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ABSTRACT BOOK

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S1_OP01

PHYTOPLANKTON AND PARTICLE ATTACHMENT DRIVE SEASONAL ABUNDANCE AND PHOTOHETEROTROPHY OF AEROBIC ANOXYGENIC PHOTOTROPHS

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Aerobic anoxygenic photoheterotrophic (AAP) bacteria are a functional group characterized by their relatively large cell size, high metabolic activity, and rapid growth rates, traits partially enabled by their ability to harvest light energy via bacteriochlorophyll-a-containing type-II reaction centers. Despite their widespread occurrence and ecological relevance, contributing 1–23% of aquatic bacterial communities, the ecological dynamics and functional roles of AAP bacteria remain insufficiently understood. To better comprehend their role in the carbon cycle and microbial food webs, we conducted three sampling studies: (1) a two-year monthly sampling campaign in a freshwater lake, (2) three seasonal sampling campaigns in the Adriatic Sea, and (3) a three-year biweekly sampling in a freshwater lake.

Our findings indicate that light-derived energy enables AAP bacteria to reduce overall community respiration while enhancing carbon uptake rates. In marine coastal waters, we observed substantially more photoheterotrophy carried out by specific AAP bacteria in the particle-attached fraction. In freshwater systems, their abundance followed a recurrent seasonal pattern for 3 years, peaking during the spring phytoplankton bloom.

These results suggest that AAP bacteria play a pivotal role in aquatic carbon cycling by recycling both dissolved and particulate phytoplankton-derived organic carbon. Their ability to thrive on carbon-rich particles while minimizing respiratory carbon loss highlights their potential to channel organic matter efficiently through microbial food webs towards higher trophic levels.

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ENVIRONMENT

S1_OP02

DELVING INTO BENTHIC BACTERIAL COMMUNITY SHIFTS UNDER CONTRASTING C, N, P, AND S POLLUTION CONDITIONS IN VILLARRICA LAKE SEDIMENTS

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Benthic bacterial communities play a critical role in nutrient cycling and are highly responsive to environmental changes. This study examines how the physiology and composition of these organisms are modulated under contrasting conditions of C, N, P, and S pollution in Villarrica Lake (La Araucanía Region, Chile), an ecosystem increasingly exposed to anthropogenic pressures. Superficial sediment samples were collected in quadruplicate during the summer of 2023 from five lake sites (NL, PuB, PoP, SL, and VB), representing varying degrees of nutrient disturbance. Each sample was analyzed for total carbon (TC), total nitrogen (TN), total phosphorus (TP), total sulfur (TS), and organic matter (OM) content. Community-level physiological profiles (CLPP) were assessed through the Average Well Color Development (AWCD) using Biolog® microplates containing over 31 C, N, P, and S substrates. The abundances of nutrient-cycling genes (including chiaA, mcrA, nifH, amoA, nosZ, phoD, pqqC, soxB, and dsrA) were quantified via qPCR. At the same time, community composition and predicted function were investigated through 16S rRNA gene sequencing using the Illumina MiSeq platform. The most impacted site (VB) showed significantly higher total nutrient content and bacterial abundance (2.96×10^{11} ± 7.85×10^{10} gene copies g^{-1}) compared to less disturbed sites. However, sites under lower anthropogenic pressure (e.g., NL) exhibited greater functional gene diversity (ranging from 8.5×10^{1} to 2.4×10^{4} gene copies g^{-1}) and higher physiological versatility (AWCD: 0.60±0.02 to 1.23±0.03). Microbial taxa, such as Pseudomonadota, Actinobacteriota, and Bacillota (accounting for more than 15%), dominated the most polluted site, while Planctomycetota and Actinobacteria were less represented, at less than 8.9%. Functional inferences showed enhanced methanotrophy and sulfate respiration at VB, but lower nitrification and sulfur-respiration potentials. These findings reveal how contrasting nutrient-polluting conditions shape the structure and metabolic scope of benthic bacterial communities. Investigating these microbial shifts can enhance the early detection of sedimentary nutrient stress and inform more effective management of lake ecosystems.

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ENVIRONMENT

S1_OP03

THE ANALYSIS OF BACTERIAL COMMUNITIES IN FLOATING TREATMENT WETLANDS (FTW) WITH INTEGRATED MICROBIAL FUEL CELLS (MFCS)

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Constructed wetlands, particularly Floating Treatment Wetlands (FTWs), are engineered systems that use plants to purify polluted water. Integrating FTWs with Microbial Fuel Cells (MFCs) is a recent innovation for bioelectricity recovery from wastewater. Bacteria play a crucial role in MFCs by converting substrates into electrical energy. Therefore this study aims to explore bacterial communities in FTW-MFCs to understand the mechanisms of energy production. FTW-MFCs reactors were planted with iris and reeds. The MFCs reactors consisted of two electrodes connected to an external circuit. Samples of the biofilm that developed on both electrodes, and rhizospheres were used for DNA extraction. After the preparation of 16S rRNA gene libraries, the DNA was sequenced using Oxford Nanopore Sequencing technology.

In the MFC reactor, the most common genera in the anode biofilm were Allistipes sp., Affixifilum sp. and Loriellopsis sp., while the cathode was mainly colonised by Cereibacter sp. In the MFCs reactor with iris, the anode microbiome had a high abundance of Allistipes sp. Affixifilum sp. and Loriellopsis sp. while the cathode microbiome had a high proportion of Thiotrix sp., Desulfosporosinus sp. and Clostridium sp. In the MFCs reactor with reeds, mainly Desulfosporosinus sp., Aquipluma sp. and Simplicispira sp. were found at the anode. The cathode of the reactor with reeds showed a high abundance of Thiotrix sp., Cereibacter sp. and Hydrogenophaga sp.

The rhizospheric bacterial population in the FTW reactors (without electrodes) was more diverse than in the FTW-MFCs reactors (with electrodes). In the case of the MFCs reactor with iris, the rhizosphere showed a high abundance of Cereibacter sp., Thiotrix sp. and Rhodobacter sp. The abundance of the genus Cereibacter sp. was significantly lower in the reactor without electrodes, and a high abundance of Loriellopis sp. was also observed in this reactor. In the MFC reactor with reeds, Hydrogenophaga sp., Acidovorax sp. and Cereibacter sp. dominated.

In general, the investigated niches were occupied by different bacterial genera, many of which are photosynthetic bacteria that can generate electricity from light. The highest voltage was found in an MFCs reactor without plants, in which Cereibacter sp. was the most abundant.

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S1_OP04

WHEREDOGGO (WHERE DOES MY GENOME GO?): PLACING PROKARYOTIC LINEAGES ON THE TREE OF LIFE.

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In about two decades since metagenomics and single cell genomics became common practice in microbial ecology, a deluge of genomes from uncultivated taxa with immense taxonomic and metabolic novelty has become available. The genomic data were accompanied by taxonomic classification approaches that use phylogenomics with concatenations of tens to hundreds of marker proteins. However, with deeper phylogenies and more divergent lineages included in the tree of life comes a host of phylogenetic systematic errors (e.g., long branch attraction, composition bias). The automated tools used by most microbiologists for the taxonomic assignment of their new lineages do not take sources of error into account, which creates issues if these phylogenies are used to formulate evolutionary hypotheses.

To address these issues, we have created WhereDoGGo (Where Does my Genome Go?), a phylogenomics pipeline that aims to address different sources of error in the placement of prokaryotic lineages on the tree of life, while being user-friendly enough to be used by researchers with minimal bioinformatics experience.

As proof of concept, we determined the placement of the class Panguiarchaeia that consists of putative symbionts with small genomes and reduced metabolic capabilities. The often-erroneous placement of Panguiarchaeia in the Thermoproteota phylum results from a combination of fast evolution, amino acid composition bias, and homologous recombination of multiple marker proteins. Instead, the Panguiarchaeia branch within Asgardarchaeota, as sister to the Heimdallarchaeia class that includes Eukaryotes. We then calculated different measures of complexity across Asgardarchaeota lineages. While Panguiarchaeia are generally the least complex lineage, the Heimdallarchaeia are not always the most complex, as we would expect from their relationship to Eukaryotes. Instead, different Asgardarchaeota clades show different degrees of complexity for the various metrics, suggesting that the evolutionary trajectories for different types of complexity are decoupled.

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ENVIRONMENT

S1_OP05

GENOMIC STRUCTURAL VARIANTS IN ADAPTIVE EVOLUTION OF SOIL CYANOBACTERIA

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Structural variants (SVs) underpin various ecological and evolutionary processes. They are widely investigated in multicellular eukaryotes, yet their role in microbial adaptation remains poorly understood. Here, we explored the importance of SVs to mechanisms of local adaptation in the filamentous soil cyanobacterium Microcoleus. Using long-read sequencing technology, we resequenced 70 strains spanning 10 species at varying levels of genetic divergence across a continental gradient. We used a genotype-environment association approach to uncover SVs affecting local adaptation to specific climatic variables. Temperature, precipitation, and UV-B exposure emerged as major contributors to overall genomic variation across the landscape. We identified 142 core insertions and deletions associated with adaptive candidate loci, including genes involved in stress response, biosynthesis of secondary metabolites, and transposable elements. Specifically, core SVs were enriched in pathways regulating methylation, transposition, DNA recombination, and damage response. These findings shed light on the evolutionary importance of SVs in soil microbial populations and underline their role in niche differentiation and adaptive divergence between species.

S1_OP06 (FT)

SEASONAL DYNAMICS OF BACTERIAL COMMUNITIES IN DEEP KARSTIC LAKES ACROSS CONTRASTING CLIMATIC REGIONS IN CROATIA

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Lakes are vital sources of freshwater and sensitive indicators of climatic and ecological changes. Environmental changes, such as variations in temperature, salinity, dissolved organic carbon (DOC), and dissolved oxygen, quickly affect microbial communities in lakes. To monitor changes in bacterial communities in freshwater lakes from contrasting climatic regions, six natural Croatian lakes were studied over the course of one year. Two continental lakes (Kozjak and Prošće) and four Mediterranean lakes (Vrana Lake on Cres, Visovac, Oćuša, and Crniševo) were sampled monthly from March 2019 to February 2020. Water temperature, conductivity, salinity, pH, total dissolved solids, and dissolved oxygen were measured in situ with a Hach HQ40D Portable Multi Meter for Water. Total nitrogen and total organic carbon were analyzed using a Shimadzu TOC-VCPH analyzer. The bacterial communities of the lakes were characterized by amplicon sequencing of the hypervariable V4 region of the prokaryotic 16S rDNA. All statistical analyses were performed using R software (R Core Team, 2021). The results revealed differences in environmental parameters and bacterial communities between climatic regions, studied lakes, and seasons. Higher temperatures and conductivity were recorded in the Mediterranean lakes. The dominant bacterial phyla were Actinobacteriota and Proteobacteria, with more pronounced seasonal shifts in Bacteroidota, Planctomycetota, and Verrucomicrobiota observed in the Mediterranean lakes. In contrast, the continental lakes were dominated by Proteobacteria and Actinobacteriota, with less pronounced seasonal changes and greater microbial community stability throughout the year. Rare taxa also showed strong seasonal dynamics, especially in winter, with a notable increase in the Mediterranean lakes, particularly in Visovac, Oćuša, and Crniševo. Continental lakes, Kozjak and Prošće, also showed seasonal patterns among rare taxa, but to a lesser extent. Functional predictions using FAPROTAX revealed distinct metabolic profiles, with continental lakes showing functional stability, while Mediterranean lakes exhibited seasonal fluctuations in carbon, nitrogen, and sulfur cycling pathways. This study provides a comprehensive overview of bacterial seasonality in deep karst lakes, highlighting how geography and climate shape microbial diversity and function. The presented research represents one of the first high-resolution studies of microbial communities across both continental and Mediterranean lake systems in Croatia.

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S1_OP07 (FT)

DISRUPTING SOIL NITRIFICATION: AMMONIA-OXIDIZER PHYSIOLOGICAL RESPONSES TO SYNTHETIC AND BIOLOGICAL NITRIFICATION INHIBITORS

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Nitrification is a crucial process within the global nitrogen cycle but also a major contributor to nitrogen loss and environmental degradation in intensively managed agroecosystems. To mitigate these impacts, nitrification inhibitors (NIs) are employed to target ammonia-oxidizing microorganisms (AOM); however, the molecular mechanisms underlying their action remain poorly understood. Here, we investigate the physiological responses of key soil AOM groups, such as ammonia-oxidizing archaea (AOA) and bacteria (AOB), to both synthetic (SNIs) and biological (BNIs) nitrification inhibitors, with a particular focus on the model soil AOA Nitrososphaera viennensis. We developed and optimized dual RNA-protein extraction protocols and established species-specific time-of-harvest methods, based on the species generation time, to capture microbial responses at both transcriptional and translational levels. Time-series experiments revealed rapid gene expression shifts within 0.5 hours and delayed proteomic changes after 6 hours of NI exposure. Following this, we investigated the physiological and molecular responses of N. viennensis to seven NIs, including the SNIs DMPP and ethoxyquin and the BNIs zeanone, sakuranetin, MHPP, and 1,9-decanediol, along with allylthiourea serving as a positive control. Our findings revealed that NIs triggered distinct physiological and molecular responses in N. viennensis, suggesting variable and potentially overlapping biochemical targets among AOM. By integrating transcriptomic and proteomic data, we provide a clearer understanding of how NIs influence microbial function and nitrifier resilience under chemical stress. This work advances our understanding of microbial nitrogen cycling and informs targeted strategies for managing soil nitrifying communities to enhance nitrogen retention and promote ecosystem sustainability.

