnrS*, *sul1*), along with the genes *intI1* and 16S rRNA; and we estimated their abundance (copies/L; copies/gr of dry soil) and relative abundance (gene copies/16S rRNA). The results show that wastewater irrigation in the soil is increasing significantly the absolute and the relative abundance of several genes (*sul1*, *intI1*, *qnrS* and *bla*OXA-58). The dynamics of microbial community in the groundwater are different, since wastewater irrigation in the groundwater in both campaigns, led to a reduction of total microbial abundance. This reduction had as an effect that the absolute abundance of those ARGs in the groundwater remained stable or lower, however their relative abundance increased after irrigation. In conclusion, wastewater irrigation is increasing the prevalence of specific genes in topsoil and groundwater (especially the genes *sul1* & *intI1)*, however with different dynamics.

Keywords: Antibiotic resistance, wastewater reuse, qPCR, soil, groundwater, ARGs

O6. PLENARY LECTURE II

Pathogens Perception by the Plant Innate Immunity System: from Basic Research to Applications

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One of the major challenges facing humankind in the 21st century is how to feed a growing population that may exceed 9.5 billion people by 2050. This needs to be done using the same or even less natural resources, because water and land are used in the increasing urbanisation of the world. This implies that plant productivity needs to increase dramatically and that preventing crop losses from diseases will therefore be crucial to ensuring global food security. Plant diseases, caused by pathogenic microorganisms, reduce the yield of the world's most important food crops. One of the main reasons that plant diseases continue to cause such damage is that disease resistance introduced by plant breeding is rapidly overcome by newly virulent pathogens. This problem is amplified by intensive crop cultivation techniques and mono-culture. In order to minimize crop losses due to plant diseases we need new knowledge of the mechanisms underpinning the molecular plant-microbe interactions. This requires understanding the biology behind the pathogenicity mechanisms of the microbes and the host components they target in plants to cause disease, as well as, the mechanisms by which plants defends themselves successfully from attack.

Up to date, how recognition of pathogen molecules activates plant defense is poorly understood. Plants recognize pathogens through an innate immunity system that monitors pathogen-associated molecules either outside or inside the plant cell. Intracellular NLR (Nucleotide-binding domain and Leucine-rich Repeat-containing) Immunity Receptors are sensitive monitors that detect pathogen invasion and are conserved in both plant and animal cells. In plants, NLRs confer recognition of diverse microbial pathogenicity components, known as "effector" proteins, associated with pathogen invasion. Our recent discoveries have provided novel insights into plant NLRs that carry fusions of other non-canonical protein domains, which play an important role in plant immunity. These composite immune receptors are thought to arise from fusions between NLRs and additional protein domains that serve as "baits" for the pathogen-derived effector proteins, thus enabling pathogen recognition.

O7. PLENARY LECTURE III

Individual cell behavior: understanding and predicting the “noisy” microbial responses in foods

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Bacterial cells within a clonal population can vary significantly in a number of phenotypic traits. The main source of this phenotypic heterogeneity is the stochastic variations associated with the genomic information flow including gene activation, transcription and translation. The fluctuations in the levels and activities of intracellular components, known as “molecular noise”, can lead to different behavior among genetically identical cells in a homogeneous environment. Heterogeneous behavior of individual cells is observed at the growth, survival and inactivation responses and should be taken into account in the context of Food Microbiology. Recent methodological advances can be employed for the study of single cell dynamics leading to a new generation of mechanistic models which can provide insight into the link between phenotype, gene-expression, protein and metabolic functional units at the single cell level. Such models however, need to deal with an enormous amount of interactions and processes that influence each other, forming an extremely complex system. In this review paper, we discuss the importance of noise and present the future challenges in understanding and predicting the “noisy” microbial responses in foods.

O8.

Effect of pistachio (*Pistacia vera* L.) on the fecal microbiota population of streptozotocin-induced diabetic rat

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Growing evidence suggests that beyond the genetic components, environmental factors, such as diet and intestinal microbiota, contribute to the onset and the development of Type 1 diabetes (T1D). Diet affects significantly gut microbiota diversity and functionality since different dietary compositions have different effects on bacterial shifts. Hence, the aim of the study was to investigate the effect of a dietary intervention with pistachio nuts on fecal microbiota populations in streptozotocin induced diabetic rat, an animal model of T1D. In this vein, male Wistar rats were randomly assigned into four groups: healthy animals which received the control diet (CD) or pistachio diet (PD) and diabetic animals which received control diet (DCD) or pistachio diet (DPD), for 4 weeks. Fecal samples were collected at the 1st (zero point) and the 4th week of the dietary intervention and microbial diversity was determined using Next-Generation Sequencing (NGS) technology. The pistachio diet resulted in significant alterations in the percentages of fecal bacterial populations. Specifically, at
genus level, increased populations of *Allobacullum* and decreased populations of *Oscillospira* and *Barnesiella* were observed in both healthy (PD) and diabetic (DPD) animals that received the pistachio diet, while percentages of lactobacilli, *Turicibacter* and *Bacteroides* were increased and *Clostridium* and *Roseburia* were reduced in PD compared to CD animals.
Moreover, percentage of bifidobacteria was increased in DPD rats, whereas *Eubacterium* was decreased compared to DCD. At phylum level, the ratio of firmicutes/bacteroidetes increased during the dietary intervention in DPD rats, but it was reduced in DCD animals. In conclusion, a pistachio supplemented diet seems to restore the fecal microbiota balance by enhancing the levels of microbial species associated with potential health benefits.

O9.

Structural variation of grape yeast communities over space and time

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Microbial biogeography supports the idea that the composition of microbial communities across space is non-random. To test this hypothesis for vineyard ecosystems we compared the grape yeast species assemblages in five major wine producing areas of Greece: Attica, Santorini (Aegean Sea), Peza (Crete) and two viticultural zones in Peloponnese (Nemea and Mantineia). Possible temporal variation of community structure was evaluated by sampling Santorini and Mantineia in two consecutive years. About 900 yeast isolates obtained from the initial stage of spontaneous fermentations were identified at the species level by PCR-RFLP and 5.8S rDNA sequence analysis. *Hanseniaspora guilliermondii, H. opuntiae, H. uvarum* and *Metschnikowia pulcherrima* were the most abundant species out of 16 total populations identified. Species richness differed among regions, ranging from 2 (Mantineia) to 11 populations (Santorini). The analysis of similarities (ANOSIM) on squared root transformed species incidences revealed significant differences ($p<0.05$) in the composition of yeast communities over space and time. The degree of structure among communities of different regions was not clearly correlated with their geographical distance. Attica and the relatively nearby Nemea region possessed the most distinct communities, while Nemea and Peza communities showed the highest resemblance. Significant differences in community composition were observed between the two sampling years in Santorini, although in Mantineia no variation was detected. Present results show for the first time spatial variability of grape yeast communities in important Greek viticultural areas. We further showed that the dissimilarity in community structure does not necessarily increase with the geographic
distance, and may be apparent even in nearby regions. Besides, the significant temporal variation in community composition observed in Santorini, a rather restricted region of minimal external influence, raises the possibility of stochastic processes as possible contributors in shaping the structure of grape yeast communities. The above results support the 'microbial *terroir'* concept for Greek vineyards, industry an importance aspect for the wine industry.

Keywords: yeasts, microbial diversity, microbial biogeography, microbial terroir, wine

O10.

Comparative evaluation of the disinfection actions of thymol and benzalkonium chloride against adapted and non-adapted to thymol *Salmonella* biofilm cells

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Nowadays there is an urgent need to develop novel efficient, safe, cost-effective and preferably eco-friendly antimicrobial methods to combat bacterial pathogenic biofilms. These methods should quickly destroy bacteria if possible, without leading to resistance development. To this direction, in this work, the disinfection actions of thymol, a natural plant-derived compound well-known for its antimicrobial action against planktonic (free-growing) cells, and of benzalkonium chloride (BC), a synthetic chemical widely used for the disinfection of surfaces in the food industry and elsewhere, were comparatively evaluated against adapted and non-adapted to thymol biofilm cells of a *Salmonella enterica* ser. Typhimurium strain. To do this, bacteria were left to form biofilm on model stainless steel (SS) coupons (type AISI 304) incubated, at 20 oC for 120 h, in 1/10 diluted tryptone soy broth (dTSB), containing or not a sub-inhibitory (equal to ½ of its MIC) thymol concentration, with medium renewal at 48 h of incubation. Following 120-h incubation and the removal of loosely attached cells by rinsing, biofilm bacteria were submitted to disinfection for 15 min at 20 oC. The remaining viable biofilm bacteria were removed from surfaces and enumerated by agar plating. In parallel, confocal laser scanning microscopy was applied to visualize biofilm structures both before and after disinfection. The minimum biofilm inhibitory and eradication concentrations (MBIC/MBEC) of the two compounds were also determined against biofilms formed on 96-well polystyrene microtiter plates. The obtained results revealed the significant anti-biofilm action of thymol and its superiority to that of BC. However, the reduction in the susceptibility of adapted to thymol biofilm bacteria is still of concern. Overall, the obtained knowledge assists the continuous efforts towards the development of novel biocides to combat biofilms, multicellular robust structures well-characterized by their increased hardiness against many stresses and antimicrobials.

Keywords: *Salmonella enterica*, biofilm, food industry, disinfection, thymol, carvacrol, CLSM

O11.

Exploring the microbial ecosystem of Geremezi cheese using culture-dependent and -independent approaches

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Geremezi is an artisanal Greek soft cheese with a slightly sour taste, manufactured usually from sheep or goat milk in Aegina island. Geremezi is poorly characterized, thus its full microbiota fingerprinting using culture-dependent and -independent approaches is of interest. The cheese sample was initially subjected to classical microbiological analysis using selective growth media. The isolates were grouped using the genotyping technique of rep-PCR and representative bacterial and yeast isolates of each group were identified at the species level by sequencing the 16S rRNA gene and ITS DNA region, respectively. Afterwards, total DNA was extracted from the cheese sample using a novel protocol developed in our laboratory and the results obtained from the sequencing of 16S rRNA gene and ITS DNA region were analyzed using advanced bioinformatics tools. Using culture-dependent techniques, two bacterial genera, i.e. *Staphylococcus* and *Enterococcus* and two yeast genera, i.e. *Pichia* and *Kluyveromyces,* were mainly identified. On the contrary, the results of the metagenomics analysis revealed a vast diversity of bacterial and yeast genera. The dominant bacterial genus identified was *Lactobacillus* (approximately 93%), followed by *Streptococcus* (1,3%) and *Leuconostoc* (0,5%). *Kluyveromyces* was the dominant yeast genus (72%), followed by *Kazachstania* (24%) and *Penicillium* (0,1%). The analysis we present here is the first attempt to explore the microbiome of Geremezi cheese.

O12.

Monitoring the effect of food spoilage bacteria on the photocatalytic activity of a TiO2 nanoparticle based surfactant against biofilms formed by foodborne bacteria

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Both pathogenic and spoilage microorganisms adhere to the surfaces in food processing environments, survive, grow and form biofilms. Microbial interactions play an important role in the initial cell adhesion and endurance of biofilm to disinfectant stresses. The aim of the present study was to evaluate the disinfecting activity of an innovative photocatalytic surfactant based on TiO_2 nanoparticles, against various biofilms. To this respect, *Salmonella* Enteritidis, *Listeria monocytogenes* and *Escherichia coli* were left to form biofilm in mono- or co-cultures with food spoilage bacteria on stainless steel (SS) coupons immerged in TSB at 20°C for 6 days. The spoilage bacteria used for co-cultures were belonged to the genera *Leuconostoc*, *Lactobacillus*, *Serratia*, *Citrobacter*, *Hafnia*, *Proteus*, *Pseudomonas* and *Brochothrix***.** After biofilm formation, the SS coupons were immersed in the disinfectant and afterwards exposed to ultraviolet radiation for 2h for each side separately. Biofilm population was enumerated by bead vortexing-plate counting method. From the results, it was evident that the exposure to ultraviolet radiation reduced the population of biofilm cells below the detection limit of the method in most cases, whereas the use of the disinfectant strengthened the antimicrobial activity of UV. Furthermore, it was observed that the presence of more than one species affected both cell biofilm ability and their resistance to ultraviolet radiation and disinfectant. In conclusion, the use of the surfactant with the photocatalytic TiO_2 agent as an alternative way of cleaning contaminated surfaces presents an intriguing case that may provide powerful solutions regarding biofilm disinfection within the food processing environments.

Keywords: biofilm, disinfection, nanoparticles

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O13. PLENARY LECTURE IV

Interactions in the microbial world – from microtiter plates to the field

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SHORT BIO

Antonis Chatzinotas, Group Leader Microbial Systems Ecology Department of Environmental Microbiology, Helmholtz Centre for Environmental Research-UFZ Leipzig, Germany. He received his PhD in 2000 from Swiss Federal Institute of Technology Zürich (ETH), Switzerland. Nowadays, his research aims at understanding the diversity and functioning of microbial communities (bacteria, protists, phage/virus, predatory bacteria) in natural and engineered ecosystems (e.g. lakes, rivers, aquifers, soils, wastewater treatment plants, urban environments, animals). More information may be found at *https://www.ufz.de/index.php?en=39070*
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O14.

Identification of microbial communities by RNA stable isotope probing and 16S rRNA sequencing from the Benguela coastal upwelling system

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The Benguela coastal upwelling system is characterized by the highest primary productivity compared to other upwelling regions, episodic occurrence of free hydrogen sulfide gas and formation of an oxygen minimum zone (OMZ). RNA-based stable isotope probing (SIP) was used to identify nitrate and sulfate reducing microorganisms from three different sediment sampling stations using 13C acetate as labelled substrate.

Labelling patterns of microbial communities, as assessed by high-throughput sequencing of 16S rRNA, varied across SIP incubations and depended on sampling station. When no external electron acceptor was added, an increase in the relative abundance of Epsilonproteobacteria was observed at two stations but of Gammaproteobacteria at the third station, which had a much lower water depth. In addition, an increase in Epsilonproteobacteria was observed both when nitrate or sulfate were added.

It can be concluded that nitrate stimulated nitrate-reducing, sulfide-oxidizing bacteria, and inhibited the growth of sulfate-reducing bacteria. Furthermore, sulfate addition did not enhance the abundance of known sulfate-reducers, such as Deltaproteobacteria. This could be attributed to the competition for electron donors between nitrate-reducers and sulfate-reducers, to the inability of certain sulfate-reducing bacteria to use acetate as an electron donor or to the short duration of the incubations.

Keywords: Oxygen minimum zone (OMZ), RNA SIP, sediment, Illumina MiSeq, acetate, 13C, nitrate, sulfate

O15.

Stable microbial communities in the Thermopyles geothermal springs over a six-year period as revealed by shotgun metagenomics

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Microbial communities in geothermal springs provide the opportunity to directly access the stability and functions of subsurface microbial communities. These environments are considered stable in terms of environmental conditions, and continuously fed by subsurface water, so that changes in microbial diversity are expected to be restricted. However, the stability of microbial communities in these ecosystems has not been test as studies over long time periods are lacking. Toward closing this gap, we applied whole metagenome sequencing to 17 water samples, collected between 2010 and 2016, two to four times annually, from the Thermopyles geothermal spring, in central Greece. As revealed based on16S rRNA gene fragments recovered from the metagenomes, *Proteobacteria* dominated in all samples (>80% of total). Morisita similarities between samples ranged from 30% to 99%, which was substantially higher than similarities seen in surface water systems such as Kalamas River (Meziti et al., Env. Microb 2016). However, a group of eleven samples was characterized by >95% Morisita similarities and was characterized by *Epsilonproteobacteria*-related operational taxonomic units (OTUs). The rest of the samples were characterized by the co-dominance of *Epsilonproteobacteria, Gammaproteobacteria* and *Alphaproteobacteria*. On the other hand, similarities between samples regarding functional gene content were much higher, with all samples being >70% similar when using third level seed subsystems.

Distinctive pathways between distinct groups of samples/communities were related to respiratory complex I, TCA and glyoxylate-serine cycle and methionine and aminoacids biosynthesis but not to the season that the sample was taken. Most groups were not similar between 16S rRNA gene taxonomy and function revealing that taxonomy was not always correlated with functions. Comparisons with other freshwater habitats on functional level revealed the significantly higher abundance of CRISPRS, sulfur oxidation and of Complex I related proteins in Thermopyles vents, with the latter proteins allowing for more flexible metabolism switching between autotrophy and heterotrophy.

Keywords: Whole metagenome sequencing, geothermal springs, microbial communities

O16.

Microbial arylamine *N*-acetyltransferases: From xenobiotic detoxification to antibiotic biosynthesis.

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Microorganisms use xenobiotic metabolizing enzymes to fight poisonous chemical influences from the environment. Arylamine *N*-acetyltransferases (NATs) are enzymes known to detoxify potentially harmful compounds, including drugs, carcinogens, agrochemicals and industrial pollutants. We study the evolution and functional diversity of this enzyme family in microorganisms. We assessed the xenobiotic metabolizing potential of 93 free-living bacteria of broad taxonomic range, using the model environmental contaminant 3,4-dichloroaniline (3,4-DCA). Cultures of each isolate were assayed by thin layer chromatography for bioconversion of 3,4-DCA to its acylated metabolites. Although the results of our recent differential scanning fluorimetry experiments support the typical acetyl-CoA dependent *N*-acetylation of xenobiotics by some bacterial NAT proteins, parallel work of our group also indicates remarkable functional divergence within this family. Our comparative *in silico* structural and phylogenetic analyses demonstrate that NAT enzymes belong to the superfamily of cysteine proteases, with the ancestral microbial homologue diverging into the families of NATs and transglutaminases. Another intriguing example is a previously identified *NAT* homologue of the antibiotic-producing actinomycete *Amycolatopsis mediterranei* which has been reported to act as a rifamycin amide synthase, catalyzing an unexpected acyl-CoA independent reaction. To examine the possible role of other bacterial NAT enzymes in antibiotic biosynthesis, 1820 genomic sequences were retrieved and analyzed with antiSMASH software for *NAT* localization within gene clusters of secondary metabolism. This bioinformatics analysis predicted 237 *NAT* homologues located in putative biosynthetic gene clusters across bacterial genomes, mainly of actinobacteria. A similar line of investigation is underway for eukaryotic microbial genomes, so far identifying 100 fungal *NAT*-bearing clusters of secondary metabolism. To understand the potential functional divergence of fungal *NAT* homologues, we have further generated and use as model a collection of single (*ΔNAT1*, *ΔNAT2*, *ΔNAT3*), double (*ΔNAT1*/*ΔNAT2*, *ΔNAT1*/*ΔNAT3*, *ΔNAT2*/*ΔNAT3*) and triple (*ΔNAT1*/*ΔNAT2*/*ΔNAT3*) knockout strains of the plant-pathogenic sordariomycete *Fusarium verticillioides*.

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O17.

Host selection differentiates intraradical endomycorrhizal communities colonizing endemic plants of sand dune ecosystems in Greece.

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Seasonal drought and low nutrient availability may limit plant growth in numerous regions of the Mediterranean basin. Costal sand dunes are typical ecosystems in which these conditions prevail. We studied the endomycorrhizal communities in the roots of four endemic plants from several costal dunes in Greece (*Pancratium maritimum, Otanthus maritimus, Medicago marina* and *Eryngium maritimum*). The intraradical arbuscular mycorrhizal fungal (AMF) communities were analysed via MiSeq-Illumina 2x300 bp sequencing, using semi-nested PCR-amplified root DNA samples targeting the SSU region of the 18S rRNA gene. Plant roots were generally highly colonized by taxon-rich AMF assemblages, and host plants appear to be critical determinants of the composition of the AMF-colonizer communities. On the contrary, spatial differentiation effects deriving from the different sand dune locations and surrounding ecosystems were not observed. This indicates that plants had access to a similar AMF pool independent of the sampling site, a status potentially deriving from uniform environmental filtering among sand dune sites. Overall, host selection leads to clearly diversified, taxon-rich intraradical AMF assemblages, which are however, dominated by a few highly abundant taxa in at least two of the examined plants, that may be critical for endemic plant survival in the sand dune ecosystems.

Keywords: arbuscular mycorrhizal fungi, sand-dunes, MiSeq Illumina, *Pancratium maritimum, Otanthus maritimus, Medicago marina and Eryngium maritimum*

O18.

Hydrolytic microorganisms isolated from an immobilized cell bioreactor treating red-pepper processing wastewater

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The red pepper (*Capsicum annuum* L.) is a common food commodity around the globe. Based on FAO statistics, the global pepper market accounts annually for 34.5 million tons, with most of such quantities being produced in Asia, Africa and in the Mediterranean basin. In particular, Greece produces annually 150.000 tons, making it the second most cultivated kind of vegetable in our country. On the other hand, the generated wastes and residues of pepper-processing industries are suitable sources for biotechnological applications and innovation. In this work, the biotechnological potential of hydrolytic microbiota isolated from pepper processing wastes were investigated through the implementation of dilution plating method in the Siran beads of an immobilized cell bioreactor treating this processing wastewater, followed by assays for the determination of the various enzymes involved in the hydrolysis of cellulosic and hemicellulosic compounds. The estimation of xylanase and glucanase activities resulted in the detection of high enzyme activities for certain microbial isolates, thus indicating their potential in the valorization of hemicellulose and cellulose. For instance, xylanase and glucanase activities in certain microbial isolates were as high as 89.9 and 18.5 U/mg protein. It is concluded that red-pepper microbiota can be considered as new sources for biotechnological application and biomass valorization.

Keywords: red-pepper processing wastewater; immobilized cell bioreactor; xylanase;
glucanase; biomass valorization

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O19. PLENARY LECTURE V

Mining the Ocean microbiome: from the known to unknown

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The Ocean Sampling Day (OSD) series of global sampling events aim to capture a “snapshot” of the ocean’s microbial diversity. The first OSD event took place on June 21st, 2014, and was performed by ~150 teams of marine scientists. These teams used standardized protocols to collect samples across European, North American, Asian, Australian, Arctic, and Antarctic coastal waters. As a result, OSD2014 produced one of the most extensive and intercomparable coastal sample collections available, allowing us to better understand the coastal microbiome and to develop a new set of bioinformatic and analytical procedures.

Within the OSD2014 data set, we detected a vast repertoire of open reading frames (ORFs) with unknown function, a common issue in most of the metagenomic studies. These ORFs usually constitute around 40-60% of the predicted ORFs in a metagenomic dataset. In most studies, this unknown fraction is ignored, alongside potentially novel insights into the ocean’s biology. To approach this mysterious unknown fraction, we have developed both a conceptual framework and a bioinformatic pipeline to first structure and then explore the functionally unknown portion of the protein universe. To structure this space, we partitioned millions of predicted ORFs from metagenomic data sets into three groups: knowns (ORFs of known function, present in sequenced genomes), genomic unknowns (ORFs of unknown function, present in sequenced genomes) and environmental unknowns (ORFs of unknown function, not present in sequenced genomes). Next, we used numerical ecology approaches to explore their relationship with environmental variables, their occurrence in the Genomic Taxonomic Database (GTDB) and their presence in biosynthetic gene clusters. In doing so, we identified clusters of unknowns that were ubiquitously distributed across the different metagenomic data sets. As they appear strongly conserved, these clusters may encode fundamental microbial functions similar to those of their characterized counterparts, present in almost every genome and ecosystem. Furthermore, we distinguished a set of clusters that are highly abundant, but not ubiquitous, that might be indicators of “adaptive”, “organism-specific” or “habitat-specific” functionality.

Getting a better understanding of these unknown entities is becoming one of the top priorities in microbiome research as they will reveal new biological and ecological insights and pave the path towards the discovery of new biosynthetic potential.

O20. PLENARY VI

Bioelectrochemical Systems for Carbon-Neutral Biofuel and Bioenergy Production

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Bioelectrochemical systems (BESs) are a promising technology for both biofuel/bioenergy production and resource recovery within a carbon-neutral framework. The presentation will briefly review factors affecting BES performance and will summarize remaining challenges needed to be addressed before such systems are considered for industrial applications. Two current BES studies will be presented.

a) H_2 production: This study assessed the biotransformation pathways and inhibitory effect of two representative furanic (furfural, FF; 5-hydroxymethylfurfural, HMF) and three phenolic compounds (syringic acid, SA; vanillic acid, VA; 4-hydroxybenzoic acid, HBA) in a MEC bioanode. Biotransformation of the five compounds occurred via fermentation, resulting in the production of acetate, which became the main electron donor for exoelectrogenesis. The extent of transformation was higher for the furanic compounds (67% of FF and 64% of HMF) than for the phenolic compounds (50% of SA, 14% of VA and 10% of HBA). The phenolic compounds transformed via a sequence of demethylation and decarboxylation reactions verified by the detection of metabolites by LC/MS-MS. Catechol and phenol were persistent transformation products of VA and HBA, respectively. All five parent compounds were inhibitory to exoelectrogens (IC_{50} = 1.9 - 3.0 g/L). Individual, non-inhibitory concentrations of the five compounds, when in mixture, resulted in severe inhibition. Microbial bioanode community analysis showed the presence of known degraders of furanic and phenolic compounds, fermentative bacteria, and exoelectrogens in syntrophic partnerships.

b) Biocathodic Conversion of CO_2 to CH_4: The objective of this study was to develop and test BESs to directly convert CO_2 to CH_4 for anaerobic digester biogas upgrading. BESs were developed with acetate-fed bioanodes and CO_2-fed biocathodes, maintained at -0.8V vs. SHE. The methane production rate with a biocathode inoculated with a hydrogenotrophic methanogenic culture was 3.8-fold higher than that of another biocathode inoculated with a mixed methanogenic culture. The archaeal communities in both biocathodes converged primarily on a single methanogenic phylotype (*Methanobrevibacter arboriphilus*). The biocathode bacterial community was enriched in *Proteobacteria*, exoelectrogens and putative producers of electron shuttle mediators, indicating an important role for Bacteria in biocathode methanogenesis. Addition of up to 3% v/v H_2S initial concentration along with CO_2 in the cathode headspace resulted in up to a 2-fold increase in CH_4 production due to the transport of H_2S to the anode compartment where was oxidized donating electrons to the anode. However, CH_4 production declined above 3% v/v/ H_2S, indicating an inhibitory effect. Microbial community analysis of four BESs with biocathodes at different conditions (control, H_2S-amended, ZVI-amended, and H_2S- and ZVI-amended) showed the effect of biofilm, ZVI and H_2S on both the anode and cathode microbial communities. Additionally, this study evaluated biocathode performance using biogas produced by a laboratory anaerobic digester to guide future design of biogas upgrading bioelectrochemical systems.

O21.

A tool for examining the bioenergetic diversity of microbiomes

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The gut microbiome has lately emerged as an important factor in health and disease. However, a lot of questions on how the diversity of the microbiome affects our health still remain unanswered. One aspect that has been largely overlooked is the diversity of the microbiome with regards to bioenergetic pathways. We recently published a study [1] in which we re-examined published datasets of human gut microbiota, focusing on a set of species which represent the full bioenergetic diversity of prokaryotes across all the major bacterial and archaeal lineages. Our results indicate that a number of species are present in the human gut of both adults and infants, which normally derive their energy from methanogenesis, iron oxidation, iron reduction, sulfate and arsenate reduction. These pathways can affect the dynamics of the human gut microbial community, as well as the bioavailability of elements such as iron, arsenic and sulfur to the host. We aim to extend our analysis to cover more datasets, from humans as well as animals of economic importance, to examine associations of bioenergetic diversity with disease. To this end, we have created a database of bioenergetic pathways for the identification of homologous relationships and "diagnostic" genes for each pathway. We use this information to assess the bioenergetic diversity of published metagenomics data. The ultimate aim is to automate this process to enable fast annotation of metagenomes in terms of bioenergetics. The database can also be mined to extract information on the evolution of the different bioenergetic pathways across prokaryotes, an analysis which is hampered by their patchy distribution across lineages [2], and the fact that different pathways share multiple homologous core complexes.

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O22.

The role of staphylococcal-specific *glyS* T-box domains in transcription regulation and antibiotic binding

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T-box riboswitches are tRNA-dependent regulons found in all Gram-positive human pathogens and attractive molecular targets for new antibiotics. Although they share a phylogenetically conserved architecture, T-boxes also exhibit species-specific structural variation and synchronize the expression of genes that are either metabolically related or are involved in a balancing act of specific amino acids utilization for different cellular processes.
Staphylococcal *glyS* T-boxes posses a unique structural feature in the terminator/antiterminator stem termed stem Sa, which distinguishes them from their counterparts in bacilli. In *S.aureus glyS* T-box controls the expression of a sole *glyS* gene which supplies with glycine both the ribosomal translation and cell wall formation, through the aminoacylation of five tRNAGly isoacceptors. Not long ago, we have shown the direct diverse effects of mainstream antibiotics on the wild type *glyS* T-box-mediated transcription and that stem Sa is a hotspot for antibiotics binding. Structural probing and *in vitro* analysis of a series of mutants containing swaps of stem I or apical loop between bacilli and staphylococci revealed that structural differences reflect on the ability of the mutants to induce transcription. Moreover, deletion of the staphylococcal specific stem Sa reduced the *in vitro* transcription independently of the presence or absence of antibiotics, suggesting that stem Sa is important for T-box-mediated transcription. Recently, we have solved the cryo-EM and crystal structure of the *glyQS* T-box from bacilli which supports the important role of stem III in sensing the aminoacylation status of the tRNAs. Our data suggest that stem Sa is important for the equilibrium of transcription termination/antitermination conformations when all tRNA isoacceptors compete for T-box riboswitch binding and accommodates binding of mainstream antibiotics. In addition, when stem Sa is deleted, antibiotics prefer to bind on stem III, thus interfering with transcription and inhibiting it. Most importantly, the available T-box structure informs for the rational design of novel species-specific antibacterials.

O23.

Characterization of RNA silencing pathways and physiological roles in the model diatom *Phaeodactylum tricornutum*

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Diatoms are a group of highly diverse unicellular, eukaryotic algae responsible for up to 20% of the earth's oxygen. Diatoms play a pivotal role in marine food webs and present promising applications in biotechnology. RNA silencing is a conserved mechanism that regulates gene expression and involves a set of key proteins including the RNases Dicer (DCR) and Argonaute (AGO), and RNA dependent RNA polymerases (RdRP). In animals and plants, RNA silencing plays an important role in growth and development, maintenance of genome integrity via the repression of transposable elements and transgenes, defense against viruses, and acclimatory response to abiotic stress. To date, no diatomaceous DCR, AGO and RdRP homologue has been cloned, while evidence for their respective contribution and mode of action in RNA silencing, as well as, for the importance of RNA silencing in diatom physiology, is still lacking.
We performed a phylogenetic analysis across a wide range of diatom species which revealed changes in DCR/AGO/RdRP gene repertoire coincided with major evolutionary transition in diatom sexual reproduction and locomotory traits. DCR/AGO/RDR homologues of the model diatom species *Phaeodactylum tricornutum* were cloned, YFP-tagged, and ectopically expressed for determination of their subcellular localization. Functional characterization of DCR was first carried out by heterologous expression approach in the yeast model *Saccharomyces cerevisiae* and the plant model *Nicotiana benthamiana.* In a second approach, DCR-KO lines were generated by CRISPR-Cas9 mutagenesis while their transcriptomes of large and small RNAs are being investigated by high-throughput RNA sequencing. With this study, we aim to provide seminal insights into key molecular mechanisms that control endogenous gene and transgene expression in diatoms, to better understand their acclimatory responses in the marine environment and to facilitate their utilization in blue biotechnology.

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O24.

Nuclear behaviour during colony initiation and conidial fusion in *Verticillium dahliae*

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The asexual ascomycete *Verticillium dahliae* is the causative agent of Verticillium wilt disease in a wide range of economically important plants. Its great genetic diversity has been attributed to the parasexual cycle which includes: hyphal anastomosis, heterokaryon formation, karyogamy and haploidization. Colony initiation in this fungus involves the first events that take place upon germination of their mitotically produced spores (i.e. conidia) to enable the formation of developing fungal colonies. Germ tube elongation, hyphal branching and anastomosis (fusion) between hyphae and/or conidia of the same or different genotypes are important aspects of this process. In this study we aimed at the analysis of nuclear behaviour during colony initiation, focusing on the interaction between genetically different nuclei. To this end, we selected representative *V. dahliae* strains of different Vegetative Compatibility Groups (VCGs; these are thought to refrain from fusing and forming viable heterokaryons), we labeled their nuclei with GFP- or mCherry-tagged histone 1 and used time-lapse live-cell microscopy to characterize the dynamics of the process. Conidia of *V. dahliae* are predominantly unicellular and uninucleate, and the first nuclear division occurs after the initiation of germination but before formation of the first hyphal septum. Cellular compartments of young hyphae remain exclusively uninucleate and only the nuclei of the apical cells retain mitotic activity, in contrast to other fungi. Conidia of the same or different genotypes can fuse through Conidial Anastomosis Tubes (CATs) in 45-80 min, under proper environmental conditions, after a distinct homing phase of mutual attraction. Following fusion, one of the nuclei occasionally divides and migrates to the interacting cell through the CAT (7-15 min), while the resident nucleus is usually degraded. Conidial anastomosis was also frequently observed between incompatible strains, but in most of these cases this was followed by a heterokaryon incompatibility reaction, i.e. nuclear degradation, membrane shrinkage and cell death. In this report we describe important developmental aspects of *V. dahliae*, which are relevant to a better understanding of genetic interaction between strains and emergence of new pathogenicity phenotypes.

Keywords: nuclear dynamics, conidial anastomosis tubes (CATs), heterokaryon incompatibility, time-lapse microscopy

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O25.