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S1_OP08 (FT)

PLANT SURVIVAL IN SERPENTINE SOILS: THE ROLE OF RHIZOSPHERIC MICROBIAL COMMUNITIES IN HEAVY METAL TOLERANCE

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Serpentine soils are harsh environments for most plants. Regardless of their great variability, these soils are characterised by a low calcium to magnesium ratio, limited availability in macronutrients (N, P and K) and particularly high concentrations of heavy metals. Serpentine soils, although covering about 1% of the Earth's surface, support more than 3000 endemic plant species. They are characterized by high rates of endemism, and distinctive floral composition, and differ in comparison to those thriving in neighboring non-serpentine soils. This study investigates the hypothesis that one of the strategies that assist plants to tolerate and accumulate heavy metals in serpentine soils is the associations with their native rhizospheric microbial communities that have co-adapted with these plant species.

Two serpentine-adapted, Ni-hyperaccumulating Brassicaceae plants, Noccea thymphaea and Odontarrhena chalcidica were sown in pots filled with a 4:2:1 v/v mixture of sand, vermiculite, and one of three soil types: (1) conspecific serpentine rhizospheric soil, (2) serpentine non-rhizospheric soil, and (3) non-serpentine rhizospheric soil from a non-hyperaccumulating Poaceae species. Three levels of Nickel were added in each treatment 0, 300, 1500 mg kg-1 in the form of $NiSO_4$. At harvest, plant height, number of leaves, fresh and dry biomass of roots and shoots were measured.

Preliminary analysis reveals that both soil origin and Ni concentration significantly affect plant growth, particularly in N. tymphaea. Statistical analysis demonstrated notable interactions between soil origin and Ni level. These findings support the hypothesis that rhizospheric microbial communities contribute to plant performance under high concentrations of Ni, potentially influencing nutrient uptake and heavy metal tolerance. Ongoing ICP-OES analysis and potential soil enzyme activities will provide insights into nutrient and metal accumulation patterns.

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S5_OP34

GENOMIC AND ECOLOGICAL CHARACTERIZATION OF NOVEL CULTIVATED BACTERIOPHAGES INFECTING ABUNDANT BACTERIAL FRESHWATER LINEAGES

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Freshwater bacteriophages remain poorly understood due to the limited cultivability of many abundant hosts and the low host prediction rate (~20%) of current metagenomic methods; combined with metaviromics' limitations in host-assignment or detection temporal patterns, the phage-host dynamic and their ecological roles remain largely unknown. In this context, our newly established culture collection, comprising some of the most abundant freshwater bacterial lineages (e.g., Planktophila, Flavobacterium, freshwater SAR11), enabled the isolation and sequencing of over 200 novel phages from the Římov Reservoir (Czech Republic). These phages targeted 16 bacterial species from six phyla. Phylogenetic analysis of the terL gene from the isolated phages together with their closest cultured relatives revealed that most belong to newly cultured families. We placed special focus on phages predicted to infect the phylum Actinomycetota, particularly the global abundant genome-streamlined genus Planktophila. The 109 isolated phages were classified into 1 family and 12 genus-level clusters. Several clusters exhibited high recruitment across geographically distant metagenomic datasets: nearly 20-fold genome coverage was observed in an Asian lake and 1.2-fold in North American samples, highlighting a rare and potentially global distribution among freshwater phages. Other phages showed recurrent seasonal presence in the Římov reservoir. Interestingly, several Planktophila-infecting phages lacked the whiB gene, a hallmark gene of phages infecting Actinomycetota. Our findings provide new insights into the population structure, behavior, and ecological functions of freshwater bacteriophages and highlight the benefits of combining culture-based methods with environmental meta-omics.

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S5_OP35

NOVEL INSIGHTS IN THE DIVERSITY OF TUBER SPECIES IN GREECE: TWO NEW SPECIES FOR SCIENCE, 22 WELL-DEFINED TAXA AND 19 UNDESCRIBED PHYLOSPECIES ARE REVEALED

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Members of the genus Tuber (Ascomycota, Pezizales) include emblematic truffle-producing fungi, with significant economic, social and environmental impacts. Since Tuber species form obligate ectomycorrhizal associations with various plants, they are essential factors in the conservation and regeneration/restoration of natural ecosystems. Irrespectively of whether ascomata are being harvested from the wild or from truffle orchards, they provide significant additional income since they are used (fresh or processed) as food of high gastronomic value. Despite the rich biogeographical history of the Balkan peninsula in terms of speciation events, relatively few data are available regarding truffles diversity in this region. In the frame of the present study, more than 400 collections of Tuber ascomata originating from various areas of Greece were obtained with the collaboration of citizen scientists and truffle hunters, and were examined by employing a combination of morphoanatomic, molecular and phylogenetic approaches. As a result, the existence of over 40 phylogenetic species was evidenced, as opposed to the 26 species previously known (only 15 of them were molecularly confirmed). More specifically, two new species for science were described, i.e., Tuber aereum and T. leptodermum [1,2], while the rest of the findings included 22 well-defined taxa, seven of which (T. anniae, T. buendiae, T. conchae, T. dryophilum, T. monosporum, T. regianum, and T. zambonelliae) constitute first national records [2]. In addition, 19 distinct phylogenetic entities could be potentially described as new species. This study significantly adds to the knowledge of the diversity of genus Tuber in Greece, and together with the other pertinent scientific results paves the way for the improvement of existing (and/or the development of novel) applications of truffles.

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EXPLORING NOVEL MICROBIAL LINEAGES IN ANOXIC LAGOON SEDIMENTS

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Coastal lagoons are vital ecosystems and their microbial communities are drivers of biogeochemical processes. This study examined the microbial diversity within the anoxic sediments of Etoliko Lagoon, a unique environment in Western Greece characterized by anoxia, high sulfate concentrations, and a persistent thermocline. Using next-generation amplicon sequencing prokaryotic and eukaryotic communities were characterized, revealing a significant proportion of unassigned sequences across all domains, strongly suggesting the presence of potentially novel phyla and other taxonomic levels.

Among the bacteria, 205 out of 861 Operational Taxonomic Units (OTUs) remained unclassified, particularly at finer taxonomic resolutions. Sequencing of the full-length 16S rRNA gene improved taxonomic assignment but still revealed a considerable portion of unknown bacteria, emphasizing the untapped microbial diversity. Similarly, for the archaeal community, 129 out of the 388 total OTUs were unassigned. Eukaryotic analysis was challenging, with nearly half of the OTUs remaining unassigned (178 out of 344), indicating a vast unexplored realm of microbial eukaryotes, including potentially novel "dark matter fungi" and protists.

To identify putative new taxonomic levels, unassigned reads were grouped together at a similarity threshold of 80%, and phylogenetic analysis was performed. The sequences were aligned with well-curated sequences to assign them to novel taxonomic levels. This analysis revealed many putative new phyla across all microbial kingdoms. Up to four new putative phyla were identified within the bacteria. For each cluster formed by grouping the unassigned OTUs, one representative sequence was used to find the closest relative using the NCBI database. The percentage identity between unidentified sequences and the database ranged from 86% to 75%. The number of reads per cluster varied from 263 to 11730 reads per new candidate phylum. The presence of new phyla and unknown taxa in Etoliko Lagoon indicates unique adaptations and metabolic capabilities. These groups of microorganisms could have essential but undefined roles in the biogeochemical cycles of lagoons. This research emphasises Etoliko Lagoon as a reservoir of microbial novelty with significant implications for environmental microbiology and biogeochemistry. Further exploration using metagenomic, metatranscriptomic, and cultivation-based methods is essential to clarify the potential and impact of these newly identified microbial lineages.

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A THOUSAND METERS DEEP: VERTICAL PROFILING OF THE SUBTERRANEAN MICROBIOME OF GOURGOUTHAKAS CAVE

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Caves are lightless environments with stable conditions of temperature and humidity, low primary productivity and allochthonous nutrient sources. They are a niche of unique biodiversity and adaptations, such as the cave-adapted fauna, i.e troglofauna, which has been studied for centuries. Yet, the subterranean microbiome remains less explored (1). Here, we characterize the microbiome of Gourgouthakas Cave (Lefka Ori, Crete, Greece) at a depth of 1100 m, currently the deepest of Greece and ranked 78th worldwide. During the "Gourgouthakas 2022" expedition (2), nine samples were collected along the cave's depth, yielding in total 820 bacterial isolates. Of these, 360 strains have been taxonomically identified, and five have been assembled with whole genome sequencing using an approach of long and short reads. Pseudomonas was the most prevalent genus (156 isolates), peaking at -900 m depth, followed by Bacillus (57), particularly abundant in deeper zones. Other genera included Stenotrophomonas, Paenibacillus, and Brevibacillus. Diversity increased with depth, highlighting microbial stratification. Selected strains exhibited antifungal and anti-bacterial activity in vitro and in planta. In specific, several strains were tested for in vitro inhibition assay against the Gram-negative pathogenic bacteria Xanthomonas campestris pv. campestris, Paracidovorax citrulli, Ralstonia solanacearumand the Gram-positive pathogen Clavibacter michiganensis, as well as the Verticillium dahliae fungus and the Phytopthora nicotianae oomycete. Interestingly, all the identified Brevibacillus strains showed an inhibition of the growth of at least one pathogen and in some cases of all tested pathogens. These findings reveal the unexplored biotechnological potential of cave microbiomes and their relevance to applied microbiology.

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MOLECULAR TINKERING AND FUNCTIONAL CONSERVATION OF REPLICATION PROTEIN A IN ARCHAEA

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In all lifeforms, single-stranded DNA-binding proteins (SSBs) are essential components of the DNA replication machinery that primarily protect the exposed single-stranded DNA (ssDNA). They play a vital role in nearly all aspects of DNA metabolism and also act as platforms onto which DNA-processing enzymes can assemble. In Bacteria, this role is fulfilled by the homotetrameric SSB. In Eukarya and some Archaea, the ssDNA binding function is primarily achieved by the heterotrimeric Replication Protein A (RPA) complex. Composed of three protein subunits, RPA contains multiple OB-folds with different DNA-binding properties. Over the past two decades, structural and biochemical studies have mostly been focused on the eukaryotic RPA.

Although archaeal chromosomes are similar to bacterial ones, their replication machinery shares greater resemblance with eukaryotic systems, making Archaea valuable models for unravelling the function and evolution of complex eukaryotic replication complexes. Despite recent studies focusing on the structure and function of heterotrimeric archaeal RPA complex, most archaeal lineages exclusively encode atypical monomeric (RPA1-like) and/or dimeric (RPA1/RPA2) RPAs. It is unknown how many alternative architectures have emerged and whether they preserve the heterotrimeric RPA's functionality. No structure of an atypical archaeal RPA has been determined so far.

Here, we incorporate evolutionary thinking into biochemistry and integrated structural biology to study the alternative architectures of Methanosarcina acetivorans RPAs (MacRPAs) and their resulting functional repertoire. We have performed a phylogenomic analysis demonstrating the origin of heterodimeric RPAs before the ancestor of Methanosarcinia, along with subsequent gene losses and domain substitutions. Biochemical assays with ssDNA and structural predictions show that MacRPAs have maintained the DNA-binding mechanism and protection despite tinkering through structural rearrangements and missing subunits.

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BACTERIAL DIVERSITY CHANGES AFTER DIESEL OIL ADDITION TO SEAWATER MICROCOSMS

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Marine oil spills are considered an environmental pollution problem worldwide. Several microbiota can degrade diesel oil since they can use this compound as a sole carbon source. In this study we examined the effect of diesel on sea-water bacterial communities, as well as their potential recovery, by using conventional and molecular microbiology techniques. Microcosms of sterile seawater (C) and sterile seawater with low diesel concentrations (0.5%) (D) using seawater as inoculum in a final 1/10 dilution were prepared in triplicates and were incubated at 28 0C for eight days. Samples for cfu counts were collected from C and D microcosms every two days and were spread on Marine Agar and Marine Agar with diesel plates respectively, followed by colony counts 24 hours later. After eight days, corresponding at the end of the stationary growth phase, DNA extraction from both colonies (P) and microcosms' water (F) was performed. In parallel, new microcosms cross inoculating water from D microcosms to sterile seawater (DC) and from the C microcosms to low diesel sterilized seawater (CD) were prepared and the same experiment was performed for eight days. Our results indicated higher diversity in all F samples compared to P, indicating for once more the inability to isolate and cultivate the majority of microorganisms found in the environment. The C microcosms were characterized by the prevalence of Paracoccaceae, Flavobacteriaceae and Alteromonadaceae communities, while in the D microcosms bacterial communities were mostly dominated by different Alteromononadaceae representatives and more specifically from the genera Glacieola and Alteromonas that are known for degrading diesel hydrocarbons. Results from DC microcosms compared to D ones, indicated decrease in diversity after diesel removal. Paracoccaceae presence was enhanced, even exceeding initial concentrations and Alteromonadacae, the most favored group after diesel addition, showed only minor declines. Flavobacteriaceae never recovered in any of the experiments. Results from CD microcosms were very similar to results from D microcosms indicating that responses of established control communities were similar to responses of untreated seawater. Overall, this study showed that several marine microbes might be able to degrade diesel oil, while communities can partially recover after cleaning.

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THE PATCHWORK OF NUCLEOID-ASSOCIATED PROTEINS IN ARCHAEA

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Genomic DNA is one of the most important molecules in living organisms and viruses and it is structured by architectural proteins. Eukaryotes encode histones and Bacteria encode nucleoid associated proteins (NAPs). Archaea can use either or both histones and NAPs. NAPs are small basic proteins that bridge, wrap, or bend the DNA. HU and IHF (the HU/IHF superfamily) are among the most abundant NAPs in Bacteria and they are also sparsely distributed in Archaea.