The *in silico* reconstructed protein-protein interaction network of *Streptomyces lividans*

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Streptomycetes exhibit unique metabolic diversity and enzymatic capabilities. They can secrete secondary metabolites that are valuable for industrial and pharmaceutical purposes making them a valuable resource for synthetic biology and metabolic engineering applications. The latter can lead to the production of minimal strains with increased cell stability in long-term fermentation and enhanced metabolic potential for the biosynthesis of heterologous molecules [1,2]. Still, the incorporation of information from omic analyses and the reconstruction of gene regulation, protein and metabolic networks in the context of systems biology approaches, are expected to enhance the knowledge about the *S. lividans* regulatory machinery, providing significant leads for directed metabolic engineering actions.

This is one of the major objectives of the collaborative European FP7 project STREPSYNTH “Rewiring the *Streptomyces* cell factory for cost-effective production of biomolecules”, a task of which is the presented study. In this context, we proceeded to the *in silico* reconstruction of the *S. lividans* PPI network, following a bioinformatics workflow that could be accordingly applied in any bacterium with no experimental PPI data available. It comprises a step in which an experimentally supported PPI network for *S. lividans* is reconstructed based on comparative genomic analysis with phylogenetically close bacteria for which PPI networks reconstructed from high-throughput experiments exist. The *in silico* reconstructed PPI network of *S. lividans* comprises 8982 direct PPIs for 1001 UniProt IDs. The reconstructed network can form the basis for validation studies of key PPIs and be used in the interpretation of omic datasets and the design of synthetic biology experiments.

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O26. PLENARY LECTURE VII

Drugs & Bugs: unexplored relationships

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Do drugs impact our gut residential flora, and if so is this restricted to antibacterials? Are there any general principles behind drug-drug interactions, and if so, can such principles help us identify effective drug combinations against multi-drug resistant (MDR) bacterial infections? Here I will present how systematic and quantitative approaches can give us insights into these questions.

We recently established that non-antibiotic drugs have a strong and broad impact on the human gut microbiome. This opens new paths for optimizing drug efficacy and mitigating side-effects, but also has direct implications on the spread of antibiotic resistance. We are now dissecting the collateral damage of antibiotics on the gut microbes, identifying unexpected activities and resistance mechanisms, and discovering ways to bypass this collateral damage.

At a second level, we have performed a large screen of ~3,000 pairwise combinations of different antibiotics, selected human-targeted drugs and food additives in 3 prominent Gram-negative pathogens. This has allowed us to draw conclusions on the conservation of drug interactions, identify mechanistic biases of synergies and antagonisms, and discover potent synergies that are effective against MDR pathogens.

Finally, we have adapted thermal proteome profiling (TPP) in bacteria, which allows to allows us to probe the thermostability of proteins *in vivo* and *in vitro* in a proteome-wide fashion. TPP can be used for antimicrobial target deconvolution and for mapping antibiotic resistance mechanisms. In addition, it provides unique insights into cellular physiology, including protein complex architecture and metabolic pathway activity.

O27. PLENARY LECTURE VIII

Diversity, Structure, Function, Assembly and Engineering of Bacterial Microcompartments

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Bacterial microcompartments (BMCs), are widespread among Bacteria; they are multienzyme-containing proteinaceous organelles bounded by a selectively permeable protein shell [1]. For example, the carboxysome is a self-assembling metabolic module for CO_2 fixation found in all cyanobacteria. These large (~100-500 nm) polyhedral bodies sequester Carbonic Anhydrase and RuBisCO within a protein shell, thereby concentrating substrates and protecting RuBisCO from oxygen generated by the light reactions. In general, BMCs sequester segments of metabolic pathways, sequester toxic and/or volatile intermediates and, essentially function as bacterial organelles. Bioinformatically, we have shown that these organelles are widespread among the Bacterial Kingdom [2]. Because carboxysomes and other BMCs function to organize reactions that require special conditions for optimization, including the sequestration of substrates, cofactors, or toxic intermediates and the protection of oxygen sensitive enzymes, they have received considerable attention as templates for synthetic nanoreactors in bioengineering and as metabolic modules for programming synthetic microbial consortia.

O28.
Sequence and cultivation study of *Muribaculaceae* reveals novel species, host preference, and functional potential of this yet undescribed family.

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Bacteria within family S24-7 (phylum Bacteroidetes) are dominant in the mouse gut microbiota and detected in the intestine of other animals. Because they had not been cultured until recently and the family classification is still ambiguous, interaction with their host was difficult to study and confusion still exists regarding sequence data annotation. We investigated family S24-7 by combining data from large-scale 16S rRNA gene analysis and from functional and taxonomic studies of metagenomic and cultured species. A total of 685 species was inferred by full-length 16S rRNA gene sequence clustering. While many species could not be assigned ecological habitats (93,045 samples analyzed), the mouse was the most commonly identified host (average of 20 % relative abundance and eight co-occurring species).
Shotgun metagenomics allowed reconstruction of 59 molecular species, of which 34 were representative of the 16S rRNA gene-derived species clusters. In addition, cultivation efforts allowed isolating five strains representing three species, including two novel taxa. Genome analysis revealed that S24-7 spp. are functionally distinct from neighboring families and versatile with respect to complex carbohydrate degradation. We provide novel data on the diversity, ecology, and description of bacterial family S24-7, for which the name Muribaculaceae is proposed.

O29.

The suitability of Brewers' Spent Grain (BSG) for 2nd generation bioethanol production

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Brewers' spent grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total by-products generated. BSG is a lignocellulosic material containing about approximately 25% cellulose, 35% hemicelluloses, and 20% lignin. BSG is available in large quantities throughout the year, but its main application has been limited to animal feeding. Recently, attempts have been made to use BSG in biotechnological processes for the production of chemicals and biofuels.

In the present work, we performed a thorough biochemical and economic assessment on the suitability of BSG for the production of 2nd Generation Bioethanol. The production of ethanol from lignocellulosic substrates is a step-wise process that involves the mild physicochemical pretreatment of the raw material, followed by the enzymatic hydrolysis of cellulose and hemicelluloses into C6 and C5 sugars, and finally, the fermentation of the latter into ethanol by a suitable microbial strain.

The mild alkaline and acid pretreatment of BSG were optimized with respect to pretreatment time and temperature as well as NaOH/H2SO4 load, using a full 3x3x3 experimental design. As the dependent variable for the optimization we used, not only the total sugar release after the enzymatic hydrolysis of the pretreated material, but also the pretreatment cost per unit sugar released, following the development of appropriate cost functions for every pretreatment condition. Our results showed that there is a different set of optimum pretreatment parameters for each dependent variable selected. In both cases though, NaOH/H2SO4 load and pretreatment temperature showed the strongest effects.

The suitability of the optimally pretreated BSG hydrolysates for bioethanol production, was subsequently examined using the bacterium Zymomonas mobilis 8b. This particular strain is genetically modified in order to ferment both glucose and xylose into ethanol. Two fermentation modes, namely Separate Hydrolysis and Fermentation (SHF) and Single Vessel Approach (SVA) were evaluated. Z. mobilis 8b was able to rapidly ferment at high yields the BSG sugars. This performance was the same for both SHF and SVA without any observable inhibition from pretreatment residuals. Considering the latter, the fact that it is possible to produce ethanol from BSG in the same Pretreatment/Hydrolysis/Fermentation vessel provides a significant step forward for the economical production of bioethanol from BSG.

O30.

Bisphenol–A interactions with the gut and feces microbiome of rainbow trout (*Oncorhynchus mykiss*)

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Bisphenol-A (BPA) is among the endocrine disrupting compounds (EDCs) that exert adverse effects and bio-accumulate on aquatic organisms and humans. BPA can enter the bloodstream through dermal contact or ingestion, causing endocrine and neurodevelopmental disfunctions that may be mediated by gut microbiota. In this study, an *in vitro* evaluation of the efficacy of BPA leading to possible alterations in the composition of gut and feces microbial communities of the freshwater fish *Oncorhynchus mykiss* was investigated. The bacterial communities were tested in regard to their potential ability to survive and/or metabolize this pollutant, when provided as the sole carbon source. The BPA degradation products examined, were 4-hydroxy acetophenone (HAP), 4-hydroxy benzoic acid (HBA), Hydroquinone (HQ) and 4-iso-propenylphenol (4-ISO). Each sample was grown on mineral salts medium containing BPA (50 μg/mL) in two experimental series, with or without supplementation with 0.1% yeast extract.

Cultures of gut and of feces bacteria were grown at 13oC and 37oC. Culture supernatant aliquots were aseptically collected at 0, 2, 4, 6, 8, 24, 48, 72 and 96 hours, centrifuged, filtered and analyzed with HPLC, using n-Octylphenol as the internal standard. Cells were collected at the end of the experiment (96h) and part of the cell pellet was subjected to 16S rRNA amplicon sequencing and the remaining to homogenization, SPE and HPLC analysis. Bacterial growth was observed only with yeast supplementation. Although BPA degradation was evident, there were significant differences among experimental series conducted at different temperatures as well as between feces and gut bacterial samples. The metabolite 4-ISO was detected only in gut samples at 37oC from 0 to 24h with its concentration being time-dependent, while HAP was detected in gut samples at 13oC and in feces samples at both temperatures, from 0 to 96 hours. Our results demonstrate different BPA degradation rates among bacterial communities, suggesting a potential BPA-dependent shift in the structure of microbial communities.

Keywords: gut, feces, microbiota, Bisphenol-A, HPLC

O31.

Dissection of the lignocellulose degradation potential of soil microbial communities through diversity and targeted functional meta-omics approaches

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The enzymatic system of several soil-dwelling fungi, especially those of the Basidiomycota division, renders them particularly suitable organisms for the degradation of lignocellulose, a process of distinct ecological significance with promising biotechnological implications (e.g. regarding biofuel production). In this study, we optimized and combined targeted meta-genomics and -transcriptomics methods to gain insight into the lignocellulose degradation process at the soil microbial community level, focusing on forest mainland and island habitats of Greece. Microbial diversity and distribution were investigated with high-throughput rDNA analyses (ITS for fungi and 16S for bacteria/archaea) and compared with the results from custom metagenomics assays targeting conserved domains of peroxidase enzymes involved in lignocellulolysis. In addition, we used metatranscriptomics to gain a better understanding of the relative functional significance of different taxa/enzymes in lignocellulolysis in complex populations. The structure of the studied communities shows distinct patterns of response to environmental parameters, including the season, soil depth and vegetation type. Overall, the most abundant fungi were members of the orders Agaricales, Russulales, Gomphales, Geastrales, Hysterangiales and Trechisporales (Basidiomycota), and Pezizales, Sordariales, Xylariales and Eurotiales (Ascomycota); the most common bacteria were assigned to the phyla Proteobacteria, Actinobacteria, Acidobacteria and Verrucomicrobia. By using optimized “universal” PCR primers that target the peroxidase-catalase enzyme family, we obtained many novel sequences from various basidiomycetes (including members with low abundance) and performed a detailed structural analysis of polymorphisms and functionally relevant amino-acid residues on a phylogenetically broad level. The combination of targeted meta-genomics and -transcriptomics uncovered an important role in lignocellulose degradation of hitherto understudied orders of Basidiomycota, such as the Hysterangiales and the Gomphales, along with the Polyporales, while also suggesting the auxiliary activity of Actinobacteria, Acidobacteria, Verrucomicrobia, Gemmatimonadetes and other Bacteria. The application of next-generation sequencing-based meta-omics methods allows a better understanding of the complex process of lignocellulolysis at the community level and the identification of candidate taxa and genes for targeted functional investigations and desired genetic modifications.

Keywords: targeted metagenomics/transcriptomics, phylogenetics, peroxidases-catalases, population dynamics, lignocellulolysis.

O32. PLENARY SPEAKER IX

Develop *Zymomonas mobilis* as a Synthetic Chassis for Lignocellulosic Bioproducts

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A key barrier for economic production of desirable lignocellulosic bioproducts is the development and deployment of robust microbial biocatalysts with high productivities and yields. *Zymomonas mobilis* is a natural ethanologen with many unique physiology characteristics, which makes it an ideal industrial microbial biocatalyst. I will briefly discuss our efforts to understand the hydrolysate inhibitor tolerance mechanisms using classical genetics and systems biology approaches, and progress for efficient xylose utilization. I will also present our work to develop *Z. mobilis* for other bioproducts using lignocellulosic biomass such as 2,3-butanediol, isobutanol and lactate, and our current effort to develop *Z. mobilis* as a chassis for synthetic biology practice including systematical identification and characterization of biological parts such as promoters and 5' UTR, as well as regulatory network by characterizing the global regulators and their interactions. In addition, efforts to develop tools for genome minimization of *Z. mobilis* including native Type I-F CRISPR-cas systems will also be discussed.

P1.

Functional metagenomic analysis of biobed systems: an invaluable source of genes for the degradation of pesticides

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Biobeds are on-farm systems used for the depuration of pesticide-contaminated effluents. Such high and regular exposure of the biobed microbial community to a range of pesticides can lead to the evolution of novel catabolic pathways. A soil functional metagenomic approach was employed to isolate novel pesticide biocatalysts without the cultivation bias, from the packing material of a biobed having a long history of exposure to pyrethroids, triazoles, strobilurines, glyphosate and organophosphates and exhibiting enhanced biodegradation of isoproturon and abundance of the *pdmAB* genes known to be involved in the demethylation of isoproturon. A fosmid library containing 40 kbp DNA fragments was constructed; 20.000 clones were obtained and screened via different phenotypic assays for the detection of esterases, aromatic monoxygenases and demethylases-monoxygenases and via PCR for *pdmAB* genes in order to explore its evolution. The 12 and 4 positive clones obtained from the phenotypic and PCR assays respectively were sequenced and assembled. The fosmids carried genes annotated as esterases, carboxyesterases, monoxygenases or involved in pathways of aromatics compounds. Further *in vitro* tests will aim to elucidate the involvement of those genes in the degradation of pesticides. Metagenomics-derived *pdmAB* positive clones showed one amino acid substitution each compared to the homologous genes isolated from different isoproturon-degrading strains like *Sphingomonas* sp sH. Metagenomics- and

Sphingomonas sH derived *pdmAB* were isolated, overexpressed and purified in heterologous hosts along with a recombined ferredoxin complementing the catabolic activity of PdmAB. In vitro assessment of the activity of these multi-component enzymes against isoproturon and other phenylurea herbicides will verify our hypothesis that the polymorphisms observed in the translated product of the metagenomic-derived *pdmAB* might have implications for the substrate specificity of the enzyme.

Keywords: biobeds, soil functional metagenomic, fosmid library, novel pesticide biocatalysts, *pdmAB* genes

Acknowledgements: This work was funded by the IAPP-FP7-MSCA project LOVE-TO-HATE and the project “Synthetic Biology: From omics technologies to genomic engineering (OMIC-ENGINE)” (MIS 5002636), funded by the Operational Programme "Competitiveness, Entrepreneurship & Innovation" (NSRF 2014-2020) and co-financed by Greece and European Union.

P2.

Agroindustrial wastewater treatment with simultaneous biodiesel production in attached growth systems using a mixed microbial culture

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One current challenge is to develop economically feasible technologies for treating wastewaters as a biomass feedstock and, ideally produce useful byproducts such as biodiesel. Biological treatment of wastewaters is considered to be a more environmentally friendly and cost-effective approach especially when using an algal/cyanobacterial-bacterial consortia. This consortium can aid environmental mitigation as well to simultaneous production of lipids suitable for third generation biofuels. In this work, an attached growth system was employed using a mixed microbial culture dominated by *Leptolyngbya* and *Limnothrix* species. Under non aseptic conditions the ability of the mixed culture removing organic and inorganic pollutants from various agroindustrial waste (winery, dairy, mixed winery-raisin waste), for producing biodiesel was investigated. A review of the literature shows that not extensive research has been carried out on mixed cyanobacterial-based flocs on support materials for treating raw agroindustrial wastewaters coupled with production of biodiesel. For this purpose lab- scale experiments were performed on photobioreactors with attached growth system (using glass rods) under batch mode and aerobic conditions. Various initial nutrient concentrations (C, N, P) treated in order to determine the rates of nutrient removal, maximum oil accumulation and biomass production. Significant nutrient and organic component removals were observed in all tested substrates (73-97.1% TN, 10.2-80.8% PO_4^{-3} and 65.5-95% d-COD) with the winery waste substrate exhibiting the highest d-COD removal of 97.4%. Maximum attached biomass productivity reached up to 5.03, 4.12 and 3.08 g m^{-2} d^{-1} for dairy, mixed and winery waste respectively, while the highest percentage of oil was obtained from winery substrate (23.2 % g of oil / g of biomass). For dairy and mixed waste the attached lipids reached 19% and 17.4%, respectively.

The resulting attached microbial biomass from each substrate contained 10-23% lipids that were dominated by saturated and monounsaturated fatty acids indicating its suitability for biodiesel production. The above results show that the proposed attached photobioreactor can efficiently treat agroindustrial wastewaters and simultaneously produce biomass suitable for biodiesel production, reducing significantly the costs and environmental impacts.

P3.

Expanding the use of biobeds: Dissipation and adsorption of pesticides contained in effluents from seed-coating, bulb disinfestation and fruit-packaging activities

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Agro-food industries that use pesticides constitute significant point sources for the contamination of natural water resources. Nevertheless, little is known about the treatment of their pesticide-contaminated effluents and biobeds could be a possible solution. In this frame we explored the dissipation and adsorption of pesticides used in seed-coating (carboxin (CBX), metalaxyl-M (MET-M), fluxapyroxad (FLX), fludioxonil (FLD)), bulb-dipping (FLD,chlorothalonil (CHT), thiabendazole (TBZ)) and fruit-packaging activities (FLD) on an organic biobed packing material and in soil. Their dissipation was tested individually and in mixtures relevant to their industrial use, while FLD was tested at different concentrations (10, 20 and 150 mg kg-1) representing the dose rates used by the different industries. CBX, FLD and CHT, when applied individually, and all pesticides when applied in mixtures, dissipated more rapidly in biobed packing material than in soil. In most cases pesticides application in mixtures retarded their dissipation. This was more pronounced in soil than in biobed packing material especially for MET-M and FLD. CHT had the most prominent inhibitory effect on the dissipation of TBZ and FLD when co-applied. FLD dissipation showed a dose-dependent pattern with its DT50 increasing from 42.4 days (at 10 mg kg-1) to 107.6 days at the highest dose rate. All pesticides showed higher adsorption affinity in the biobed packing material (Kf = 3.23 - 123.3 g ml-1) compared to soil (Kf = 1.15-31.2 g ml-1). We provide first evidence for the potential of biobeds to remove pesticides from effluents produced by various agro-industries.

Keywords: pesticide-contaminated effluents, agro-food industries, biobeds, dissipation adsorption

P4.

High pressure experimentation for the study of deep sea oil spills

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High hydrostatic pressure is characteristic of most of the biosphere given that 79% of the volume of the ocean lies below 1000 m. Pressure perturbs biological structures and processes, and varying degrees of adaptation to pressure among deep sea organisms is widespread.

Microbial activities related to organic matter degradation are pressure-sensitive; biopolymer hydrolysis and bacterial production in natural deep water communities in the Mediterranean have been reported to be considerably higher at *in situ* pressure compared to that following decompression. The effect of pressure on hydrocarbon degradation specifically has only been studied in monocultures of isolated bacteria. It is obvious that our knowledge on the effect of pressure on the microbial degradation of hydrocarbons is remarkably limited particularly in view of the petroleum industry's trend of increasing oil and gas production at depths exceeding 1500 m. an advanced experimentation system developed at the host institute will be used to simulate the discharge of oil and gas at pressure and the formation of a hydrocarbon plume in deep waters. Two collaborative projects have recently been funded by the Hellenic Foundation for Research and Innovation Call for the support of Postdoctoral Researchers and hosted at the BEEB lab (School of Environmental Engineering, TUC) to 1) develop, and improve already existing, high pressure sampling and experimentation devices without disruption of the pressure continuum and 2) study the fate of hydrocarbons in a deep water plume and the response of the microbial community to oil release at depth. Planned research involves sampling at 1000 m depth in the Eastern Mediterranean and experimentation onboard in high pressure vessels and in the lab using BEEB's high pressure bioreactor without decompression at any stage. Experiments will be carried out under different remediation scenarios including commercially-available chemical dispersants as well as biosurfactants and biostimulation techniques to enhance the natural capacity of the ecosystem to recover.

P5.

Assessment of the risk of spoilage for evaporated milk exported to the Mediterranean region based on the effect of storage temperature on *Geobacillus stearothermophilus* growth

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A predictive model for the effect of storage temperature on *Geobacillus stearothermophilus* growth was applied to assess the risk of evaporated milk spoilage in Mediterranean region. *G.stearothermophilus* growth in evaporated milk was evaluated during a shelf-life of one year based on historical temperature profiles covering 23 Mediterranean capitals for five years (2012-2016) obtained from Weather Underground database. Totally, 115 scenarios were tested simulating the distribution and storage conditions of evaporated milk in Mediterranean region. The highest growth of *G.stearothermophilus* for 2012-2016 was predicted for Marrakech, Damascus and Cairo with mean values of 7.2, 7.4 and 5.5 logCFU/ml, respectively, followed by Tunis, Podgorica and Tripoli with mean growth of 2.8, 2.4 and 2.3 logCFU/ml, respectively. For the rest 17 capitals the mean growth was <1.5 logCFU/ml. Podgorica, Cairo, Tunis and Ankara showed the highest variability in the growth during 2012-2016 with standard deviation values for growth of 2.01, 1.79, 1.77 and 1.25 logCFU/ml, respectively. The predicted extent and variability of growth during shelf-life were used to assess the risk of spoilage which was visualized in a geographical risk map. The growth model evaluated adjustments of the evaporated milk expiration-date which can reduce the risk of spoilage. The resulting quantitative data can assist the food industry to evaluate the microbiological stability of these products throughout distribution and storage at a reduced cost and assess whether and under which conditions will be able to export a product to a country without spoilage problems. This decision support may lead to a significant benefit for both the competitiveness of the food industry and the consumer.

Keywords: *Geobacillus stearothermophilus*, evaporated milk, risk of spoilage, Mediterranean countries

Acknowledgments

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P6.

Exploring the microbial ecosystem of Polynesian yogurt using culture-dependent and -independent approaches

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Yogurt is one of the most popular fermented milk products in the world and it has been consumed for thousands of years by different civilizations. Nowadays, encouraging the production of low cost, environmentally friendly, and sustainable dairy foods, rich in proteins, is crucial. Yogurt is rich in vitamins and minerals and an excellent source of calcium and protein. Besides the nutritional benefits, consumption of yogurt that contains beneficial viable microorganisms that compete with pathogenic bacteria for nutrients and space shows promising health benefits. Today, yogurt is prepared by fermenting cow, sheep or goat milk using a symbiotic culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which shape the organoleptic characteristics of the final product. However, the ecosystem of artisanal yogurt may be more complicated. In the present study, the microbiota of an artisanal yogurt prepared from Polynesian cow milk was explored using culture-dependent as well as -independent approaches. For this purpose, yogurt was subjected to conventional microbiological analysis using selective growth media. The isolates were grouped using the genotyping technique of rep-PCR. Representative bacterial and yeast isolates of each group were identified at the species level by sequencing the 16S rRNA gene and ITS DNA region, respectively. Furthermore, total DNA was extracted from the yogurt sample using a novel protocol developed in our laboratory and the results obtained from the sequencing of 16S rRNA gene and ITS DNA region were analyzed using advanced bioinformatics tools. Using culture-dependent techniques, one bacterial and one yeast genus, i.e. *Lactobacillus* and *Candida*, respectively, were mainly identified. The results of the 16S metagenomics analysis revealed the presence of mainly four lactic acid bacteria genera, namely *Lactococcus* (64.3%), *Acetobacter* (24%), *Leuconostoc* (9.2%) and *Lactobacillus* (2.1%). On the other hand, ITS metagenomics analysis showed a higher biodiversity in the fungal community by identifying 59 different fungal genera. Among them, *Galactomyces* (35.3%), *Debaryomyces* (13.3%) and *Sporobolomyces* (8%) were the most abundant. The analysis we present here is the first attempt to explore the microbiome of Polynesian yogurt.

P7.

The response of the soil and phyllosphere microbial community to repeated application of the fungicide iprodione: Selection for biodegradation or toxicity?

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Although pesticide effects on the soil microbial communities have been intensively studied in the late years, little is known about other environments such as the phyllosphere. The mechanisms driving these interactions are well established in soil but not in other pesticides-exposed ecosystems. We tested the hypothesis that soil and phyllosphere microbial communities respond to the fungicide iprodione. Pepper plants received five repeated (30-d intervals) foliage or soil applications of iprodione. The development of accelerated biodegradation of iprodione was determined via measurement of its presence and transformation in soil and leaves. The response of the soil and epiphytic microbial communities to the regular pesticide exposure was determined via amplicon sequencing. Iprodione-degrading bacteria were isolated from leaves and soil and the transformation pathway was determined via HPLC-PDA. Epiphytic and soil microbial communities responded to iprodione.
Repeated applications led to a gradual acceleration in the degradation of iprodione. Bacterial and Archaeal communities showed little disturbance by iprodione. Whereas the fungal community was significantly altered, especially in soil where *Sordariomycetes, Agaricomycetes* were stimulated and *Dothideomycetes* were inhibited. Differential abundance analysis highlighted (a) the stimulation of epiphytic *Arthrobacter* known as exclusive degraders of iprodione in soil and (b) the inhibition of the soil ammonia-oxidizing Crenarcheon *Candidatus Nitososphaera*. Enrichment cultures resulted in the isolation of the same iprodione-degrading *Paenarthrobacter* strain from soil and leaves which hydrolyzed iprodione to 3,5-dichloraniline via the formation of 3,5-dichlorophenyl-carboxiamide and 3,5-dichlorophenylurea-acetate.

Keywords: Iprodione, phyllosphere, soil, biodegradation, microbial diversity

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P8.

In vitro evaluation of the inhibitory effect of Quinone Imine- the main oxidation derivative of Ethoxyquin- on nitrification

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Nitrification inhibitors (NIs) are used in agricultural practice to improve nitrogen use efficiency and reduce nitrogen losses from agricultural ecosystems. In addition to characterized NIs, other agrochemicals can also affect the organisms responsible for nitrification. Ethoxyquin (EQ) is an antioxidant used as a preservative in the fruit-packaging industry. Recent *in vitro* and *in situ* studies of our research group showed that EQ is rapidly transformed to quinone imine (QI) (major metabolite, low persistence) and 2,4-dimethyl-6-ethoxyquinoline (EQNL) (minor metabolite, long persistence), and suggested that EQ and probably more potently QI, had a major inhibitory effect on the growth and activity of soil nitrifiers. Here, we investigated the spectrum of inhibitory effects of QI on the activity (nitrite production/consumption) and growth (*amoA/nxrB* gene abundance) of a range of terrestrial nitrifying strains including ammonia-oxidizing bacteria (AOB) (*Nitrosomonas europaea*, *Nitrosospira multiformis),* ammonia-oxidizing archaea (AOA) (*Ca.* Nitrosotalea sinensis, *Ca.* Nitrosocosmicus franklandus), and a nitrite-oxidizing bacterium (NOB) (*Nitrobacter* sp. NHBI) in liquid cultures, comparatively to widely used NIs (DCD, nitrapyrin, and DMPP). AOA were the most sensitive group of nitrifiers tested, with QI ≥ 0.27 μM suppressing their growth and activity compared to ≥ 135 μM required for AOB and NOB inhibition. *N. multiformis* and *N. sinensis* were the most sensitive strains of AOB and AOA, respectively. Compared to the other NIs tested, QI was more potent against AOB than DCD, with the EC50 values for the tested compounds increasing in AOB cultures in the order nitrapyrin<DMPP<QI<DCD. Studies on the effect of DCD, nitrapyrin and DMPP on AOA are on the way. The differential sensitivity of AOA and AOB to QI may reflect their contrasting biological or physiological features and/or differences in their ammonia oxidation pathways/mechanisms which would be further explored via proteomics. Overall, our findings could be exploited for the development of novel, universal NIs.

Keywords: quinone imine, soil nitrifiers, nitrification inhibitors

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P9.

Lactic acid production via High Gravity Enzymatic Hydrolysis and Fermentation by Lactic Acid Bacteria

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Lactic acid (LA) is a valuable compound with various applications in food, pharmaceutical and chemistry industries. Moreover, it attracts great interest for its biodegradable polymerized form—polylactic acid (PLA), a biodegradable and biocompatible polymer with a multitude of applications. LA can be produced by either chemical synthesis or microbial fermentation. A biological method has the advantage that an optically pure LA can be obtained by employing specific strains of lactic acid bacteria (LAB), whereas chemical synthesis always results in a racemic mixture of LA. Low-cost, renewable, non-edible materials, especially lignocellulosic biomass from agricultural, agro-industrial and forestry sources, are of great interest for lactic acid production. Using lignocellulosic biomass to produce chemicals is a promising way to alleviate significant bottlenecks in the supply of energy resources; however, efficient bioconversion of biomass to LA still faces considerable challenges, including the difficulty of using lignocellulosic biomass due to its complex structure and recalcitrance. In the present work, lignocellulosic biomass was used as a substrate for the production of highly-concentrated sugar streams able to support the growth of LAB strains and the efficient yield of LA. Mild oxidative organosolv pretreatment of biomass using aqueous solutions of organic solvents took place as an initial step in order to remove lignin fraction, disrupt the rigid structure of the material and render it more amenable to enzymatic hydrolysis. Simultaneous saccharification and fermentation (SSF) using Cellic® CTec2 (Novozymes) enzyme and *Lactobacillus delbrueckii ssp. bulgaricus* was used for the production of fermentable sugars and their subsequent bioconversion to LA respectively. To make the production of LA economically viable and at the same time reduce the environmental impact of the process, high initial solid concentration of the substrate (15% initial dry matter) during saccharification and fermentation was investigated.

Keywords: Lignocellulosic biomass, high gravity processes, Lactic acid bacteria, SSF

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P10.

Functional analysis of transporters encoded in the RutR regulon of *Escherichia coli*

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One third of all transporter genes in the model bacterium *E. coli* K-12 are still orphan in the sense that their substrates are not known experimentally. Deorphanization of such transporters would enhance our understanding of several aspects of bacterial metabolism that are not yet clear. Substrates of functionally unknown transporters are often hypothesized from phylogenetic analysis or from the metabolic pathway encoded in a related operon. However, such transporters may fall in homology clusters that include no known representing member or the operon encoding a transporter may be regulated in concert with several other operons and subject to multiple metabolic controls, making sequence- and operon-based predictions difficult. We studied one example, concerning two currently unknown transporters encoded in a purine/ureide/glyoxylate (*gcl*) catabolic operon. The transporters (YbbW and YbbY) belong to known nucleobase-cation symporter families (NCS1 and NCS2, respectively) but represent distinct homology clusters that do not include functionally known homologs. The related *gcl* operon is subject to repression by RutR, a global transcription regulator that interconnects genes for purine, pyrimidine, dicarboxylate and amino acid metabolism but is primarily considered to be a master regulator of pyrimidine metabolism and its major effector is uracil.
As a superimposed control, the *gcl* operon is repressed by AllR, which is affected by allantoin and glyoxylate. We analyzed the properties of the two transporters of the *gcl* operon using amplified gene expression in appropriate *E. coli* K-12 hosts, and found that YbbW is a high-affinity transporter for uracil/5-FU/hypoxanthine/guanine, and YbbY is a high-affinity transporter for adenine/hypoxanthine/guanine. The finding that YbbW transports both pyrimidines and purines, together with our recent finding that RutG (encoded in the *rut* operon for pyrimidine catabolism which is also under control of RutR) is also a transporter for both pyrimidines (uracil/5-FU/thymine) and purines (xanthine/oxypurinol), point to a key role of RutR in maintaining metabolic balance between pyrimidines and purines. Not only both a pyrimidine (*rut*) and a purine (*gcl*) catabolic operon are subject to similar regulation by RutR, but also transporters encoded in each one of these operons (RutG, YbbW) can both supply the cell with either purine or pyrimidine nucleobases to feed either one of the two catabolic pathways.

Keywords: pyrimidine/purine catabolism, RutR regulon, membrane transporters

P11.

Symbiosis and dysbiosis in the snail gastrointestinal tract - Rebiosis by probiotic administration

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It is now well known the important role of the intestinal commensal microbiota on the health and disease in the gut of vertebrates. Symbiotic host-microbe interactions play a fundamental role on the structural and functional integrity of intestinal tract, maintenance of immune homeostasis and enhancement of immune defense mechanism against pathogens. On the other hand, the disruption of the normally occurring gut microflora, known as dysbiosis, could lead to infectious diseases, metabolic and immune disorders and cancer. However, little is known about the interactions of gut commensal microbes with the gastrointestinal mucosa and innate immunity on health and disease in invertebrates, such as snails. In this study we investigated the host-microbe interactions that are associated with the symbiosis and dysbiosis on the edible-farmed snails *Cornu aspersum* maxima.Twelve commensal symbiotic lactic acid bacterial strains, previously isolated from the intestinal tract of the farmed snails exhibiting presumptive probiotic activity were administrated to snails and their immunomodulatory activity was determined. Eight strains exhibit increased chemotactic and phagocytic activity. We demonstrate that the strain Sgs14 showing the highest activity enhance antibacterial activity of snail haemolymph and expression of TLRs. We have isolated and characterize for the first time snail pathogenic bacterial strains that belong to the genus *Listeria* and *Pseudomonas*. Pathogenic activity is characterized by 60% snail mortality and by phenotypic markers such as the alterations on the haemolymph cellular population and feces composition that are reversed after treatment of snails with the presumptive probiotic strains *L. plantarum* Sgs 14 and *L. plantarum* Sgm B alone or as cocktail. In addition probiotic administration in snails results in acceleration of snail growth to commercial availability by a month. These data indicate that the strains Sgs 14 and Sgm B fulfill the criteria to be characterized as presumptive probiotic strains and further experimentation is required for their application in snail farming units.