HU homologs usually form homodimers that bind to DNA without sequence specificity. IHF is mostly heterodimeric and recognizes and binds to a 13-nucleotide consensus sequence. These proteins are responsible for bending DNA, maintaining DNA supercoiling and compact structure, while participating in key processes, including replication, recombination, gene regulation, and repair in Bacteria. Their functional repertoire in Archaea is not widely studied with the exception of the HU of Thermoplasma acidophilum that has been recently shown to function as an archaeal histone analog, in lieu of absent histones.

Here, we set out to determine the evolutionary origin, functional, and structural repertoire of HU/IHF proteins in Archaea. Through a phylogenomic approach, we found 327 HU/IHF homologs across 16 archaeal phyla. All homologs were originally acquired from Bacteria through multiple independent horizontal gene transfer events. Most transfers were very recent, resulting in only one archaeon acquiring one HU/IHF.

Exceptions exist in the Thermoplasmata, Halobacteria, and some DPANN superphylum lineages, where HU/IHF genes were each acquired through a more ancient transfer from Bacteria, followed by vertical inheritance and intradomain transfers. The Halobacteria homologs in particular correspond to a unique monomeric HU/IHF resulting from a duplication of the horizontally acquired gene, followed by the two copies fusing together and a with a segment encoding an N-terminal Twin-Arginine signal peptide. To further explore the function of the monomeric HU/IHF we have begun to biochemically characterize and solve the structure of the homolog in Halorubrum aquaticum.

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BACTERIAL FUNCTIONAL GROUPS: CLASSIFICATION AND CULTIVATION CHALLENGES IN FOOD WEB RESEARCH

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Understanding microbial contributions to aquatic food webs is critical, yet functional classification of bacteria remains underdeveloped compared to phytoplankton and zooplankton. Functional assignments are constrained by scarce trait-based data and poorly understood interactions with other trophic levels; cultivation of representative strains – especially at the biomass levels needed for experiments – is often unsuccessful or ecologically uninformative; and their abundance is difficult to quantify in absolute terms. As part of a broader effort to evaluate the predictive power of network models, our work focuses on the role of bacteria in freshwater mesocosm communities. We aim to selectively enrich defined functional groups and track changes in community composition and ecosystem responses following perturbation. These efforts are designed to ultimately allow comparison between observed outcomes and model predictions. In parallel, we explore unresolved issues in defining bacterial roles such as nutrient cycling, decomposition, and predator susceptibility. Our work seeks to stimulate discussion around the methodological and conceptual challenges of integrating bacteria into food web experiments.

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UNIQUE MICROBIAL COMMUNITY OF A SHALLOW SODA LAKE (LAKE VELENCE, HUNGARY) REVEALED THROUGH CO-OCCURRENCE NETWORK ANALYSIS

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Lake Velence is one of the largest soda lakes in the Carpathian Basin, characterized by a distinctive microbial community shaped by its alkaline pH and brackish salinity. Ongoing global warming makes the lake increasingly vulnerable to water-level decline, potentially leading to more frequent ecological disturbances such as fish kills. To enhance our understanding of microbial processes under these changing conditions, we conducted biweekly monitoring throughout 2024.

Sampling efforts included measurements of water chemistry (nutrients, pH, and other physico-chemical parameters) and indicators of microbial biomass and activity (ATP concentrations). In parallel, we applied high-throughput environmental DNA (eDNA) metabarcoding, targeting 16S and 18S rRNA gene amplicons, to profile the diversity of prokaryotic and eukaryotic microorganisms. This multi-faceted approach allowed us to characterize Lake Velence's microbial community structure and function with unprecedented resolution.

The microbial communities were dominated by bacterial taxa including Balneolaceae, Parcubacteria, Pseudohongiella, and Rhodobacteraceae. Among microeukaryotes, Ochromonadales, Acanthoecida, and various ciliates were most prevalent. Network analyses highlighted specific taxa potentially acting as keystones, critical to maintaining ecological stability. Additionally, network topology metrics correlated significantly with environmental parameters, demonstrating sensitivity to ecological fluctuations.

Variability in network topological properties revealed clear patterns in response to environmental gradients. Certain indices exhibited linear trends linked to physico-chemical parameter shifts, underscoring the potential of microbial network characteristics to serve as indicators of ecological change. Given the vital role microbial communities play in aquatic ecosystem health—and their responsiveness to environmental stressors—these findings emphasize the importance of detailed microbial monitoring for predicting and managing ecological disturbances.

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FORECASTING MICROBIAL TRAJECTORIES WITH DEEP LEARNING

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In most environments, microorganisms coexist within complex communities, interacting with each other and their environment. These communities play fundamental roles in ecological and physiological processes, such as the degradation of organic matter or the health of humans and animals. Predicting the trajectories of microorganisms over time is crucial for understanding community function. In this study, we predict microbial trajectories based on the current state of the community with a deep learning framework.

We focus on forecasting individual microbial trajectories, using hundreds of real-world longitudinally sampled microbiome datasets as training data. We develop separate deep learning models for each microbe, trained to predict changes in the abundance of a given microbe at the next time point. Hyperparameters are optimized for each model independently.

Our models elucidate which microbes increase or decrease in abundance over time within a microbial community. This innovative approach can find direct applications, such as disease progression prediction caused by individual microorganisms, or understanding the mechanisms of invasion of new microbial species in a stable community.

Our models provide insights into the impact of time on the evolution and trajectory of microbial communities, benefiting predictive microbiome modelling.

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FOREST DISTURBANCES AND MANAGEMENT SHAPE MICROBIAL COMMUNITIES ACROSS DIVERSE EUROPEAN FORESTS

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Soil microbial communities are critical to forest ecosystem resilience, yet their responses to forest management and natural disturbances remain insufficiently understood at larger spatial scales. Here, we present an overview of findings from the LifeSystemic (https://www.lifesystemic.eu/) and HoliSoils (H2020, https://holisoils.eu/) projects, where microbial responses to natural disturbances (e.g. windthrow, wildfire) and forest management (e.g. clearcutting, thinning) were studied across 26 forests in 11 European countries. These sites cover a broad environmental gradient, including boreal peatland, temperate and Mediterranean forests, encompassing different disturbance types and management intensities. Sampling was carried out with standardized protocols for collecting soils for microbial and biogeochemical analyses. Amplicon sequencing (16S rRNA and ITS2) was used to assess bacterial and fungal community composition, complemented by soil chemistry analyses (C, N, pH, available P). At selected sites, metagenomics, microbial biomass and soil respiration measurements were also conducted. In some forests, sampling over three consecutive years allowed insights into temporal shifts in microbial communities.

Ectomycorrhizal (ECM) fungi showed consistent declines in disturbed forests, with relative abundances often more than double in undisturbed controls compared to clearcut sites. Bacterial community composition remained comparatively stable, though metagenomic profiles indicated functional shifts under severe disturbances. Natural disturbances in managed forests further amplified microbial responses. Root exclusion via trenching revealed significant reductions in microbial biomass and ECM fungi, underlining the role of plant-microbe interactions. Close-to-nature management resulted in minimal microbial disruption, emphasizing its value for sustaining belowground biodiversity and ecosystem functioning across European forests.

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PROPHAGE DIVERSITY AND METABOLITE ANALYSIS OF VIRAL INDUCTION IN LYSOGENIC GROUNDWATER BACTERIA

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Viral lysis of bacterial hosts results in the release of metabolites and cell lysates that promote energy and material fluxes, a process known as the viral shunt. Viruses can lyse their host immediately after infection (lytic cycle) or replicate their genome alongside the host genome (lysogenic cycle). Under certain conditions (e.g., environmental stress), the integrated viral genome (i.e., the prophage) can be induced to enter the lytic cycle. While these processes have mainly been studied in marine systems, the role of prophage diversity and virus-driven cell lysis in terrestrial food webs, particularly in groundwater environments, is not well understood.

We investigated the diversity of prophages in lysogenic groundwater bacteria and the release of dissolved organic matter (DOM) following prophage induction. Induction tests with mitomycin C on 146 groundwater bacteria indicated that 37 strains had inducible prophages; phage production was confirmed by counting the released virus-like particles (VLPs). Using nanopore long-read sequencing technology we identified 120 complete or partial phage sequences in genomes of the lysogenic groundwater bacteria, with some isolates harboring up to 13 prophages. All phage sequences belonged to 59 unique vOTUs, all of which were classified within the Caudoviricetes. Annotation of genes in the prophage genomes revealed several potential auxiliary metabolic genes (AMGs) that code for essential microbial functions in oxic and anoxic groundwater systems such as nitrification, dissimilatory nitrate reduction to ammonium, and denitrification.

We further analyzed the dissolved organic matter (DOM) released after prophage induction and lysis using direct injection mass spectrometry (DI-MS). Mass spectrometric analysis of the DOM showed consistent metabolic responses across many isolates, indicating that 766 out of 11,291 molecular species consistently increased or decreased following induction. Variance of partitioning indicated that 34% of the metabolic responses can be attributed to species-level factors or species affiliation, and that 2% of the differences can be explained by the treatment, regardless of species identity. The combined analysis of prophage genomes, metabolites and cell lysates may provide a basis for identifying potential biomarkers of viral lysis and offer new insights into the lysis-driven nutrient transfer in groundwater systems.

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METAGENOMIC RECONSTRUCTION AND FUNCTIONAL ANALYSIS OF MICROBIAL GENOMES FROM RED TIDE EVENTS IN A COASTAL ECOSYSTEM

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Harmful algal blooms (HABs), such as red tides, are complex ecological phenomena that significantly alter marine microbial community function. The aim of this work is to present a comprehensive and robust bioinformatic pipeline to retrieve Metagenome Assembled Genomes (MAGs) from red tide events caused by Noctiluca scintillans in Thermaikos Bay, Greece. Seven environmental DNA (eDNA) samples were collected during different red tide events, and high-throughput shotgun sequencing was implemented. The optimized pipeline to retrieve high quality MAGs consisted of the following steps: Initial raw reads were subjected to quality assessment with FastQC, followed by adapter removal and trimming of low-quality bases with Fastp, ensuring high-confidence input data for downstream analyses. Then, metagenomic contigs were generated using MEGAHIT, a fast and memory-efficient assembler optimized for large and complex metagenomes. The contigs were grouped into draft genomes utilizing MetaBAT2 and MaxBin2, which implement distinct algorithmic strategies for binning. Results from both tools were integrated using DAS Tool, selecting the most representative bins. The quality of resulting MAGs was evaluated using CheckM2, which overcomes limitations of marker gene-based methods by using genome-wide features and neural network-based models trained on diverse taxonomic groups, thereby offering more accurate and generalizable predictions, especially for uncultivated lineages. Only the MAGs meeting the standards of >50% completeness and <10% contamination were retained. However, in non-red tide marine samples a completeness of at least 80% is recommended as a minimum threshold for high-fidelity MAG retrieval. Taxonomic classification was performed using GTDB-Tk, a robust tool that leverages the updated GTDB reference database to assign prokaryotic genomes within a standardized phylogenomic framework. The retrieved MAGs belonged to the genera Crocinitomix (family Crocinitomicaceae), Planktomarina, HIMB11, and LGRT01 (family Rhodobacteraceae), all members of the dominant marine phyla Bacteroidota and Alphaproteobacteria. Finally, MAGs and contigs were annotated using Prokka, a specialized annotation pipeline for prokaryotic genomes. Genes involved in carbohydrate degradation were shared across samples, highlighting glycolytic and pentose phosphate pathway activity. Genes related to ammonium regeneration and nitrogen turnover were also identified, supporting the involvement of the retrieved MAGs in nitrogen recycling, which is essential in N. scintillans red tides persistence.

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SEASONAL SEDIMENT WATER FLUX DYNAMICS IN COASTAL AQUACULTURE PONDS: IMPLICATIONS FOR PRIMARY PRODUCTION

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Traditional extensive aquaculture ponds systems offer high value products and are an integral part of the sociocultural heritage in coastal areas, such as the Bay of Cadiz. In these ponds, the growth and quality of fish and oysters depends on the in-situ productivity and overall food availability. In order to improve the management of these systems, it is important to understand which factors affect sediment-water fluxes, microalgal productivity, and functioning of the system. To address this, we conducted a high-resolution, year-long study in an oyster (OY) and a fish (FI) shallow aquaculture pond culture system in Cádiz Bay, Spain. Temperature in both ponds peaked in late spring and summer, with FI consistently warmer than OY ($p < 0.001$). On average, OY was net autotrophic, exhibiting a 29% higher oxygen fluxes under light and 30% lower under dark compared to FI. In contrast, FI was net heterotrophic. Chlorophyll a concentration in the sediment showed no significant differences between ponds or clear seasonal peaks, remaining relatively stable throughout the year. Interestingly, in OY, the spring– summer microphytobenthos (MPB) biomass—reflected by high Chl c:a—, coincided with reduced oxygen fluxes under light-suggesting possible photoinhibition, a phenomenon noted in other southern European estuaries. Nutrient fluxes were generally low and exhibited no clear temporal or spatial trends. Sediment bacterial abundance did not show seasonal coupling with oxygen fluxes. In the water column, bacterial abundance increased during summer as cell size decreased, and the maximum abundances of picoplankton groups-including two eukaryotic groups and Synechococcus-varied throughout the year. Despite pronounced climatic seasonality, the system as a whole did not display the expected variability in benthic fluxes. Oxygen flux dynamics proved challenging to explain based on individual biological or environmental drivers, underscoring the nonlinear and complex nature of benthic metabolism. This complexity constrains our ability to predict ecosystem responses to climate change, highlighting the need for further research to better understand and anticipate shifts in benthic flux dynamics under future climatic extremes

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S9_OP72 (FT)

REVEALING NOVEL MICROBIAL DIVERSITY ACROSS THE HELLENIC VOLCANIC ARC THROUGH METAGENOME-ASSEMBLED GENOMES (MAGS)

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The Hellenic Volcanic Arc, a region consisting of several active volcanic centers along the South Aegean, represents a unique pilot site for investigating microbial responses to multiple co-occurring stressors, since the marine environment of the area is simultaneously influenced by hydrothermal fluxes and several anthropogenic activities. We aimed to explore the taxonomic and functional microbial diversity of these multi-stressed coastal environments using shotgun metagenomic sequencing of water and sediment samples and targeting the reconstruction of metagenome-assembled genomes (MAGs). Bioinformatic analysis of 11 water and 5 sediment samples from hydrothermal and radon-rich environments produced 181 medium- and high-quality MAGs, including 66 high quality ones, meeting the criteria of ≥95% completeness and ≤5% contamination. Taxonomic classification of the high-quality MAGs revealed novel microbial diversity, with several MAGs lacking valid taxonomic classification or being only partially assigned across taxonomic ranks. A wide range of bacterial phyla was revealed, including Pseudomonadota (dominated by Gammaproteobacteria-related MAGs), Bacteroidota, Thermodesulfobacteriota, and Campylobacterota along with four candidate or provisionally defined phyla, as designated in the Genome Taxonomy Database (GTDB). The retrieved groups are known to include metabolically diverse organisms adapted to hydrothermal environments. For example, Zetaproteobacteria belonging to Pseudomonadota and Thermodesulfobacteriota are often associated with sulfur and hydrogen metabolism, thermophily and tolerance to redox variations. The functional annotation of the MAGs revealed diverse metabolic capabilities, reflecting adaptation to the hydrothermal environment. Pathways, related to sulfur oxidation, carbon fixation, methane and nitrogen metabolism, link these microorganisms to key biochemical processes within the hydrothermal environment. Additionally, genes associated with polyamine biosynthesis, terpenoid production, and other secondary metabolites suggest microbial strategies for coping with environmental stress. This work sets the stage for the detection and formal description of novel microbial genomes and enhances our understanding of microbial community adaptations to the multi-stressed coastal systems of the Hellenic Volcanic Arc.

Acknowledgments

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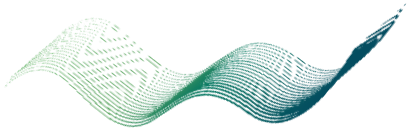

S9_OP73 (FT)

LONG-TERM ADAPTATION OF AN ABUNDANT MARINE DIATOM TO GLOBAL CHANGE DRIVERS

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Anthropogenic climate change is reshaping marine microbial ecosystems, yet the role of evolutionary history and biogeographic origin in modulating phytoplankton adaptation remains poorly understood. We investigated how long-term exposure to elevated temperature and CO_2 influences the ecological fitness of the coastal diatom Skeletonema marinoi under simulated future ocean conditions.

Two genetically distinct strains of S. marinoi - isolated from northern (S8) and southern (S16) Norway - were exposed for 12 months to combinations of temperature (13°C, 19°C) and pCO_2 (400 ppm, 1000 ppm), reflecting present-day and end-of-century scenarios. These evolved strains were then inoculated into controlled mesocosms containing natural seawater, where temperature and CO_2 levels replicated several environmental future scenarios.

Over a two-week period, we monitored chlorophyll dynamics and characterized community composition via 16S and 18S rRNA gene metabarcoding. This experimental setup enabled us to assess whether prior adaptation to global change stressors enhanced performance in complex communities and whether geographic origin constrained adaptive potential.

Our results reveal pronounced strain-specific differences in ecological performance and interaction patterns, highlighting how evolutionary background shapes competitive dynamics and community outcomes under environmental change. These findings emphasize the need to integrate microbial evolutionary processes into ecosystem models to improve the prediction of future ocean functioning.

Acknowledgments

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S2_OP09

DECIPHERING THE BIOLOGICAL ROLE OF OXIDATIVE CHITIN-ACTIVE CAZYMES IN THE LIFE CYCLE OF THE PLANT PATHOGEN USTILAGO MAYDIS.

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The fungal cell wall (FCW) plays a crucial role in survival and adaptation of fungi to various environmental conditions (1). It is mainly composed of structural polymers such as glucans and chitin, which can be enzymatically modified by carbohydrate-active enzymes (CAZymes; (2)) during the fungal life cycle. While the functions of some synthesis and hydrolytic CAZymes on FCW polysaccharides has been investigated (3,4), the function of oxidative CAZymes remain largely unknown. Here, using the plant pathogenic fungus Ustilago maydis as a biological model, we studied the biological role of some of its oxidative enzymes active on chitin (as for its unique lytic polysaccharide monooxygenase (LPMO; 5) or its two chitooligossacharides oxidases UmAA7A and UmAA7B) during plant infection. To this end, we used wet enzymology to probe the enzymes substrate specificity (6) and reverse genetics (using CRISPR-Cas9) to investigate their involvement in fungal growth and plant infection.

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S2_OP10

SYNERGISTIC INTERACTIONS BETWEEN INTRODUCED AND NATIVE PLANT-BENEFICIAL MICROORGANISMS IN THE RHIZOSPHERE IMPROVE MAIZE PERFORMANCE AND RESILIENCE UNDER DROUGHT CONDITIONS

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Inoculants that contain plant-beneficial microorganisms (plant-BMs) can enhance crop performance. However, their observed effects under field conditions can be inconsistent, suggesting that environmental factors or farming practices may influence their efficacy. To investigate how these conditions influence plant-BM inoculation effects, we conducted a field inoculation experiment using a plant-BM consortium comprising two bacterial strains (Bacillus atrophaeus ABi03, Pseudomonas sp. RU47) and one fungal strain (Trichoderma harzianium OMG16). We root-inoculated maize (Zea mays cv. Benedictio) grown under various agricultural practices, collected rhizosphere samples, and performed 16S rRNA gene and ITS amplicon sequencing. Inoculation significantly increased plant biomass and iron uptake in 2020 during severe drought compared to the average precipitation, but not in 2021. Plant-BM inoculation increased the relative abundance of several amplicon sequence variants (ASVs), most classified as Comamonadaceae spp., in 2020 but not in 2021. Therefore, we hypothesized that inoculated plant-BMs improved maize resilience during drought conditions via interactions with resident plant-BMs in the rhizosphere. To test this hypothesis, we isolated resident rhizosphere plant-BMs from maize grown in the same field. We selected a single isolate based on a) its sequence homology to the differentially abundant Comamonadaceae ASVs, b) its in vitro plant-beneficial traits, and c) its capability to grow in filter-sterilized cultures of ABi03 and RU47. We performed a greenhouse inoculation experiment on maize by re-introducing the selected isolate in the maize rhizosphere in the presence or absence of the bacterial plant-BM inoculum (ABi03 & RU47). Combining the resident isolate with the inoculants improved maize resilience under drought, but not under well-watered conditions. Moreover, the plant-BM inoculants promoted the relative abundance of the re-introduced taxon under drought, as inferred by 16S rRNA gene amplicon sequencing and statistical modeling. In conclusion, drought promotes the synergistic positive effects of inoculated and resident rhizosphere plant-BMs on crop resilience.

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S2_OP11

WHEN TRADITIONAL CULTURING MET WITH MODERN MALDI-TOF MS TO UNCOVER THE SO-CALLED RUMEN 'UNCULTURABLES'

Theano Stoikidou[1], Arthur Bourguet[2], Milka Popova[2], Ziming Wu[1], James Pickup[1], Fernanda Godoy Santos[1], Gillian Scoley[3], Diego Morgavi[2], Chris Creevey[1], Sharon A. Huws[1]

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Rumen microbiologists have joined efforts to isolate and characterise novel rumen microbes, employing both MALDI-TOF Mass Spectrometry (MS) and sequencing for microbial identification. MALDI-TOF MS is a rapid, cost-effective, and high-throughput tool, enabling efficient screening of large microbial isolate collections. However, its identification accuracy relies on the reference database's comprehensiveness. Our work delivers a three-part solution: an expanded culture collection of rumen microbes, a rumen-specific MALDI-TOF MS reference database, and a standardised R pipeline for spectral preprocessing and clustering, enhancing rumen microbiome research. We used a culturomics approach to broaden cultured rumen bacterial diversity and isolate previously uncultured strains. Rumen fluid was collected from conventional and intensively managed dairy cattle at 12, 28, and 49 weeks of age. Two isolation methods (direct plating and dilution-to-extinction) and two media types (Hobson's M2 and BHI with rumen fluid) were employed under anaerobic conditions at 39°C. Isolates were identified via Sanger sequencing of the 16S rRNA gene. A custom MALDI-TOF MS database was developed using the obtained bacterial isolates, four Methanobrevibacter (M. ruminantium, M. smithii, M. wolinii, M. formicicum), and one Methanomicrobium (M. mobile) species. An optimised R pipeline was designed for spectral preprocessing, consensus spectrum generation, and hierarchical clustering. The culturomics approach yielded 331 pure rumen bacteria. A BLAST search against the Greengenes2 database showed that isolates belong to 9 phyla—Actinomycetota, Bacillota A, Bacillota C, Bacillota I, Bacteroidota, Desulfobacterota, Fusobacteriota, Pseudomonadota, and Spirochaetota—encompassing 59 species. Notably, 62 isolates showed <97% similarity, suggesting they may represent novel rumen strains and help close gaps in microbial taxonomy and ecology. The development and construction of a custom MALDI-TOF MS rumen database increased identification accuracy from 18% to 97%. The R pipeline enabled spectral processing, including baseline correction, noise reduction, peak detection, and consensus spectrum generation. Hierarchical clustering revealed taxonomically coherent groupings, validating the pipeline's discriminative power for microbial fingerprinting. This study increased the number of available rumen isolates, enhancing rumen microbiome understanding, and delivers a valuable MALDI-TOF MS database for high-throughput identification of rumen isolates. The R pipeline for spectral processing and clustering is applicable across ecosystems and prokaryotes, with plans to extend to eukaryotes.

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S2_OP12

UNVEILING THE GENOMIC AND FUNCTIONAL ARSENAL OF BACILLALES ENDOPHYTES FROM HALOPHYTES AND OLIVE TREES

Nikolaos Arapitsas[1,2], Christos A. Christakis[1,2], Savvas Paragkamian[1], Stefanos Sultatos[1,3], Franziska Reden[4], Chrysianna Psarologaki[1], Emmanouil Avramakis[5], Alexandros Stamatakis[4,6,7], Emmanouil A. Markakis[3], Panagiotis F. Sarris[1,2,8]

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The ongoing environmental crisis requires sustainable farming to ensure food security. Endophytes, and in particular endophytic species of the Bacillales order, exhibit promising agricultural potential by promoting plant growth, controlling challenging plant pathogens, and producing a substantial variety of secondary metabolites. Unraveling the phylogeny of this order in conjunction with genome mining and comparative genomics provides unique insights into the evolutionary relationships among its members and can reveal novel traits that will be crucial for future biotechnological and agrifood applications. We thoroughly study 25 novel endophytic Bacillales strains that we isolated from various halophytic plants and olive trees on Crete and Chrysi island. We evaluate the ability of these isolates to grow under increased salinity, to inhibit the growth of economically important phytopathogens in vitro, and enhance plant tolerance against biotic and abiotic stress. We employ a hybrid sequencing approach that combines Illumina short-reads and PacBio long-reads, to accurately reconstruct the complete genome of each isolate. Genome mining and comparative genomics analyses identify genes that are associated with plant-growth promotion, production of secondary metabolites as well as antimicrobial compounds, and an increased genetic novelty among the genomes of our isolates. Furthermore, we infer a well-supported phylogeny that includes our isolates as well as publicly available Bacillales representatives. We identified four putatively novel species and show that these isolates and even isolates of already well-studied species (e.g., Bacillus thurigiensis) may harbor yet unidentified protein-coding genes and secondary metabolites. Our study highlights the increased genetic, functional, and taxonomic novelty that reside within endophytic strains of a well-studied order and emphasizes the value of deep exploration of endophytic microbial genomes and communities for microbiology, ecology and agriculture.

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S2_OP13

LET THE INHIBITOR OUT OF THE FLASK: CULTURE–SOIL DISPARITIES IN BNI EFFICACY

Chrysovalantou Moutzourelli[1], Eleftheria Bachtsevani[2], Evangelia Papadopoulou[3], Graeme Nicol[2], Christina Hazard[2], Dimitrios Karpouzas[1]

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Biological nitrification inhibition (BNI) has emerged as a promising strategy to enhance nitrogen use efficiency and reduce nitrogen losses via leaching and nitrous oxide emissions. While various plant-derived BNIs have shown potential, their efficacy under field-relevant conditions remains poorly characterized. In this study, we assessed the inhibitory activity of six BNI compounds —zeanone, 2-methoxy-1,4-naphthoquinone, sakuranetin, MHPP, 1,9-decanediol, and MBOA — against a mixture of two synthetic nitrification inhibitors (SNIs), ethoxyquin and DMPP. Experiments were conducted using three distinct experimental systems: (1) single-strain cultures of ammonia-oxidizing archaea (AOA) and bacteria (AOB), (2) short-term potential nitrification assays in soil slurries, and (3) soil microcosm incubations using two agricultural soils. Our results indicate that BNI efficacy is strongly influenced by the targeted ammonia oxidizers (AOA vs AOB) and the experimental context. Although robust inhibition was observed in culture systems, these effects did not consistently carry over to soil-based assays. Soil microcosms showed the lowest levels of inhibition, likely due to rapid compound degradation, as suggested by previous findings. Notably, even the most effective BNIs — MHPP and MBOA — were significantly less potent than the SNI mixture. These findings highlight the critical role of biotic and abiotic soil factors in shaping BNI performance and underscore a potential disconnect between laboratory efficacy and field relevance. Our results, emphasize the need to evaluate BNI strategies under conditions that closely mimic natural soil environments.