P12.

In vitro and *in vivo* selection of potential probiotic bacteria isolated from the *Cornu aspersum* (Muller 1774) gastrointestinal tract

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Probiotics are health-promoting microorganisms exhibiting specific properties that include certain cell surface traits as well as immunomodulatory activity in vertebrates. However, little is known concerning immune responses and probiotics in invertebrates. *Cornu aspersum* (Müller, 1774) snail gut commensal bacteria isolates (126) were examined for their *in vitro* probiotic properties such as surface traits that permit their survival and adhesion to gastrointestinal tract as well their immunomodulatory activity. Especially, the traits that were examined are autoaggregation, hydrophobicity and biofilm formation that are linked with the ability to colonize the intestine. The ability to survive in pH=3, in gastric mucus, pedal mucus and gastric juices was also examined. Application of Principal Component Analysis (PCA) distinguished 18 isolates named 1, 11, 18, 19, 20, 21, 5A, 19A, 20A, 13AMP, cam7, cam8, cam10, cam11, cama8, cama9, cama11, cama15 exhibiting the highest values. The above strains were further investigated for their *in vivo* immunomodulatory activity such as their capacity to increase the chemotactic and phagocytic activity of snails' haemolymph cells. Our data indicate that the isolates named 1, 5A and cam10 exhibited the highest chemotactic and phagocytic activity and further investigation is required to demonstrate their beneficial activity in snail homeostasis in order to be characterized as presumptive snail probiotics.

P13.

Development of a probiotic potential prediction model by using classification tree analysis

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In the process of screening for snail probiotic strains there are not clearly established bacterial phenotypic markers, which could be used for the prediction of their immunomodulatory capacity. In this study we applied Principal Component Analysis (PCA) in association with Classification and Regression Tree analysis (CART) using phenotypic markers that characterize *in vitro* probiotic properties of snail *Cornu aspersum* gut bacterial strains to distinguish and characterize their immunomodulatory capacity. For this purpose 233 bacterial strains isolated from snail gastrointestinal tract were used. PCA was initially carried out to discriminate the bacterial strains depending on their *in vitro* tolerance capacity to pH 3.0, gastric mucus, pedal mucus and gastric juices as well as on their cell surface traits such as autoaggregation, hydrophobicity and biofilm formation. Then, a classification tree analysis was performed to classify the strains on an ordinal measurement scale as GroupA (32 strains), GroupB (39 strains) and GroupC (162 strains). According to the classification tree results, it can be concluded that gastric mucus, hydrophobicity and biofilm are the most significant explanatory variables for the classification of GroupA and groupB. Based on this classification tree, 11 GroupA strains, 9 GroupB strains and 11 GroupC strains were examined for their capacity to increase the chemotactic and phagocytic activity of snail haemolymph cells. This analysis revealed that all strains of GroupA (except one) and GroupB but only 5 strains of GroupC exhibited immunomodulatory activity suggesting that the gastric mucus tolerance in combination with high hydrophobicity and biofilm formation traits may be used as adequate parameters to discriminate presumptive snail probiotic strains exhibiting immunomodulatory activity. Furthermore, food-administration in snails of one of the above strains (characterized as *L. plantarum*) proved to be beneficial by exhibiting adhesive capacity to intestinal epithelium, antibacterial activity, and acceleration of growth rate supporting the application of PCA and CART analyses to discriminate snail gut microbes exhibiting probiotic properties.

P14.

Post-harvest agricultural wastes control N_2O emissions through denitrifying and bacterial community composition and abundance

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There is an emerging need to reduce N chemical fertilizers and using agricultural organic wastes as organic amendments could be a valuable strategy to reduce GHG emissions and sustain agro-ecosystems productivity. An incubation experiment was performed to examine the effects of agricultural wastes application on soil bacterial community as well as CO_2 and N_2O direct emissions. Untreated soils compared with soils received the same amount of N (100 μg/g soil) in the form of mineral fertilizer and organic agricultural waste. In particular, soils were incubated with three different organic agricultural wastes, orange (OP), mandarin (MP) and banana peels (BP) and ammonium nitrate (NH_4NO_3) after adjusting soil water at 70% of its holding capacity. We described the microbial community structure using next generation sequencing of amplified 16sRNA and found that both treatment and time had a strong effect on the microbial assemblages. Agricultural wastes stimulated the growth of copiotrophic bacterial groups like Proteobacteria and Firmicutes while the abundance of most bacterial Phyla detected was suppressed. We found that direct soil N_2O emission is positively associated to bacterial diversity in soil while the highest cumulative emission was calculated in fertilized treated soils. Interestingly, soils received organic amendments exhibited a substantial reduction of the abundance of bacterial species that have been associated with N_2O emissions. Apparently, the lower N_2O emission found in soils incubated with agricultural organic waste is related to lower substrate availability and possibly due to the proliferation of N_2O reducers. Indeed, the incorporation of post harvest agricultural wastes significantly suppressed *nir*K gene abundance and increased the abundance of *nos*ZII community. This study represents a basis for future research regarding the impact of different organic amendments on soil denitrifying community and bacterial community networks and how pivotal key genes are influenced upon their application in soils.

P15.

Effect of different strains of arbuscular mycorrhizal fungi on the growth and nutrient content of cowpea plants in association with symbiotic nitrogen fixing bacterium *Sinorhizobium meliloti*

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Cowpea is one of the most important grain legume crops in sustainable agriculture due to its ability to fix atmospheric nitrogen and form effective associations with both nitrogen fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF). In this current study, we examined the interaction of four different mycorrhizal inocula (Inc3, Inc5, Inc9, Inc21) isolated from sand dunes in Greece, with nitrogen fixing bacterium *Sinorhizobium meliloti* on cowpea plants. Pot experiment was conducted in a complete randomized design (CRD) system, where 70 cowpea plants were grown using 14 different treatments. These treatments came up by mixing the four mycorrhiza inocula in different combinations (Inc3+Inc21, Inc21+Inc9, Inc9+Inc5, Inc3+Inc9, Inc21+Inc5, Inc3+Inc5, Inc3+Inc5+Inc9+Inc21), with and without nitrogen fixing bacterium. The effect of the different treatments on plant biomass and nutrient content was assessed. Different treatments had significant effect on AMF colonization, which was NFB dependent. NFB presence had positive impact on AMF colonization. NFB inoculation caused significant increase on cowpea biomass irrespectively of AMF inoculation, except the AMF combinations Inc21+Inc5 and Inc21+Inc9. Nitrogen content on plant biomass was significantly affected by NFB inoculation. The correlation between colonization and nitrogen content was significantly negative in plants with NFB inoculum. In addition, a significant positive correlation between colonization and phosphorus content of plants without NFB inoculum was noticed. This study suggests that the tripartite symbiosis of cowpea plants with AMF and NFB improves plant biomass growth and nutrient content. Also the percentage of root colonization depends on the combination of different AMF strains inocula as well as the presence of NFB. This coexistence between NFB and AMF seems to be antagonistic depending on AMF inoculum. More research should be done on the selection of AMF strains that enhance legumes growth and productivity.

P16.

Nutrient management schemes in olive orchards and the effects on microbial denitrification communities

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Olives are among the most widely grown crops in the Mediterranean region with an immense agro-economic value. The application of mineral fertilizers in orchard farms has become an integral part of soil fertility management, aiming at maximizing productivity and crop yield efficiency. Nitrogen (N) is the most commonly applied fertilizing element in olive orchards. Many studies have reported shifts in microbial community structure under N fertilization, however, information is lacking on the functional microbial communities that directly associate with N cycling. Nitrous oxide (N_2O) is an important greenhouse gas, released as a by-product of the denitrification process. N availability in soils is the main driver of N2O emissions, thereby constituting a crucial component of sustainable management practices to address climate change. The present study investigated four N fertilization schemes during a field trial performed in an olive orchard located in northwest Cyprus. These included chemical fertilizer applications (NH_4NO_3), organic soil amendments such as animal manure and compost, and green manure (clover as a cover crop). No fertilization was used as a control. The treatments were arranged in a randomized complete block design with six replications and applied in a ring of 40 cm. Three soil samples were collected from each ring on regular time intervals over a period of 225 days and analyzed for chemical properties (organic matter, NO_3^- and NH_4^+ content), as well as the abundance of total bacteria (*16S rRNA* gene) and functional groups of N transforming bacteria (*nirS, nirK, nosZ* clade *I* and *II* genes) using quantitative PCR. Organic fertilizer applications led to higher organic matter content compared to other fertilization schemes, while chemical N-fertilized treatments substantially increased NO_3^- and NH_4^+ levels at early time points. Furthermore, all fertilization schemes had clear effects on total bacteria and denitrifier community composition throughout the course of the field trial. The results of this study provide novel insights into the responses of microbial functional groups to long-term N input in olive orchards under different nutrient management schemes.

P17.

Temporal and spatial variations of biotic parameters in the sediment of lake Pamvotis

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Biotic parameters in the water column of lake Pamvotis in Ioannina have been thoroughly studied in the last decades, however, little is known on variations that occur in its sediment. Thus, important biotic and abiotic parameters were studied in lake's sediment at three depths (0-1, 2-3, 4-5 cm) during the transition from the warm to the cold period of the year (October to December, 2018), in order to determine their temporal and spatial variability. In particular, bacterial abundance was measured using epifluorecence microscopy after staining with DAPI. Photosynthetic pigments were measured using spectrophotometry after extraction with 90% acetone. The Loss On Ignition (LOI) method was used to estimate organic matter content in the sediment. In parallel, chlorophyll a concentration as well as basic physicochemical parameters (salinity, TDS, conductivity, temperature, and pH) and dissolved oxygen concentration were measured in the water column. Sediment chlorophyll a concentration was higher in the top layer, reaching a maximum of 1733 mgm-3 in late November, while concentration in the deeper layers was <527 mgm-3. Chlorophyll α concentration was higher than pheophytin concentration only in the top layer and pheophytin concentration varied less with depth. The percentage of organic matter in sediments (dry weight, g) increased in early November in all depths and then decreased, reaching its previous levels. Top layer samples showed the largest variability with values ranging from 0,7 to 1,5 % (labile), 0,4 to 1,4 % (refractory) and 1,1 to 2,8 % (total). Water bacterial abundance decreased from October to November (~3*105 bacteria ml-1) and increased slightly in December (~$4*10^5$ bacteria ml^{-1}). The top layer sediment bacterial abundance peaked in October ($9,2*10^6$ bacteria cm^{-3}) and decreased almost two orders of magnitude in November. Bacterial abundance in the deeper layers was always lower than in the surface and maxima occurred in December ($1*10^6$ bacteria cm^{-3}). Water temperature decreased from 19 to 10.9^oC during the study period while salinity, TDS and conductivity continually increased. Water chlorophyll α concentration ranged from 20,9μg L^{-1} (November) to 13,9 μg L^{-1} (December). Preliminary results showed that both bacterial abundance and chlorophyll a concentration were higher in the sediment and did not appear to follow the variation patterns observed in the water column. Bacterial abundance showed a vertical gradient probably associated with the availability of organic matter and nutrients in the sediment.

P18.
Grass lawn as a feedstock for second generation biofuels

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Lignocellulosic biomass including agricultural and forestry residues, perennial crops, softwoods and hardwoods, can be used as feedstock for biofuels production. Although being abundant and almost zero cost feedstocks, the main obstacles of their use are the low efficiencies and yields attained, due to the recalcitrant nature of their lignocellulosic content. However, with the application of a pretreatment process, depolymerization of cellulose and hemicellulose and breaking the lignin seal can be achieved facilitating the liberation and subsequent uptake of simple sugars (hexoses and pentoses), that can be converted to biofuels in consecutive steps, by microorganisms, leading thus to higher yields. In the present study, various pretreatment methods such as thermal, alkaline, acid and Liquid Hot Water treatment were applied on one perennial crop, grass lawn. The effect of each pretreatment method in carbohydrates' solubilization and fractionation of the lignocellulossic content (cellulose, hemicellulose, lignin) was evaluated. Complete analysis of the liquid fractions obtained after pretreatment was also conducted. A detailed characterization of the pretreated feedstocks through techniques such as scanning electron microscopy (SEM) and IR spectroscopy was carried out. In the sequel, the pretreated feedstocks were further evaluated for 2nd generation biofuels production (biohydrogen, biogas and bioethanol). For all experiments, the whole slurry (containing both the liquid and the solid fractions) or separately the solid or the liquid fraction obtained after pretreatment, were used. Fermentative hydrogen production was performed at meshophilic conditions, using heat treated mixed anaerobic sludge, as microbial inoculum, with the addition of commercial enzymes via a SSF (simultaneous saccharifiacation and fermentation) process. Bioethanol production from pretreated feedstocks was studied in batch experiments at 30oC, using the xylose -fermenting yeasts of *Pichia stipitis* or *Pachysolen tannophilus* or the traditional glucose -fermenting yeast of *Saccharomyces cerevisiae* either in a SSF or a SHF (separate hydrolysis and fermentation), concept. Anaerobic digestion of pretreated biomass was studied in batch experiments and the biochemical methane potential (BMP) of all pretreated feedstocks, was assessed. The experiments showed that for enhancing the anaerobic digestion process, alkaline pretreatments are the most suitable, since a lignin reduction is effected, which is negatively correlated to its BMP. On the other side, for enhancing fermentations such as bioethanol or biohydrogen production processes, acid pretreatment methods are more promising, since hemicellulose reduction and xylose release are carried out.
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P19.

Cyprus Soil Microbial Genetic Resources Bank: a National Infrastructure for conserving, exploiting and exploring soil microbial diversity and functioning

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The evolution of biotechnology industry and its application to agriculture and environment ultimately depends upon our ability to exploit and use the potential of microbial diversity. This is the reason why EU supported a knowledge-based bioeconomy emphasizing that, biological sciences and particularly microbes are adding value to a multitude of products and services (OECD Report The bio-economy to 2030: designing a policy agenda). Under this concept, soils microbes are not only key players in ecosystem processes by driving the biogeochemical cycles but also directly and indirectly influencing plant, animal, and human welfare. 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 establishement 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 is able to 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. The aim of this poster is to present the technical and informatic/bioinformatic tools, as well as the scientific objectives within MAGNET infrastructure. Finally, the capabilities of storing soil genetic resources in the Eastern Mediterranean region are presented.

P20.

Microplastic occurrence in aquatic environments and their potential role as microbial vectors

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The accumulation of plastic in the aquatic environment has been a serious environmental issue, mainly due to the durability and wide dispersal of plastic debris. Microplastics (<5mm, MP) in the marine environment in particular, are of great concern since due to their special characteristics, they can be ingested by marine organisms and enter the food web. Additionally, they provide a substrate for attachment of microorganisms and can act as vectors for their transportation, therefore leading to further potential ecological or human health hazards. Within this context, we investigated (a) the occurrence and characteristics of MPs in the sediment of a beach in Peloponnese, Greece using density separation methods and (b) the potential of microplastics as vectors for the transportation of microorganisms in aquatic environments. For this, the presence and abundance of attached bacteria, on floating plastic debris (membranes) sampled from a lake were studied using epifluorescence microscopy. In addition, microcosm experiments were conducted with sampled plastics to investigate whether attached bacteria can be transferred and grow in the water column. Our results showed microplastic pollution in the sediment of the beach we studied. The predominant category of microplastics observed was filaments with presence of foamy plastic and membranes. The microcosm experiments showed that a) plastic debris can act as a substrate for attached microorganisms, and b) transfer of bacteria from MPs to the aquatic medium and exponential growth may occur within 45h, after MPs are released in the environment. Bacterial abundance in the microcosms reached 13.34 x 10^5 cells ml^{-1} at 69h, the respective growth rate being 0.13 h^{-1}, and generation time 5.32 h. Our findings suggest that microplastics can act as vectors for microorganisms and provide a relatively stable environment to sustain their viability, therefore posing a potential ecological threat.

Keywords: microplastics, microorganisms, bacterial growth, aquatic plastic pollution, beach sediment

P21.

Microbial spoilage of gilthead seabream during storage under modified atmospheres at different temperatures

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Microbial and enzymatic activity is the major cause of fish spoilage. The shelf-life of perishable food commodities, such as fish, is expected to be short hardening their distribution, and resulting frequently in consumer dissatisfaction.
The aim of this study was the monitoring of microbial growth of fish during storage under modified atmosphere packaging conditions and at different temperatures. The storage experiment was carried out using gilthead seabream (*Sparus aurata*), a Mediterranean fish of high commercial interest in Greece. Specifically, whole ungutted fresh fish was stored under modified atmosphere packaging (MAP; 40% CO_2, 30% N_2, 30% O2) and at isothermal conditions (0, 4 and 8°C) for a maximum time period of 19 days. At regular time intervals during storage, duplicate samples (i.e. different fish) were subjected to microbiological analyses to allow for an efficient kinetic analysis of microbial growth. Two independent experimental replicates (i.e. different times and fish batches) were conducted (n=4), and a total of 174 different samples were analyzed. The microbiological analyses involved the enumeration of total viable counts (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria (LAB), bacteria belonging to the family Enterobacteriaceae, H2S-producing bacteria and yeasts. The primary model of Baranyi was fitted to the derived microbiological data (log CFU/g) and the growth kinetic parameters for some of the aforementioned microbial groups were estimated. The maximum specific growth rate (μmax, h-1) of TVC was 0.012, 0.041 and 0.061 at 0, 4 and 8^{o}C, respectively. Similar μmax values were estimated at the corresponding storage temperatures for *Br. thermosphacta* which, along with *Pseudomonas* spp. and H2S-producing bacteria, contributed the most to fish spoilage at all applied storage temperatures. On the other hand, LAB, Enterobacteriaceae and yeasts did not appear to be considerably associated with the
microbial spoilage of gilthead seabream.
The results of this study demonstrate that *Br. thermosphacta*, *Pseudomonas* spp. and H_2S-producing bacteria are the dominant spoilage microorganisms of gilthead seabream under MAP conditions, and that MAP in conjunction with low-temperature refrigerated storage is expected to constitute an efficient means of retention of fish microbiological quality.

Keywords: fish, microbial spoilage, modified atmospheres

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P22.

Evaluation of novel plant origin materials for the cleaning - protection of caves belonging to natural and cultural heritage of Greece

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The current study concerns the serious issue of interior alteration observed in many caves of Greece due to the development of various microorganisms. This phenomenon has as direct consequence the significant aesthetic and functional degradation of the caves belonging to natural and cultural heritage sites. Literature results have indicated that the organisms normally grown in these cases are non-photosynthetic and/or photosynthetic microflora visible as green or dark color on the cave walls. The main subject of the current study was to address the above problems by replacing chemical bleaches with herbal biocides. For this purpose, a series of essential oils and hydrosols of plant origin extracted from various Greek plants i.e. *Citrus aurantium* (1), *Citrus limon* (2), *Citrus sinensis* (1), *Citrus reticulata* (2), *Citrus x paradisii* (2), *Juniperus drupacea* (2), *Juniperus phoenicea* (2), *Satureja thymbra* (1), *Foeniculum vulgare* (1), *Salvia triloba* (1), *Laurus nobilis* (1), *Origanum vulgare* (2), was evaluated. The above specimens were tested *in vitro* in 96-well-plates in various concentrations against 35 bacterial and 24 fungi isolates (previously isolated from Greek caves) and the antimicrobial activity was evident through the absorbance changes in a microplate reader apparatus. The results showed that *Origanum vulgare* (2) and *Satureja thymbra* (1) essential oils, exhibited the highest efficacy against microorganisms, followed by specimens with medium or low efficacy that belonged to essential oils of *Citrus limon* (1), *Laurus nobilis* (1), *Juniperus drupacea* (1). The rest specimens that were either essential oils or hydrosols of *Citrus aurantium, Citrus limon, Citrus sinensis, Citrus reticulata, Citrus paradisii, Juniperus drupacea, Juniperus phoenicea, Foeniculum vulgare* and *Salvia triloba* showed no efficacy against the tested microorganisms in the highest evaluated concentration (1% v/v). The current study demonstrated that herbal biocides may replace chemicals, thus, presenting intriguing products with significant prospects for commercial exploitation.

Keywords: caves, natural antimicrobials, microorganisms
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P23.

Molecular characterization of *Pseudomonas* spp. isolates from sporocarps of oyster mushrooms with yellow blotch disease

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Oyster mushroom (*Pleurotus ostreatus*) is the most extensively cultivated mushroom species worldwide. Cultivation and production of this species can be strongly affected by outbreaks of blotch forming diseases. A major causative agent of the yellow blotch disease has been identified the species *Pseudomonas tolaasii*, with its pathogenicity being manifested via the secretion of the toxin tolaasin. The aim of this work was the identification of *Pseudomonas* spp. occurring in decaying sporocarps through the utilization of culture-dependent molecular techniques. Five samples of infected oyster sporocarps were analyzed. After enumeration, a percentage of colonies which grew on the selective medium for *Pseudomonas* were isolated and subjected to Random Amplification of Polymorphic DNA (RAPD) for differentiation. Species specific PCR using specific primers PT1A and PT1D1 was applied for identification of *P. tolaasii.* The rest isolates were subjected to 16s rRNA and *rpoB* sequencing, respectively, for species characterization. From a total number of 51 isolates were recovered by the infected oyster sporocarps, five groups and four individual profiles were obtained after RAPD analysis. Four out of five groups (40 isolates in total) were characterized as *P. tolaasii*. The rest isolates were assigned to *Pseudomonas* genus. The results of this study indicate that *P. tolaasii* plays a determinant role in the development of yellow blotch disease in oyster mushrooms.

Keywords: oyster mushrooms, tolaasin, yellow blotch disease

P24.

Isolating bacteria able to rapidly degrade fungicides used in fruit packaging industry: Tailored made inocula for the treatment of relevant agro-industrial effluents

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Fungicides are used by fruit-packaging industries to control fungal infestations of fruits during storage. The applicant of dense fungicide solutions on stored fruits results in the production of large wastewater volumes which according to EU regulation should be treated on site before environmental release. Despite that these agro-industrial effluents in most cases are either land spread in adjacent fields or discharged in the municipal wastewater treatment systems. Both practices result in the extensive contamination of soil (levels of 12 g/kg have been monitored in such disposal sites) and surface water systems due to the limited capacity of the indigenous soil microbial community and the sewage treatment plants to degrade the fungicides contained in the effluents of fruit-packaging plants. Since 2013 our group has isolated from contaminated soil bacteria able to rapidly degrade pesticides contained in those effluents like thiabendazole (a proteobacterial consortium), iprodione (*Paenarthrobacter* sp.), ortho-phenylphenol (*Sphingomonas haloaromaticamans*), diphenylamine (*Pseudomonas putida*) with the potential to develop tailored-made inocula to be used in biological engineered systems for the treatment of effluents from fruit-packaging plants. Currently the group has established enrichment cultures inoculated (i) with contaminated soil collected from wastewater disposal sites of fruit-packaging plants in Greece (ii) solid remnants from the drenchers used for the treatment of fruits aiming to isolate bacteria able to rapidly degrade the fungicides imazalil and fludioxonil, the only two fungicides for which pesticide-degrading bacteria are not available in our inventory. Enrichment cultures are on-going and the outcome of our isolation efforts will be presented in the conference. Further efforts of the group focused on the use of these inocula for the bioaugmentation of (i) a soil from a wastewater disposal site of a fruit-packaging plant in Cyprus heavily contaminated with thiabendazole and (ii) a biobed system receiving discharges of thiabendazole-contaminated effluents. In both cases the thiabendazole-degrading consortium was able to remove thiabendazole residues and effective decontaminated soil and effluents.

Key words: pesticides biodegradation, fruit-packaging industry, wastewaters, microbial inocula

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P25.

Exploiting marine fungal biodiversity of the mesophotic zone for biodegradation of organic pollutants

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The marine environment is an untapped source of microbial diversity, showing various characteristics valuable for biotechnological applications, including bioremediation, especially considering that a great part of the earth's pollution appears in the oceans. An exceptionally under-investigated source of marine biodiversity is the fungal symbionts of invertebrates (e.g. ascidians, cnidarians, and sponges). The mesophotic zone, in particular, is an underexplored marine habitat, probably due to the fact that it is below depths (30-100 m) reached with traditional SCUBA diving techniques. The biodiversity of mesophotic coral systems is considered a potential source of novel symbiotic microorganisms, which can contribute to the enzymatic arsenal of Biocatalysis and specifically biodegradation of recalcitrant pollutants. Chlorophenols (CPs) are introduced in the environment as metabolites of herbicides and other chlorinated xenobiotics or through anthropogenic activities as effluent discharge of industrial processes. Polychlorinated biphenyls (PCBs) are produced through the fusion of two benzene rings in the presence of chlorine gas and are considered persistent organic pollutants (POPs). PCBs in wastewaters derive from leaching runoff, leaching from landfills and improper disposal of chemical waste.

Bioremediation is the use of living organisms in order to remove pollutants from soil and water; a method that is considered more cost-effective and environmentally friendly than the conversional techniques. Microorganisms – mainly bacteria and fungi – indigenous to the contaminated regions are potential candidates for the task, benefiting from their acquired enzymatic arsenal, aiming to use the contaminants as food, ideally towards their complete mineralization. Fungi are robust organisms and most of them are usually more tolerant to high concentrations of pollutants compared to bacteria. In the present work, we study the potential of 66 symbiotic fungi that were isolated from marine Mesophotic invertebrates through TASCMAR H2020 project to bioconvert the aromatic chlorinated pollutants 2,4-dichlorophenol (DCP) and PCB29. The metabolites of DCP detoxification by the most efficient fungi were elucidated using mass spectrometry and activities of ring-cleaving dioxygenases were measured. Regarding PCB29 bioconversion, enzymatic activities implicated in this process were measured. In both cases, novel enzymes (catechol dioxygenase and laccase) were isolated and biochemically characterized.

P26.

Metagenomic characterization of bacterial communities on ready-to-eat vegetables and effects of household washing in their diversity and composition

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Ready-to-eat (RTE) leafy salad vegetables are considered as foods that can be consumed immediately at the point of sale without further preparation or treatment. The aim of the study was to investigate the bacterial community composition of RTE salads at the point of consumption and the changes in bacterial diversity and composition associated with different household washing treatments. In particular, the bacterial microbiomes of the leaves of rocket and spinach salads were examined with the use of 16S rRNA gene high-throughput sequencing. Overall, 886 different OTUs were detected in the salads' phyllospheres. Proteobacteria was the most diverse high-level taxonomic group (comprising of 67.4 % of the total number of OTUs) followed by Bacteroidetes (13.3 %), and Firmicutes (5.1 %). Rocket salad showed different bacterial community composition than spinach salad, according to Bray-Curtis dissimilarity, although they were processed at the same production facilities. The most notable difference between the salads was found to be in the contribution of Proteobacteria and Bacteroidetes to the total number of OTUs, with Proteobacteria comprising 61.4 % in rocket and 78.3 % in spinach, while Bacteroidetes comprised 20.5% of the total number of OTUs in rocket and only 8.4 % in spinach. The tested household decontamination treatments proved inefficient in the removal of bacterial laods from both RTE salads. Furthermore, storage duration of the salads at refrigeration temperatures affected the bacterial composition, by decreasing the richness of the microbiome, and promoting the dominance of psychtrophic bacteria. Finally, both salads microbiomes were found to be a reservoir of some antibiotic resistant and opportunistic human pathogens and washing methods usually available at home proved to be inefficient in the removal of such bacteria.

P27.

Microbial desulfurization Microbial desulfurization of petroleum products

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The petroleum industry is facing the demands considering the radical reduction of sulfur dioxide emissions (SO_2) occuring from the combustion of organic compounds of various different petroleum fuels. The sulfur compounds in petroleum are divided into two categories, inorganic (SOx, H2S) and organic (aromatic or aliphatic thiols, sulfides or heterocyclic compounds). Between the above, the aromatic heterocyclic compounds such as dibenzothiophene (DBT) and its derivatives, 4,6 bimethyl (DBT) and its derivatives, 4,6 bimethyl-dibenzotheophene (4,6-DMDBT) for example, the main sulfuric compounds of Gasoil and thus of Diesel are of great importance. Due to the new legislation concerning the reduction of fuel sulfur levels (10ppm for liquid fuels as in Directive 2009/30/EC and 0.5% w/w in ship fuels as in Directive 2012/33/EU instructions), the exploration of new, low cost desulfurization processes is becoming imperative. The main process used today for the disposition of organic sulfur is hydrodesulfurazation (HDS), a process conducted in high temperature and pressure, conditions that become even more extreme if the target levels are to be implemented. Biological desulfurization (BioDeSulfurization - BDS) has emerged lately as an environmentally friendly and low energy process of petroleum cuts. Selected microbial strains are able to decompose sulfur compounds in petroleum by consuming the sulfur inside them. The process is accomplished by both aerobic and anaerobic microorganisms. There are two basic metabolic pathways of theophene decomposition that have been studied, the Kodama and the 4S pathway. Only the 4S pathway shows technological interest, because it leads to complete removal of sulfur, while fuel's calorific value remains intact.
In this study the *Rhodococcus rhodochrous* IGTS8 bacterium was used and the factors effecting the biomass growth with high desulfurization ability were studied. The experiments were conducted in a biphasic system of aqueous/organic phase and dodecane was used as the organic phase and the target compound was dibenzotheophene (DBT). Specifically, the rate of bacterial growth in different growth mediums, the effect of growth in different growth mediums, the effect of inoculums on the rate of biomass growth and on the rate of biomass growth and the rate of DBT desulfurization, the desulfurization ability of the strain at different DBT concentrations (0.5,1,5,10,20 and 30mM) and the effect of the way oxygen is supplied and distributed in the system, were studied.

Η εργασία υλοποιήθηκε στο πλαίσιο της Δράσης ΕΡΕΥΝΩ Η εργασία υλοποιήθηκε στο πλαίσιο της Δράσης ΕΡΕΥΝΩ – ΔΗΜΙΟΥΡΓΩ ΔΗΜΙΟΥΡΓΩ - ΚΑΙΝΟΤΟΜΩ και συγχρηματοδοτήθηκε από την Ευρωπαϊκή Ένωση και εθνικούς πόρους μέσω του Ε.Π. και συγχρηματοδοτήθηκε από την Ευρωπαϊκή Ένωση και εθνικούς πόρους μέσω του Ε.Π. και συγχρηματοδοτήθηκε από την Ευρωπαϊκή Ένωση και εθνικούς πόρους μέσω του Ε.Π. Ανταγωνιστικότητα, Επιχειρηματικότητα & Καινοτομία (ΕΠΑνΕΚ) (κωδικός έργου:Τ1ΕΔΚΑνταγωνιστικότητα, Επιχειρηματικότητα & Καινοτομία (ΕΠΑνΕΚ) (κωδικός έργου:Τ1ΕΔΚΑνταγωνιστικότητα, Επιχειρηματικότητα & Καινοτομία (ΕΠΑνΕΚ) (κωδικός έργου:Τ1ΕΔΚ--02074).

P28.
Cyanobacteria as a source of novel bioactive compounds

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Cyanobacteria represent an ancient group of oxygenic, photosynthetic prokaryotes that produce a variety of functionally diverse and structurally complex natural compounds, some of which have already been used for pharmaceutical purposes. As part of our ongoing efforts to discover novel bioactive compounds with pharmaceutical interest, we evaluated the antimicrobial and antileukemic potential of filamentous non-heterocytous and heterocytous cyanobacteria against Gram-positive, Gram-negative and fungal potential pathogens and acute myeloid leukemia MOLM-13 cell line. Extracts showing antibiotic and/or antileukemic activity were subject to reversed-phase HPLC and individual fractions were re-evaluated for their ability to kill the abovementioned pathogens and/or induce cell death in MOLM-13 cell line. Compounds present in the active HPLC fractions were analysed by UPLC/ESI/Q-TOF apparatus and were identified based on publicly available data and several up-to-date scientific articles. New bioactive compounds, new variants and a number of yet unidentified metabolites exhibiting antimicrobial and/or antileukemic activity are reported herein. In detail, new merocyclophanes and a number of new merocyclophane variants were present in the active fractions of *Nostoc* sp. CENA69. The heterocytous cyanobacterium *Aliinostoc* sp. CENA513 produces the recently discovered metabolite nocuolin A, a compound with antimicrobial and antiproliferative properties, and unidentified lipid compounds that show only bacteriocidal activity against *B. cereus*. Interestingly, none of the abovementioned strains had any effect on the growth of Gram-negative pathogens. *Planktothrix agardhii* UHCC 0018 and *Anabaena* sp. UHCC 0187 strains showed only antileukemic activity. The majority of the bioactive fractions deriving from these two strains contained either lipids or pigments and their derivatives. The remaining active HPLC fractions contained a great number of unidentified compounds. Further studies are required in order to identify the unknown compounds and purify the new merocyclophanes and antibacterial lipids. These results clearly show that Cyanobacteria are an emerging source of bioactive metabolites that can be used in drug development process or act as a source of inspiration for the production of synthetic drugs.

Keywords: cyanobacteria, bioactive compounds, antimicrobial activity, MOLM-13

P29.