Acknowledgments

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S2_OP14 (FT)

SYNONIM: SYNTHETIC COMMUNITY DESIGN BASED ON FUNCTIONAL REPRESENTATION

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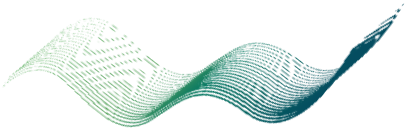
Environmental microbial communities are inherently complex, making it difficult to experimentally assess the roles of individual members, their interactions, and their responses to perturbations. Synthetic microbial communities (SynComs) simplify this complexity by capturing key metabolic traits while minimizing confounding diversity. However, designing SynComs that accurately represent natural communities remains a challenge.

SYNONIM (SYNthetic cOmmunity designN via approxIMation) addresses this issue using a variety of complementary strategies. It employs a function-first, bottom-up approach that integrates different optimization techniques to design SynComs that best approximate a reference functional profile derived from metagenomic data. This allows for the creation of SynComs that not only reflect essential metabolic functions but also account for ecological principles such as functional redundancy, complementarity, and community size control.

Building on existing strategies for SynCom design, SYNONIM offers flexibility by allowing users to tailor constraints to specific needs—including the ability to focus on the coverage of key functions or controlling the diversity of the community. These capabilities enable the generation of reductionist, yet representative, SynComs that retain critical functions and dynamics, facilitating hypothesis-driven experiments that isolate the contributions of individual traits.

As a proof of concept, SYNONIM has been used to design SynComs with varied denitrification pathway configurations. Ongoing experiments are assessing how these differences affect soil nitrous oxide emissions. Ultimately, SYNONIM's versatile approach paves the way for more predictable, engineered ecosystems and provides valuable insights into microbial ecology.



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S2_OP15 (FT)

DIGESTATE AS A FERTILIZER: EFFECTS ON SOIL MICROBIAL AND NEMATODE COMMUNITIES

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Digestate, the byproduct produced from the anaerobic digestion of organic materials like food waste, manure, and crop residues in biogas plants, is increasingly recognized as a sustainable fertilizer. Despite its rising use, the effects of digestate on soil microbial communities are not yet fully understood. This study aimed to evaluate how digestate application influences soil microbial communities using molecular techniques. We compared its effects to those of conventional fertilizer and a combined (mixed) fertilizer treatment, after one growing season of maize in an agricultural field. Soil bacterial and fungal communities were assessed using 16S rRNA and ITS amplicon sequencing, respectively. Our results showed that digestate application did not significantly alter microbial diversity or community composition when compared to conventional or mixed fertilization. These findings are consistent with prior mesocosm research suggesting that short-term digestate application may have limited impact on microbial communities (Nikolaidou et al., 2024). The stability observed underscores the resilience of native soil microbes, which appear to maintain their structure and function despite the introduction of exogenous taxa (Mola et al., 2024). To further explore potential microbial shifts, we identified specific microbial biomarkers and examined functional implications. In the digestate and mixed fertilization treatments, fungal biomarkers associated with dung and bacterial taxa typically found in biogas plant environments were detected. Additionally, we analyzed soil nematode communities using microscopy. Unlike microbes, nematodes responded more noticeably to fertilization since digestate increased bacterivorous nematodes and reduced herbivorous ones. In summary, digestate appears to be a promising alternative to conventional fertilizers. It supports sustainable agriculture by maintaining microbial balance and potentially improving soil health, without causing ecological disruption.

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S2_OP16 (FT)

HOW RUMINANT MANURE ORIGIN AND REGIMES AFFECT THE MICROBIAL ECOLOGY DURING EARLY COMPOSTING

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Composting of ruminant manure is a microbially driven process influenced by substrate characteristics and environmental conditions such as moisture. This study investigates how manure type (dairy cow vs. sheep) and hydration regimes (dry vs. wet) shape microbial community structure and diversity during early composting. Using 16S rRNA metabarcoding, we tracked bacterial succession across four time points spanning dry and rewetting phases. Microbial communities were dominated by Bacillota, Actinomycetota, and Pseudomonadota, with notable differences in relative abundance between manure types. Alpha diversity was initially higher in sheep manure, likely due to greater substrate heterogeneity, but declined rapidly following rewetting, suggesting strong environmental filtering. Beta diversity analyses revealed significant effects of both manure type and moisture changes, with distinct microbial communities and greater compositional variability in sheep manure. Venn diagram and abundance analyses highlighted differential taxonomic turnover, with sheep manure exhibiting higher richness and bacterial turnover across moisture conditions. Differential abundance testing identified moisture-responsive taxa, including drought-adapted genera during the dry phase, such as Solibacillus (ASV365) and UCG-005 (ASV263), and copiotrophic taxa, including Thermobacillus, Melghirimyces, Ornithinibacillus, and Saccharomonospora, which were enriched after rewetting. These findings govern the role of substrate origin and moisture in structuring microbial communities and suggest that moisture pulses selectively favour fast-growing taxa, reshaping diversity and dominance patterns during composting.

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S3_OP17

ASSESSING THE POSSESSION OF STRESS SURVIVAL ISLET 1 IN LISTERIA MONOCYTOGENES STRAINS ISOLATED FROM SMOKED SALMON PRODUCTION

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Listeria monocytogenes is a foodborne pathogen that continues to cause numerous outbreaks worldwide.

This ubiquitous microorganism can withstand a broad range of hostile conditions, including refrigeration, high salinity, oxidative stress, and acidic pH, making its control particularly challenging within food production environments (Ferreira et al. 2014). This remarkable ability to survive might be further enhanced by the possession of stress-related genes. One example is Stress Survival Islet 1 (SSI-1), a five-gene segment (lmo0444-lmo0448) that promotes the survival of L. monocytogenes under low pH and osmotic stress conditions (Ryan et al. 2010).

The purpose of this study was to assess the presence of SSI-1 in 49 L.monocytogenes strains previously isolated from a smoked salmon processing facility. Strains were subtyped by Multi Locus Sequence Typing (MLST) as described by Ragon et al. (2008), whereas the possession of SSI-1 was determined by PCR according to Ryan et al. (2010).

MLST typing revealed six Sequence Types (STs): ST5 (24.49%), ST6 (8.16%), ST7 (8.16%), ST9 (53.06%), ST14 (2.04%), and ST155 (4.08%). SSI-1 screening showed that 85.71% of strains harbored the entire five-gene islet, whereas the remaining isolates carried only a partial set of genes.

These findings highlight the widespread occurrence of SSI-1 among the tested strains, suggesting a possible enhancement in their ability to survive in food processing environments, particularly under acidic and osmotic stress conditions. Future investigations focusing on the expression of this five-gene set will provide a deeper understanding of the mechanisms underlying SSI-1 activation and its role in L. monocytogenes stress adaptation.

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FOOD & NUTRITION

S3_OP18

SPOILAGE-INFORMED QUANTITATIVE MICROBIOLOGICAL RISK ASSESSMENT

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This study aimed to introduce a framework for spoilage-informed risk assessment by integrating quantitative microbiological risk assessment (QMRA) for a pathogen, with quantitative microbiological spoilage risk assessment (QMSRA) for a specific spoilage organism (SSO) into a single combined model to provide a more realistic estimation of foodborne illness risk, taking into account the variability in consumer perception of spoilage. The combined model was developed for Listeria monocytogenes and Lactic Acid Bacteria (LAB) in sliced deli meats. Microbiological testing of deli meat samples and historical data were collected for the prevalence and concentration of the pathogen Listeria monocytogenes and the specific spoilage organism (SSO) i.e. LAB in prepackaged sliced deli meats from Greece and Poland. Product physicochemical properties (pH, water activity, and nitrites concentration) influencing bacterial growth were analysed. Domestic storage temperature data was collected and consumer behaviour regarding consumption frequency and storage practices were surveyed via questionnaire with n=200 participants. Probability distributions were fitted to the collected data to describe variability of the model inputs. The predictive microbiology model for Listeria monocytogenes and LAB in meat products developed by Mejlholm and Dalgaard was used to estimate microbial growth during product storage. A dose-spoilage response model derived from consumer sensory testing, was integrated into the QMRA model, to describe the variability in spoilage perception among consumers. Sensitivity analysis was performed on the final combined model. Distribution fittings, Monte Carlo analysis and sensitivity analysis were performed in R programming language. The probability of illness and the probability of spoilage were then by means of conditional probability (P[illness|not perceived spoiled]) to calculate the spoilage-informed risk of listeriosis. The combined risk assessment model yielded a more realistic estimation of foodborne illness risk. The inclusion of the spoilage module resulted in a lower estimated combined risk for listeriosis, indicating the significance of accounting for spoilage in risk assessment. Integrating QMRA and QMSRA methodologies for pathogens and spoilage microorganisms in the framework of conditional probabilities can enhance the accuracy of foodborne illness risk estimation. This approach provides a more holistic understanding of food safety risks, acknowledging the impact of spoilage and consumer spoilage-perception.

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S3_OP19

GROWTH DYNAMICS AND OCHRATOXIN A PRODUCTION OF FOUR ASPERGILLUS CARBONARIUS STRAINS: A CASE STUDY IN MAIZE-MODEL MEDIUM

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Aspergillus carbonarius is a potential producer of ochratoxin A (OTA), a mycotoxin with severe health implications. Although this species is frequently associated with grapes, its emerging occurrence in maize renders to an increased concern. Temperature is a critical factor affecting both fungal growth and OTA production. The current study evaluated growth kinetics and OTA production of four A. carbonarius strains isolated from wine grapes produced in various Greek regions (F24 from Peloponnese, F70 and F71 from Crete, F80 from Attica). Aliquots of 10 µL per strain (of a 10^7 spores/mL suspension) were centrally placed in 90 mm Petri dishes containing maize-model medium (water activity: 0.93; pH: 6.8). All samples were incubated at 15°C, 20°C, 25°C, 30°C, and 35°C for max. 75 days. Two independent experimental batches, each with two replicates (n=4) were conducted. Growth was daily monitored through colony diameter measurements and OTA was quantified via HPLC-FLD at three key time points: onset of sporulation, and when colony diameters reached 45 mm and 90 mm. Maximum specific growth rate (µmax; h-1) and lag phase (λ; h) were calculated using Baranyi equation, which demonstrated a high degree of fit across all datasets (R^2> 0.97). Temperature significantly affected µmax (p< 0.001), with the minimal growth observed at 15°C (µmax: 0.064–0.085 h-1), while the optimal was demonstrated at 30°C (µmax: 0.331–0.391 h-1) regardless of strain. Due to rapid initial expansion along with the limitation of daily sampling interval, lag phase was not estimated. OTA production was significantly influenced by temperature and sampling time point (p< 0.001). OTA highest values were quantified between 20°C and 25°C, while all strains showed reduced production at 35°C. Strain F24 produced the lowest OTA (~5 ppb at 35°C; ~1350 ppb at 25°C), whereas F71 consistently recorded the highest (~71 ppm) and most variable production, potentially suggesting strain-specific metabolic responses to environmental conditions. These findings highlight the dual influence of temperature on A. carbonarius growth and toxin production, with moderate temperatures favoring OTA accumulation. Understanding such thermal responses is critical for assessing OTA contamination risks and developing effective mitigation strategies in maize.

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S3_OP20

A COMPARATIVE STUDY OF RADIO FREQUENCY AND CONVENTIONAL THERMAL INACTIVATION MECHANISMS OF SALMONELLA TYPHIMURIUM AND LISTERIA MONOCYTOGENES IN BROTH

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Radio Frequency (RF) is a novel dielectric heating technology recently gaining attention for microbial inactivation. Nonetheless, RF inactivation mechanisms have not been deciphered, especially concerning RF-specific (non)thermal effects. This study adds valuable insight to the inactivation mechanisms of (Gram-negative) Salmonella Typhimurium and (Gram-positive) Listeria monocytogenes by using similar dynamic temperature profiles in both RF heating and conventional thermal treatments. To compare the inactivation mechanisms of the two heating methods, the effects of each treatment were evaluated initially on a macroscopic level by measuring inactivation and sublethal injury, followed by a microscopic analysis of membrane and DNA damage. Inactivation kinetic parameters were estimated using the Geeraerd et al. (2000) model with Bigelow-type temperature dependency. Membrane integrity and DNA denaturation were assessed by fluorescence methods and agarose gel electrophoresis was used to further determine DNA damage. Overall, RF demonstrated a higher inactivation rate and higher sublethal injury than conventional treatments for both Salmonella Typhimurium and L. monocytogenes. Additionally, RF treatments caused more membrane damage than conventional treatments in both microorganisms, although differences could be possibly linked to viable cell density differences. No significant DNA denaturation differences were observed between RF and conventional treatments for each microorganism, indicating that occurring DNA damage was purely thermal. For L. monocytogenes, potential DNA damage due to RF treatments was observed via agarose gel electrophoresis. In conclusion, while the existence of RF-specific inactivation mechanisms is possible, they are probably only related to membrane damage based on the results of this study. The use of additional techniques (e.g., flow cytometry, next-generation sequencing) is recommended to enhance the understanding of RF inactivation mechanisms for the specific foodborne pathogens.

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FOOD & NUTRITION

S3_OP21

MYCOLOGICAL FERMENTATION OF PLANT-BASED SUBSTRATES FOR BLUE CHEESE ANALOGUE PRODUCTION

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The growing demand for sustainable and ethical alternatives to traditional dairy products has accelerated innovation in the development of plant-based cheese analogues. Among these, the formation of plant-based alternatives to mold-ripened dairy products, has proven to be an important challenge for the food industry (Fabiszewska et al., 2024). Especially the blue cheese analogues present a unique challenge due to their complex organoleptic characteristics, traditionally derived from microbial activity during ripening. In this study, Penicillium roqueforti isolated from traditional Roquefort cheese together with lactic acid bacteria, were employed to ferment cashew and tofu-based substrates for the development of blue cheese analogues.

FTIR analysis demonstrated that the plant-based cheese analogues exhibited spectral similarities to dairy Roquefort, particularly in regions associated with fatty acids and proteins, indicating successful lipolytic and proteolytic activity by Penicillium roqueforti, producing key compounds linked to blue cheese aroma/flavor. The titratable free acidity and pH values of plant-based blue cheese analogues were found to be similar to those of dairy Roquefort (26.87% acidity, pH 5.76), although some variation was observed. The cashew-based variants exhibited acidity values ranging from 19.19% (w/w) to 20.47% (w/w), with pH values between 5.06 and 5.25, while tofu-based variant showed an acidity of 20.5% (w/w) and pH value of 6.36. In terms of fat content, dairy Roquefort exhibited a fat level of 38.81% (w/w), whereas the plant-based alternatives contained lower amounts. The cashew-based cheese ranged from 34.11% to 34.78% (w/w) fat, while the tofu-based cheese had a notably lower fat content of 14.45% (w/w). Moreover, image analysis further revealed that the blue vein percentages were 0.81%–1.10% for the cashew-based cheese analogues and 0.23% for the tofu-based cheese, reflecting the extent of the growth and sporulation of Penicillium roqueforti in cheese cavities.

These findings highlight the potential of plant-based substrates in replicating the complex characteristics of mold-ripened blue cheese, while also emphasizing the influence of substrate composition on fermentation dynamics.

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S3_OP22 (FT)

OMICS APPLICATIONS FOR STUDYING FOOD MICROBES AND MICROBIOMES: FROM DATA ANALYSIS TO TECHNOLOGICAL PROPERTIES

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Food microbiology is being revolutionized by the advent of omics technologies combined with advanced bioinformatics tools. The data produced by these approaches is often overwhelming, but it sheds light on unprecedented details concerning the biology and technological properties of foodborne microorganisms and microbiomes. In the first part of the study, the application of a multi-omics approach is demonstrated, providing the means to tackle questions about fermented foods of dairy, plant and meat origin. This approach involves culturomics, metagenomics and metabolomics to highlight the intricate interactions between the microbial members of the fermenting ecosystems, as well as between their metabolites. The presence of technologically important microbial strains and their gene pools can be identified. This includes starter and non-starter lactic acid bacteria (LAB), but also spoilage microorganisms and foodborne pathogens. In the latter case, virulence and antibiotic resistance genes can also be detected revealing important aspects of the safety of the products. The second part of the study concerns the application of whole genome sequencing combined with transcriptomics on pure bacterial cultures to provide information about the mechanisms underpinning the adaptation of spoilage microorganisms and foodborne pathogens, in different food matrices. The examples to be presented indicate how these approaches can lead to an understanding of the functional properties of specific microorganisms before they are considered as part of a foodborne microbiome. Overall, the continuous improvement and simplification of omics technologies and analysis tools facilitate their integration into routine food quality control and safety monitoring at the industrial scale, thereby paving the way for more precise, predictive, and sustainable food microbiology practices.

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S3_OP23 (FT)

A CLEAN-LABEL APPROACH: COMBINING HHP AND NISIN TO CONTROL LISTERIA MONOCYTOGENES IN PLANT-BASED MILK ALTERNATIVES

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Plant-based milk alternatives (PBMAs) are gaining popularity due to the high prevalence of lactose intolerance and their role as carriers of bioactive compounds and functional ingredients. High Hydrostatic Pressure (HHP) is a promising non-thermal food processing technology used to extend shelf life and improve microbial safety. When combined with clean-label antimicrobials like nisin, HHP offers potential for enhanced pathogen control, process optimization, and more sustainable food production.

This study aimed to investigate the potential synergistic antimicrobial effect of HHP and nisin against Listeria monocytogenes in five commercially available PBMAs derived from oat, almond, soy, coconut, and hazelnut. PBMAs were inoculated with a five-strain Listeria monocytogenes cocktail to a final concentration of ~10^7 CFU/mL. Samples were subjected to HHP at 300, 350, and 400 MPa for 10 min, either alone or in combination with nisin at 500 IU/mL. Microbial inactivation was quantified immediately after treatment and all experiments were conducted in three independent biological replicates. Statistically significant differences between the PBMAs were assessed using post-hoc analysis ($p < 0.05$).

At 400 MPa, the combined HHP-nisin treatment achieved >5 log reduction in oat and almond PBMAs, and 3.4–4.8 log in soy, coconut, and hazelnut. The highest synergistic effect was observed in oat and hazelnut PBMAs (>0.85 and 1.1 log, respectively). HHP alone achieved significantly higher ($p<0.05$) inactivation in almond and oat PBMAs (5.0 and 4.6 log, respectively), while soya-based matrix offered a protective effect (2.7 log), highlighting matrix-dependent variability in HHP efficacy.

This study provides novel insights into HHP-nisin combination as an effective clean-label strategy for L. monocytogenes control in PBMAs. Synergism between HHP and nisin may allow for reduced processing intensity and energy use, supporting the development of minimally processed, refrigerated dairy alternatives. Future research should explore shelf life, sensory impact, and consumer acceptance for industrial application.

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S3_OP24 (FT)

IN VITRO CHARACTERIZATION OF ADHESIVE CAPABILITY OF POTENTIALLY PROBIOTIC STRAINS TO HT-29MTX INTESTINAL EPITHELIUM CELL LINE.

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The adherence of lactic acid bacteria to epithelial cells and mucosal surfaces has been considered as a potential probiotic marker along with other desirable attributes as it stimulates the host-microbe interactions, promotes their persistence time in the gut, and provides protection to the intestinal barrier by various mechanisms.

Six potentially probiotic strains (Lacticaseibacillus paracasei subsp. paracasei E93, E94, Lactiplantibacillus pentosus E104, E108, Lactiplantibacillus plantarum E10,E69), isolated from naturally fermented olives were screened for their adherence potential to intestinal epithelial cell line HT-29MTX. Two comercial probiotic strains Lacticaseibacillus casei Shirota and Lacticaseibacillus rhamnosus GG were used as controls. Two different concentrations of LAB strains were examined, 6 & 7 log CFU/mL, for two different time points, 30 and 120 min of bacterium-host cell contact.

Comparing colonization data (expressed as Δlog CFU/cm2) from 6 potentially probiotic strains in two different concentrations (6 & 7 logCFU/mL) and two different time points 30 & 120 min, all the strains showed an overall greater colonization capability ($p>0.05$) at the lowest concentration (6 logCFU/mL) for 120 min. Specifically, Lactiplantibacillus plantarum E10 and Lactiplantibacillus plantarum E69 showed the highest colonization, close to zero ΔLog, (values equal to -0.155 and -0.176 respectively) in HT-29MTX cell line after 120 min when the initial inoculum was 6 logCFU/mL. On the contrary, Lactiplantibacillus pentosus E104, Lactiplantibacillus pentosus E108 and Lacticaseibacillus paracasei subsp. paracasei E94 showed low colonization trend. Lactiplantibacillus plantarum E10 and Lactiplantibacillus plantarum E69 showed higher colonization after 120 min also when an initial load of 7 log CFU/mL was tested (Δlog = -0.23 and -0,24 respectively).

This in vitro study underlines the impact of six potentially probiotic strains on direct adhension to the intestinal cell model HT-29MTX in two different concentrations and two different time points. These findings highlight the potential of LAB strains to colonize the gut effectively, which is crucial for their selection and application in promoting gut health.

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S4_OP25

SELECTIVE OXIDATION OF 5-HYDROXYMETHYLFURFURAL BY A NOVEL FUNGAL GLYOXAL OXIDASE FOR BIOPOLYMER PRECURSOR SYNTHESIS

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Lignocellulosic biomass is a renewable and abundant resource for producing high-value chemicals as sustainable alternatives to petroleum-derived products [1]. Among these, 5-hydroxymethylfurfural (HMF), generated through catalytic dehydration of biomass-derived sugars [2], serves as a pivotal intermediate for synthesizing compounds like 2,5-furandicarboxylic acid (FDCA), a promising biopolymer precursor [3].Biocatalysis offers an environmentally friendly and selective approach to such transformations, surpassing traditional chemical methods [4-6].This study explores the enzymatic oxidation of HMF utilizing a novel fungal glyoxal oxidase (GlGlyOx) from Ganoderma lucidum, belonging to the Auxiliary Activity family AA5 of the CAZy database. The enzyme was identified through genome mining, heterologously expressed in Pichia pastoris, purified, and evaluated for its activity on model furans. GlGlyOx efficiently converted HMF to 5-hydroxy-2-furancarboxylic acid (HMFCA), furan-2,5-dicarbaldehyde (DFF) to 5-formylfurancarboxylic acid (FFCA) and FFCA to FDCA.Further assessments were conducted using HMF derived from actual lignocellulosic hydrolysates produced from OxiOrganosolv-pretreated wheat straw via enzymatic saccharification and isomerization, combined with mild catalytic dehydration [1]. Various homogeneous and heterogeneous acidic catalysts were assessed for sugar dehydration efficiency. The balance between Brønsted and Lewis acid sites significantly influenced product distribution and reaction selectivity. This work underscores the potential of GlGlyOx in the biocatalytic upgrading of HMF, contributing to the development of sustainable pathways for producing green chemicals and biopolymer precursors.

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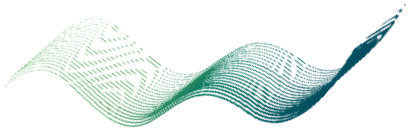

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BIOTECHNOLOGY

S4_OP26

PRODUCTION OF EXOPOLYSACCHARIDES BY HALOPHILES ISOLATED FROM ARMENIAN SALINE ENVIRONMENTS USING COST-EFFECTIVE WASTE SUBSTRATES

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Hypersaline environments, such as salt mines, salterns, and salt lakes, are rich sources of extracellular polysaccharide (EPS)-producing halophiles with significant biotechnological potential. This study aimed to investigate the production, structure, and chemical composition of EPSs from two halophilic strains: Haloarcula japonica SST1, isolated from a subterranean salt deposit, and Halomonas elongata N1T, isolated from the salt lake Aghi Lich in Armenia.

Microbial biomass and EPS production were assessed to determine the optimal cultivation time and carbon sources. For H. japonica SST1, the highest yield of extracellular product (EP) (628.4 mg L^{-1}) and specific production (1.5 g g^{-1} dry cells) were achieved at the late stationary phase (120 h) in sucrose-supplemented media under optimal growth conditions (20% NaCl, 35 °C, pH 7.0). The EP showed a high carbohydrate content (48.5%), low protein content (4.32%), minimal nucleic acid content (0.97%), and a significant proportion of uronic acids (10.8%). When molasses was used as the carbon source, the EP yield was 304.8 mg L^{-1}, with a carbohydrate content of 20.8%. Gel filtration chromatography estimated the EPS molecular weight at approximately 900 kDa. Structural analyses using GC-MS, HPAE-PAD, and NMR revealed that the EPS was a heteropolymer composed of mannose, galactose, and glucose.

For H. elongata N1T, EP yields on sucrose reached 91 mg L^{-1} in unbuffered media and 934 mg L^{-1} in buffered media at the stationary phase (144 h) under optimal conditions (8% NaCl, 35 °C, pH 7.5). The EP exhibited a carbohydrate content of 16.33%, low protein content (8.6%), low nucleic acid content (2.94%), and 3.27% uronic acids. When molasses was used instead of sucrose, the EP yield was 527 mg L^{-1} under the same conditions. The EPS composition, analyzed by TLC, HPAE-PAD, GC-MS, and NMR, identified a heteropolysaccharide comprising glucose, fructose, arabinose, glucosamine, ribose, rhamnose, and mannose. Genomic analyses of both strains revealed the presence of genes associated with monosaccharide activation, biosynthesis, assembly, and secretion pathways involved in EPS production. The successful utilization of molasses highlights both strains as promising and cost-effective EPS producers, supporting their potential application in a circular bioeconomy.

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S4_OP27

BIOCHEMICAL AND COMPUTATIONAL TOOLS FOR THE STUDY AND VALORIZATION OF XYLOGLUCAN-DEGRADING ENZYME SYSTEMS OF WHITE-ROT FUNGI.

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Lignocellulosic biomass is a composite material consisting of cellulose, hemicellulose and lignin. Xyloglucan is a complex, highly substituted plant hemicellulose, covering cellulose fibrils. Xyloglucanases, the enzymes responsible for its degradation, can be utilized for the design of efficient bioprocesses by incorporating them in enzyme cocktails that target cellulose-containing substrates. From a biochemical and structural perspective, these enzymes are of particular interest because, unlike other endoglycosidases, they are able to act on a heavily substituted substrate. In order to shed light on the enzymatic degradation of xyloglucan by white-rot fungi, xyloglucanases from the basidiomycete Abortiporus biennis were studied. Two genes corresponding to xyloglucanase enzymes were heterologously expressed in the methylotrophic yeast Pichia pastoris, and purified. Biochemical characterization revealed that both enzymes were of mesophilic nature, acting on moderate temperatures and lightly acidic pH, while they both showed relatively strict substrate specificity, using xyloglucan as their main substrate. Activity was demonstrated for a few other polysaccharides but not cellulose. Also, both enzymes significantly enhanced the activity of cellulases, enabling the saccharification of lignocellulosic substrates. In order to determine the tolerance of both enzymes for polysaccharide substitutions, extensive synergism assays were performed with enzymes of known specificity. Additionally, the molecular determinants of substitution tolerance were investigated with computational methods, employing tools such as molecular modelling with AlphaFold 2.0, molecular dynamics (MD) simulations in the GROMACS MD engine, substrate docking with Rosetta GlycanDock and ligand binding free energy calculations using the MMPBSA method. Our results indicate that, harnessing the complementarity of biochemical and computational methodologies could help accelerate structure-function studies and provide essential information for the computational redesign of tailored biocatalysts for specific applications.