Alternative sources of organic N in greenhouse tomato crops: the case study of inoculation of cowpea with *Bradyrhizobium* and endophytic bacteria

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In organic greenhouse tomato inorganic N fertilizers cannot be applied. Since the maximum amount of N delivered through farmyard manure is insufficient for the crop, alternative sources of organic-N are needed. To this end, two greenhouse experiments on organic tomato were conducted aiming to evaluate whether green manure with a legume crop, applied in addition to farmyard manure (FYM) and beneficial bacteria, can benefit tomato crop in terms of soil N availability and yield. An additional objective was to assess the efficiency of compost and green manure with legume crops inoculated with rhizobia as sole sources of N. In Treatment 1 (T1, control) of Exp1, no other source of N was applied except for the FYM. In T2, T3 and T4 additional N was provided through green manure, by sowing cowpea. In T2, the seeds of cowpea were not inoculated with any rhizobia. In T3, the seeds of cowpea were inoculated with *Bradyrhizobium* sp. VULI11, while in T4, the seeds of cowpea were inoculated with a mix of *Bradyrhizobium* and endophytic bacteria (*Enterobacter* sp. C1.2, *Enterobacter* sp. C1.5, *Enterobacter* sp. C3.1, and *Lelliottia* sp.). In Exp2, FYM was applied only in T1 (control). In T2 of Exp2, only olive-mill waste compost was applied. In T3 of Exp2, a green manure crop of cowpea inoculated with a mix of *Bradyrhizobium* and endophytic bacteria, similarly to Exp1 was used as sole source of N. Finally, in T4 of Exp2, the same compost as in T2 was applied, in combination with cowpea green manure as in T3. The results showed that the inoculation of cowpea seed with *Bradyrhizobium* sp. VULI11 increased appreciably the above-ground biomass of cowpea plants used as green manure, while the inclusion of endophytic bacteria to the *Bradyrhizobium* inoculum provided no additional benefits to biomass production. In addition, the inoculation only with *Bradyrhizobium* sp. VUL11 had a significant impact on total-N, and δ15N air of the cowpea tissue.

Keywords: Organic, tomato, cowpea, rhizobia, nitrogen

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P30.

Impact of Plant Growth Promoting Rhizobacteria in grafted tomato plants subjected to combined nutrient and water stress

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Inoculation of tomato roots with Plant Growth Promoting Rhizobacteria (PGPR) could potentially improve plant growth and total yield, thereby mitigating water and nutrient demands. Two consecutive soilless culture experiments were conducted on a commercial tomato hybrid ('*Belladonna*'), studying five different PGPR strains: C1.2 (*Enterobacter ludwigii*), C1.5 (*Enterobacter cloacae*), DN1.2 (*Paenibacillus sp.*), C3.1 (*Enterobacter mori*) and D2.4 (*Lelliottia amnigena*). In the 1st experiment, C1.2 and C1.5 were inoculated as a mix onto tomato roots and plants were either self-grafted or grafted onto rootstock M82. In the 2nd experiment, plants were either self-grafted or grafted onto rootstock Buffon and inoculated with either C1.2 or C1.5. The strain DN1.2 was not included in the second study. Plants of both experiments were either optimally fertigated or exposed to combined water and nutrient (N, P shortage) stress. In both experiments, PGPR enhanced plant growth but had no impact on total fruit yield. In the 1st experiment, plants inoculated with C3.1 showed the highest biomass under non-stress conditions while plants inoculated with a mix of C1.2 and C1.5 gave the highest biomass under stress conditions. In the 2nd experiment, plants inoculated with C3.1 and grafted onto the commercial rootstock Buffon showed the highest biomass. However, in the self-grafted plants, the C1.2 strain appeared to produce the higher biomass compared to the other treatments. Finally, no inoculation of tomato roots with PGPR resulted in the lowest biomass production. Strain C3.1 was selected as the most promising strain to be used for metabolomic analysis and was compared to the non-inoculated treatment. Samples also included non-stressed and stressed plants as well and self-rooted and grafted plants. The applied bioanalytical and bioinformatics protocols resulted in a strong discrimination among the various treatments, with the metabolite profiles of plants treated with PGPR being distinct from that of untreated plants.

Keywords: hydroponics, rootstock, endophytics, metabolomics, biostimulant

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P31.

Characterization of non-rhizobial bacteria from root nodules of cowpea grown in Greece

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Rhizobia are responsible for root nodulation and nitrogen fixation in most legume species. However, legume nodules are occupied by other endophytic bacteria, called non-rhizobial bacteria. Recent studies have shown that a high diversity of non-rhizobial bacteria exist with nodules and they may encompass up to 99% of the total bacterial population. Growing evidence indicates that some of these non-rhizobial bacteria could be beneficial to their legume hosts by enhancing plant growth, fixing atmospheric nitrogen, solubilizing phosphate and improving the nodulation and N2 fixation of legume–rhizobia symbionts. In contrast to these positive effects, some non-rhizobial bacteria may be able to reduce the fitness of nodulating rhizobia or to trigger host defence responses resulting in the prevention of the infection process. Nevertheless, the biological significance and the agronomic implications of nodule endophytism are still not well understood. This study aimed to characterize the diversity of bacteria associated with root-nodules of cowpea plants collected from different locations in Greece and to assess their ability for promoting plant growth in order to select potential PGPR that perform higher plant growth promotion for use as biofertilizers. Nodule endophytic bacteria were isolated from field grown cowpea plants and were grouped into four clusters by BOX-PCR. Representative isolates of each cluster were subjected to multilocus sequence analysis (MLSA) using five housekeeping genes (16S rRNA, *gyrB*, *rpoB*, *atpD* and *infB* genes) to assess their phylogenetic affiliation and were screened for various plant growth promoting traits. Phylogenetic analysis showed that the nodule endophytic isolates were related to *Enterobacter cloaceae*, *Enterobacter ludwigii*, *Enterobacter mori*, and *Lelliottia amnigena*. All the test isolates possessed multiple plant-growth promoting characteristics *in vitro*: phosphate solubilization, production of phytohormone indole acetic acid and siderophores, and plant growth promoting activity. Moreover, the isolates showed swarming ability and exhibited high salt tolerance (up to 8%). The results are in line with previous studies reported that certain *Enterobacte*r spp. possess multiple growth promoting activities. These isolates could be developed as an eco-friendly biofertilizers for cowpea and probably for other important plant species in future.

Keywords: Plant Growth Promoting Rhizobacteria, nodule endophytes, cowpea, phylogeny

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P32.

Characterization of microsymbionts isolated from root nodules of *Vicia faba* grown in Greek soils

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Vicia faba L. is an important leguminous crop cultivated worldwide for food and feed. Considering the European guidelines towards sustainable agriculture, and the agro-economic importance of *V. faba*, the selection and the application of efficient rhizobial inoculants is a crucial lever for increasing legume crop yields. Despite that *Rhizobium leguminosarum* sv. *viciae* (*Rlv*) is the most common symbiont of *V. faba* worldwide, genetic differences in specificity among strains of *Rlv* have been reported. Other rhizobial species have also been reported as microsymbionts of faba beans. Considering the lack of systematic studies of rhizobia nodulating *V. faba* in Greece, this study aimed to reveal the genetic diversity and phylogeny of indigenous rhizobia nodulating diverse varieties of faba beans grown at different locations in Greece. This study is expected to offer rhizobial germplasm for selection of highly efficient symbiotic bacteria associated with faba bean and adapted to the local conditions.

Phenotypic, biochemical and molecular approaches were used to estimate the diversity of rhizobia nodulating various field-grown faba bean genotypes. The genetic diversity of the rhizobial isolates was assessed by applying the BOX-PCR fingerprinting technique and the phylogenetic affiliation was assessed by multilocus sequence analysis (MLSA) of housekeeping and symbiotic genes. A total of 273 rhizobial strains isolated from faba bean nodules were analyzed by BOX-PCR and were divided into 30 clusters. Representative isolates of each cluster were further analyzed. Most of the isolates were effective microsymbionts of *V. faba* and were classified into *Rlv* group based on the phylogeny of housekeeping and symbiotic genes. However, some strains revealed genetic differences in housekeeping and symbiotic genes indicating that they might represent novel genomic lineages.

Keywords *Vicia faba*, Faba bean, Rhizobium, Symbiosis, Nodulation, Phylogeny

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P33.

Diverse bacteria isolated from root nodules of common bean grown in different ecoregions of Greece

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Phaseolus vulgaris (L.), commonly known as bean or common bean, is a legume species that is cultivated worldwide as a grain or vegetable crop, and is a promiscuous host that forms root nodules with a wide range of symbiotic nitrogen-fixing bacteria belonging to the genera *Rhizobium*, *Ensifer* and *Burkholderia*. This is the first report on the characterization of bean-nodulating rhizobia in Greece. The goals of this research were (i) to isolate and characterize rhizobia nodulating local common bean genotypes grown in six different edaphoclimatic regions of Greece; and (ii) to assess their competitiveness and tolerance to environmentally stressful conditions. Therefore, selection of highly efficient rhizobia adapted to the local conditions will serve as resources for the production of inocula. Phenotypic, biochemical and molecular approaches were used to estimate the diversity of bacteria within nodules collected from field-grown common bean genotypes. The genetic diversity of the rhizobial isolates was assessed by BOX-PCR and the phylogenetic affiliation was assessed by multilocus sequence analysis (MLSA) of housekeeping and symbiotic genes. A total of 133 fast-growing rhizobial strains were isolated and grouped into 15 clusters by BOX-PCR. MLSA of representative isolates of each cluster revealed that some of them were affiliated to defined *Rhizobium* species whereas others might represent novel genomic lineages. Interestingly, a high occurence of isolates belonging to the *Caballeronia* genus were found within bean nodules. Although *Caballeronia* species have been isolated from root nodules, there is no evidence that any of the defined *Caballeronia* species is able to nodulate legumes. The phylogenetic affiliation of the *Caballeronia*-like isolates and their ability to nodulate common bean is under investigation.

Keywords: Common bean, Rhizobium, Symbiosis, Nodulation, Phylogeny

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P34.

Irradiation effect on the structure of bacterial communities associated with the oriental fruit fly, *Bactrocera dorsalis*

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The role of symbiotic microbes in insects, especially the beneficial character of this interaction, has received much attention in recent years as it has been related to important aspects of the host insects' biology such as development, reproduction, survival, and fitness. Among insect hosts, tephritid fruit flies are well known to form beneficial associations with their symbionts. To control these destructive agricultural pests, environment-friendly approaches, like the sterile insect technique (SIT) as a component of integrated pest management strategies, are currently successfully implemented against the Mediterranean fruit fly *Ceratitis capitate,* the Mexican fruit fly *Anastrepha ludens* and Melon fly *Zeugodacus cucurbitae* or are under consideration for other species. In this study, changes in the bacterial profile of mass-reared oriental fruit flies, *Bactrocera dorsalis* (Hendel) (Tephritidae) were examined in both larval and adult stages and also after irradiation by employing a 16S *rRNA* gene-based Illumina sequencing approach. Proteobacteria were the prevalent bacterial phylum in non-irradiated adults and larvae. Alphaproteobacteria were the most abundant class in larvae but almost absent in adults, which were dominated by Gammaproteobacteria. Firmicutes were present in both developmental stages but at lower relative abundance. At genus level, *Acetobacter* prevailed in the larval stage and members of the Enterobacteriaceae family in adults. Irradiated samples exhibited higher diversity and richness indices compared to the non-irradiated oriental fruit flies, whereas no significant changes were observed between the two developmental stages of the non-irradiated samples. *Lactobacillus*, members of the Orbacecae family and *Morganella* were detected but to a lesser degree upon irradiation, whereas the relative abundance of *Lactococcus* and *Orbus* increased. The bacterial profile of larvae appeared to be different compared to that of adult *B. dorsalis* flies. The subsequent application of irradiation at the pupal stage led to the development of different microbiota between treated and untreated samples, affecting diversity and operational taxonomic unit composition. Controlling and exploiting the microbiota during all developmental stages may improve the performance of irradiated males and therefore the efficiency of SIT.

P35.

Effect of tetracycline and vitamin composition on the microbiota of tsetse flies

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Tsetse flies (Diptera: Glossinidae) are the cyclical vectors of trypanosomes that cause parasitic disease to humans (sleeping sickness) and animals (nagana). The Sterile Insect Technique (SIT) is a powerful, environment-friendly method used to control tsetse fly populations. It relies on the mass-rearing of sterile flies intended to mate with the natural population, causing infertile crosses which lead to the suppression or even the local elimination of the targeted population. Since bacterial symbionts influence the fitness and behaviour of their hosts, they can be used to improve the quality of mass reared insects and therefore the application of SIT. In this study, we investigated the effect of four different diets, enriched with B-vitamins or Vanderzant vitamin mixture, with or without Tetracycline treatment, on the bacterial profile of three laboratory colonies of *Glossina* species: *Glossina morsitans morsitans* (*Gmm*), *Glossina pallidipes* (*Gp*) and *Glossina palpalis gambiensis* (*Gg*). Total DNA was extracted from ovaries, testes and guts of teneral and 16-day old flies. To examine the bacterial diversity, the set of primers 341F and 805R was used to amplify the 16S *rRNA* V3-V4 hypervariable regions. Raw sequencing reads was pre-processed using usearch11. Alignment, phylogenetic tree and taxonomy assignment against SILVA database were performed on Qiime2. Calculation of alpha and beta diversity was executed in R MicrobiomSeq packages and Rhea scripts respectively.

Differences in OTU distribution based on diet revealed *Sodalis* as the most dominant taxon in diets with B-vitamins and Vanderzant vitamin mixture, while diets with Tetracycline/B-vitamins and Tertacyline/Vanderzant vitamin mixture, contained *Empedobacter* as the most dominant followed by *Sodalis*. Regarding *Wigglesworthia* (the primary endosymbiont in *Glossina* sp.), is less abundant in diets with only vitamins especially for B-vitamin diets, whereas it is totally absent in diets containing tetracycline. Overall, the *Glossina* samples reared on Tetracycline diets exhibited higher richness and diversity indices. Importantly, in all three *Glossina* species we observed statistically significant differences between samples fed on diets containing Tetracycline and those fed only on vitamins, except for *G. p. gambiensis* where no statistical difference was observed between B-vitamins and Tetracycline/B-vitamins diets. In addition, tsetse flies kept on B-vitamin or Vanderzant vitamin mixture did not show any statistical significant difference in the bacterial profiles of the three studied *Glossina* species. Results on co-occurrence network analysis will be presented as well.

P36.

Cholesterol lowering properties of *Lactobacillus* strains isolated from infant faecal microbiota

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Hypercholesterolemia is considered the main factor for cardiovascular disease. Supplementation with probiotics interfering with cholesterol metabolism may contribute todisease prevention. The cholesterol lowering ability of some probiotic strains can be attributed to the bile salt hydrolase (BSH) activity and / or the ability for cholesterol assimilation and the subsequent removal of cholesterol from the intestinal lumen.
Aim: To test potential probiotic *Lactobacillus* strains (Harokopio University Culture Collection) for BSH activity and for their ability to assimilate cholesterol from the culture medium.
Materials and Methods: Thirty four *Lactobacillus* strains (*L. gasseri*, *L. crispatus*, *L. acidophilus, L. rhamnosus*, *L. fermentum*, *L. salivarius*, *L. paracasei paracasei* and a strain of *Lactobacillus* spp.), which have been isolated from the intestinal microbiota of healthy neonates, were tested for BSH activity. *L. acidophilus* DSM 20079 was used as a positive control strain. Activated cultures were incubated in MRS agar supplemented with 0.5 % (w/v) sodium taurodeoxycholate for 72 h. Only the strains that exhibited positive activity of BSH were tested for cholesterol assimilation from the culture medium. In detail, activated cultures were anaerobically incubated for 24 hours in modified MRS Broth supplemented with oxgall 0.3% (w / v) and polyoxyethanyl cholesteryl sebacate. At the end of incubation, dry biomass of the bacteria was measured and the amount of cholesterol remaining in the supernatant was determined photometrically.
Results: Four strains of *L. gasseri* (C5, C15, C32, C45), one *L. crispatus* (C1), one *L. delbrueckii delbrueckii* (AnLB40), one *L acidophilus* (AnLB16) and the strain of *Lactobacillus* spp. (C39) demonstrated BSH activity.The cholesterol assimilation assay revealed that almost all the strains, (except AnlB16 strain), exhibited significant cholesterol removal from the supernatant compared to the uninoculated substrate. Cholesterol removal for all the tested lactobacilli (expressed per gram of dry biomass), was similar to the positive control. Our results revealed the cholesterol lowering capacity of potential probiotic strains; further testing is required through *in vitro* assessment systems followed by *in vivo* procedures.

Keywords: probiotic strains, lactobacilli, cholesterol assimilation, bile salt hydrolase

P37.

Degradation efficiency of a laboratory-scale bioreactor treating wastewater of high ibuprofen concentration

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In the recent years, increased amounts of pharmaceuticals have been directed in Wastewater Treatment Plants (WWTP). This is considered as a serious threat of the aquatic life. Among such pharmaceuticals, ibuprofen is a common non-steroidal anti-inflammatory drug detected in almost all WWTP examined. Despite the fact that several studies are focused on the monitoring of ibuprofen as micropollutant, little is known about the bacterial taxa that are capable of degrading ibuprofen. In this study, a continuous flow bioreactor system was set up to investigate biosystem removal efficiency at high ibuprofen loads. The biosystem was periodically evaluated by examining its physicochemical characteristics and estimating the ibuprofen, COD and total nitrogen removal efficiencies, whereas identification of bacterial biota was examined by next generation sequencing techniques. It was found that the biosystem could result in ibuprofen removal efficiencies as high as 95% in a relatively short hydraulic retention time. Besides, the implementation of next generation techniques revealed the dominance of *Proteobacteria*, in particular the predominance of members of alpha and gamma classes of this bacterial phylum. It can be concluded that specific part of activated sludge microbiota can deal with the high ibuprofen concentration, which are not couple to co-metabolism, being capable of serving as the main constituents of biological filters specialized in non-steroidal anti-inflammatory drug degradation.

Keywords: ibuprofen degraders, wastewater treatment plants, biodegradation, activated sludge

P38.

Exploring characteristics of gut microbiota and bacterial metabolic products in autism spectrum disorder: a pilot study

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Aim: Autism spectrum disorder (ASD) is a neurodevelopmental disorder, often characterized by abnormalities in gut-brain axis communication. Specific gut bacteria and metabolites had been proposed to interfere with ASD-associated pathophysiology, though further research is necessary. This pilot study aimed to investigate quantitative alterations of selected bacteria and bacterial metabolites (Short Chain Fatty Acids, SCFAs) in children with ASD, compared to age-matched neurotypical healthy controls (4-10 years of age). **Materials-Methods:** Gut microbiota analysis was performed on stool samples of 14 children with ASD and 7 neurotypical healthy controls, based on qPCR quantification of total bacteria and previously proposed ASD-related microbes (*Bifidobacterium* sp., *Lactobacillus* group, *Prevotella* sp., *Clostridium perfringens* group and *Faecalibacterium prausnitzii*). Measurement of faecal SCFAs by gas chromatography was performed in a further sample of 8 children with ASD and 9 neurotypical healthy cases. **Results:** Gut microbiota analysis indicated no significant ASD-associated differences in levels of total bacteria or selected tested microbes. Measurement of faecal SCFAs proposed a trend for higher molar ratio (%) of acetate (45.52±3.17 vs. 41.90±3.93, p=0.055) and increased levels (μmol/g faeces) of isocaproic acid (0.25±0.06 vs. 0.20±0.05, p=0.070) in the case of children with ASD compared to age-matched neurotypical healthy controls, respectively. **Conclusions:** Our preliminary data proposed a trend for quantitative alterations in known gut bacterial metabolites, which may functionally affect ASD. Though no significant differences could be detected in terms of gut microbiota profiling in this pilot study, merely due to small sample size, implementation of greater scale, well-designed studies will shed further light into implications of gut microbiota characteristics in ASD-associated intestinal function and behaviours.

Keywords: Autism Spectrum Disorder, gut microbiota, SCFAs

P39.

Tolerance and biodegradation of Bisphenol-A by two strains of probiotic bacteria

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It is well established that probiotics confer health benefits on their host by restoring the composition of the gut microbiome, which plays a key role in the human intestine. It is also known that there is a complex interplay between gut microbes and ingested xenobiotics, resulting in regulation of their toxicity that may lead to disease. A common xenobiotic widely used in food-packaging industry is Bisphenol-A (BPA), a synthetic estrogen that is able to disrupt endocrine signaling. In this study two probiotic strains *Lactococcus lactis and Bacillus subtilis,* were initially tested for their ability to survive under stress conditions when BPA was provided as the sole carbon source and their toxicity threshold was estimated. Moreover the strains were tested for their ability to degrade BPA via the two major degradation pathways producing possible metabolites such as 4-hydroxy-benzoate (HBA), 4-hydroxy-acetophenone (HAP), hydroquinone (HQ), and 4-iso-propenylphenol (PP). Both bacterial strains were incubated in minimal medium supplemented with 0.1% yeast extract and a BPA concentration of 50 μg/mL. Higher BPA concentrations exhibit growth inhibition. Supernatant from the cultures was collected at 0, 24, 48, 72 and 96 hours, centrifuged, filtered and analyzed with HPLC using n-Octylphenol as the internal standard, for the quantification of BPA and its possible metabolites. Cells were harvested at 96 h, centrifuged and the cell pellet was subjected to homogenization, SPE and HPLC analysis.
Our results revealed that *B. subtilis* growth was highly inhibited at BPA concentrations above 50 μg/mL and only *L. lactis* showed a higher tolerance. Also yeast extract stimulated bacterial growth and BPA biodegradation. Both strains exhibited BPA removal efficiency up to 50% and HPLC analysis revealed two peaks, one related to (HAP) from supernatant samples and the other related to (PP) from cell pellet analysis for both bacteria. Our findings advocate that both probiotics uptake and degrade BPA and thus these bacterial strains may mitigate its harmful effects.

Keywords: Bisphenol-A, probiotic bacteria, biodegradation, HPLC

P40.

Rhizospheric bacterial strains isolated from stress tolerant plants as potential biostimulants for cultivated species

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In the past two decades, microbiome research and its potential applications to the field crops has received remarkable attention. The identification of rhizospheric bacterial strains that have the potential of enhancing plant growth and subsequently crop productivity and/or providing cross-protection against multiple stress factors (Plant-Growth-Promoting Rhizobacteria or PGPR) became an important scientific breakthrough towards sustainable agriculture. Along these lines, the current study focuses on isolation and characterization of new bacterial strains that can serve as PGPR. In particular, bacterial strains from the rhizosphere of stress-tolerant plant species have been collected from sampling sites of Axios Delta (salt stress) and the urban forest of Seih Su (drought stress). The samples have been collected from naturally growing native plants, aromatic (*Mentha pulegium*, *Cistus sp*, *Thymus sp*) and non-aromatic (*Atriplex sp*, *Sacrocornia sp. Critamus sp.*). To evaluate their potential effectiveness in promoting growth, all morphological different bacterial isolates were tested for their *in vitro* putative PGP traits, including auxin production, ACC deaminase activity, siderophore production, phosphorus solubilisation, and organic acid production. Based on 16S rRNA sequences on 20 selected strains with potential PGP traits, nine strains belong to the genus *Pseudomonas*, four to the genus *Bacillus*, and others to the genera *Curtobacterium*, *Pedobacter*, *Pantoea*, and *Burkholderia*. As a further step, whole genome sequencing of the isolated bacterial strains using NGS techniques will allow us to identify loci coding proteins associated with the functional traits of interest implicated in plant stress tolerance.

Acknowledgments

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P41.

Effect of olive fruit size on the fermentation process of cv. Kalamata natural black olives

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Natural black cv. Kalamata table olives of three different sizes, namely Colossal (130 pieces/kg), Jumbo (180 pieces/kg), and Superior (260 pieces/kg) were fermented according to the traditional Greek-style process. Microbial populations of the dominant microorganisms, namely lactic acid bacteria (LAB), yeasts, and *Enterobacteriaceae* were monitored throughout fermentation. Analyses for the determination of pH, titratable acidity, salt content as well as changes in color and texture of the olives were also undertaken. Results showed the dominance of LAB, followed by yeasts, indicating normal fermentation process. For Colossal olives, LAB reached 7.9 $\log_{10}$ CFU/mL and 8 $\log_{10}$ CFU/g, while yeasts ranged between 7.1 $\log_{10}$ CFU/mL and 5.5 $\log_{10}$ CFU/g in the brine and olives, respectively. For Jumbo olives, LAB counts were 8.2 $\log_{10}$ CFU/mL and 7.71 $\log_{10}$ CFU/g, whereas yeasts were enumerated close to 6.5 $\log_{10}$ CFU/mL and 5 $\log_{10}$ CFU/g in brine and olives, respectively. Finally, for Superior olives, LAB counts were 8 $\log_{10}$ CFU/mL and 7.7 $\log_{10}$ CFU/g, while yeasts were enumerated close to 7.4 $\log_{10}$ CFU/mL and 5.4 $\log_{10}$ CFU/g in brine and olives, respectively. After of 100 days of fermentation, acidity was close to 0.6% (Colossal), 1.3% (Jumbo) and 0.5% (Superior) (w/v) lactic acid, while pH was approximately 3.7-4.3 for brines and 3.8-4.3 for olives. Texture analysis for all sizes showed a decrease in Break Force, related to hardness, from 3.0-4.4 N to 1.2-1.8 N during the first 70 days of fermentation followed by a gradual increase up to 1.8-3.0 N until the 100th day of the process due to salt absorption. An increase in the lightness coordinate (L^*) was observed indicating the increase in luminosity (brightness) of the color in all sizes. The attribute a^*, corresponding to violet color, increased during the fermentation process in all olive fruit sizes within the first 100 days. The values of the parameter b^* were negative before the beginning of fermentation and changed to positive during the process, with most pronounced changes for the Superior size olives.

P42.

Monitoring of the degradation of imazalil in a laboratory-scale immobilized cell bioreactor

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The fruit processing industry produces annually large amounts of wastewaters worldwide. In particular, various effluents contain high concentrations of post-harvest fungicides, including the imidazole fungicide imazalil, which is commonly used in post-harvest handling of citrus and banana. However, the biodegradation potential of imazalil in bioreactor systems remains unknown. In the current study, the biodegradation potential of imazalil was examined in an immobilized cell biosystem through the implementation of a comprehensive physicochemical analysis in the influent and the effluent of the bioreactor. In parallel amplicon next generation sequencing was employed to monitor the bacterial community composition in the immobilized biomass. Indeed, the acclimatized biomass was capable of reducing imazalil concentration over 80% under prolonged hydraulic retention time. It is concluded that the immobilization of the slow degraders in the Siran carriers permitted the effective reduction of this systemic post-harvest fungicide.

Keywords: fruit processing wastewater, imazalil, postharvest fungicide, immobilized cell bioreactor

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P43.

Cytotoxic Effects of *Lactococcus lactis* Secretome on Colorectal Cancer cells

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Gut microflora has been correlated with human gut-associated diseases. Probiotics are the predominant inhabitants in the colon which, when administered in adequate amounts, confer a health benefit maintaining intestinal microbial balance in their host through their secreted metabolites (organic acids, hydrogen peroxide, bacteriocins). Bacteriocins are cationic, ribosomal synthesized peptides with antimicrobial activity, which led to their characterization as alternatives to antibiotics. Furthermore, selective cytotoxicity of bacteriocins secreted from other probiotic strains toward cancer cells has been documented and attributed to the different membrane properties of cancer cells. The aim of this study was to investigate the anti-proliferative effects of the 24h and 48h cell-free culture supernatant (CFS) of the probiotic *Lactococcus lactis* on human colon carcinoma cell line RKO, in a dose-response manner after 24, 48 and 72h of incubation. CFSs were collected from *L. lactis* cultures after 0, 6, 12, 16, 24, 48h of incubation and their antimicrobial activity was tested against *Enterococcus faecalis* (D 4041), with highest values observed using the 24h and 48h CFSs. The total protein levels and bacteriocin activity of each sample were determined using Bradford assay and a specifically constructed nisin-standard curve. The CFSs were filtered (0.45μm), lyophilised, dissolved in DMEM, re-filtered (0.2μm) and their cytotoxic effects were determined by colorimetric MTT assay and crystal violet cell staining. Our results demonstrate viability reduction of RKO cells, suggesting a significant cytotoxicity of *L. lactis* bacteriocins against colon cancer cells.

Keywords: probiotics, bacteriocins, cell toxicity, colon cancer, MTT

P44.

Assessment of electrocoagulation as a pretreatment method of olive mill wastewaters for biofuels production

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Olive oil mill wastewater (OMW) is the liquid wastewater that is generated during the production of olive oil from the continuous three-phase decanter process. OMW is characterized by an extremely high organic load and phenolic content, making its safe disposal a huge environmental concern. For that reason, during the last decades, increasing attention has been paid to discovering alternative ways of handling OMW aiming to the reduction of the organic load and the toxic effect. Among them, electrocoagulation (EC) is reported to be quite promising. With this technology, in situ generation of coagulant species by electrolytic oxidation of sacrificial anode materials, triggered by electric current, is carried out. The metal ions generated by electrochemical dissolution of a consumable anode spontaneously undergo hydrolysis in water, forming various coagulant species including hydroxide precipitates (able to remove pollutants by adsorption/settling) and other metallic ion species. In the present study, EC was assessed as a pretreatment method of OMW for biofuels production. Experiments were performed using Al or Fe electrodes, in an electro-coagulation reactor consisted of one anodic and two cathodic electrodes, with an effective surface area of 12 cm2 each, and a current density supply of 0.17 A/cm2. Undiluted and diluted with tap water OMW, were pretreated under different currents and the pretreated waste was either let to settle or was centrifuged, in order to be separated to different fractions. The different fractions of the combined pretreatment methodologies were assessed as substrates for methane and hydrogen production via mixed acidogenic consortia. It was shown that the centrifugation after EC altered the chemical composition of the wastewater in a favorable way for biofuels production. The maximum methane yield (0.3 L CH_4/g COD initial) was achieved for both electrodes for combined EC with centrifugation, for current values equal to 0.05 A or 0.5 A and for pretreatment time equal to 3 h. As it regards fermentative hydrogen production, the maximum achieved yield 2.26 ± 0.01 mol /mol consumed carbohydrates was observed for combined EC with centrifugation with Al electrodes, current value equal to 0.05 A and pretreatment time 3 h.

Keywords: Electrocoagulation, OMW, methane, hydrogen

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P45.

***In vitro* evaluation of the antimicrobial and nematicidal activity of multi – productive streptomycetes isolated from Greek terrestrial ecosystems**

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The Gram-positive Actinobacteria and specially the genus of *Streptomyces,* are widely known for their unique capacity to produce approximately the 75-80 % of the commercially and medically useful bioactive compounds and about the 60% of the antibiotics which are used in agriculture. Our previous work proved that due to the characteristic Greek Mediterranean micro-climate and the special geomorphological conditions of the Greek territory, the indigenous *Streptomyces* populations are multi-active by producing a rich variety of bioactive compounds. Therefore, the aim of this work was to isolate novel indigenous *Streptomyces* from seven different Greek terrestrial ecosystems and to search for producers against root-knot nematodes. 462 isolates of streptomycetes were recovered from soil samples and examined for possible inhibitory activity against 12 different microbial markers (*Bacillus subtilis* DSM 10, *Micrococcus luteus* DSM 1790, *Lactobacillus fermentum* ATCC 9338, *Escherichia coli* NEB DH 5a, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas fluorescens* DSM 50090, *Acinetobacter radioresistens* DSM 6976, *Aspergillus niger* DSM 1957, *Aspergillus nidulans* LA1, *Fusarium oxysporum* DSM 62059, *Saccharomyces cerevisiae* DSM 70449, *Cyberlinderasaturnus Minter* ATHUM 2576). 120 of the above isolates inhibited at least one of the microbial markers, and 15 of them, called multi-productive strains, inhibited the growth of at least 7 microbial indicators which in addition with another 11 isolates, displaying antifungal activity and finally selected and tested against root-knot nematode *Meloidogyne javanica*. Batch cultures of 26 isolates were carried out for 7 days incubation at 30° C and thereafter the culture supernatants were checked for nematicidal activity. Increased juvenile mortality was observed after 72 h for all strains tested. Two of the examined isolates displayed a high potential activity against nematodes. They were identified as *Streptomyces colombiensis* strain 173874 and *Streptomyces monomycni* strain NBRC 100769, respectively. The above results are discussed in relation to the threat on the agricultural production, that nematodes pose, since it is an urgent need to find environmentally friendly ways to treat the crops without spreading harmful substances at the cultivated soil and possibly a promising biocontrol treatment of nematodes.

P46.

The effect of diet and radiation on the bacterial symbionts of the melon fly, *Zeugodacus cucurbitae* (Coquillett)

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Symbiotic bacteria contribute to a multitude of important biological functions such as nutrition, reproduction, protection against pathogens and communication, affecting multiple physiological factors of their insect hosts, including fitness, longevity and survival. The melon fly, *Zeugodacus cucurbitae* (Coquillett), is an important agricultural pest of a variety of cultivated plants, mostly from the Cucurbitaceae family. It is considered invasive and widespread in many parts of the world. Several approaches are currently being considered for the management of this pest including the environmentally friendly and effective sterile insect technique (SIT), as a component of an integrated pest management strategy. In the present study, we examined the effect of diet and radiation on the bacterial symbiome of *Z. cucurbitae* flies with Illumina amplicon sequencing of the V3-V4 region of the 16S *rRNA* gene. Melon flies were reared on two diets at the larval stage, a bran-based artificial diet and on sweet gourd, which resulted in the development of significantly different bacterial profiles. Significant differentiation was also observed based on gender. The effect of radiation was mostly diet dependent, with irradiated melon flies reared on bran exhibiting a significant reduction in species diversity and richness compared to all other samples, followed by a drastic reduction in the number of sequences affiliated with members of *Citrobacter*, *Raoultella* and Enterobacteriaceae and an increase in *Enterobacter*, *Providencia* and *Morganella*. At the same time, flies reared on sweet gourd exhibited opposite responses, with irradiated males showing a significant reduction in species richness and minor differences in the relative abundance of *Enterobacter* and *Providencia*, while the irradiated females showed a significant increase in both richness and diversity compared to their respective non-irradiated controls. These results suggest that alterations caused by the irradiation treatment to the microbiota of mass-reared melon flies could be potentially reduced by enriching the diet provided at the larval stage, thus enhancing the fitness of the flies and the application of the SIT.