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BIOCATALYTIC DEPOLYMERIZATION AND UPCYCLING OF PLASTICS USING ENGINEERED YARROWIA LIPOLYTICA

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Plastic pollution, largely driven by the durability and accumulation of synthetic polymers like polyurethanes (PUs) and polyethylene terephthalate (PET), represents a pressing global environmental issue. Enzymatic depolymerization presents a promising, eco-friendly solution by breaking down these persistent materials into reusable monomers, which can subsequently be upcycled into valuable products. This study aims to develop efficient enzymatic hydrolysis strategies specifically targeting polyurethane degradation, utilizing microbial cell factories as a sustainable platform.

Through a synthetic biology approach, we employed Golden Gate assembly to construct a modular plasmid library incorporating four secretory signal peptides, six polymer binding modules, and four linkers fused to a Fusarium oxysporum cutinase. This combinatorial design yielded up to 96 enzyme variants, which were genomically integrated into Yarrowia lipolytica to ensure stable expression. Screening on a selective medium containing only the polyurethane Impranil DLN-SD enabled the identification of the most active variants, as indicated by halo formation resulting from polymer hydrolysis.

In addition to depolymerization, we investigated the upcycling of monomers such as ethylene glycol (EG), a PET degradation product, into high-value compounds. ^13C-labelling experiments demonstrated that acetate enhances EG metabolism via the glyoxylate cycle, facilitating its conversion into glycolic acid (GA). Bioreactor optimization further improved this process, achieving a GA concentration of 48.41 ± 1.4 g/L, with a molar yield of 73% and a productivity of 0.73 g/(L·h). GA is a commercially valuable molecule with applications in cosmetics, pharmaceuticals, and biodegradable materials.

Overall, this integrated strategy highlights the potential of engineered Y. lipolytica as a versatile platform for both enzymatic plastic degradation and bioconversion, supporting the advancement of a circular plastics economy.

Acknowledgements

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S4_OP29

STIMULATION OF SOIL BACTERIA TO EMIT GARLIC VOLATILE ORGANIC COMPOUNDS

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Diallyl disulfide (DADS) is a volatile organic compound that is derived from garlic and a major component of garlic (essential) oil. DADS has proven successful in reducing plant pathogen populations in the soil either directly by boosting natural enemies of pests or indirectly by activating the soil microbial community. So far, the production of DADS has only been shown for Allium plants. The initial precursor molecule, S-allyl-L-cysteine (SAC), is transformed into S-Allyl-L-cysteine sulfoxide (alliin) which, in turn, is converted to allicin that quickly transforms into DADS. However, emission of DADS by bacteria remains unknown. In this study, we investigated if soil bacteria are able to synthesize DADS from the precursor molecule SAC. For this purpose, we applied SAC in non-sterile and sterile soil. Untargeted volatilomic analysis confirmed an overproduction of DADS in the volatile blend emitted from the non-sterile soil. Other compounds commonly found in the garlic aroma, including (di-)allyl sulfur compounds and vinyl dithienes, were also significantly overproduced from the non-sterile soil. Microbial community analysis revealed an increase in the abundance of members of the Pseudomonas genus. These results suggest that the soil bacteria can metabolize SAC to DADS and other volatile compounds commonly produced by garlic, and Pseudomonadas are probably involved in this process. Future studies with pure bacterial isolates will confirm the potential of Pseudomonas strains and reveal the molecular mechanisms involved in the transformation of SAC to volatile compounds commonly emitted by garlic.

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S4_OP30

BASIDIOMYCETES AND THEIR ENZYMATIC POTENTIAL FOR SYNTHETIC POLYMER DEGRADATION

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The increasing environmental burden of synthetic polymer waste demands sustainable solutions[1]. Fungal enzymes, particularly those from white-rot fungi, are emerging as promising biocatalysts for polymer degradation due to their oxidative and hydrolytic activities[2,3]. In this study, we investigated a strain of the order Agaricales (Basidiomycota), isolated from a Greek habitat and identified through ITS rRNA gene sequencing, previously known for its plant litter decomposition capabilities but largely unexplored for plastic biodegradation. The strain was evaluated for its ability to grow on polyurethane as sole carbon source, showing efficient substrate degradation and notable biomass production. Extracellular enzymatic activities were assessed spectrophotometrically in the culture supernatants, revealing significant oxidative activity, followed by hydrolysis driven by esterases. To further explore its biocatalytic potential, the culture supernatant was applied to polyurethane powder, and changes in polymer molecular weight were analyzed by gel permeation chromatography. Gas chromatography – mass spectrometry analysis was performed to identify the biodegradation products while proteomic analysis of culture supernatants was conducted to identify and quantify enzymes involved in polymer degradation. This study highlights the promising role of this strain in developing sustainable strategies for plastic waste management and valorization.

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S4_OP32 (FT)

ACTIVATED SLUDGE AS A VALUABLE SOURCE OF HYDROLYTIC SUBSTANCES AND NUTRIENTS.

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It is well documented that the increase in the global population and the lack of a single policy for protecting the environment have led to a depletion of valuable resources, especially in highly urbanized and industrialized countries, where vast quantities of wastewater are produced. According to the United Nations (2018), more than 50% of the world's population lives in cities. That is expected to increase by 68% by the year 2050, causing a further increase in the produced wastewater and reinforcing the need for efficient treatment. Full-scale wastewater treatment plants (WWTPs), as a sign of civilization, are essential infrastructures for the protection of the environment and are increasingly recognized as valuable resource recovery facilities, going beyond their primary role of treating domestic and industrial effluents, to satisfy the criteria for safe discharge. Activated sludge, within the framework of circular economy, can serve as source for the recovery and valorization of hydrolytic enzymes, and nutrients such as nitrogen and phosphorus, so that what is so far lost considered as waste can now be recovered and reintegrated into production cycle, with the ultimate goal to efficiently minimize environmental impact, and contribute to the prevention of global climate change. In this study, a full-scale WWTP located in North Greece was monitored in terms of the hydrolytic activity of α- and β-glycosidase, lipase, and protease, as well as the presence of soluble microbial product (SMP) and extracellular polymeric substances (EPS). Moreover, the implementation of high-throughput amplicon sequencing enabled, in this work, the investigation of the microbial community structure in the aerobic and anoxic reactors and the surface foaming of the activated sludge tank. Indeed, activated sludge can serve as a significant source for recovering nutrients and enzymes within the circular economy concept.

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S7_OP49

NOVEL THERMOBACULUM STRAINS FROM HIGH-ALTITUDE GEOTHERMAL SPRINGS IN TAJIKISTAN

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Three novel thermophilic bacterial strains, designated Thermobaculum spp., were isolated from geothermal springs in the Tamdykul and Khodja-Obi-Garm regions of Tajikistan, located at elevations of 2198 m and 1835 m, respectively. Samples from these environments, with temperatures of 88°C (pH 7.4) and 93°C (pH 8.5), yielded obligately aerobic, non-spore-forming rods forming pink colonies on R2A agar. The isolates,T2p, T4pink, and KhOGp grew between 55–80°C and pH 5–10. Phylogenetic analysis of 16S rRNA gene sequences placed them within the Thermobaculum genus, showing 94.2% identity to Thermobaculum terrenum, the sole described species in the genus. Draft genome sequencing revealed genome sizes of 3.0 to 5.7 Mb, GC contents of 51.6–62.8%, and genome completeness above 97%. ANI and dDDH values (89.5%/36.5% and 89.6%/36.6%) confirmed their classification as a distinct Thermobaculum species. These isolates exhibited robust hydrolytic activity at 65°C, including degradation of cellulose, casein, starch, and activity of enzymes such as C8 esterase lipase, β-galactosidase, and α-glucosidase. These findings highlight the biotechnological potential of Tajikistan's thermophilic microbiota, representing the first thermophilic isolates reported from this region.

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S7_OP50

DISTINGUISHING SUBERINASES FROM CUTINASES: A STRUCTURE-BASED CLASSIFICATION OF CUTINASE-LIKE ENZYMES AND THEIR SUBSTRATE SPECIFICITY VALIDATED IN SILICO, IN VITRO AND IN PLANTA

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Cutin and suberin are structurally related polyesters found in plants, serving as protective barriers. While cutin coats aerial surfaces, suberin is found in roots and bark. Microbial cutinases, members of the α/β-hydrolase fold, are well-characterized enzymes that degrade cutin and various polyesters. However, the enzymatic breakdown of suberin remains poorly understood, with only one known bacterial suberinase reported to date.

In this study, we aimed to distinguish suberinases from cutinases based on structural characteristics and substrate specificity. Using AlphaFold-predicted structures of cutinase-like enzymes with reported suberin activity, we formulated a hypothesis differentiating cutinases from putative suberinases. A dataset of 160 fungal cutinase-like sequences from 22 species (assembled via UniProt and BLASTp) was analyzed. Structural relationships among proteins were examined by constructing a structure-based phylogenetic tree using FoldTree. The resulting clusters revealed a distinct sub-group of putative suberinases, independent of species origin. Multiple sequence alignments and phylogenetic analyses were conducted using MAFFT and IQ-TREE. We assessed physicochemical properties (e.g., isoelectric point, aromaticity, GRAVY index) in silico and predicted kinetic parameters (KM, kcat, Vmax) using machine learning tools (Km_prediction_function and CatPred). Statistically significant differences between the two identified clusters further supported their functional divergence. Eight representative enzymes from Fusarium oxysporum and Botryotinia fuckeliana were expressed in Escherichia coli, purified, and biochemically characterized in vitro. Substrate specificity assays revealed varied activity toward natural (cutin, suberin) and synthetic polyesters (e.g., polyethylene terephthalate). To evaluate activity in a native context, the enzymes were expressed in Nicotiana benthamiana and Lotus japonicus leaves and roots, to investigate effects on cutin and suberin integrity, respectively.

Our findings suggest that suberinases form a distinct structural and functional subclass within the broader group of cutinase-like enzymes. This distinction deepens our understanding of plant-microbe interactions and enables more precise characterization of fungal pathogenicity mechanisms. By combining bioinformatics, enzymology, and plant biology, this work provides a foundation for future studies on polyester-degrading enzymes and their roles in plant-pathogen dynamics.

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S7_OP51

EXPLORING THE EVOLUTIONARY LINKS BETWEEN MICROBIAL XENOBIOTIC AND SECONDARY METABOLISM: THE DIVERSE ROLES OF NAT GENES IN BACTERIA AND FUNGI OF ENVIRONMENTAL AND BIOTECHNOLOGICAL RELEVANCE

Sotiria Boukouvala[1], Dimitra Basdani[1], Vasiliki Garefalaki[1], Archontis Goumagias[1], Evanthia Kontomina[1], Ioannis Olbasalis[1], Dionysios Patriarcheas[1], Sokratis Zekkas[1]

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Microorganisms are capable of modifying their chemical environment through their remarkable metabolic plasticity and adaptability. Especially fascinating is their ability to engage in "chemical warfare", as they employ secondary metabolism to generate chemicals that harm competitors, while they can also protect themselves against toxic substances of natural or manmade origin through xenobiotic metabolism. Since those two metabolic functions are considered to have overlapping evolutionary histories, we became particularly interested in microbial NAT enzymes that have been implicated in both the biosynthesis and the biotransformation of bioactive compounds in bacteria and fungi. NAT catalytic activity relies on a conserved Cys-His-Asp triad that is characteristic of cysteine proteases. We performed computational comparative studies into the evolutionary origins of the NAT family, demonstrating its common ancestry with Clan_CA transglutaminases (a family of cysteine protease superfamily). We additionally screened >300,000 sequenced microbial genomes, expanding the list of formally annotated NAT genes to about 4600 new sequences in bacteria, archaea, fungi and protists. We performed advanced computational predictions and cloned representative NAT genes for recombinant protein expression-purification and enzymatic analysis, in order to explore the functional variability of NAT homologues in bacteria and fungi. The majority of microbial NAT homologues can be classified into three functional groups: the first includes the archetypal xenobiotic metabolizing N-acetyltransferases known to act on aromatic amines and hydrazines, including a range of highly toxic recalcitrant by-products of industry and farming; the second group includes homologues encoded by genes located within biosynthetic gene clusters, and those demonstrate remarkable functional variability against a range of substrates generated during the biosynthesis of potentially useful natural products, including antibiotics. Finally, certain NAT homologues in fungi have diverged to function as N-malonyltransferases allowing pathogenic Fusarium to survive the chemical defence of cereal plants during infection. Adaptation of NAT enzymatic function is, in this case, through unique dimerization of the protein which thus represents a candidate target for selective pathogen inhibition to control infection of crops.