Keywords: *Zeugodacus cucurbitae*, Sterile Insect Technique, Next Generation Sequencing, 16S rRNA, irradiation, diet.

P47.

Detection and characterization of reproductive parasites in Southeast Asian tephritid fruit fly populations

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Certain endosymbiotic bacteria infect a wide range of insect-hosts and can induce a series of reproductive abnormalities, such as cytoplasmic incompatibility (CI), parthenogenesis, feminization and male-killing. These extended phenotypes can be potentially exploited in support of integrated pest management strategies for controlling natural populations of agricultural pests. In this study, we investigated the presence of reproductive parasites in *Bactrocera*, *Dacus* and *Zeugodacus* fruit flies from Southeast Asian populations. A specific 16S *rRNA* PCR assay was used to investigate the presence of reproductive parasites in natural populations of nine different tephritid species originating from three Asian countries, Bangladesh, China and India. *Wolbachia* infections were identified in *Bactrocera dorsalis*, *B. correcta*, *B. scutellaris* and *B. zonata*, with a prevalence of 12.2-42.9%, Entomoplasmatales in *B. dorsalis*, *B. correcta*, *B. scutellaris*, *B. zonata*, *Zeugodacus cucurbitae* and *Z. tau* (0.8-14.3%) and *Cardinium* in *B. dorsalis* and *Z. tau* (0.9 - 5.8 %), while none of the species tested harbored infections with *Arsenophonus*. Prevalence in infected populations ranged from 3 to 80 % for *Wolbachia*, 2 to 33 % for Entomoplasmatales and 5 to 45 % for *Cardinium*. *Wolbachia* and Entomoplasmatales infections were found in tropical and subtropical populations, the former mostly in India and the latter in various regions of India and Bangladesh. *Cardinium* infections were identified in both countries, only in subtropical populations. Phylogenetic analysis revealed the presence of *Wolbachia* strains belonging either to supergroup B or supergroup A. *Spiroplasma* strains belonged to the citri–chrysopicola–mirum and ixodetis groups and the remaining Entomoplasmatales to the Mycoides–Entomoplasmataceae clade. *Cardinium* strains clustered with strains infecting *Encarsia pergandiella* in group A. Sequence analysis revealed deletions of variable length and nucleotide variation in three *Wolbachia* genes which were either new or similar to those of previously identified pseudogenes that were integrated in the host genome.

Keywords: tephritid, endosymbiotic bacteria, reproductive parasite, 16S Sequence Typing, *wsp*, Horizontal Gene Transfer. *rRNA*, Multi Locus

P48.

Comparative evaluation of the mushroom production performance of *Pleurotus ostreatus* and *P. eryngii* strains cultivated on by-products deriving from olive oil and wine industries

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The treatment and disposal of residues/wastes produced by wine and olive oil industries is a complex and often problematic process. For assessing the exploitation potential of these by-products, twenty strains of *Pleurotus ostreatus* and *P. eryngii* were cultivated in substrates consisting of grape marc and wheat straw (GM; 1:1 w/w), and olive leaves and two-phase olive mill waste (OL; 1:1 w/w). The GM-based substrate led to a reduction in the time required for mushroom appearance by 1 to 4 days compared to the conventional substrate (WS: wheat straw) for five out of ten *P. ostreatus* strains examined. Biological efficiency (fresh weight of mushrooms to dry weight of substrate) was higher in WS (55–97%) compared to GM and OL (up to 62%) for all strains tested. Furthermore, the total cultivation period (from inoculation to the end of second flush) lasted from 37 to 63 days in WS, whereas it was 41–71 days in GM and 61–80 days in OL. For the majority of *P. eryngii* strains studied, mushroom yields were significantly higher in the OL and GM substrates (when compared to WS) by 10 to 186% and by 4 to 86%, respectively. Substrates containing grape marc led to a reduction in total cultivation period by up to 22 days in respect to WS. In contrast, OL-based media prolonged the cultivation cycle of *P. eryngii*. Hence, by-products from wine and olive oil industries could be exploited as substrates for the production of *Pleurotus* mushrooms. Optimization of cultivation parameters (including assessment of the suitable ratios of the materials used and their effect on mushroom quality) is currently under way to generate new substrates for large-scale implementation of such bioprocesses.

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P49.

Effect of Non-Nutritive Sweeteners in Growth Kinetics of Gram (-) and Gram (+) Bacteria.

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Artificial or Non-Nutritive Sweeteners (NNS) are getting more attractive among consumers with a preference for sweet foods and soft drinks. Such products are also consumed by those with dietary preferences or metabolic disorders as a substitute to sugar. However, there are scientific reports claiming that the overuse of artificial sweeteners have a major impact on bacterial growth thus altering the gut microbiota and contributes to metabolic dysfunction. In our study, four commercially available products containing sodium cyclamate, sucralose, erythritol and steviol glycosides or mixtures of the above, were tested for their effects in growth kinetics of two microbial strains of *Escherichia coli* (E. coli 11 & E. coli 24) and two *Staphylococcus aureus* strains (S. aureus 15 & S. aureus 20) of clinical origin.

Bacteria were incubated in densities of 0,5 McFarland Units in 96 well micro-plates with Mueller-Hinton broth and with the presence of increasing concentrations (0.06 – 3.66 mg/mL) of the above commercially available NNS. During incubation the absorbance at 620nm of each well was recorded every 10 minutes and for up to 8 hours. The growth inhibition percentage and the IC50 were then calculated for all experiments and repetitions. Based on our results, a 8.4% (±4.2%) to 59.3% (±1.8%) of growth inhibition was recorded among the various bacterial strains depending on the concentration of NNS, followed by an increase up to 50% of the Laq phase in comparison to the controls. Products with mixtures of sucralose, erythritole and steviol glycosides presented an average IC50 when compared to sucralose which exhibited the highest IC50 values and sodium cyclamate the lowest. These observations are indications of a negative impact of NNS on microbial growth.

P50.

Functional complementation studies with *Escherichia coli* cytoplasmic prolyl isomerases point towards redundant and non-redundant roles during swimming and biofilm formation

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Prolyl isomerases assist in the folding of nascent proteins and induce conformational changes in folded proteins as they accelerate the *cis*/*trans* isomerization of the peptide bond preceding proline. There are three major families of prolyl isomerases, i.e. the cyclophilins, the FK506 binding proteins (FKBPs), and the parvulins, which are characterized by structurally distinct catalytic domains. Previously we have shown that the cytoplasmic cyclophilin of *E. coli* acts as a negative modulator of motility and biofilm formation ability. Here we show that FklB and FkpB, members of the *E. coli* FKBP family, also act as negative modulators of swimming and biofilm formation ability, and we further study the possible functional redundancy among different family members of the cytoplasmic prolyl isomerases. The phenotypic characterization of the Δ*fklB* and Δ*fkpB* strains showed that the deletion of either *fklB* or *fkpB* resulted in increased swimming motility and biofilm formation ability. The two enzymes modulate the swimming motility independently of their prolyl isomerase activity as the overexpression of an FklB or an FkpB enzyme variant characterized by reduced prolyl isomerase activity in the respective deletion strain restored the swimming motility phenotype to the control levels. However, the FkpB variant showing reduced prolyl isomerase activity could not restore the biofilm formation phenotype of the Δ*fkpB* strain to the control levels indicating that the prolyl isomerase activity of FkpB is necessary during certain growth conditions. The other cytoplasmic prolyl isomerases of *E. coli* appear to have redundant and non-redundant roles during swimming as the overexpression of each one of them restored the swimming motility of the Δ*fklB* strain to the control levels but the overexpression of none of them could restore the swimming motility of the Δ*fkpB* strain to the control levels. Furthermore, the overexpression of certain prolyl isomerases could not complement either of the two strains during biofilm growth suggesting that the mechanisms of action of these enzymes do not fully overlap.

P51.

Evaluation of the biological activity of the edible mushrooms *Pleurotus ostreatus* and *Ganoderma lucidum* from Greek strains with enhanced β-glucan content

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Basidiomycetes are known worldwide for their health-promoting properties. β-Glucans, a group of β-D-glucose polysaccharides naturally occurring in fungal cell walls, are considered responsible for their potential prebiotic, immuno-modulating and anti-tumour effects. It is, thus, of great importance to use indigenous fungal genetic resources in order to isolate bioactive compounds for the development of nutraceuticals. We examined the biological activity of cultivated *Pleurotus ostreatus* and *Ganoderma lucidum* mushrooms originating from Greek strains. Lyophilized mushrooms and their β-glucan-enriched extracts were examined, using the MTT method, for their ability to affect cell proliferation of human epithelial colorectal adenocarcinoma cells (Caco-2 and HT-29), peripheral blood monocytes (U937) and human peripheral blood lymphocytes (PBLs) of healthy donors. Their cytotoxic effect and the cell death mechanism were investigated via Trypan blue exclusion assay, flow cytometry (FACS) and Western blot analysis. Furthermore, pre- and anti-inflammatory cytokine production was estimated in PBLs to examine their potential immune-modulatory effect. According to our results, *P. ostreatus* inhibits cell proliferation more effectively than *G. lucidum* in Caco-2 and HT-29 cells in a dose-dependent manner, whereas only the highest concentration (200μg/ml) of the tested lyophilized samples exhibits cytotoxic activity in U937 cells. FACS and Western Blot analysis revealed that the cytotoxic effect of both basidiomycetes in Caco-2 and U937 cell lines is due to necrosis. Additionally, both lyophilized samples and matched β-glucan enriched extracts induce anti-inflammatory IL-10 levels in a dose-dependent manner. Our initial findings support the anticancer and immuno-modulating potential of *P. ostreatus* and *G. lucidum.* Ongoing investigation is expected to establish their prebiotic activity and unravel the mechanisms underlying their biological activities.

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P52.

Development of a FTIR-based chemometric model for the prediction of *Cyclocybe cylindracea* mushrooms performance on lignocellulosic substrates

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Valorization of agricultural and agro-industrial by-products remains a very challenging issue. In Greece, by-products deriving from olive-oil production (i.e. olive pruning residues and two-phase olive-mill waste) and wineries (e.g. grape-marc) are traditionally treated as waste, and they are either disposed into the surrounding environment or burned, thus creating significant pollution problems. Mushroom fungi are particularly efficient at biodegrading a wide range of lignocellulosics to produce highly nutritional value-added products. However, the suitability of alternative substrates (i.e. other than those based on wheat-straw or sawdust which are commonly used in commercial cultivation) and the way they could affect mushroom production has to be investigated through long-lasting experimentation. This study attempts to develop for the first time a fast and inexpensive method for the estimation of *Cyclocybe cylindracea* mushroom production on the basis of the biological efficiency values exhibited by the fungus when cultivated on twenty substrates composed of various plant residues and agro-industrial wastes. For this purpose, Fourier transform infrared spectroscopy (FTIR) was used, while multivariate analysis was applied on spectroscopic data to calibrate a model predicting biological efficiency. Important compositional changes taking place during the cultivation process were monitored, while high prediction scores were obtained (i.e. R2CAL: 0.95, R2CV: 0.70, RMSECV: 24%). Interpretation of regression coefficients revealed a positive correlation of biological efficiency to substrates' content in lignocellulosic compounds, and a negative correlation to proteins and phenolics. In conclusion, results indicated that this FTIR-based approach could be potentially exploited as a tool for the evaluation of cultivation substrates' suitability prior to their use. The model's predictive power and accuracy can be constantly enhanced by incorporating additional data. Furthermore, FTIR can provide valuable information concerning the structural modifications of major substrate components which take place during the mushroom cultivation process.

P53.

Cultivated mushrooms with enhanced *β*-glucan content as obtained through the valorization of agricultural and agro-industrial by-products

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Beta (*β*)-glucans are linear D-glucose polymers linked with β-glycosidic bonds, and differ with respect to their length/molecular mass, viscosity, solubility and branching structure. They occur in the cell-wall of bacteria, fungi and cereals, and they are considered as biological response modulators with a plethora of health promoting functions (e.g. immunomodulatory, anticancer and prebiotic properties). Mushrooms are relatively rich in *β*-glucans and are therefore considered as excellent sources of these compounds for humans. However, since very little is known regarding edible mushrooms and the effect of production substrates on their *β*-glucan content, 32 wild/indigenous strains belonging to seven species of basidiomycetes (i.e. *Pleurotus ostreatus*, *P. eryngii*, *P. nebrodensis*, *P. citrinopileatus*, *Ganoderma lucidum*, *Hericium erinaceus* and *Cyclocybe cylindracea*) were cultivated on conventional (wheat straw or beech sawdust) and alternative (two-phase olive mill wastes, olive tree prunings and grape marc) media. Results revealed a high intraspecific variability of *β*-glucan content ranging from 10.9 to 30.6% (d.w.) for *P. ostreatus* and from 26.3 to 37.2% for *C. cylindracea*. As regards mushrooms produced in conventional substrates, *P. eryngii* strain LGAM216 revealed the highest content in *β*-glucans (38.7%), followed by *C. cylindracea* (37.2%) and *P. ostreatus* (30.6%) strains. Finally, a significant effect was observed in mushrooms *β*-glucan content when alternative substrates were used. Indicatively, *P. eryngii* LGAM216 revealed an increase of 10% (in respect to the value previously quoted) when a substrate based on grape marc was used, as opposed to lower *β*-glucan content detected in substrates containing olive-mill waste or olive prunings. In contrast, *P. ostreatus* exhibited higher *β*-glucan content in mushrooms produced on substrates consisting of olive by-products. In general, the nature of cultivation substrate exercised a notable effect on *α- and β*-glucans content, which depended on the mushroom species/strain used.

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P54.

In vitro fermentation of *Pleurotus ostreatus* and *Ganoderma lucidum* by human gut microbiota: metabolomic analysis of the products

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Edible basidiomycetes are known for their health-promoting properties. Growing evidence supports that their immune-modulating and anti-cancer effects are mediated by their prebiotic capacity. β-Glucans, a group of β-D-glucose polysaccharides abundant in the fungal cell walls, are considered responsible for their potential prebiotic effects. The use of indigenous fungal genetic resources to develop nutraceuticals is, thus, of great importance. In the present study, the prebiotic activity of *Pleurotus ostreatus* and *Ganoderma lucidum* cultivated mushrooms deriving from Greek strains with high β-glucan content is being investigated. Hence, the whole fungus as well as β-glucan enriched extracts were tested for their ability to alter the composition of the intestinal microbe following their *in vitro* fermentation by faecal slurry of healthy volunteers. Lyophilized fungal substrates and inulin, an established prebiotic, at appropriate concentrations, were *in vitro* fermented for 24 hours. *In vitro* fermentation without any carbon source was in parallel carried out to be used as reference. The global metabolic profiling of fermented products was assessed by the use of 1H NMR spectroscopy, and metabolites resonances were assigned guided by Chenomx NMR Suite and literature data. Preliminary results revealed variations in the profile of the products as a result of the *in vitro* fermentation of *P.ostreatus* and *G.lucidum* derived substrates. A comparative survey between the above substrates, using chemometrics in combination with 2D NMR spectroscopy will be further applied, in order to identify biomarkers associated with the health promoting effects and the biological activities of *P. ostreatus* and *G. lucidum*.

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P55.

New records of ectomycorrhizal basidiomycetes associated with *Alnus glutinosa* (priority habitat 91E0) from Andros island, Greece

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Alluvial forests/woods (priority habitat 91E0*, Annex I Directive 92/43/EEC) consist of vegetation dominated by alder trees (genus *Alnus*) and occur along river banks and watercourses. Several *Alnus glutinosa* stands exist in streams and rivulets of permanent flow in Andros island (Central Aegean) and comprise the southernmost distributional limit of this habitat type in the Balkan Peninsula. Alders are involved in a tripartite symbiosis with ectomycorrhizal (ECM) fungi and nitrogen-fixing actinobacteria of the genus *Frankia*. As regards the ECM basidiomycetes, it is known that only few species form highly specialized symbiotic relationships with alder and play a crucial role for its growth and long-term survival; however, this type of fungal diversity remains largely unexplored in Greece. In the frame of an ongoing project (LIFE16 NAT/GR/000606), an extensive sampling of ECM fungi was performed aiming at the study and restoration of alluvial alder-stands of Andros. To date, over 40 pertinent samples were subjected to morphoanatomical examination and/or DNA sequencing, and have been identified to genus level as follows: *Gyrodon*, *Inocybe, Naucoria* (syn. *Alnicola*), *Lactarius*, *Paxillus*, *Russula* and *Tomentella*. Among them, seven species constitute new national records, i.e. the recently described *Paxillus olivellus*, *Naucoria celluloderma* (syn. *Alnicola inculta* sensu Moreau), *N. escharoides* and *N. subconspersa*, two alder-specific *Lactarius* spp. (namely *L. obscuratus* and *L. omphaliiformis*), and *Russula alnetorun* (syn. *R. pumila* sens. auct.). Further research is in progress to reveal the hidden diversity of alder-associated mushroom-producing fungi.

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P56.

Elimination of microbial pathogens and antibiotic resistance genes from wastewater using constructed wetland systems.

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Pathogenic microorganisms contained in wastewater entail a risk to public health, as they are considered virulent carriers of waterborne diseases. Their presence in high concentrations, resistant nature and rapid transmission illustrate the importance of their inactivation by means of effective sewage treatment. Researchers also focus on the antibiotic resistant bacteria (ARB) and their respective antibiotic resistance genes (ARGs), which may occur in high numbers in wastewater. Whether resistance may develop during wastewater treatment is currently under discussion. Links are not yet well established between the presence of antibiotics in wastewater and the favoring of resistant bacteria as well as the transfer of resistance. Constructed wetlands (CW) have been investigated as alternative low-cost systems for wastewater treatment, which could also meet the overall socio-economical and environmental requirements of small communities due to their low operational and maintenance cost. In this perspective, two systems of pilot-scale CWs (S1 & S2) were developed as tertiary wastewater treatment systems with the view a) to evaluate their potential to remove fecal bacterial indicators (*E. coli* and Enterococci), bacteriophages and enteric viruses, namely enteroviruses (EVs) and adenoviruses (AdVs), b) to assess the elimination of ARB, c) to study possible changes in bacterial antibiotic resistance profile through treatment and d) to detect target ARGs prior to and post treatment.
Results showed that these low cost systems are capable of eliminating effectively the bacterial indictors achieving removal rates over 99% for both systems. Regarding viruses, AdVs, EVs and phages were detected at all sampling points and during all seasons, and they were only partly removed in S1 and/or S2. For example, the virus load was decreased by 4 Log units for AdVs and 3 Log units for EVs in the case of S1, while the respective values were 7.5 Log units and 4 Log units for S2. Fluctuations in the antibiotic resistance profile of bacteria and in the removal levels of ARGs were also observed, depending on the employed operational conditions, as treatment processes affected the microbial response up to a certain extent. The high concentration of certain ARGs post treatment highlights the complicated behavior of CWs on their elimination. Current results showed that the macrophytes could enhance the proliferation of certain ARGs in *E. coli* and Enterococci. Although CWs may demonstrate high bacterial removal rates, effluents have to be tested assiduously before their release into water courses, as transfer of antibiotic resistance may occur.

Keywords: pathogens; wetland; antibiotic resistant bacteria; resistance genes

P57.
The effect of copper on ammonia oxidising archaea

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Ammonia oxidizing archaea (AOA) are involved in the first step of nitrification, oxidation of ammonia to nitrite. This step is catalysed by ammonia monooxygenase, enzyme which is believed to be copper-dependent. In addition, AOA are thought to have a copper-dependent respiratory chain and their genomes encode for a large number of putative copper-containing proteins. Although it has been repeatedly speculated that copper plays an important role in AOA, amazingly little is known about copper requirements in ammonia oxidisers or about mechanisms AOA use to deal with copper deficiency and toxicity. There is also lack of evidence on the role of copper in the metabolism of AOA. We tested a growth of *Nitrosocosmicus franklandus* and *Nitrosotalea sinensis* in media with various copper concentrations and the copper chelator TETA. In optimal growth conditions (500 nM Cu^{2+}, 1uM TETA), the specific growth rate of *N. sinensis* reached 0.8 d-1. 50 nM Cu^{2+} was growth limiting and 5 μM partially inhibited the growth of this organism. *N. franklandus* had a broader range of tolerated copper concentration. This strain was only limited in the presence of 10 μM TETA, and toxic concentration of copper was 50 μM. However, we observed physiological changes of *N. franklandus* growing along a gradient of copper concentration. In media with partially inhibitory levels of copper, the cells were smaller and despite acidifying nitrite production, pH of the media increased slightly. Cultures grown in the optimal copper concentration (500 nM) formed large biofilms, visible to the naked eye. These attributes suggest various strategies of niche differentiation available for *N. franklandus*. We are currently analysing the proteins expressed in cells grown in media with limiting, optimal and toxic concentrations of copper to elucidate the mechanisms underpinning the observed physiological changes and to understand how AOA deal with variable copper concentration in the environment. Series of activity assays are also being conducted in order to define which steps in nitrification are copper dependent.

Keywords: Ammonia oxidising archaea, copper, ammonia monooxygenase, biofilm formation

P58.

Diversity of ectomycorrhizal fungi from littoral sand dunes habitats with thermophilous pine forests in Greece

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The Mediterranean region is considered as a biodiversity hot-spot with increased risk of degradation due to climate change. Littoral sand dunes are among the most threatened habitats because of their marginal characteristics and the extreme conditions prevailing there. Ectomycorrhizal (ECM) lifestyle is one of the most important factors influencing and supporting growth/survival of plants in sand dunes, including Mediterranean thermophilous pines. The present work forms a part of an ongoing study on the diversity of ECM fungi in selected littoral sand dune habitats of Greece, e.g. the Schinias-Marathon and the Strofilia National Parks which are dominated by *Pinus pinea* and *P. halepensis*. Pine root tips were sampled and their morphoanatomic study revealed the existence of 96 ECM morphotypes; the identity of 45 of them was further assessed through DNA sequencing (ITS rDNA). Nine were identified to species level, i.e. *Chroogomphus confusus*, *Geopora clausa*, *Inocybe arenaria*, *I. pseudodestricta*, *I. rufuloides*, *Rhizopogon roseolus*, *Sebacina epigea*, *Suillus collinitus* and *S. mediterranensis*. Other 17 specimens were associated to six different genera (their exact identity pending verification) as follows: *Amphinema*, *Helvellosebacina*, *Inocybe*, *Sebacina* (two species), *Thelephora* (four species) and *Tomentella* (eight species). Among them, of particular interest are the ectomycorrhizae formed by *I.arenaria* and *S. mediterranensis* in association with pines which are hereby described for the first time. Of special importance are also the ectomycorrhizae assigned to the “suilloid cluster” (genera *Suillus* and *Rhizopogon*), known as exclusive pine symbionts and pioneers in ecological succession taking place in marginal habitats.

P59.

Preliminary data on the underground diversity of alder (*Alnus glutinosa*) associated fungi

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Alluvial forests with alder trees (*Alnus glutinosa*) constitute a habitat of particular interest (priority habitat 91E0*, Annex I Directive 92/43/EEC). The alder stands of Andros island (central Aegean) occur at their southernmost limit of distribution in the Balkan Peninsula. In the frame of an ongoing project (LIFE16 NAT/GR/000606), an extensive sampling of alder associated (i.e. ectomycorrhizae, endophytic and rhizosphere) fungi was performed aiming at their study and/or potential exploitation as inocula to enhance growth of alder seedlings in restoration of *A. glutinosa* stands of Andros. Several field-trips were performed in selected sites of the island during 2017-2018. Sampling took place in four mountainous (>500 m a.s.l.; Zenio, Evrousies, Vourkoti, Katakaleoi) and three coastal or low-altitude (0-250 m a.s.l.; Achla, Vori and Lefka) alder stands. A. glutinosa roots and rhizosphere soil samples were processed and their associated fungi were isolated in pure cultures by using various methods and were finally identified by morphological and molecular (DNA sequencing) approaches. So far, the identity of 25 isolates was determined; 12 of them were found to belong to the following species: *Alternaria alternata*, *Apiognomonia lasiopetali*, *Chaetomium murorum*, *Fusarium solani*, *Ilyonectria radicicola*, *Lambertella tubulosa*, *Metacordyceps chlamydosporia*, *Neurospora reticulata*, *Penicillium chrysogenum*, *Phialocephala fortinii*, *Pleurotus ostreatus* and *Talaromyce sruber*. In addition, 13 isolates were identified to genus level, i.e. *Botrytis*, *Fusarium*, *Knufia*, *Penicillium*, *Phomopsis*, *Trichoderma* and *Umbellopsis*. The majority of the materials identified are soil-borne ascomycetes which generally possess an opportunistic ecology, maneuvering between different trophic habits depending on environmental conditions.

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P60.

Taxonomic studies on the genus *Entoloma* (Basidiomycota) in Greece involving morphological and molecular approaches

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Entoloma is one of the most species-rich genera of the order Agaricales (Fungi, Basidiomycota). In many cases, delimiting intraspecific from interspecific morphological variation and accurate determination of taxa within *Entoloma* are particularly challenging. Although pertinent research in the western Mediterranean has led to the description of several species new to science, this genus remains poorly surveyed and inadequately studied in the eastern part of the Basin. This work presents the early results of a multi-disciplined approach to investigate diversity of the genus *Entoloma* in Greece. Sampling was carried out in the regions of Western Macedonia, Attica, Peloponnese and South Aegean islands, with emphasis on typical Mediterranean habitats, e.g. thermophilous pine forests, and littoral and/or insular arid scrublands dominated by *Cistus* spp. communities. Species identification was achieved by combining morphological examination of both fresh material and exsiccatae, ecological features, DNA sequencing and phylogenetic analysis. *Entoloma anthracinum*, *E. graphitipes*, *E. cistophilum*, *E. llimonae* and *E. philocistus* are reported for the first time in Greece; the former two are rather widely distributed in Europe, while the other three are confined in the Mediterranean and are known to be associated with *Cistus* spp. In addition, one collection from Paros island is of particular interest since it is phylogenetically distinct and represents an undescribed species. Moreover, the outcome of this study confirmed that *Entoloma cettoi* is a synonym of *E. neglectum* and assessed the presence of the rare for Greece *E. nitens*.

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P61.

Detection and characterization of *Spiroplasma* and *Wolbachia* in *Glossina tachinoides*

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Blood feeding tsetse flies (Diptera: Glossinidae) are among the most important insects in sub-Saharan Africa since they are vectors of the African trypanosomiasis, caused by hemoflagellate trypanosomes that kill humans and domestic mammals. Sustainable management of their populations can be achieved through the application of environment-friendly techniques for vector control such as the sterile or incompatible insect techniques(SIT and IIT). Both SIT and IIT are considered increasingly important components of area wide integrated pest management programs for certain key insect pest species. Reproductive parasites like *Spiroplasma* and *Wolbachia*, endosymbiotic bacteria capable of inducing reproductive alterations to their insect-hosts, are used as central components of such techniques. *Spiroplasma* is a genus of wall-less bacteria belonging to the class Mollicutes, associated with a variety of plants and arthropods. *Spiroplasma* strains are grouped into three major clades based on the 16S rRNA gene as well as multi locus sequence typing (MLST) approaches. They usually exhibit a dual life, with a capacity to live intracellularly in a variety of tissues or extracellularly, in the hemolymph. They have developed a wide range of symbiotic associations, producing diverse effects on insect evolution, ecology, reproduction and sex determination with the induction of male-killing.*Wolbachia* is a widespread group of obligatory intracellular maternally transmitted bacteria belonging to Alphaproteobacteria. *Wolbachia* mainly reside in the reproductive tissues of arthropods and cause a number of reproductive alterations including cytoplasmic incompatibility (CI), thelytokous parthenogenesis, feminization of genetic males, and male-killing. In this study, we investigated the presence of *Spiroplasma* and *Wolbachia* in a wild population of *Glossina tachinoides* from Burkina Faso with a 16S rRNA gene PCR approach. As a result, we found that approximately half of our population was infected with *Spiroplasma*, whereas *Wolbachia* was totally absent from our samples. Moreover, molecular genotyping was carried out through the MLST characterization of the *Spiroplasma* strain found in *G. tachinoides*. These findings provide useful information for enhancing the application of the previously mentioned techniques for controlling this devastating insect species.

Keywords: *Glossina tachinoides*, *Spiroplasma*, *Wolbachia*, Sterile Insect Technique (SIT)

P62.

Volatile aroma profile of *Pleurotus ostreatus* and *P. eryngii* mushrooms cultivated in substrates based on grape marc or olive mill wastes. Preliminary results.

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Wine and olive oil production are amongst the principal agricultural activities in the Mediterranean region, generating big volumes of wastes and by-products. Within the concept of recycling and sustainability, several attempts have been made to exploit these agroindustrial wastes for the production of value-added products, like mushrooms. Mushrooms' consumption and production is constantly increasing because of their health benefits and organoleptic characteristics. Volatile aroma compounds are determinants for the flavour of mushrooms. *Pleurotus* species are widely commercialized worldwide. Hence, the present work aimed to study the effect of alternative cultivation substrates on the volatile compounds of *Pleurotus eryngii* and *Pleurotus ostreatus* strains grown on wheat straw (as control), grape marc, olive leaves and two-phase olive mill wastes, in mixture. Harvested mushrooms were kept frozen until analysis. Volatile aroma compounds were isolated by headspace solid phase microextraction (HS-SPME) and identified by gas chromatography - mass spectrometry (GC - MS), after samples' equilibration in thermostated saturated NaCl solution. More than 60 volatile compounds were identified by means of mass spectra libraries and were semi-quantified using 4-methyl-1-pentanol as internal standard. Amongst the strains of *Pleurotus* mushrooms studied, 1-octen-3-ol (the so-called "mushroom alcohol") predominated comprising the 33-52 and 61-82% of volatiles in *P. ostreatus* and *P. eryngii*, respectively. Other C-8 alcohols, aldehydes and ketones were also determined in all samples. The concentrations of C-8 alcohols ranged from 430-1470 μg/g DW in *P. eryngii* and 520-1450μg/g DW in *P. ostreatus*, being higher in mushrooms grown on grape marc or olive mill wastes compared to those cultivated on wheat straw. The concentrations of C-8 ketones and C-8 aldehydes were 3-40 times and 5-130 times lower than C-8 alcohols, respectively. Additional analyses and data evaluation will be carried out.

KEYWORDS: *Pleurotus*, Volatile aroma profile, HS-SPME-GC-MS

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P63.

Effect of culture conditions on microalgae grown on pure and biodiesel-derived crude glycerol

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Microalgae have received increased attention in recent decades due to their ability to produce a wide range of high value compounds (pigments, antioxidants, proteins, polysaccharides, lipids, fatty acids, vitamins), used as bulk commodities in different sectors such as cosmetics, agriculture, pharmaceutical, health sector. Crude glycerol, an inexpensive substrate readily available in significant quantities from the onsite production of biodiesel, can be used as substrate for microalgae growth under heterotrophic or mixotrophic conditions. The aim of the present work is to examine the effect of various culture conditions on the growth of *Chlorella vulgaris*, *Scenedesmus obliquus* and *Scenedesmus almeriensis* on glycerol as sole organic substrate. To achieve this goal microalgae were grown in Bold's Basal Medium (BBM) supplemented with 5 g/l pure or crude glycerol, with either sodium nitrate or yeast extract as nitrogen sources at a C/N ratio of 7/1. All experiments were performed at a constant agitation rate of 150 rpm and the initial pH and temperature were 7.2 and 27oC, respectively. Mixotrophic cultures were grown under 16h light (3000 Lux intensity) and 8h dark regime, while heterotrophic growth was carried out under darkness. Higher values of specific growth rate were observed when *C. vulgaris* was cultured heterotrophically, rather than grown under mixotrophic conditions. In both cases yeast extract's presence yielded to higher specific growth rates. Biomass production was also promoted when yeast extract was used, especially under mixotrophic conditions. pH values slightly increased when $NaNO_3$ was added, while in the presence of yeast extract became acidic. Maximum specific growth rates for *S. obliquuus* and *S. almeriensis* were obtained when the microalgae were grown heterotrophically, especially in the cultures with yeast extract. Higher biomass production was also recorded when yeast extract was used, with no significant differences between mixotrophic and heterotrophic cultivations. pH values ranged around 7.0, excluding the mixotrophic cultures grown on NaNO3, where pH turned to alkaline. Overall, *S. obliquus* grown on glycerol yielded the highest biomass concentrations among the three algae. In all cultures the use of yeast extract as nitrogen source, resulted in higher biomass production under both mixotrophic and heterotrophic conditions.

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P64.

Isolation and characterization of endophytic fungi from Greek olive tree cultivars

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Fungal endophytes are specified as microorganisms which reside in the internal tissues of living plants without causing any disease. Plant growth promoting fungi (PGPF) are non-pathogenic fungi with beneficial effects on plants. PGPF are being increasingly studied for their ability to promote plant health by various benefits, protecting their hosts from plant diseases. In the present study, 83 endophytic fungi from leaves and root of asymptomatic Greek olive tree cultivars (Kalamata, Koroneiki and Amfissa) isolated and categorized in 36 morphotypes. One of each group was selected and identified using the ITS rDNA molecular marker. Moreover, isolates were tested *in vitro* for their antagonistic activity against important phytopathogens (*Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *radicis-lycopersici, Verticillium dahliae* and *Colletotrichum acutatum*). In addition, fungal strains were further evaluated for their plant growth promoting traits such as indole-3-acetic acid, siderophore production, phosphate solubilization and extracellular enzyme production (protease, urease and cellulase). Finally, fungal isolates screened *in vitro* for growth promoting activity on model plant *Arabidopsis thaliana* ecotype Col–0. Thus, four of them showed remarkable beneficial effect on plant growth, individuating them as potential antagonistic agents against severe phytopathogens.

Keywords: Endophytic fungi, PGPF, Greek olive tree cultivars, antagonistic agent.

P65.

Identification of endophytic fungi from medicinal plants and their potential applications

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Fungal endophytes associated with plants have a potential role to promote plant growth through different mechanisms. They protect their host from biotic and abiotic stresses as they enable nutrient uptake, which is important for plant growth. However, the biological and ecological roles of fungal endophytes are still totally unexplored. In this study, 56 endophytic fungi were isolated from plant tissues of medicinal plants *Salvia fruticosa* and *Nigella sativa* and classified into morphotypes according to their colony morphology and mycelium forms. A total of 22 representative fungal isolates were selected and further characterized identified by using ITS rDNA amplification and sequencing. These isolates were also tested for their antagonistic activity against severe phytopathogenic fungi as *Rhizoctonia solani, Fusarium oxysporum* f. sp. *radicis-lycopersici, Verticillium dahliae and Colletotrichum acutatum* through dual culture method. In addition, these strains were evaluated for plant growth promoting features such as IAA production, siderophores and phosphate solubilization. Furthermore, the strains were tested for their ability to produce extracellular enzymes, especially urease, cellulase and protease activities. Moreover, these fungal isolates were evaluated *in vitro* for their growth promoting ability in *Arabidopsis thaliana* (Col-0). Finally, four distinguished fungal isolates showed equally remarkable antagonistic activity and enhanced plant growth of *Arabidopsis thaliana* seedlings.

Keywords: Endophytic fungi, antagonism, plant growth promoting, medicinal plants.

P66.
Virome characterization in aphids and association with the bacterial profile

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Aphids constitute a major threat in phytopathology, since they transmit a wide range of phytopathogenic viruses causing incalculable damage in crops worldwide. Any effort for the effective control of aphid-transmitted viruses in plant production, prerequisite a fast, easy, low-cost, powerful molecular tool that it will be able to detect the presence of parasitic viruses. Previous studies focused on the detection of plant viruses indirectly by screening the plant tissues of host. Moreover, the flexible genome of the viruses renders more difficult to design suitable – universal primers which being capable for the effective detection of each group of plant viruses. In this study we suggest a combination of three multiplex PCRs using twenty-four primers, aiming to characterize the aphid virome in forty-seven aphid populations (three samples per population). We targeted nine groups of plant DNA-viruses with aphids being their main vector (*Soymovirus, Rosavirus, Nanovirus, Cavemovirus, Caulimovirus, Capulavirus, Badnavirus, Babuvirus* and *Tungrovirus*). Our data indicate that *Rosavirus, Nanovirus* and *Caulimovirus* were the most dominant in all analyzed samples, while *Capulavirus, Babuvirus* and *Tungrovirus* were detected in fewer samples. Additionally, the correlation between the bacterial and viral profile was examined, in order to decipher any positive or negative relationship that could potentially contribute in an integrated pest management.

Keywords: DNA-plant virus, detection, multiplex PCR, integrated pest management

P67.
Symbiont profile and density in natural aphid populations

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Microbiota play an important role in the biology, ecology and evolution of insects including aphids. The bacterial communities in aphids vary among species and seem to play a vital role in vectorial capacity. Aphids have established complex symbiotic relationships. First of all, *Buchnera aphidicola* is the primary symbiont found in almost all aphid species providing them with essential amino acids lacking from their phloem diet. Occasionally, aphids harbour secondary symbionts that can have positive effects on the aphid host such as *Hamiltonella defensa* and *Regiella insecticola* which can protect aphids against parasitoids, as well as *Serratia symbiotica* which is implicated in heat tolerance. Aphid microbiota can be exploited for the development of innovative tools and strategies for vector and disease control. So far, a limited number of studies have aimed to characterize the aphid symbiont density variations in natural populations. In this study we estimated the bacterial density of *Buchnera, Serratia, Arsenophonus* and *Rickettsia* in forty-seven natural aphid populations from Greece using qPCR. Bacterial density varied depending on the aphid and plant host species. Specifically, the higher *Rickettsia* density was estimated in *Aphis cytisorum*, the higher *Serratia* density in *Aphis hederae*, the higher *Arsenophonus* density in *Aphis craccivora*, and the higher *Buchnera* density in *Aphis fabae*. Our data indicate exclusion phenomena between the four bacterial taxa examined, with the exception of *Buchnera* and *Rickettsia* which a positive interaction was characterized.

Keywords: aphids, qPCR, bacterial density

P68.

Plant growth promoting effects of endophytic bacteria from *Teucrium polium* and *Hypericum hircinum* on *Solanum lycopersicum*

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Sustainable agriculture needs effective and environmentally-friendly solutions, such as the use of Plant Growth Promoting Microorganisms (PGPMs) or Plant Probiotics. They can act directly through growth enhancement or indirectly through biocontrol activity. Among the PGPMs, Plant Growth Promoting Endophytes (PGPEs) hold an important position. Endophytes, in general, are defined as microorganisms that colonize internal living plant tissues, part or all their life cycle, without causing disease. Medicinal plants seem to harbor endophytes with special characteristics. For that reason, we have isolated endophytic bacteria from surface sterilized leaves and roots of asymptomatic medicinal plants *Teucrium polium* and *Hypericum hircinum*. Bacteria that exhibited plant growth promoting characteristics at biochemical assays and the model plant *Arabidopsis thaliana* were selected for further study in the economically important plant *Solanum lycopersicum*. In this study, we investigated the ability of the bacteria to induce early germination with the method of seed biopriming. Furthermore, we examined their capability as *in vitro* biological control agents of the phytopathogenic fungus *Botrytis cinerea*.

P69.

Effect of endophytic bacteria from *Calendula officinalis* in plant growth of tomato (*Solanum lycopersicum*)

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Plant growth promoting bacteria (PGPB) can be found in the rhizosphere, on root surfaces and inside plant tissues (Plant Growth Promoting Endophytic Bacteria, PGPEB) applying either direct or indirect mechanisms which aim to suppress phytopathogenic microorganisms, increase plant resistance and eventually enhance plant growth. Endophytic bacteria are beneficial microorganisms that colonize the internal tissues of their host plants without causing any disease. In our studies we isolated and identified endophytic bacteria from roots, leaves and flowers of the pharmaceutical plant *Calendula officinalis*. Bacterial strains were studied *in vitro* for their ability to inhibit growth of the phytopathogenic fungus *Botrytis cinerea*, analyzing possible mechanisms of their action with production of several compounds like antibiotic compounds and enzymes. Furthermore we inoculated selected bacteria strains on tomato seeds (*Solanum lycopersicum*) evaluating their colonization ability and effect in early seed germination *in vitro*.

P70.

Metagenomic analysis reveals specific ciclosporin-related effects on human gut microbiota after bone marrow transplantation.

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Considering the close cross talk between gut microbiota and the immune system, an emerging role has been increasingly attributed to microbiota after bone marrow transplantation. Growing evidence suggests that host-microbiota interactions have an impact on the development of the main complications following allogeneic stem cell transplant, as Graft versus host disease and infections. In the present study, we aimed to investigate the relationship between the gut microbiota and the immunosuppressive ciclosporin therapy in recently bone marrow (BM) transplanted patients. Serial fecal specimens were collected from recent bone marrow transplanted patients treated with a ciclosporin based therapy (n=15) and from healthy drug-free controls (n=18). High-throughput sequencing of the V3-V4 hypervariable region of bacterial 16S rRNA gene was performed using Illumina MiSeq platform to identify putative impact of immunosuppressive treatment on gut microbiota composition and abundance. Amplicon sequence analysis was mostly performed using the QIIME2 pipeline, R and bioperl scripts. Our systematic characterization of the gut microbial composition showed major differences of taxa distribution at phylum level. Faecal *Bacteroidetes* abundance was significantly higher in control group (percent abundance mean±SD: 58.07±8.28) than in BM group (30.24±18.94) (p-value <0.01). Faecal *Proteobacteria* was more abundant in BM transplanted group (34.15±18.94) than in control group (4.2±2.7) (p-value <0.01). Interestingly, *Actinobacteria* was distinguished only in control group (1.91±0.91) (p-value<0.05). By comparing species diversity profiles, a statistical analysis of comparative high-throughput data demonstrated that according to the degree of Shannon diversity (alpha diversity) at the taxonomic level, there is a highly significant difference between bone marrow transplanted patients and control group (p< 0.01) suggesting a higher diversity within healthy subjects rather than bone marrow transplanted patients. Our findings indicate specific ciclosporin-related effects on fecal microbiome of BM transplanted recipients compared to healthy subjects. A better understanding of the role of intestinal bacteria in immune reconstitution and in the risk of infection after allogeneic transplantation could constitute a new approach for preventing side effects of bone marrow transplantation.

P71.

The contribution of the mitochondrial genome in the strain identification of the entomopathogenic fungus *Metarhizium*

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Metarhizium is a well known genus of entomopathogenic fungi commonly used as fungal biological control agent (BCA) for pest control, due to its pathogenicity against a broad range of hosts. Taxonomically, the genus belongs in the phylum *Ascomycota*, in the family of *Clavicipitaceae,* but the phylogenetic relationships of each species are quite unresolved. Taking into account both the role of *Metarhizium* to the crop protection and the difficulty in identifying the species within this genus, its study attracted the scientific interest of many researchers who focus mostly in finding the appropriate molecular markers for typing. Until now, mitochondrial (mt) molecular markers have been often used successfully to the characterization of fungal species and strains, due to their higher evolutionary rates compared to the nuclear genome. The main aim of this work is the species and strain characterization of 14 new potential BCA strains of *Metarhizium* spp., using the mitochondrial genome as a possible source of molecular markers. A comparative genome analysis of the seven known and available fungal mitochondrial genomes of *Metarhizium* has been performed, in order to locate diverse regions ideal for species/strains discrimination. Seven such regions were located, both intergenic domains and gene fragments. After PCR amplification, the samples were sequenced and several SNPs were detected among these 14 strains, after their *in silico* analyses. These SNPs are able to sum these strains in several different groups. A phylogenetic tree was produced using a concatenated matrix of all sequences from the seven examined regions, in order to clarify the groupings. The tree was based on the Bayesian method, and showed good support values of most topologies. Despite the ambiguity of species characterization for some of these strains, the detection of these differences using mitochondrial molecular markers may be extremely useful in strain differentiation. This differentiation may lead to strain typing which is necessary in further pathogenicity studies concerning these strains. Thus, mitochondrial genome is a useful source of molecular markers for extrapolating primers for the typing of strains which are to be used in mass production as BCAs.

Keywords: *Metarhizium,* mitochondrial genome, genus, entomopathogenic fungi, BCAs, genetic fingerprinting

P72.

Mitochondrial ribosomal RNA genes unveil the phylogenetic relationship among fungi

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Mitochondria play an important role in cells, due to their leading role in cellular respiration and cell detoxification. It is well known that in fungi, they have usually their own circular genome which includes genes that encode the necessary proteins involved in oxidative phosphorylation, as well as rRNA and tRNA genes. These genes and their intergenic regions are often used as molecular markers in taxonomic, phylogenetic and evolutionary analyses. In this study, the ribosomal RNA genes in mitochondrial genome of fungi, i.e., the 16SrRNA of the small mitoribosomal subunit (*rns*) and the 23SrRNA of the large mitoribosomal subunit (*rnl*) were examined. A shared feature of these genes is that they have some domains with high levels of identity (homologous regions) interrupted by intensively differentiated regions. This attribute renders both *rns* and *rnl* genes as promising molecular markers of high sensitivity which may be used in order to successfully clarify the evolutionary relationships in a noteworthy range of taxonomic ranks within the Fungal Kingdom. In order to create the matrix to be analyzed, 129 fungal species which belong to 45 different orders were selected. The most serious problem was that these genes are not well characterized in literature, and this resulted to a thorough analysis of their secondary structures, their gene borders (5' and 3' ends) and their intronic sequences before proceeding to phylogeny. In detail, the rRNA sequences were aligned using MAFFT. The conserved regions of the aligned matrix were separated from badly aligned domains of large sizes. These domains were identified as introns with open reading frames, usually mitochondrial homing endonucleases. This result combined with the analysis of the secondary structure of the rRNA genes led to the clarification of the genes' exons. Thus, the concatenated *rns* and *rnl* nucleotide matrix was used in order to construct phylogenetic trees by employing Neighbour-Joining and Bayesian methods. The trees produced based on this matrix showed the clustering of species belonging to the same Order with good support values as shown by the Bootstrap and Posterior Probability. Moreover, through the topologies of the different fungal groups, the evolutionary routes of the mitochondrial rRNA genes within the Kingdom of Fungi were revealed.

Keywords: mitochondrial rRNA genes; fungal phylogeny; gene evolution

P73.

Characterization of the technological microbiota of PDO Galotyri market cheeses

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Galotyri is a traditional Greek PDO acid-curd cheese produced in Epirus and Thessaly regions from sheep's or goat's milk or their mixtures. According to its PDO description, the Galotyri cheese milk is naturally acidified and ripened, while addition of lactic starters is permitted. In this study, the microbial diversity of two commercial Galotyri cheese PDO varieties produced by dairy plants in Epirus was evaluated by culture-dependent methods. Initially, the cheeses were subjected to microbial quantification analyses for total viable bacteria, mesophilic and thermophilic lactic acid bacteria (LAB), enterococci, coliforms, total and coagulase-positive staphylococci, yeasts and molds plus detection of *Listeria* and *Salmonella* contamination by culture enrichment. Afterward, a total of 160 cheese LAB and yeast isolates were identified and grouped by biochemical criteria, REP-PCR and RAPD-PCR, and representative isolates of each LAB or yeast group were subjected to species identification by sequencing the V1-V3 variable region of the 16S rRNA gene and ITS region, respectively. In addition, multiplex PCR amplification targeting *rec*A gene derived primers was performed for the differentiation of LAB isolates assigned to the *Lactobacillus plantarum/paraplantarum/pentosus* genomic group. Microbial enumeration showed that the technological microbiota consisted of several thermophilic and mesophilic LAB and yeast types at population levels of 4-9 log cfu/g and 6-7 log cfu/g, respectively, while all cheese samples (pH 3.8-4.1) were safe. The LAB isolates were assigned to *Streptococcus thermophilus, Lactobacillus delbrueckii, Lactobacillus plantarum*, *Lactobacillus paracasei, Lactococcus lactis, Leuconostoc mesenteroides*, *Enterococcus faecium* and *Enterococcus faecalis;* the former two species were predominant because apparently were the primary LAB starters used, while all others were subdominant in both cheese varieties. Yeasts were assigned to *Kluyveromyces marxianus, Pichia fermentans, Candida* sp., *Debaryomyces hansenii, Candida zeylanoides* and *Pichia membranifaciens.* Significant genetic similarities appeared to occur within the isolates from the cheeses of each plant, while similarities were occasionally noted between cheese isolates from both plants. The present culture-dependent identification results are considered important as a basis to determine unique biotechnological characteristics of different PDO Galotyri market cheeses.

Keywords: Galotyri cheese PDO, microbial diversity, culture-dependent identification

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P74.

Bacterial profile of natural and aquaculture populations of *Sparus aurata*

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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 environment, social interaction and diets. Changes in gut microbiota composition attributed to captive-state have been frequently reported. Our study is focused on the characterization of the cultivable gut microflora of *Sparus aurata* from two wild and two aqua-cultured populations. The selection media used were TSA and LB and incubation was performed in two temperatures (25^{o}C & 37^{o}C). Bacterial strain characterization was performed by 16S rRNA gene sequencing. All sequences used in this study were aligned using MUSCLE as implemented in Geneious and manually edited. Phylogenetic analyses were based on Bayesian Inference. Bayesian analyses were performed with MrBayes. For the wild populations 192 bacterial strains were isolated, representing 95 different operational taxonomic units (OTUs). The identified OTUs were classified into 5 phyla, 5 classes, 14 orders, 19 families and 27 genera. The majority of the detected taxa belonged to phylum Proteobacteria. Natural populations were dominated from *Pseudomonas*, *Shewanella*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, *Vibrio*, *Psychrobacter* and *Photobacterium*. On the other hand, for the domesticated populations we isolated 144 strains, which grouped in 45 OTUs and were classified into 5 phyla, 5 classes, 6 orders, 11 families and 14 genera. The majority of the detected taxa belonged to phylum *Firmicutes*. The dominant genera reported were *Bacillus*, *Micrococcus*, *Staphylococcus*, *Psychrobacter*, *Dietzia* and *Photobacterium*. Gaining a greater understanding of the fish bacteriome and its associated components will improve our ability to manipulate and fortify fish gut bacterial communities to enhance fish health and aquaculture productivity.

Keywords: seabream, 16S rRNA, microbial diversity, culture-dependent

P75.

Development of a bioinformatic approach to discern between high-throughput genetic data of metazoan and prokaryotic origin for complex holobionts

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Sponges are organisms with simple body plan, without true tissue differentiation. Moreover, they are notorious for hosting rich, regulated symbiotic bacterial communities, thus creating the sponge holobiont. These traits, combined with the expansive and diverse nature of the poriferan phylum and the fact that only two sponge species have been sequenced, imposes challenges to genomic approaches. For example, although transcriptome assembly facilitated through NGS can provide sound answers to ecological questions, a *de novo* assembly approach is required for non-model organisms, due to absence of a reference genome. This becomes more challenging in sponges as it is often difficult to discriminate between signals of metazoan and prokaryotic origin, thus introducing biases to interpretation. To overcome this impediment, we developed and present a novel bioinformatics pipeline that efficiently separates between bacterial expressed genes from those of eukaryotic origin, overcoming this challenge. This method was used towards the transcriptome acquisition for the common Mediterranean bath sponge *Spongia officinalis*. The pipeline involves standard read pre-processing steps and incorporates extra analyses to identify and filter prokaryotic reads out of the analysis. Following multiple quality control filters, the surviving reads were scanned for non-sponge sequences. First, the reads were mapped against the bacterial sequences available in the NCBI RefSeq database with the tool ribopicker. The unmapped reads were consequently used for the transcriptome reconstruction. The resulting draft transcriptome assembly was annotated with standard pipelines and the annotation was used to notify further on the presence of prokaryote data. As a final filtering step, the top blast hits of each contig were scanned and the taxon identity of the corresponding organisms were retrieved. Contigs with top hits taxon identities that matched bacterial sequences were eliminated to end up with a high-quality, prokaryote-free assembly. The proposed pipeline can be followed to overcome the technical RNASeq problems characteristic for symbiont-rich metazoan organisms with low or non-existent tissue differentiation, such as sponges and cnidarians. At the same time, however, it can be valuable towards the development of targeted experimental approaches that specifically examine the symbiotic communities of these organisms. This is highly important, since as is often the case, several important functions and properties of these complex holobionts cannot be confidently attributed to the host metazoan or the symbiont community.

This research was implemented through an IKY scholarship funded by the action “Reinforcement of Postdoctoral Researchers” of the operational programme "Human Resources Development, Education and Life Lifelong Learning" with priority axes 6, 8, 9 and was co-financed by the European Social Fund (ESF) and the Hellenic Republic.

P76.
A useful technical tip for improving DNA extraction from streptomycetes

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Streptomycetes are Gram-positive bacteria and their cell wall consists of peptidoglycan layers densely functionalized with anionic glycopolymers called wall teichoic acids. In order to achieve an effective DNA extraction with adequate yield and purity, one must make sure to completely break that cell wall and isolate genomic DNA for further molecular manipulations. Microwave apparatus is a form of non-ionizing electromagnetic radiation with a higher frequency of radio waves than the ordinary, but lower than infrared light. The microwave ovens that are in the market usually are manufactured of 2.45 gigahertz (GHz), a wavelength of 12.2 centimeters. Water, fat, and other substances absorb energy omitted in the form of microwave radiation and are rapidly heated. In this study we used the known and well-established phenol-chloroform DNA extraction technique in members of genus *Streptomyces,* during which, for the first time, the step of thermal shock was elaborated in a microwave in order to increase DNA yield and purity and reduce time consumption. Conventional DNA extraction method requires heat shocking of the bacterial cells in a water bath at 65° C over the course of a total of two hours. This time consuming step could be avoided by the use of a microwave. Extensive research on our streptomycete isolates and in combination with multiple other parameters have resulted in the optimum number of intervals, time duration and power of microwave radiation applied to the cell mix before moving to the DNA isolation steps of the process.

P77.

The presence of Non-Ribosomal Peptide Synthase (NRPS) and Isopenicillin N synthase (IPNS) genes in Greek *Streptomyces*

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It is known that the *Streptomyces* genus is abundant in nature and is recognized as the best candidate for commercially production of bioactive compounds used in medicine. For instance non ribosomal peptide synthetases (NRPS) are biosynthetic systems involved in the synthesis of a range of important biologically active compounds (like antibiotics, antiparasitic agents, antifungals, anticancer drugs, toxins and immunosuppressants) produced by several members of streptomycetes. In addition isopenicillin N synthase (IPNS) which mediates the oxidative conversion of the linear δ-(L-α-amino-adipate)-L-cysteine-D-valine (ACV), to isopenicillin, the precursor of all penicillins and cephalosporins, which are broad spectrum β-lactam antibiotics and protagonists against microbial infections for over 80 years, is present in streptomycetes as well; researchers reported that there are seven IPNS gene sequences which share the highest (70-80%) similarity in *Streptomyces* when compared with other groups of microorganisms. This study focuses on screening NRPS gene in rare streptomycete strains, isolated from diverse soil habitats of Greece, in order to select not only the producer strains, but also those which they do not exhibit antimicrobial productivity, when tested against the specific agents, but host the NRPS gene; for the latter strains, we aimed to induce the present gene for production of new bioactive compounds by using several parameters which are discussed in this work. In the case of the IPNS gene, the aim was to screen the genome of all our isolates (more than a hundred unknown streptomycetes isolated from various Greek soil habitats) for the presence of the prolonged gene. Our results suggested that the IPNS gene was hosted in more than 30% of the isolated strains and data are discussed in relation to relative parameters.

P78.
Effects of endomycorrhizal symbiosis on plant-plant interactions

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Plants are engaged in a diverse set of biotic interactions with soil microorganisms, that may affect coexistence with their plant neighbours. Arbuscular mycorrhizal fungi (AMF) constitute a major monophyletic group (Glomeromycota) of microbial plant symbionts. They colonize the roots and form symbiotic relationships with the majority of terrestrial plant species. There is overwhelming evidence that AM fungi alter plant–plant interactions. In this study we used three plants that represent functionally diverse groups, (a C3 broadleaf plant, a C4 grass, and a legume). Our hypothesis is that for each plant, growth in tri-partite plant community microcosms would influence AMF colonization and sporulation dynamics differently, compared to growth in monospecies microcosms, while *vise versa,* the presence of AMF would alter the plant growth characteristics between monospecies and tri-partite plant microcosms. The three plants that we used are: *Capsicum annum* (common pepper), a C3 plant; *Zea mays* (sweet corn variety), a C4 plant; and *Vigna unguiculata*, a legume. The AMF inoculum that we used is an autochthonous *Funneliformis mosseae,* AMF strain, previously isolated, that is continuously propagated in our lab. The experiment was carried out in greenhouse conditions and plants for both microcosms (monospecies and tri-partite) were placed in different nylon mesh bags (30μm) to allow hyphae translocation and soil solution exchange but to prevent the penetration of plant roots across bags. Several plant traits were monitored, while AMF colonization and sporulation and rhizobium nodulation were determined at harvest (after 90 days). The results will be presented at the conference.

P79.

The role of microorganisms in the degradation and transformation of the anthelminthic veterinary drug albendazole

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Helminths such as gastrointestinal nematodes (GEN) are the major pathological factors for goat and sheep livestock, affecting their health and productivity. To prevent and treat infections from GEN, synthetic anthelminthics (AHs) are used. AHs are poorly metabolized in the animals and are excreted to faeces which are then applied as manure in agricultural soils leading to the further environmental dispersal of AHs. We examined the hypotheses that (a) soil microorganism have a major role in the degradation and transformation of albendazole and (b) the regular exposure of soils to excreta from treated animals will result in the selection of soil microbes with advanced catabolic activities against albendazole leading to their enhanced biodegradation. To address these issues, we collected soils from different areas of livestock farms in the island of Lesvos which had been using or not using albendazole. The role of soil micro-organisms in the degradation and metabolism of albendazole and its major metabolites, albendazole sulfoxide and albendazole sulfone, which also present anthelmintic activity, was determined in sterilized and non-sterilized samples from each soil. The presence of enhanced biodegradation was examined by comparing the degradation rates of albendazole in soils collected from farms which had been using albendazole vs farms not administering albendazole. Finally, we determined possible correlations between the soil physico-chemical characteristics of the different soils and the degradation of albendazole. The results generally showed rapid degradation of albendazole in most soils with DT50s between 0.1 - 4.2 days in soils with a history of contamination with albendazole and DT50 = 1.2-3.2 days in soils with no usage history. Soil sterilization in both cases partially slowed down the degradation of albendazole with DT50s = 0.22-6.9 days and 1.6-13.7 days in non-sterilized and sterilized soils respectively, demonstrating the role of both biotic and abiotic processes in the degradation of albendazole in soil. When the total toxic residues of albendazole were considered (albendazole + albendazole sulfoxide + albendazole + sulfone) DT50 and DT90 values > 365 days were observed in sterilized samples. Regarding non-sterilized samples, in those without a history of albendazole use, total toxic residues appeared more persistent (DT50 = 1.12 to >365 days) compared to soils with a systematic use of albendazole (DT50 = 1.6 to 4.5 days).

The results of this study demonstrate the role of both biotic and abiotic processes in the degradation of albendazole in the soil. However, there is not strong evidence for the occurrence of enhanced biodegradation of albendazole in soils studied and further studies under controlled conditions will investigate the possibility of adaptation of the soil microbial community under realistic treatment regimes (soil application of AHs via manuring or faeces).

Keywords: anthelminthics, albendazole, biodegradation, enhanced biodegradation albendazole sulfoxide, albendazole sulfone, soil

P80.

Determinants of intraradical arbuscular mycorrhizal fungi diversity in Greek olive tree cultivars

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Arbuscular Mycorrhizal Fungi (AMF) are widespread soil microorganisms that form mutualistic symbiosis with the majority of terrestrial plants, including olive trees. In exchange for photosynthetic carbon supplied by the host plant, AMF enhance the capacity of the plant to acquire nutrients from soil and to tolerate abiotic stresses (e.g. drought and salinity). Olive trees (*Olea europaea* L.) are spread across the arid or semi-arid ecosystems of the Mediaterrean basin. They are adapted to drought and low soil fertility and their cultivation has had historically an important role in the economy of olive oil producing the countries, like Greece. Under the framework of the National Emblematic Action “ROADS OF OLIVE” we aim to shed light on the factors that determine the AMF diversity in the roots of selected Greek olive trees varieties.

To address this aim, we followed two sampling strategies: (i) To determine whether different varieties of olive trees harbor distinct AMF communities, we sampled roots from eleven different varieties cultivated at the same experimental field located in Southern Peloponnese. (ii)To explore the role of soil conditions and location, we sampled roots from trees of the emblematic Greek variety (Koroneiki), derived from the same propagation origin and cultivated at six different locations across Greece. In both cases, samples were collected in spring and autumn allowing us to consider seasonality as an extra factor that might affect AMF intraradical diversity. The AMF diversity in all samples was determined by amplicon sequencing of the large ribosomal subunit via Illumina MiSeq 2x300 bp paired-end analysis and the outcome of the analysis will be presented at the conference.

Our study constitutes the first attempt to elucidate the factors determining AMF community assemblage in olive tree roots, and to expand our knowledge on the role of this symbiotic relationship.

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P81.

Effect of operating parameters on cyanobacterial biotreatment of brewery wastewaters

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Brewery wastewater is generated from the beer brewing process in large amounts (4-8 m3 per m3 of beer produced). It consists of high organic matter content, significant nitrogen and phosphorus concentrations and easily biodegraded compounds. Even though most biological treatment technologies applied to brewery wastewaters include the use of bacteria, cyanobacteria (photosynthetic microorganisms) constitute attractive means for sustainable and low cost wastewater treatment producing high biomass concentration, which could be utilized as feedstock for various biotechnological applications (such as biofuels, fertilizers, food supplements etc). In this study, a mixed culture of a filamentous cyanobacterium *Leptolyngbya* sp. was used to treat brewery wastewaters. A range of operating conditions (pH, temperature, pollutant concentration) was examined in an attempt to optimize the rate of pollutant removal and biomass production. Moreover, the produced biomass was characterized in terms of carbohydrate, protein and lipid concentration in order possible applications to be investigated. The experiments were conducted in batch mode under non-sterile conditions in lab-scale photobioreactors. The removal rates of nitrate, ammonium, orthophosphates, total phosphorus and chemical oxygen demand (COD) were achieved in high percentages for all parameters tested, while the biomass produced consisted of approximately 40% carbohydrates, 35% proteins and 12% lipids. Therefore, the treatment of brewery wastewater using cyanobacteria species could be effective, while the cyanobacterial biomass could be used in numerous fields for diverse applications.

Keywords: Cyanobacteria, Wastewater treatment, Brewery wastewater, Biotechnology, Environment

P82.

The effects of arbuscular mycorrhizal fungi and rhizobia co-inoculations in *Lotus japonicus*

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Plants establish symbiotic relationships with soil bacteria or fungi, which colonize the plant root and provide the plant with inorganic nutrients, in exchange for photosynthetic products. Of the many associations formed between plants and microbes, arbuscular mycorrhizal symbiosis seems to be the most ancient and widespread. Legume plants associate with arbuscular mycorrhizal fungi (AMF), but also, with the nitrogen-fixing soil bacteria called rhizobia. During the legume-rhizobium symbiosis, biological nitrogen fixation takes place in specific plants organs formed on the root and called nodules. Using a biological system that enables the simultaneous study of many different microbial interactions, the model legume *Lotus japonicus*, we study the establishment of the tripartite symbiosis legume-rhizobia-AMF. In order to simulate the natural conditions, where rhizobia and AMF co-exist in soils, we examine how AMF and rhizobia affect one another during the colonization of the same legume root, by performing co-inoculations. Moreover, we monitor the effect of the different co-inoculations on the general plant performance. We test the implication of different AMFs, like *Claroideoglomus lamellosum* and *Funneliformis mosseae*, on nodulation and *vice versa*. According to our results, rhizobia and AMFs do affect one another in different ways. The effect of rhizobium on AMF root colonization depended on the AMF strains used. On the other hand, all different AMF strains tested were found to have a positive effect on the formation of root nodules. The legume-rhizobia-AMF tripartite symbiosis was also monitored under abiotic stress conditions. This study aims to enhance our understanding on how and when the plant selects, combines and controls its symbionts, in order to make a more efficient use of legume plants in agroecosystems.

P83.

In silico reconstruction of the protein-protein interaction network of *Moorella thermoacetica,* used for the non-photosynthetic CO_2 bioconversion into useful chemicals

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For many years, metabolic engineering research has focused on the study and evolution of photosynthetic microorganisms and algae for the bioconversion of CO_2 emissions into products of industrial interest. As the yield of these processes and the cost of their maintenance did not prove positive for their use in pilot studies at industrial-scale, there has been in the recent years an increased interest in the use of extremophiles, such as, *Moorella thermoacetica* [1] and *Clostridium ljungdahlii* [2], as the CO_2 assimilation organisms, for the non-photosynthetic CO_2 assimilation into high-value chemicals and biologics. The selection of these two microorganisms was based on their higher metabolic flexibility compared to other extremophiles possessing the non-photosynthetic CO_2 assimilation pathway, and mainly on the availability of reconstructed metabolic models and extensive molecular physiology datasets. In the context of a project investigating the usefulness of the non-photosynthetic bioconversion of CO_2 emissions in lignite units of the Greek electricity producing company PPC SA-ΔΕΗ into high-value chemicals (BIOMEK – Τ1ΕΔΚ00279), we focused our studies on *M. thermoacetica* as it is an obligate anaerobic, fully sequenced, thermophilic and Gram-positive acetogen with a small sized genome, thus flexible for laboratory studies and synthetic biology applications. Its genome-scale metabolic network was reconstructed in 2015 [3] however there has been no study focusing on the reconstruction of its protein-protein interaction (PPI) network, which could enhance our understanding about the physiology of this microorganism and improve the analytical capability of multi-omic studies investigating its physiological boundaries under particular CO_2 availability conditions. In light of the availability of extensive and systematic microbial genome databases enabling large comparative genomic analyses, in the present study we aimed at reconstructing the PPI network of this microorganism from available experimental PPI data for evolutionary adjacent microorganisms. To achieve this goal, we mined mainly the microbial genome database "Integrated Microbial Genomes" database [4] to identify the neighboring organisms with experimentally reconstructed PPI networks and then the homologous protein interactions in these microorganisms that could be potentially active in *M. thermoacetica* too. To extend the recovered PPI network, we repeated the process comparing *M. thermoacetica* with the most studied and modeled bacterium *Escherichia coli*. We identified a number of PPIs as potentially active in *M. thermoacetica*. Ongoing work focuses on further enriching the existing data with genome comparisons with other microorganisms with experimental PPI networks and integrating the reconstructed PPI to the metabolic network of *M. thermoacetica* to further our understanding about its molecular physiology underlining its potential in industrial biotechnology.

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P84.

Effect of microcystin-rich irrigation water on radish (*Raphanus sativus* L.) and its associated soil microbiota

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Microcystins are the most widespread toxins produced by aquatic cyanobacteria, thriving mainly in freshwaters. Irrigation from these water sources induces food chain contamination via bioaccumulation of microcystins in edible plant tissues. Although the toxin bioaccumulation and its subsequent impact on plant performance are well documented, the effect of microcystins-rich irrigation water on soil microbiota is understudied. In this frame we investigated a) the changes on the dynamics of functional microbial groups (via q-PCR) driving key processes in soil N (ammonia-oxidizing microorganisms (AOM)) and S cycling (sulfur-oxidizing bacteria (SOB)), b) the diversity of the soil microbial community (via amplicon sequencing) c) plant growth characteristics and d) toxin bioaccumulation during experimental growth of radish plants irrigated with natural and artificial freshwater containing high concentration of microcystins. We used the following irrigation treatments: (i) water from the Karla reservoir containing 2μg/L of MC-LR, (ii) tap water containing 2μg/L MC-LR, (iii) tap water with 12 μg/L of MC-LR, (iv) water from the Karla reservoir, spiked with MC-LR to reach a concentration of 12 μg/L and v) tap water as control. In the Karla water containing treatments the lowest weight of the edible tissue, i.e. root, and the highest amounts of accumulated microcystins were observed. Similar pattern of microcystins accumulation was found in the leaves. Regarding the soil microbiota ammonia-oxidizing Archaea (AOA) dominated over ammonia-oxidizing Bacteria (AOB) in all treatments. MC-LR applied at 12 μg/L in tap water induced a significant decrease in the abundance of AOA and AOB at 70 days after the commencement of the treatment, however this inhibition did not concur with any significant effects in the concentration of NO_3^--N and NH_4^+-N measured. In contrast MC-LR did not affect the abundance of SOB. Our findings suggest that microcystin bioaccumulation can impose health risk as it can be transferred to the radish edible tissue while no clear effect on the abundance of key soil functional groups was found, suggesting its neutral impact on soil microbiota. Ongoing analysis of amplicon sequencing data for total soil bacteria, archaea and fungi will shed light into the effects of MC-LR on the soil microbial diversity.

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P85.

Soil solarization effects on microbiomic networks with emphasis to soil-borne fungal pathogens

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Soil solarization is an agricultural practice, developed in recent decades for soil-borne pathogen control and weed elimination. In this work, we examined under real-field conditions the dynamics of the soil microbiome, focusing on the influence of soil solarization on prokaryotes and fungi over time. We applied a completely randomized experimental design, with ten plots (10 m^2 each) covered by a special mulch (ORGASUN, Crete Plastics SA) during July and August 2016 and ten left uncovered as controls. The soil temperature was monitored hourly and plots were sampled at 10 and 20 cm depth before and after solarization. DNA was extracted from soil samples and the microbiome diversity was revealed by amplifying the genomic V3-V4 region of
bacteria and the ITS2 region of fungi, followed by sequencing on an Illumina MiSeq sequencer. All possible associations among the recognized genera (or species) were evaluated by Spearman correlation, using the corresponding “OTU” values. Networks were constructed using the significant interactions as links and the microbe species or genera as nodes. Network analysis showed an effect of solarization on some of the network centralities, which relate to small, denser and more fragmented networks, implying a loss of interactions among microorganisms. Focusing on plant pathogens we found that some fungi had more significant correlations to certain soil bacteria, than to each other, and that their degree (number of associations) was affected by time and/or solarization. Overall, our work shows that pathogenic fungi may be an active component of the soil microbiome and that network construction and analysis may reveal useful information, not only on the associations among microbes of interest but also on the effects of specific factors on the structure and composition of the microbiome.

P86.
Electrostimulation: effects on kinetics and dynamics of fungal growth.

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The extended use of antibiotics against infectious diseases as well as for various agricultural and environmental purposes has been on the verge of abuse. This tendency reinforced the natural resistance mechanisms of microbes, resulting in low efficacy and environmental burden. Therefore, the need for novel amenities for better and more affordable cures, improving the quality of life and the yield and environmental footprint in agriculture have incited research for alternative approaches. Electrostimulation (ES) in different modalities promises cost-effective, adaptable and flexible solutions either complimentarily or alternatively to antibiotics. Its introductory use has been in wound healing without administering drugs. However, the precise role of ES in microbial growth has not been determined yet. This work centers on pilot experimentation of conductive ES by standard medical-approved instrumentation applied onto solid cultures of *Candida parapsilosis*, *Cryptococcus neoformans* and *Colletotrichum gloeosporioides* at room temperature. Measurement of each colony diameter and photographs *for* estimating the surface, were the main metrics of dosage differentiation. The results show that (i) dose-result curves are individualized per fungus tested; (ii) galvanotaxis may or may not be manifested, as *Candida parapsilosis* showed prominent galvanophilia whereas *Cryptococcus neoformans* showed noneon SDA when challenged with 1mA daily for 5 min; and (iii) amperage between 0.5 and 5 mA (which amounts to an order of magnitude of current amplitude) still produces inductive effects.

P87.
The effect of lactose hydrolysis on sheep milk yogurt properties

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Milk and dairy foods are nutrient-dense foods supplying energy and significant amounts of protein and micronutrients. The consumption of dairy products in human's diet contributes to a balanced diet. Consumers in Greece prefer yogurt, made from ovine milk, due to its higher nutritive and organoleptic value. However, many consumers are unable to enjoy the benefits of these product consumption due to lactose intolerance, which detracts from wider consumption of dairy products caused by an insufficiency of the enzyme β-galactosidase (lactase) in the digestive tract. The objective of this work was to study the main characteristics of a low fat, lactose-free sheep yogurt during a storage time of 21 days. For this purpose, four yogurt batches were prepared from low fat ovine milk with a different degree of lactose hydrolysis: T1 (50% hydrolysis), T2 (75% hydrolysis), and T3 (100% hydrolysis), and the M (0% hydrolysis). These products were examined for pH, acidity microbial flora, syneresis, the rheological properties and finally evaluated organoleptically. Also, the used milk was checked on its composition, the freezing point, pH, acidity and total microbial flora. The results showed that the degree of hydrolysis does not affect significantly any of the features studied. However, significant statistical differences were observed in relation to the above characteristics during the storage time. Specifically, increasing the storage time led to a reduction of pH, and the corresponding increase in acidity. Also, increased syneresis was observed on the first day of storage and the population of lactic acid bacteria slightly reduced at 21 days preservation. In addition, samples had more viscous than elastic behavior the 1st day after production. Finally, the free lactose yogurt samples T1 and T2 were more accepted by tasters, because of the stability of all quality characteristics. In conclusion, the degree of hydrolysis did not affect negatively the technological characteristics of yogurt but highlighted it as a pleasurable product with good rheological characteristics, sweet taste, and possibly more functional due to reduced lactose content.

Keywords: lactose-free yogurt; sheep yogurt; lactose hydrolysis

P88.

The effect of 'free lactose' sheep yogurt environment on pathogenic growth susceptibility

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The traditional set-type Greek yogurt from sheep milk is still produced under small scale manufacturing in an open production system, which remains favorable for contamination with potentially pathogenic microorganisms. Furthermore, to promote the purchase of sheep yogurt in larger population groups, we produced a set-type free-lactose yogurt from homogenized low-fat sheep milk and studied its susceptibility to pathogenic growth. We investigated the survival of three pathogens, *L. monocytogenes, S. aureus* and *E. coli,* in the lactose-free sheep yoghurt environment during preparation and storage at 4°C. Two different microbial challenge studies were performed using the above pathogens as contaminants; one contaminated the lactose-free sheep milk before the yogurt preparation (fermentation), and one contaminated the ready to eat free-lactose yogurt (post-fermentation contamination). The results obtained were shown that the yoghurt environment has been an inhibitory factor in the survival of the three pathogens, as there has been a decrease in this population over a relatively short period of time in both types of yoghurt. However, the free-lactose yogurt environment shown to slightly favor the survival of pathogens, especially in the case of milk contamination before yogurt fermentation, as the time it took for them to stop being detected in the free-lactose product was slightly larger than normal yogurt. Overall, the findings show a positive effect on the safety of yogurt as a food, but certainly further scientific research is needed, particularly regarding lactose hydrolyzed sheep yogurt, whose results are new data.

Keywords: Lactose-free yogurt; sheep yogurt; pathogens susceptibility

P89.

The nutritional status of the endophytic *Fusarium solani* strain K, and its association with fungal symbionts in legume roots.

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Fusarium solani strain K is a root endophyte isolated from the roots of tomato plants. It protects the plant against biotic and abiotic factors (Kavroulakis et al 2007; 2018; Garantonakis et al 2018; Pappas et al 2018). FsK has a broad range of plant hosts, as it also colonizes model legumes. In their natural habitat, plant roots interact with a large microbial spectrum: symbionts, endophytes, pathogens etc. Beneficial microbes colonize plant roots mostly to engage in a bilateral allocation of nutritional sources with their hosts. An experimental procedure was set with primary focus to gain insight into the growth traits of FsK under various conditions. In addition, a tripartite system was established which involved the endophyte, a fungal symbiont (*R. irregularis*), and a model legume (*L. japonicus*). Microbial root colonization quantification was conducted and main plant phenotypic traits were recorded. We will present: 1) the nutritional 'demands' of FsK in the absence of a host, and 2) the tripartite interaction system of FsK with an Arbuscular Mycorrhizal Fungus within the root of model legumes. Results of this work will aid further research concerning FsK optimal growth and potential nutritional sources acquisition from the plant in the presence/absence of other interacting microorganisms.

Keywords: *Fusarium solani*, endophytes, legumes, Arbuscular Mycorrhizal Fungi

P90.

Degradation and assimilation of a plant-protectant natural amino acid analogue by soil fungi

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L-Azetidine-2-carboxylic acid (AZC) is a non-proteinogenic L-proline (L-Pro) [1] analogue produced by members of the Liliaceae and Fabaceae families and by several *Beta vulgaris* species [2], [3]. AZC in plants serves as a defensive mechanism to poison predators and thus protecting against infections and consumption. This defense mechanism relies on the stereochemical similarity of AZC with L-Pro that leads to misincorporation of AZC resulting in deleterious accumulation of misfolded proteins [4]. Mechanisms of AZC resistance have been described in plants, fungi and bacteria [5]–[8]. In fungi, Σ1278b strain of *Saccharomyces cerevisiae* has been shown to be resistant to AZC [9] and it was reported that the Mrp1/2 acetyltransferases are required for this detoxification [10]. Additionally, a Pseudomonas strain isolated from soil derived from the roots of the AZC-producing *Convallaria majalis*, a Liliaceae flower [11] has been shown to be resistance to ACZ and an AC-hydrolase was reported to be responsible for this detoxification and assimilation of ACZ. In this study, we investigate the mechanisms underlie resistance of soil fungi to AZC using the genetically tractable fungus *Aspergillus nidulans*. Our results show that *A. nidulans* possesses the ability to not only detoxify AZC, but also to use it as a nitrogen and/or carbon source. For this ACZ detoxification and accumulation, orthologues of Mpr1/2 acetyltransferases of *S. cerevisiae* and an orthologue of the Pseudomonas AC-hydrolase, named Ngn2 and AzhA respectively, are necessary and sufficient. In addition, our results provide evidence that AZC, is assimilated via the GABA metabolic pathway in *A. nidulans*. Importantly, phylogenetic analysis shows that strict homologues of the AzhA AZC hydrolase are present in nearly all species of soil fungi tested, including several phytopathogenic ones. As these species are also pathogenic for AZC-producing plants of significant economic value (such as sugar beets and beet roots), our results indicate novel targets for the development of treatment strategies for fungal contaminated ACZ-producing plants.

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P91.
Bioprospecting for biodesulfurization bacteria in a unique Greek environment

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In recent years, the petrochemical industry is facing increasingly tough limits on the sulfur content of its products (10ppm for liquid fuels as in Directive and 0.5% w/w in ship fuels as in Directive 2012/33/EU instructions). As a result, the cost of desulfurization of petroleum products is constantly increasing, and the need for new and low-cost desulphurization technologies at the refinery level is becoming imperative. The current technology for the removal of sulfur compounds from petroleum products is HydroDeSulfurization (HDS), which is carried out in the presence of inorganic catalysts at high temperatures and pressures. HDS has several drawbacks, but the most important is the fact that although it almost completely removes aliphatic sulfur compounds, it is not as effective for the recalcitrant heterocyclic sulfur compounds, such as thiophene (TP) and its derivatives dibenzothiophene (DBT) and 4,6-dimethyl-dibenzothiophene (4,6-DMDBT). The removal of these compounds through HDS, requires energy intensive conditions that increase costs to potentially prohibitive levels. BioDeSulfurization (BDS) appears as an environmentally friendly and energetically efficient method for the deep-desulphurization of the various oil fractions, following HDS. During BDS, selected microbial strains are employed, that can break-up under mild process conditions the recalcitrant sulfur compounds in oil, without downgrading the caloric content of the processed product. In BDS, certain bacterial species are employed, that can utilize the sulfur in thiophene derivatives transforming them in sulfur depleted biphenolic compounds, compatible for biodiesel. But the rates and volumetric capacities of BDS are still quite low, a fact that fuels the search for novel and more efficient biodesulfurization microbial catalysts. In the present work, we are focusing on the analysis of the aerobic bacterial desulfurization potential within a unique environment in Greece (Keri lake, Zakynthos island). The area is a marsh type wetland, where there are many high sulfur oil leaks to the surface. This activity in the area lasts for at least 2.5 millennia, a period which is likely to be sufficient for the development of specialized and highly reactive metabolic pathways for the assimilation of organic sulfur. Soil samples from the area have been collected and used as inoculum in enrichment cultures with various carbon sources and DBT as the sole sulfur source. Following five successive enrichment cycles, the process has resulted in the isolation of two desulfurizing strains. The characterization and physiology of these two novel isolates are presented and discussed.

P92.

Citrus medica and *Cinnamomum zeylanicum* Essential Oil Mixture as Potential Biopreservative Agent Against Low Alcohol Wine Spoilage

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Today, low alcohol wines represent a new steadily rising trend in the global wine market driven mainly by the major awareness about serious long-term effects of alcohol consumption, as well as social and economic reasons. Since low alcohol products are sensitive to spoilage, the use of natural agents with antimicrobial activity is considered a promising alternative to chemical preservatives. Thus, the aim of the present study was to investigate possible antimicrobial action of *Citrus medica* and *Cinnamomum zeylanicum* essential oils (EOs) and assess its commercial potential in the wine industry. The main constituents identified by GC/MS analysis were limonene (38.46 %) and linalool (35.44 %) in *Citrus medica* EO, whereas *trans*-cinnamic-aldehyde (63.58 %) was the dominant compound in *Cinnamomum zeylanicum* EO. The antimicrobial properties were initially verified by the disk diffusion assay and subsequently the minimum inhibitory, non-inhibitory and minimum bactericidal concentration values of an EO mixture against common wine spoilage microbes were determined, applying a previously published model that combined absorbance measurements with the common dilution method and non-linear regression analysis to fit the data. The efficiency of the EO mixture was further validated in low alcohol wine products and in products deliberately spiked with *Gluconobacter cerinus, Oenococcus oeni*, *Pediococcus pentosaceus*, *Dekkera bruxellensis*, *Candida zemplinina*, *Hanseniaspora uvarum*, *Pichia guilliermondii* and *Zygosaccharomyces bailii*, separately and stored at room temperature. Wine supplementation with the EO mixture resulted in significant delay of spoilage and extension of the products' shelf-life, as well as in microbial growth inhibition after deliberate inoculation, indicating the potential of the EOs as effective biopreservatives in the wine industry.

Keywords: essential oils, low alcohol wines, biopreservatives, *Citrus medica*, *Cinnamomum zeylanicum*, spoilage

P93.

Selective Magnetic Separation to concentrate bioactive compounds from microalgae

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Bioactive compounds from various natural sources (plants, fruits, fungi, bacteria, algae etc.) have been attracting more and more attention, owing to their broad diversity of functionalities and benefits to the human health. The extraction of bioactive compounds from plant materials is the first step in the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, food ingredients, pharmaceutical, and cosmetic products. However, many of these compounds often exist at extremely low concentration in a mixture so that new extraction methods are required to obtain several high-quality bioactive compounds by lowering, at the same time, the cost production and the energy consumption. The innovative solution proposed to refine and enhance the purity of organic molecules extracted from natural sources (plants, fruits, fungi, bacteria etc.) is based on the use of small Magnetic Particles or beads (MPs) functionalized with surfactants and ligands able to specifically bind only one class of target molecule. The magnetic separation is mainly designed to increase the purity of enriched solutions (>80%). The main principle is based on non-covalent and/or ionic binding of a specific organic molecule to a short oligopeptide, antibodies, metal ions and other chemical groups. Herein, we present preliminary results for the extraction of astaxanthin. This molecule belongs to the family of carotenoids which is very attractive for important industrial markets, such as food grade coloring and antioxidant agent. Our investigation is focused in the use of organic binders which present many advantages such as easy and inexpensive synthesis, the possibility of thousands of combination that will result in at least one molecule able to bind the target compound and the fast screening. In the present work, different astaxanthin extracts preparations from heamatococcus pluvialis (microalgae) were used as target compounds to be bind on magnetic nanoparticles. Effects of time, concentrations and temperature on astaxanthin recovery were investigated. After an extraction time of 60 min, maximum recovery was reached at room temperature.

P94.

Comparative treatment of bacterial food pathogens with Bdellovibrio bacteriovorus and T7 bacteriophage in vitro and in food matrices

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A comparative study was carried out to compare the effectiveness and applicability of the use of Bdelloovibrio bacteriovorus and T7 phage in the elimination of food pathogens and the biological disinfection of food products. In vitro tests were carried out in liquid synthetic medium with single cultures of target cells (Escherichia coli, Salmonella typhimurium, Campylobacter jejuni) and co-cultures of target cells with Bdellovibrio (cell lysates) and/or T7 phage (cell lysates). The results showed a significant decrease of up to 1,5-2 log cfu/ml in the culture medium during a 48-h culture, especially in the case of E. coli and C. jejuni, who responded very well (were effectively killed) after use of either Bdellovibrio and T7 phage. S. typhimurium was less affected by both Bdellovibrio and T7, since its population was reduced by about 0,5 log cfu/ml. Notably, the predatory activity of Bdellovibrio could be maintained after 1-month preservation of the cell lysates under refrigeration, while in the case of T7 phage, a long refrigerated storage reduced the number of viable phages and thus its bioprotective capacity. In food products such as fresh meat and fresh salad the reduction of the above pathogens was similar to the in vitro tests and a minimum reduction of 1 log/cfu/g was observed in the Total Plate Count, which favors the storability of each product and the retardation of spoilage. In foods, the use of phage was probably more convenient, since it leaved no traces of by-products or undesirable flavor, while in the case of Bdellovibrio a change in flavor, probably due to by-products of its metabolism, could be observed in the case of meat treatment. The above approach for raw food disinfection with the use of predatory bacteria such as Bdellovibrio bacteriovorus and bacteriophages such as T7, holds promise for use in food, taking into account the limitations for the use of each microorganism.

Keywords: Bdellovibrio, T7 bacteriophage, bioprotection, phage therapy, food safety and hygiene, food pathogen

P95.

An expanded molecular toolbox for efficient genome engineering of the plant-pathogenic fungus *Verticillium dahliae*

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Fungal biology has been revolutionized in the last years with an ever-increasing number of genome sequences being released. This underlines the need for efficient methods that would enable mycologists to take full advantage of the rapidly accumulating information by applying it in targeted functional investigations. *Verticillium dahliae* is a notorious fungal plant pathogen that infects a wide variety of economically important plants worldwide. Here we report the development and optimization of efficient methods for transformation, plasmid propagation, gene knocking-out and tagging in *V. dahliae* and other filamentous fungi. In this context, we also introduce -for the first time in *V. dahliae*- a highly efficient CRISPR/Cas9-based system for gene tagging. In addition to the standard transformation methods, i.e. protoplast-mediated and *Agrobacterium tumefaciens*-mediated methods, both of which are routinely used in our laboratory, we recently developed a high-efficiency conidial transformation method that relies on treatment of spores with lithium acetate (LiAc) and heat shock. The experimental procedure is significantly easier and faster than the former ones, does not require specialized or expensive equipment and consumables, and it performs consistently across various *V. dahliae* strains as well as a range of other filamentous fungi. Furthermore, we adopted the *Aspergillus nidulans* AMA1 replicator element, transformed *V. dahliae* with appropriately constructed AMA1-based vectors and assessed plasmid stability. This led to a 25-fold increase in the efficiency of transformation (compared to integrative vectors) and the plasmids generally persisted as autonomous replicons under proper selective pressure. Based on these advancements we next developed a novel CRISPR/Cas9-based system for targeted modifications of the *V. dahliae* genome and optimized it for the *in situ* N-terminal fluorescent tagging of the *atg8* homolog of *V. dahliae*, which encodes a useful protein marker of autophagy. Using this method, we obtained high tagging efficiencies (12-75% depending on the length of homologous flanks) without integration of selection markers. In this report we present highly optimized methods that facilitate targeted genetic engineering of *V. dahliae* and other filamentous fungi and render high-throughput analyses of these organisms feasible.

Keywords: fungal transformation, plasmid stability, CRISPR/Cas9, gene tagging

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P96.

Construction and study of a new species-specific shuttle vector for the biofuel-producing *Zymomonas mobilis*

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Zymomonas mobilis is an alphaproteobacterium utilized in the production of first and second generation bioethanol and high added value compounds that derive from its powerful Entner-Doudoroff glycolytic pathway. The application of *Z. mobilis* in various bioprocessing procedures often requires its metabolic broadening, which is achieved via genetic engineering and foreign gene introduction. Shuttle vectors that have been hitherto used for the engineering of *Z. mobilis* are, in their vast majority, based on a scarce number of suitable broad-host-range plasmids or on native cryptic plasmids, small in size (1.6 to 2.7 kb) and contributing mostly with their replication functions. In this work, we created alternative shuttle vectors for *Z. mobilis* that make use of a 4.5-kb native plasmid of the *Z. mobilis* wild-type strain NCIMB 11163, namely plasmid pZA1003 (GeneBank acc. no. CP001725). pZA1003 harbors an α-proteobacterial type replicator initiator protein (RepB), a plasmid addiction stabilization system (DinJ-YafQ toxin-antitoxin system), and a four gene-member mobilization region (*mobA-mobD*), homologous to those of colicinogenic plasmids. The shuttle vectors created (initial construct named pJC1 and final, pJAD) are fusion products between pZA1003 and a 2.7-kb derivative of pBluescript II KS (+) that, for reasons of compactness, lacks the f1 *ori*. They additionally carry a chloramphenicol resistance gene (*catE*) that is suitable for plasmid selection in *Z. mobilis*. They proved to be extremely stable in the two most applied and patented *Z. mobilis* strains, industrial strains ZM4 and CP4, and highly transferrable to *E. coli* and *Z. mobilis* recipients via TraP-mediated mobilization, at respective frequencies of 10-1 and 10-5 transconjugants per recipient. Importantly, they stably co-exist with plasmids of the broad-host-range pBBR1MCS and RSF1010 lineages in *Z. mobilis*, thus enabling their simultaneous use for gene stacking purposes. Current optimization of pJAD entails: (i) the introduction of the powerful *Z. mobilis* pyruvate decarboxylase (*pdc*) promoter in the plasmid polylinker for gene overexpression purposes, (ii) the substitution of *catE* with other suitable for *Z. mobilis* selection markers, and (iii) the depletion of the pZA1003 plasmid addiction region, in hopes of reducing vector size without compromising its stability.

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P97.

Purification and characterization of lignin modifying enzymes: The case of a multicopper oxidase and a DyP type peroxidase from *Pseudomonas kilonensis*

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Lignin represents the most abundant bio-renewable source of aromatic moieties in the biosphere. The exploitation of lignin from residual lignocellulosic biomass is a sustainable alternative approach to the production of added-value fine chemicals, currently produced from petroleum-based processes. A restrictive factor in the valorization of lignocellulose is the recalcitrant nature of its matrix, composed of crystalline cellulose, hemicellulose and the heterogeneous fraction of lignin. As a result, its exploitation requires the concerted action of a number of enzymes of various specificities. In this work, we investigate the ligninolytic potential of two oxidoreductases from the aerobic, mesophilic bacterium *Pseudomonas kilonensis* ZKA7, isolated from the Keri Lake in Zakynthos Island, Greece. This area has been known since the ancient times due to its asphalt springs which release crude oil, rich in aromatic hydrocarbons, and has nowadays evolved to a coastal marsh with increased biomass degradation, mainly composed of reeds. The bacterial strain was isolated from enrichment cultures of surface soil samples using organosolv lignin as the sole carbon source, and was extensively studied for its ability to grow on lignin and model aromatic compounds. *In silico* analysis of its genome revealed the presence of genes possibly encoding lignin modifying enzymes. Two genes were selected for further study, yielding a multicopper oxidase and a DyP type peroxidase. Both enzymes were heterologously expressed in *E. coli* BL21 and purified through affinity and gel filtration chromatography. In order to study the two enzymes' ligninolytic activity, the soluble fraction of lignin derived from alkali pretreated corn stover was used as an enzymatic substrate. To further assess the enzymes' properties, we examined their oxidative activity towards diverse aromatic monomers related to lignin, as well as their decolourization activity towards various recalcitrant synthetic dyes. The biochemical characterization of these enzymes revealed their ability to degrade lignin and a wide spectrum of aromatic substances and dyes, suggesting their application potential in lignin biorefinery processes.

P98.

Optimization of bacterial cellulose production using the Corinthian currant finishing side-stream as substrate and physicochemical/textural characterization

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A Corinthian currant possessing company produces a large amount of a lower quality side-stream (up to 5 tn/day), with ~70% invert sugar content. In Greece, this side-stream is mainly used for vinegar production and to a lesser extent for syrups production. This nutritional side-stream has a huge potential for biotechnological exploitation as substrate to produce a variety of added-value products (fermented foods, single cell protein, and valuable microbial metabolites). The solid residues can also be valorised, in a biorefinery manner, through the recovery of functional food formulations (antioxidant polyphenols, prebiotic fibre, etc.). The aim of this work was to develop a bioprocess for the production of bacterial cellulose (BC), a high-value microbial product, using the BC producing strain *Komagataeibacter sucrofermentans* DSM 15973 and the industrial side-stream of Corinthian currants finishing (CFS) as substrate. The need for nitrogen sources addition (peptone and yeast extract) was initially evaluated at different pH values and sugar concentrations of the substrate (CFS extracts). To further optimize the conditions of BC production, a mathematical model was developed using the Response Surface Methodology (RSM), based on the Central Composite Design (CCD), in order to correlate the dependent variable (BC yield) with three independent variables [sugar concentration of the CFS extracts (20, 50, and 80 g/L), pH (4, 6 and 8), and temperature (20, 25 and 30° C)]. The statistical analysis showed that 98% of the BK yield variability is interpreted by the independent variables studied (R2=0.98), and the Rj2 Ad=0.97 indicates the reliability of the model. Among the possible combinations of the studied factor values to maximize the BC yield, the best was: 28.07°C, pH 6.42, and 46.2 g/L sugar concentration. The produced BC displayed different forms and textures, depending on how it was processed (convectively dried, freeze-dried, etc.). The physicochemical/textural characteristics of the produced BCs were analysed by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Nitrogen adsorption-desorption porosimetry analysis, Fourier Transform Infrared Spectroscopy FT-IR), and Thermogravimetric/Differential Thermal Analysis (TGA-DTA).

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Keywords: Bacterial cellulose, Corinthian currant finishing side-streams, RSM/CCD, SEM, XRD, FT-IR, BET, TGA/DTA

P99.

Assessment of fruit juices as alternative food "vehicles" for probiotic delivery. Recent advances and prospects.

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Nowadays, the term "probiotic" refers to products that contain viable and abundant bacteria in particular numbers, i.e. 106-108 colony-forming units (CFU) per gram or ml of the product, in order to exhibit health benefits. Probiotics have become available as nutrition supplements and are mainly delivered through dairy products, such as cheese and yogurt. Even though, health benefits of probiotics are fulfilled by dairy products, increased lactose intolerance, cholesterol content, allergic milk proteins and increase in vegetarian consumers worldwide are limiting factors in growth of dairy probiotics. Likewise, food industry is obliged to search for dairy alternatives with high level of nutrients along with health promoting factors such as vegetables, fruits and cereals. Among them fruit juices are getting more attention due to their high nutritional value. Lactic acid-fermented fruit juices from carrot, potato, pomegranate, orange, grapes, apple, pear, and cashew apple are some examples of fruit substrates used the last years. Current review discusses the various factors affecting the survival of probiotics throughout fermentations and cold preservation of various fruit juices, the nutritional value and the overall acceptance of the products.

P100.
The microbiome of Kariki cheese produced in Tinos Island

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Kariki is an artisanal Greek hard cheese prepared in Tinos Island (Cyclades), which has a long tradition in the production of a big variety of local cheeses. Kariki cheese analyzed in the present study was produced from pasteurized cow milk, without the use of starters. After coagulation, the curd was left inside the whey at room temperature for about 24 h for acidification. It was then transferred into a cheese cloth and left in a cool place (16-18 oC) for a 5 h natural drainage, until it was finally pressed for 12 h. Subsequently, it was kneaded by hand, dry salted (2-3% salt) and transferred into small molds. Finally, the small molded cheeses were left for ripening (10 months, at 16 oC) inside an empty dry local pumpkin called Kariki, hermetically sealed. The final product had a pH of 4.92, moisture 17% and fat in dry matter 58%. Taking into consideration the exceptional ripening way, the Kariki cheese microbiota deserves a deeper look. In our study, we employed a metagenomics approach. Total DNA was extracted from the cheese sample using a novel protocol developed in our laboratory and the results obtained from the sequencing of 16S rRNA gene and ITS DNA region were analyzed using advanced bioinformatics tools. The results of the metagenomics
analysis revealed a high biodiversity with the presence of 34 bacterial and 50 yeast genera. The dominant bacterial genus identified was *Lactobacillus* (53.5%), followed by *Leuconostoc* (25.1%) and *Streptococcus* (14.6%). At the species level, *Lactobacillus brevis* (43.2%), *Leuconostoc pseudomesenteroides* (21.8%) and *Streptococcus parauberis* (14.6%) dominated the bacterial microbiota. Moreover, *Penicillium* (58.3%), *Kluyveromyces* (10.2%) and *Galactomyces* (9.6%) were the main yeast genera found in Kariki cheese with *Penicillium commune* (24.5%) and *Kluyveromyces lactis* (10.1%) being the dominant species. This is the first attempt to record the technology and explore the microbial ecosystem of Kariki cheese.

P101.

Biofilm formation on stainless steel surfaces in a marine Recirculated Aquaculture System.

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The adhesion of microorganisms on surfaces and the consequent biofilm formation is a major issue in the aquaculture sector. This is the case with submersed sensors which are widely used in Recirculated Aquaculture Systems (RAS) for water monitoring purposes. The biofilm formation can affect the function of these sensors that may lead to misinterpretation of the acquired measurements. The aim of this study was to determine qualitative as well as quantitative the formation of biofilms on stainless steel surfaces under seawater conditions. Sterilized stainless steel coupons were placed in an empty aquarium tank, part of an experimental marine RAS. Water temperature, salinity, pH, oxygen concentration, nitrite and total ammonia nitrogen (TAN) were monitored. Sampling was conducted at 24h, 48h, 72h and every three days thereafter until the 30th day. Tank water and SS coupons (in triplicates) were sampled. SS coupons were rinsed by pipetting with 10 mL MRD to remove loosely attached cells and were transferred in plastic tubes with 6 mL MRD and 10 glass beads. Biofilm cells were detached from the SS coupons by vortex at maximum speed for 2 min and the bacterial solution was sampled. Enumeration of viable biofilm cells and planktonic cells from the water samples was performed by plating after 10 fold dilutions on Marine Agar plates incubated for 72h at 25^{o}C.

Water physicochemical parameters were maintained stable during the experimental period. The results of the microbiological analysis of the tank water revealed that the water microbial load ranged from 3,8 to 5,4 $\log_{10}$CFU/mL, with a mean of 4,5±0,5 $\log_{10}$CFU/mL throughout the month. The stainless steel surfaces were colonized and within 24h of exposure the biofilm cells on the SS coupons reached 4,5±0,05 $\log_{10}$CFU/cm2. It needs to be noted that the maximum microbial load (5,2±0,03 $\log_{10}$CFU/cm2) was achieved within the 6th day and this population remained constant until the 30th day.

Keywords: biofilms, aquaculture, RAS

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P102.

Microbiological and physicochemical changes of industrially fermented green olives by the Spanish method

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Green olives of the varieties Conservolea and Halkidiki were fermented according to the Spanish-style. Microbial populations of the dominant microorganisms namely, lactic acid bacteria (LAB), yeasts, and *Enterobacteriaceae* were monitored. Analyses for the determination of pH, titratable acidity, salt content as well as changes in color and texture of the olives were assessed. Results showed the dominance of LAB followed by yeasts indicating normal fermentation process. For cv. Conservolea, LAB reached 7.3 $\log_{10}$ CFU/mL and 5.5 $\log_{10}$ CFU/g, while yeasts ranged between 5.6 $\log_{10}$ CFU/mL and 5.1 $\log_{10}$ CFU/g in the brines and olives, respectively. For cv. Halkidiki, LAB counts were 6.8 $\log_{10}$ CFU/mL and 6.9 $\log_{10}$ CFU/g, while yeasts were enumerated close to 4.5 $\log_{10}$ CFU/mL and 5.6 $\log_{10}$ CFU/g in brines and olives, respectively. Acidity was close to 0.6% (cv. Halkidiki) and 0.7 % (cv. Conservolea) (w/v) lactic acid, while pH was approximately 3.5-3.6 for brines and 3.9 for olives in both cultivars. Texture analysis for cv. Halkidiki showed a decrease in Break Force (F*break*) which is related to hardness from 13.5N to 5.8N during the first 15 days of fermentation followed by a gradual increase up to 11.7N until the end of the process due to salt absorption. For cv. Conservolea F*break* was close to 12.6N and remained at this level throughout fermentation. An increase in the lightness coordinate (L^*) was observed indicating the increase in luminosity (brightness) of the color in both cultivars. The attribute a^* corresponding to green color was decreased (negative values) during the process for cv. Halkidiki, whereas it increased (positive values) for cv. Conservolea after the first 30 days. The values of the parameter b* were maintained positive during the process with small changes indicating the prevalence of yellow color. This was also confirmed by the values of parameter h^* (hue) which ranged between 85-90° corresponding to yellow hues.

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P103.

Microbiological quality of chicken patties during storage at different temperatures

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Meat and poultry preparations gain increasing popularity among consumers, with various types of products being currently available in the market. Specifically, prepared chicken patties are commonly preferred among consumers as a practical and healthy food option. The objective of this study was the evaluation of the microbiological quality of chicken patties during storage at different isothermal conditions. Chicken patties, consisting of 76% minced chicken meat (and fresh vegetables, rusk and various herbs) were obtained from a local manufacturer, and stored aerobically at different temperatures (0, 4 and 8 °C). At regular time intervals during storage, duplicate samples were subjected to microbiological analyses and pH measurements. Two independent experiments were carried out, and 109 samples were analyzed in total. The conducted microbiological analyses involved determination of the populations of total viable counts (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria (LAB), bacteria of the family Enterobacteriaceae and yeasts. The initial (day-0 of storage) TVC of chicken patties was 5.04 log CFU/g, and the initial pH value was 5.95. The microbial groups contributing mainly to the microbial spoilage of chicken patties were *Pseudomonas* spp., *Br. thermosphacta* and LAB. With regard to the relative contribution of these bacterial groups to spoilage (in terms of attained populations during storage), this appeared to depend on the applied storage temperature. Indeed, LAB appeared to dominate over *Pseudomonas* spp. and *Br. thermosphacta* with increasing storage temperature, an observation which was also supported by the measured pH values of the samples; at the end of storage at 0, 4 and 8 °C (corresponding to a TVC of *ca.* 8.5 log CFU/g), the pH of chicken patties was 5.45, 4.75 and 4.47, respectively. The spoilage microflora of chicken patties during aerobic storage appears to be strongly delineated by the storage temperature, and to be different from that expected in unprocessed poultry commodities, most likely due to the presence of additional ingredients.

Keywords: chicken patties, microbial spoilage, storage temperature

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).

P104.

Satellite bacteria of *Tetraselmis suecica* and *Tisochrysis lutea* cultures

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Satellite bacteria of algae play an important biochemical and ecological role in the aquatic environment. They are living in a very close proximity to the external walls of algal cells and efficiently metabolize photosynthetically produced dissolved organic substances. In addition, they refuel primary production via remineralisation of nitrogen and phosphorous and other inorganic elements. Moreover, they sustain algal auxotrophy with the provision of vitamins and trace minerals. Such biological relationships between bacteria and algae are known to occur both in natural and under experimental conditions. The aim of the present study was to compare the community structure of satellite bacteria in *Tetraselmis suecica* and *Tisochrysis lutea* cultures grown in polythelyne bags and photobioreactors. Samples from the culture media at the start and the end of each culture were taken. The bacterial communities of the samples were analyzed with 16S rRNA gene diversity. The dominant Phylum in the start and the end of *T. suecica* culture in bags was *Proteobacteria* with 73.99% and 68.1% relative abundance, respectively. The dominant Phylum in the start and the end of *T. suecica* culture in photobioreactor was *Proteobacteria* in 85.87% and 63.43%, respectively. Nevertheless, the dominant Phylum in the start and the end of *T. lutea* culture in bags was *Cyanobacteria* in70.01% and 61.07%, respectively, and in the start and the end of the culture in photobioreactor was 74.68% and 63.79%, respectively.The abundance of OTUs in the photobioreactor culture of *Tisochrysis lutea* was statistically different between the beginning and the end of the growth period. All the treatments had six operational taxonomic units OTUs in common. In *T. suecica* cultures the OTUs were more abundant than in the *T. lutea* cultures. Furthermore, the OTUs in photobioreactor cultures were more than that of the bags and the start of every culture revealed less OTUs than the end. In conclusion, no essential differences were found between the two types of algal growth regarding their microbiota structure.

P105.

Potential prebiotic effect of mushrooms and extracts of *Pleurotus ostreatus* and*Ganoderma lucidum* strains on human gut microbiota of healthy and osteopenic women

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Aim: To investigate the effects of selected *Pleurotus ostreatus* and *Ganoderma lucidum* strains and their bioactive compounds in compositional and metabolic parameters of gut microbiota of healthy and osteopenic women and to evaluate their potential prebiotic effect.

Materials-Methods: *Pleurotus ostreatus* IK 1123 and *Ganoderma lucidum* LGAM 9720 were cultivated in wheat straw and beech sawdust respectively. Lyophilized mushroom powder (2% w/v) from each strain and their *β*-glucan enriched extracts (1% w/v) were evaluated in six-plicate for their potential prebiotic effect based on an *in vitro* batch-culture fermentation model, using faecal inoculum (20% v/v) from healthy (n=3) and osteopenic (n=3) women. Positive (inulin) and negative (no substrate) controls were also included. Gut microbiota parameters analysis [qPCR-based quantification of selected bacteria, measurement of Short chain Fatty Acids (SCFAs)] were performed at baseline (0h) and after 24h of fermentation, and 24h-prebiotic indexes were calculated.

Results: *In vitro* fermentation models positively highlighted the treatments '*P. ostreatus* IK 1123 mushroom on wheat straw' (POWS) and 'extract of *G. lucidum* LGAM 9720 mushroom on beech sawdust' (GLBSE), based on gut bacterial enumeration and calculation of prebiotic indexes. These treatments were characterised by positive prebiotic indexes on both groups of bone health status, with POWS exerting a significant bifidogenic effect in the osteopenic group. Changes in butyrate- producers were also evident after 24h of fermentation, with a significant increase in *Faecalibacterium prausnitzii* in POWS treatment and a rather universal decrease in *Roseburia* sp.-*Eubacterium rectale* in all cases, except positive controls. Although total concentration of SCFAs was quite low at baseline (0h) (2-4 μmol/ml culture) in all cases, at the end of fermentation (24h) all substrates were characterised by significantly greater SCFAs concentration ($p<0.05$) compared to negative control, with the highest SCFAs levels detected in POWS and its *β*-glucan enriched extract treatments.

Conclusions: *In vitro* data highlighted the potential prebiotic effect of tested mushrooms and their extracts on human gut microbiota.

Keywords: *Pleurotus ostreatus*, *Ganoderma lucidum*, prebiotic effect

P106.

Hydrolytic potential of microbiota treating orange juice processing waste in a methanogenic bioreactor

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Orange juice industries represent a valuable sector of the Greek economy, accounting for 1.5% of orange juice production worldwide. This processing sector generates significant amounts of wastes, which are composed of various polymeric soluble and insoluble carbohydrates, such as pectin, cellulose and hemicellulose, which exceeds 40% of orange's d.w. Moreover, orange juice processing wastewaters are characterized by high organic load, considering these residues amenable for anaerobic digestion. However, the slowly hydrolyzed biomolecules, i.e. lignocellulosic substrates, resist biodegradation, reducing energy recovery gain during anaerobic digestion process. In this work, orange juice processing wastewater was digested in an anaerobic digestion system at mesophilic conditions and the hydrolytic potential of microbiota in the methanogenic reactor was investigated through the determination of both intracellular and extracellular polygalacturonase, endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase, β-1,4-D-glucosidase, endo-1,4-β-xylanase and 1,4-β-xylosidase activities. In particular endoglucanase, exoglucanase, β-xylosidase and β-glucosidase activities were limited, whereas reasonable β-endoxylanase activities were determined. It is concluded that induction of xylanases was favored due to the high hemicellulose content of such waste and the slow hydrolysis of β-glycosidic bond.

Keywords: orange juice processing wastewater; anaerobic digestion; cellulases; xylanases; pectinases

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This work was carried out in the frame of the research project "Optimizing the energy recovery of waste from oranges juice industries using specialized native microorganisms as a starting culture, code MIS (OPS) 5006203, which is implemented through the Operational Program "Human Resources Development, Education and Lifelong Learning" and is co-financed by the European Union (European Social Fund) and Greek national funds.

P107.

Gut microbiota composition of farmed sea bass (*Dicentrarchus labrax*) and the significance of individual variability

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The current state of next-generation sequencing technologies has prompted the rapid research on intestinal microbiota field considering its crucial role in host health. Studies on human gut microbiota have focused on the variation of bacterial communities among people in order to be associated with health implications. Composition of gut microorganism communities undergoes substantial changes as it is influenced simultaneously by numerous factors thus enhancing the uniqueness and heterogeneity of microbiota across individuals. Despite the recent interest of aquaculture in microbiota influence on physiology due to alternative protein sources in aquaculture feeds, individual variability of fish gut microbiota has yet to be examined. The aim of this study is the assessment of the importance of individual variability on species presence and their abundance as well as the estimation of the suitable number of biological samples that is less affected by inter-individual microbiota differences. Illumina sequencing of the V3–V4 region of the 16S rRNA gene was used to examine gut microbial communities of European sea bass (*Dicentrarchus labrax*) individuals fed three insect based diets (*Tenebrio molitor*, *Hermetia illucens* and *Musca domestica*) and a fishmeal control diet. A comparative analysis of microbial community composition between individuals of each of the four different diets was performed. Specifically, abundance and operational taxonomic unit (OTU) data retrieved from 12 individuals per diet were randomized in possible pairs of 3 (220), 7 (792) and 11 (12) and statistical analysis was performed. As expected, no significant differences between pairs of 11 medians were observed in none of the four dietary treatments. Regarding pairs of 3, two dietary treatments showed significant differences in pair's medians, while a small but non-negligible number of pairs displayed statistically significant differences. In conclusion, gut microbiota of different individuals within the same diet, display inter-individual differences regarding the presence and abundance of bacterial species that may outbalance the overall outcome. Thus, a suitable number of samples that eliminates individual variability should be taken into consideration in future studies.

P108.

GAP-FILLING WITH THE ATLAS OF BIOCHEMISTRY TO RESOLVE METABOLIC GAPS IN *E. coli*

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Advances in medicine and biotechnology rely on the further understanding of biological processes. Despite the technological advances and increasing available types and amounts of omics data, significant biochemical knowledge gaps remain uncharacterized. We necessitate methods that enable systematically analysing the growing sets of data and identifying the knowledge gaps. Several approaches [1][2] have been developed during the past decades to identify missing metabolic annotations in genome-scale models (GEMs), which are biochemical databases for an organism. However, these approaches suggest missing metabolic reactions within a limited set of already characterized metabolic capabilities. In this study, we propose a workflow to identify, classify and characterize missing metabolic capabilities in GEMs using the ATLAS of Biochemistry [3]. The ATLAS of Biochemistry, which involves more than 130,000 possible enzymatic reactions between known biological compounds, represents the upper bound of missing biochemistry and is hence a guide to fill the gaps. We apply our gap-filling approach to the latest genome-scale model of *Escherichia coli* (iML1515) [4] and develop a database of top suggested biochemistry that can indicate its missing metabolic capabilities. Interestingly, some gaps cannot be filled with the ATLAS of Biochemistry and represent biochemical bottlenecks for further analysis. Overall, our approach will be a reference and valuable tool for the reconstruction and refinement of metabolic networks, and our results will accelerate experimental studies toward fully annotated genomes.

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P109.
Understanding and Expanding the Cyanobacterial Potential for Bioproduction

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Cyanobacteria are a promising platform for the renewable production of high-value chemicals, as theycombine photosynthetic growth with simple cellular organization; however their potential has not been fully explored. There are two major limitations preventing such biotechnological applications from reaching their full potential: the lack of detailed knowledge of the effects of genetic manipulations of the cyanobacterial metabolism, and the lack of synthetic biology tools for rapid strain generation. In this talk, I will describe two approaches targeted at these specific challenges. First, I examine the effects of introducing two heterologous biosynthetic pathways – a terpenoid and a cyanogenic glucoside – in the cyanobacterium *Synechocystis* sp. PCC 6803 are examined using targeted metabolite analysis and computational modelling. The results highlight the robustness of the cyanobacterial metabolism and show some unexpected off-target effects of the genetic manipulations. Second, I present the generation of a cyanobacterial Golden Gate toolbox which—together with the plant and the recently published algal MoClo kit—provide the research community with several genetic engineering options for photosynthetic organisms. I will conclude by presenting some upcoming work at the University of Athens, where I will continue exploring the biosynthetic potential of photosynthetic microbes.

P110.

Study of biofilm development on marine plastic litter and interactions with an organic pollutant

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Marine pollution by plastics (e.g. the common single-use plastics Low Density Polyethylene (LDPE) and Polyethylene Terephthalate (PET)) has been reported in many studies. Their amount in the global ocean is concerning with negative impacts on marine and on non-marine species (e.g. humans) (Takada and Karapanagioti, 2019). Plastic surfaces are colonized by marine microorganisms. Biofilms formed provide microbes advantages such as stability and ease of nutrient intake (Rummel et al., 2017). Via ocean currents micro/macroplastics travel in huge distances from their source to different ecosystems. Both plastics and biofilm/plastics have the ability to sorb/desorb pollutants. In the present study, LDPE and PET strips (1×7 cm) without and with biofilm of marine microorganisms were used to investigate the sorption kinetics of phenanthrene as a model organic pollutant. A bioreactor set up was carried out with synthetic sea water (SSW) and marine microorganism to create the biofilm on the plastic strips. Diffuse Reflectance Spectroscopy (DRS), a spectroscopic technique in UV–vis region, was used as a new method for studying biofilm formation on plastic surfaces. Visual observations of the plastic surfaces were performed using scanning electron microscope (SEM) and optical microscope. At a later stage, batch reactors with SSW and phenanthrene were carried out to examine the sorption behavior of the organic pollutant using fluorescence data. DR spectra indicated similar appearance of peaks within samples of the same material but different between LDPE and PET. This points out that the type of biofilm formed is affected by the plastic type used and is a function of the corresponding biomass attached to each plastic. Also, the type of plastic is closely related to the quantity of biofilm and the sorption kinetics. In the first 90 minutes, sorption of phenanthrene follows 1st order kinetics. The order of increasing 1st order rate constant k per g of plastic (min-1 g-1) was PET(biofilm) < PET and LDPE(biofilm) < LDPE, with sorption capacity 0.016, 0.063, 0.106, and 0.193 μg/cm2 for PET(biofilm), PET, LDPE(biofilm), and LDPE, respectively. In conclusion, it was found that DR spectroscopy can be used for the determination and evaluation of biofilm which develops in a specific way on plastic surfaces. The type of plastic was observed to be related to the growth of biofilm as well as to the sorption kinetics.

Keywords: Environment, Marine Pollution, Biofilm on Plastic Surface

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P111.

Fine mapping of the bacterial diversity in the gastrointestinal tract of *Ceratitis capitata*

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Wolbachia, is widespread among arthropods and can induce reproductive abnormalities such as male-killing, feminization, parthenogenesis and cytoplasmic incompatibility (CI). Recent evidence suggests that *Wolbachia* is not restricted in reproduction. This symbiont has been implicated in host nutrition and metabolism, apoptosis and immunity. Furthermore, *Wolbachia* can affect fitness traits including fecundity, fertility, locomotion, feeding, life span, as well as mating behavior with potentially catalytic effects in speciation. These unique biological properties fostered *Wolbachia* as a promising tool in support of the sterile insect technique (SIT) for the population control of insect pest species such as the Mediterranean fruit fly *Ceratitis capitata* (medfly). The medfly is a major agricultural pest worldwide. The diversity and structure of the medfly gut associated bacterial communities have been acquired using culture-dependent and independent approaches. Members of the Enterobacteriaceae family were shown in previous studies to dominate the gut of wild medflies and particularly *Klebsiella* spp., *Pantoea* spp, *Enterobacter* spp., *Pectobacterium* spp., *Citrobacter* spp. and *Pseudomonas* spp. with enterobacterial communities most likely exhibiting pectinolytic and diazotrophic activities. Given the importance of the gut-associated microbiota in this study we mapped the midgut, hindgut, crop and rectum associated bacterial communities using 16S rRNA gene next generation sequencing approach. We also investigated the potential effect of *Wolbachia* infection on the diversity and structure of the medfly microbiota. Characterization of the Vienna 8 GSS gut community indicated that Gammaproteobacteria is the dominant class in all tissues examined. More specifically, *Serratia* exhibited a higher relative abundance in hindgut while *Plurallibacter* in midgut. In the rectum the most dominant taxon was *Acinetobacter*. Finally, our results indicated that the Vienna 8 GSS infected with *Wolbachia* exhibited significant lower relative abundance of *Sphingomonas, Plurallibacter* and *Providencia* when compared with the non-infected line, indicating that *Wolbachia* has the capacity to alter the relative abundance of other gut- associated bacteria.

P112.

High-throughput amplicon sequencing reveals potential impact of maintained Tacrolimus therapy on fecal microbiome after kidney transplantation.

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Gaining long-term graft function and patient survival remain a critical challenge following kidney transplantation. Commensal microbiota plays a significant role in the immunomodulation of transplant recipient responses. It is therefore tempting to hypothesize a correlation between the composition and abundance of resident gut bacterial taxa and the physiological parameters of the host. Here we aimed to investigate the relationship between the gut microbiota and the immunosuppressive tacrolimus therapy in stable kidney transplanted patients. Serial fecal specimens were collected from kidney transplant patients (n=40) treated with a Tacrolimus based therapy (1-5 mg/day), and from healthy control subjects (n=18) after signing informed consent forms. Next-generation sequencing of the 16S V3-V4 hypervariable region was performed using Illumina MiSeq platform. We characterized the composition and diversity of gut microbiota within both groups using QIIME2 pipeline. Finally, alpha diversity analysis has been computed to estimate and compare bacterial ecosystem's richness and diversity. Our systematic characterization of the gut microbial composition showed that both study groups shared five common most abundant phyla: *Bacteroidetes, Firmicutes*, *Proteobacteria, Verrucomicrobia* and *Actinobacteria*. Faecal *Bacteroidetes* abundance was higher than the other identified phyla in both TAC and control groups 54.5%±5.6%. Interestingly, taxonomic genera belonging to the phylum *Proteobacteria* (mainly *Echerichia-Shigella*) were highly more abundant within Tacrolimus (TAC) group compared with control group (p-value<0.005). Statistical analysis of comparative high-throughput data demonstrated a higher microbial diversity within healthy subjects than kidney transplanted patients (Shannon p-value:< 0.01; Simpson p-value =0,0005). Alpha diversity analysis shows also a higher richness index within healthy controls compared to TAC group (p<0.01) suggesting a higher richness of gut bacterial ecosystem within control group compared with kidney transplanted patients. Metabolomic data obtained by bioinformatic analysis of nuclear magnetic resonance (NMR) profiles will be presented. Our study, indicated specific tacrolimus-related effects on fecal microbiome of renal transplant recipients compared to healthy subjects. Our findings suggest that the analysis of the gut microbial community could represent a new tool to better evaluate the effects of drugs currently employed in organ transplantations.

P113.

The role of tRNA genes and their intergenic regions in the structure, diversity and evolution of fungal mithochondrial genomes

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tRNA genes (*trn*) are substantial components of every organism's genome, due to the crucial role of their transcriptional products to the transfer of the correct amino acid at the newly-synthesized protein in the ribosomes, thus, controlling the overall protein synthesis process. In fungal mitochondria, the full set of tRNAs responsible for the translation of the organelle's few protein-coding genes found in the mt genome, requires approximately 25 *trn* genes. However, this number varies greatly among different fungal taxa, an immediate result of significant events these organelles have undergone during their evolutionary history. In this study, the diverse presence of *trn* genes in the fungal mitochondrial genomes was examined in order to reveal any possible implications with the organelles' and organisms' evolutionary pathways and relationships. The first step towards this goal was to analyse the presence and clustering of *trn* genes, at different major taxonomic units, like Orders and Genera. An *in silico* analysis of 120 fungal mitochondrial genomes was performed, including representative taxa from all known orders of the phylum Ascomycota. The clustering of the majority of the *trn* genes into 3 main groups and several scattered *trn*s was easily identified. However, the composition of these clusters as well as their intergenic regions varied immensely. While the sequence of the *trn* genes is conserved, their synteny as well as their intergenic regions present a variability which can be further correlated to the taxonomic level of the organisms compared. This genome shuffling seems more extensive at higher taxonomic levels and more limited within species, indicating a higher evolutionary stability of mitochondria in the recent times compared to more extended ancient rearrangements. Both *trn* genes and their intergenic regions composed a matrix which was the template for the Neighbour-Joining and Bayesian based phylogenetic analyses. The trees produced with both methodologies were highly similar and the topologies showed groupings which in cases revealed the evolutionary pathways of these taxa from their common fungal ancestor. Furthermore, the mechanisms of this genome shuffling at the *trn* clusters, i.e., homologous recombinations, duplications and/or horizontal gene transfers are analysed in order to decipher the mt genome synteny of the fungi under scrutiny.

Keywords: *trn* gene; synteny; genome shuffling; evolutionary mechanisms; phylogeny

P114.

Epiphytic bacteria affect volatiles emitted by bean plants and spider mites behavior

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Epiphytic bacterial colonization may affect the emission of plant volatiles and as a consequence the behavior of herbivorous insects and mites. In this study, we explored the effects of three bacterial strains namely *Pantoea agglomerans, Pseudomonas syringae*, and *Pseudomonas putida* inoculated bean plants on the leaf volatile organic compounds (VOCs) emitted 24 h after plant inoculation. Further, we examined egg production, leaf damage and responses to VOCs of the two spotted spider mite *Tetranychus urticae* after bacterial colonization. Our results exhibit that the bean plants emanate different quantities of VOCs depending on the bacterial strain. More specifically, the quantities of VOCs 1-undecanal and z-3- hexen-1-ol significantly increased after *P. syringae* inoculation, while methyl salicylate and anisol was increased mainly in response to *P. agglomerans*. The responses of *T. urticae* females to VOCs emitted from colonized plants was significantly lower than those from the uncolonized plants. Leaf damage caused by *T. urticae* females was 3-fold higher in plants inoculated by *P. agglomerans* than by *P. syringae.* In addition, the number of eggs laid on leaves inoculated by *P. syringae* was significantly lower than those on inoculated by *P. agglomerans* or on the uncolonized ones. It is concluded that different epiphytic bacteria may differently affect the emission of plant volatiles and the respective behavioral and developmental responses of the spider mite *T. urticae.* Further experiments are needed to elucidate the plant - spider mite -bacteria interplay on the phyllosphere, and to explore whether epiphytic bacteria can be used in the future for the biological control of spider mites.

P115.

The coevolution of fungal mitochondrial introns and their Homing Endonucleases (GIY-YIG and LAGLIDADG)

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Fungal mitochondrial genomes consist of conserved genes which are often enriched with introns. Mitochondrial introns can be divided into the self-catalyzing group I and group II introns. Group I introns are folded into conserved secondary structures which help their discrimination to five main subgroups (i.e., IA, IB, IC, ID, IE). Self-splicing introns include Open Reading Frames (ORFs) of Homing Endonucleases (HEs). HEs are enzymes that recognize site-specific DNA targets and are mostly encoded by ORFs in group I introns. The ORF is mainly positioned in loops of the intron that do not interfere with its splicing efficiency. HEs may also be found as free standing ORFs or in a few other cases, within group II introns. HEs are thought to assist in transposition and/or in the splicing of the host intron. The most common HEs in the fungal mitochondrial genomes are GIY-YIG (GIY) and LAGLIDADG (LD) endonucleases. This study presents the characterization and analyses of the introns and intronic ORFs (HEs) found in 163 fungal complete mitochondrial genomes from all up to date known fungal Orders. Furthermore, phylogenetic trees based on amino acid matrices of the HEs (with NJ and BI methodologies) showed mostly identical topologies which combined with data like genetic code, the type of host introns and the mt genes which carry them, presented evidence for the coevolution of HEs and their introns. The results indicate a parallel ancestry of certain categories of introns and HEs, like group II introns with LD ORFs and the novelty of others, such as IC introns with HEs in Pezizomycotina. Additionally, free standing forms of GIY endonucleases seem to be ancestral versions that gradually gave rise to respective genes located in an intron. Certain gene clusters surrounding the free standing HEs seem to be conserved. The target sequences of those introns display a substantial level of identity to the recognition sites of the HEs. Based on that, it is suggested that any homology in target sequences could have motivated the transposition of those elements in a plethora of similar mt regions. Finally, Horizontal Gene Transfer events were found and completed the evolutionary patterns of the introns and their HEs.

Keywords: mitochondrial introns; Homing endonucleases; evolution

P116.

Advancing crop protection of olive trees through metabolomics: olive anthracnose

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Olive tree represents one of the most important cultivated plant species in Greece. However, sporadic disease outbreaks represent a major obstacle towards further development of the sector. During 2016-‘17, heavy fungal infections were recorded in many olive-growing areas, causing significant qualitative and quantitative yield losses, worth of multi-million euros in damages. Recent developments in metabolomics has enabled the dissection of fungal metabolism and its monitoring in response to biotic and abiotic stresses. Within this context, we have undertaken the task to isolate and identify the cause(s) of the infections and study the pathogenesis and susceptibility of the isolates to fungicides employing advanced metabolomics analyses. Olive fruit samples from Lakonia and Messinia were examined. Results confirmed that the main cause of the infections was the fungus *Colletotrichum acutatum,* with various of its morphotypes being isolated. We studied the pathogenicity of these morphotypes to flowers and fruits of the varieties “Koroneiki” and “Kalamon” and their sensitivity to registered plant protection active ingredients (a.i.) belonging to strobilurins and triazoles. The isolated morphotypes being studied were capable to infect flowers and fruits, causing the typical symptoms of the disease. Moreover, the results revealed that different morphotypes exhibit variable sensitivity to the applied a.i.. Additionally, metabolomics analysis was employed for the dissection of the isolated morphotypes’ metabolism. The metabolomics/bioinformatics analyses revealed changes between the metabolomes of the isolated morphotypes, in which their differences in pathogenicity and resistance could be attributed. These results highlighted the potential of metabolomics for the in-depth study of fungal metabolism towards the design/development of alternative crop protection strategies. This study represents the first report on the pathogenicity and susceptibility of *C. acutatum* morphotypes to registered in olive tree plant protection a.i., and these results should be taken into consideration during the design of plant protection programs.

Keywords: *Colletotrichum acutatum,* fungal metabolomics, olive anthracnose, pathogenicity

P117.

Distance-decay relationship of aquatic unicellular eukaryotes in mountainous vernal pools in Thessaly, Central Greece

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In recent years, research has been increasing to study biogeographical patterns of unicellular eukaryotes. Vernal pools are dynamic freshwater ecosystems that dry during summer and refill during autumn and winter. The aim of this study was to investigate whether the distance-decay relationship (DDR) apply for the unicellular eukaryotes communities. It has been observed that the greater is the distance between the ecosystems, the smaller is the similarity in taxa composition. For this purpose, sampling was performed in 30 mountainous ponds, one water reservoir and two sea points in Thessaly, an administrative region at Central Greece, were sampled in March 2016 and May 2017. Sampling sites had different hydromophological characteristics (altitude 124 m to 655 m, depth 0,3 m to 1 m, surface area 19 m^2 to 0.12 km^2) and geographical distances (0.03 km to 48.4 km). Community composition of aquatic unicellular eukaryotes was determined by Illumina sequencing. Sequences were aligned by using MOTHUR against the PR2 database. The distance-decay relationship were estimate using the Sorensen index between two samples for presence/absence data for all OTUs and also presence/absence data for abundant and rare OTUs. Also, the habitat specialization was calculated using Levins' niche index (B) with arbitrary cut-off values. From a total of 46,757 sequence reads were obtained 4282 OTUs and from 31,211 sequence reads were obtained 3,082 OTUs of aquatic unicellular eukaryotes from March 2016 and May 2017 respectively. OTUs were mainly assigned to five super-groups: Sramenopiles, Alveolata, Rhizaria, Archaeplastidia and Opisthokonta. The results revealed that the aquatic unicellular eukaryotes community is not affected by geographical distance. Moreover, in neither case of abundant and rare OTUs were found community similarity to be related either positively or negatively to geographic distance. Finally, the pattern between two sea points and vernal pools is not affected by geographical distance. Levins' niche index showed that ~91% of OTUs were habitat specialists and only 1% were habitat generalists. The great quota of specialists display that may environmental filtering is stronger than geographical distances to spatial turnover of eukaryotes community. Additional research is required to check the role of environmental factors in conformation community structure of aquatic unicellular eukaryotes.

P118.

Physiological and morphological studies of the medicinal fungi *Ganoderma lucidum* for bioprocess optimization and enhanced polysaccharide and mycelium production in submerged cultures

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Ganoderma lucidum produces several bioactive substances, of which the exocellular and endocellular polysaccharides are of outmost importance, due to their various pharmaceutical properties (immunostimulating, anticancer, antioxidant, anti-inflammatory, hypocholesterolemic, hypoglycemic, hepatoprotective, etc). The effect of bioprocess parameters (pH, agitation rate) and substrate composition (carbon and nitrogen sources) during submerged culture of the pharmaceutical mushroom *Ganoderma lucidum* were studied, with view to identifying optimal conditions for mycelium growth, exopolysaccharide and endopolysaccharide synthesis. Also, the size, shape, clumpiness and other morphological characteristics of mycelia, which vary from loose filaments to compact pellets, were studied in relation to the bioprocess parameters and associated with mycelium and polysaccharide biosynthesis. The results showed that optimal conditions for biomass growth and polysaccharide accumulation may be distinct. For instance, peptone was the best nitrogen source for mycelium growth and stimulated intracellular accumulation of polysaccharides, although inorganic nitrogen sources resulted in higher concentration of extracellular polysaccharides. Glycerol did not enhance polysaccharide formation, in contrast to other polysaccharide bioprocesses, and seemed to hinder growth and reduce the viscosity of the process medium. The latter (viscosity) is a reflection of both mycelium growth and exopolysaccharide concentration. A process pH of 5 was optimal for growth and endo- and exo-polysaccharide production in terms of maximum concentration, although the highest productivity of exo-polysaccharides was achieved at pH 6.5. The highest concentration of exo-polysaccharides (~38 g/l) occurred at 250rpm, which led to the highest viscosity of process medium (and apparently highest molecular weight of the biopolymers), while a milder agitation of 150 rpm was preferable for mycelium growth and endo-polysaccharide production. In general, under optimal conditions, total exo-polysaccharide concentration could surpass the concentration of total biomass, of which the endo-polysaccharides represented a fraction of ~10-30%. In terms of mycelium morphology, exopolysaccharide roduction was more associated with a clamped pellet morphology, while filaments of large area and diameter were associated with high biomass and endopolysaccharide production. Based on the above results, different process conditions can be chosen and different morphological criteria can be used for optimizing bioactive exopolysaccharide biosynthesis, or mycelium growth and intracellular accumulation of medicinal biopolymers, depending on the molecules that need to be isolated and their proposed use in foods or pharmaceuticals. For example, exopolysaccharides are easier to isolate from the process medium and used as purified pharmaceutical biomolecules, while filtered mycelia containing bioactive biopolymers in the cytoplasm could be better used as crude food supplements.

Keywords: *G. lucidum*, bioactive polysaccharides, submerged fermentation, fungal physiology, image analysis

P119.

Do Biochar and arbuscular mycorrhizal fungi cooperate in improving lettuce (*Lactuca sativa* L.) growth and nutrition in a saline soil?

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Biochar is considered to improve soil fertility and aid climate change mitigation through soil carbon storage. However, its effects in saline soils have received less attention. In this study the potential synergy between biochar and arbuscular mycorrhizal fungi (AMF) in a saline soil was investigated. A completely randomized pot experiment with lettuce (*Lactuca sativa* L.*)*, two levels of biochar (with or without) and three levels of inoculum (autoclaved, native soil, *Funneliformis mosseae*) and a Syros island saline soil, was conducted. Lettuce biomass, AMF and PGPR colonization and macro-micro nutrients concentration will be measured. Preliminary data indicate that the autoclaved soil treatment had the lowest lettuce leaf biomass production, while there was no interaction between AMF and biochar, except for P. Biochar can be beneficial for plants in saline soils and a potential aid in saline soil reclamation.