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BIOTECHNOLOGY

S7_OP52

OPTIMIZATION OF CULTIVATION CONDITIONS AND THE RECOVERY OF HIGH-ADDED VALUE TERPENES FROM GENETICALLY ENGINEERED YEAST STRAINS

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Advances in molecular biology have enabled the engineering of microorganisms for the sustainable production of high-added value compounds such as terpenes and terpenoids. Among other yeast hosts (i.e. Saccharomyces cerevisiae), Yarrowia lipolytica offers distinct advantages due to its ability to metabolize a wide range of substrates, including low-cost, industrial by-products (e.g., glycerol, waste oils), and its highly active peroxisomes, which provide an ideal environment for terpenes/terpenoids biosynthesis. This study assesses how various cultivation parameters influence the physiology and metabolic activity of engineered yeast strains producing terpenes (i.e. limonene or squalene). Batch and fed-batch fermentations were conducted in flasks using different carbon sources (e.g., glucose, xylose, glycerol, and acetic acid), varying carbon-to-nitrogen (C/N) ratios, and the addition of an organic overlay. Cell growth and viability were monitored via optical density measurements (600 nm) and flow cytometry, respectively, while carbon substrate consumption and by-product formation were analyzed using HPLC. Under optimized conditions, the recovery of terpenes was assessed by quantifying its distribution between the different phases of both conventional and two-phase fermentation systems. In conventional fermentations, the majority of limonene is retained in the biomass fraction. The spatial distribution of terpenes was also correlated with the portion of lysed/non-lysed cells to provide insights concerning downstream recovery methods. The optimization of the cultivation medium and conditions pave the way towards validation of terpenes production by engineered yeast strains in a lab-scale bioreactor.

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S7_OP53

INNOVATIVE SOLUTIONS FOR SUSTAINABLE AGRICULTURE: WATER AND NUTRIENT RECOVERY FROM CONSTRUCTED WETLANDS THROUGH BIONANOTECHNOLOGICAL APPROACHES

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Nature-Based Solutions (NBS) are recognised as alternative treatment methods for water reclamation and recovery of valuable resources (e.g. nutrient-rich biomass). In this context, CIRQUA, a 3-year PRIMA project focusing on upgrading constructed wetlands (CWs) within the framework of circular economy concept, aims to valorize non-conventional nutrient resources for precision irrigation and fertilization, which are key pillars of sustainable agriculture. CIRQUA develops new wastewater treatment approaches with higher removal efficiency, reduced energy consumption and a lower carbon footprint. It adopts a hybrid approach that integrates three cutting-edge technologies to improve water quality and efficiency, and increase soil fertilization. Special focus is placed on improving soil fertility by converting biomass into nutritional nanoparticles, to be used as soil improvers and fertilizers. The high nitrogen content of CW biomass, combined with bionanotechnological practices, enables the production of an innovative biomass-derived nanostructured fertilizer. This fertilizer can be applied in the agricultural sector both as a soil enhancer and a plant protection agent, exhibiting beneficial effects on soil microbial structure and activity.

Keywords: Constructed wetlands; biomass; soil fertility; water quality; soil microbiota; bioeconomy

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S7_OP54 (FT)

EXPLOITING A NOVEL NON-CONVENTIONAL ZYGOSACCHAROMYCES BAILII STRAIN TO ENHANCE WINE AROMA VIA COMMENSAL INTERACTION WITH SACCHAROMYCES CEREVISIAE

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Non-conventional wine yeasts represent a diverse group of species that differ significantly from the well-studied Saccharomyces cerevisiae. Among them, Zygosaccharomyces bailii is known for its ability to spoil food and beverages. However, significant variability in this phenotype at strain level provides an opportunity to discover efficient, non-spoilage strains with potential as wine starters. The aim of this study was to investigate the effect of microbial interactions between indigenous strains of Z. bailii and S. cerevisiae on the aromatic and sensory profiles of the produced wines. Towards this direction, sequential and simultaneous co-inoculation modalities were compared to pure cultures of Z. bailii and S. cerevisiae. Fermentation kinetics were monitored by measuring CO_2 production, while microbial populations were enumerated by plate counting. Additionally, High-Performance Liquid Chromatography (HPLC) analysis was performed during alcoholic fermentation in order to monitor sugar consumption and the production of some metabolites (e.g. acetic acid, lactic acid). Oenological characteristics of the final products were assessed using official methods of International Organization of Vine and Wine (OIV). The wine's aromatic profile was assessed via Gas Chromatography-Mass Spectrometry (GC-MS), while sensory analysis provided a holistic evaluation of inoculation modality effect. Overall, fermentation kinetics and microbial enumeration throughout the process suggests a commensal relationship between Z. bailii and S. cerevisiae. Notably, Z. bailii exhibits remarkable alcohol tolerance, fructophilic behavior, and the ability to metabolize malic acid, uncommon yet advantageous traits for the wine industry. Moreover, the sensory and aromatic profiles of the resulting wines are significantly shaped by the presence and activity of Z. bailii. The timing of S. cerevisiae inoculation in mixed fermentations emerges as a crucial factor, influencing both fermentation dynamics and wine composition. Among the inoculation strategies evaluated, co-inoculation proved most effective, yielding wines comparable to those fermented with pure S. cerevisiae cultures but with enhanced fruity, citrus, passion fruit, and floral characteristics. This study highlights a recently isolated strain of Z. bailii with promising oenological potential and suggests a type of commensal interaction with S. cerevisiae, opening new perspectives for the winemaker.

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ACTIVATION OF BACTERIAL SECONDARY METABOLISM BY NON-TARGETED COMPETITION OF STRAINS OR TARGETED ENGINEERING OF BIOSYNTHETIC GENE CLUSTERS PREDICTED TO GENERATE ANTIBIOTICS

Dimitra Basdani[1], Sokratis Zekkas[1], Evanthia Kontomina[1], Vasiliki Garefalaki[1], Christina Stavraki[1], Theofilos M. Vetsikas[1], Dorothea Evmorfidou[1,2], Konstantina C. Fylaktakidou[2], Giannoulis Fakis[1], Tamás Felföldi[3], Károly Márialigeti[3], Sotiria Boukouvala[1]

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Secondary metabolites (SMs) play a key role in microbial communities by mediating adaptation and interspecies competition. Microbial genomics combined with computational mining of Biosynthetic Gene Clusters (BGCs) reveal the immense biosynthetic potential of microorganisms, while promoting the discovery of new natural products for medicinal purposes. Co-cultivation of bacterial strains can stimulate microbial competition and has been shown to activate cryptic BGCs to produce SMs. However, this approach is non-targeted compared with direct cloning and heterologous expression of BGCs via genetic engineering methods. In the first part of this project, binary co-cultures were performed using isolates from our in-house taxonomically broad collection of non-pathogenic, free-living bacteria, documenting growth inhibition patterns between strains. To assess the possible production of antimicrobial SMs, the co-culture media was subjected to LC-MS analysis, providing proof-of-concept results for the bacterium Bacillus siamensis grown either with another bacterium (Lysinibacillus sphaericus) or with a fungus (Fusarium verticillioides), detecting SMs with well-documented antibacterial or antifungal properties, respectively. The second part of this project involved whole-genome Nanopore sequencing of 84 isolates, followed by computational prediction of BGC distribution using antiSMASH and BiG-SCAPE. This highlighted the biosynthetic capabilities especially of streptomycetes that possess 20–70 BGCs per genome. To enable targeted BGC activation, two different cloning strategies are currently being pursued. The first is employed to clone BGCs of 20-40Kb, predicted to produce antimicrobial compounds, using long-accurate PCR and in vitro Gibson assembly ligation into a BAC vector for transformation in Escherichia coli. As proof-of-concept, a BGC predicted in the genome of Streptomyces pilosus M97 to generate a metabolite similar to albaflavenone was cloned, and efforts for cloning additional BGCs are currently underway. In an alternative approach, in-gel Cas9-assisted targeting of chromosome segments (CATCH) combined with Gibson assembly is being optimized for 20Kb BGCs (e.g. for 7-prenylisatin), prior to application for larger BGCs. The long-term objective of this study is the heterologous expression of cloned BGCs to identify new antimicrobial SMs. *The research project was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the "2nd Call for HFRI Research Projects to support Faculty Members & Researchers" (Project Number: 3712).*

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S7_OP56 (FT)

EFFECTS OF BIOCHAR ON METHANE YIELD AND MICROBIAL DYNAMICS IN ANAEROBIC DIGESTION UNDER INCREASING ORGANIC LOADS.

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Biochar is a promising but debated additive in anaerobic digestion, potentially enhancing methane yield under specific conditions like high doses and substrate loadings (Hu et al., 2023). This study aimed to identify optimal biochar doses in batch tests and evaluate their effects on methane yield and microbial community dynamics in continuous systems with rising organic loading rates (OLRs).

Six wood-chip biochar concentrations (0–20 g/L) were tested via Biochemical Methane Potential (BMP) assays at 3 $gVS \cdot L^{-1} \cdot day^{-1}$. The top-performing doses (10 and 15 g/L) were used in mesophilic Continuous Stirred-Tank Reactors (CSTRs): R1 (control), R2 (10 g/L), and R3 (15 g/L), operated for 110 days across two OLRs (3 and 4 $gVS \cdot L^{-1} \cdot day^{-1}$). Methane production, pH, VFA concentration, and biogas composition were monitored. Reactors' microbial communities were analyzed via 16S rRNA sequencing.

Biochar improved methane yield in batch mode, but not in continuous operation, possibly due to biochar washout or poor microbial immobilization. All reactors showed methane yield decline at higher OLR without VFA or pH instability. Instead, elevated CO_2 levels indicated increased fermentation due to substrate overloading.

Initially, Firmicutes dominated, but at higher OLR, Synergistota, Planctomycetota, and Verrucomicrobiota became more abundant, likely due to higher influx of complex cellulosic compounds. The increase in OLR favored the abundance of methanogens Methanosarcina, hydrogenotrophic Methanoculleus, and putative syntrophic acetate-oxidizing bacteria (SAOBs), suggesting a shift toward hydrogenotrophic methanogenesis via syntrophic acetate oxidation (Zhang et al., 2022). However, CO_2 from SAOBs overwhelmed hydrogenotrophic methanogens' capacity.

Findings highlight that while biochar boosts methane in batch tests, its benefits in continuous systems may depend on better retention strategies to support microbial colonization. Further research is needed to optimize biochar application for long-term performance.

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S6_OP41

CLIMATE CHANGE METAGENOMIC RECORD INDEX (CCMRI) AND SAMPLE MATCHER: LEVERAGING METAGENOMIC DATA FOR CLIMATE CHANGE RESEARCH

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Climate Change (CC) is reshaping ecosystems globally. Metagenomic data can uncover the impact of CC on microbial communities and the role of the latter in these transformations. However, the vast amount of environmental genomics data complicates finding related studies. The Climate Change Metagenomic Record Index (CCMRI) aims to harvest metagenomic records related to CC and to provide researchers with a pertinent curated database. We are constructing such a database through, initially, the manual curation of all aquatic and terrestrial studies in Mgnify (assessing study titles, descriptions, and linked abstracts for CC clues). To keep future curation efforts scalable, we are developing an automated system that gathers and pinpoints new CC-related studies for a final manual inspection. To this end, text-mining, machine-learning, rule-based classification, and large language model (LLM) methods are being explored. A web platform will offer database access and email notifications upon new CC-studies.

In parallel, the Sample Matcher is an algorithm developed to facilitate the comparison and clustering of metagenomic samples based on their taxonomic and functional profiles. Building on MGnify data, Sample Matcher creates a global taxonomic and functional space in which samples can be compared to each other using similarity metrics, like euclidean and cosine distances. Dimensionality reduction techniques refine the analysis and improve clustering accuracy. In the CCMRI context, the Sample Matcher serves as a means to locate climate-change-related studies and samples based on taxonomy and functional data (and not just text-based input). Nonetheless, Sample Matcher is adaptable and could be employed for broader applications.

Acknowledgements

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S6_OP42

AEROBIC RESPIRATION KINETICS IN THE OXYGEN MINIMUM ZONES OF THE TROPICAL PACIFIC OCEAN

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Deoxygenation is one of the main environmental threats of the last century, with a direct impact on biogeochemical cycles and marine ecosystems. This loss of oxygen has contributed to the formation and expansion of oxygen minimum zones (OMZs), while hypoxic and anoxic events in coastal areas have become increasingly frequent. Microorganisms inhabiting these environments have evolved adaptations to perform aerobic respiration at low oxygen levels. Aerobic terminal oxidases, enzymes responsible for the final step in aerobic respiration, have a molecular diversity with different affinities for oxygen. While Eukaryotes have low-affinity terminal oxidases (LATO), prokaryotes might also have high-affinity terminal oxidases (HATO) and then a potential advantage under low oxygen conditions. The simultaneous expression of both terminal oxidases allows them to modulate their affinity for oxygen. The study of aerobic respiration kinetics through the kinetic parameters (maximum respiration rates and the Km) provides insight into how microbial communities adapt to decreasing oxygen conditions. Here we explore the respiratory kinetics of natural communities from coastal and oceanic areas in the OMZs located in the East North and South Tropical Pacific Ocean. We performed dark incubations exposing the community to a wide range of oxygen concentrations, from full saturation to nanomolar levels, measuring the oxygen consumption in water samples collected throughout the oxycline. In both types of OMZs, respiration rates showed a dependence with the available organic carbon pools (both dissolved and particulate forms), decreasing rates with depth. The apparent Km values of the community respiration showed a general pattern of decreasing values with oxygen concentration until reaching suboxic levels, suggesting an increase in the affinity for oxygen and an adaptation to low-oxygen concentrations. However, the apparent Km values increased again below this threshold, approaching values typically associated with LATO rather than HATO and then contrasting with the expected behaviour of increasing affinity at very low oxygen levels. These findings reveal the complex regulation of the terminal oxidases and the non-direct link between the presence of HATO in the genome and its use at low and trace oxygen levels, with relevant implications for understanding how microbes use oxygen in a deoxygenating ocean.

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S6_OP43

PRIORITIZATION OF MICROBIOLOGICAL HAZARDS IN FRESH PRODUCE UNDER CLIMATE CHANGE

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Climate change is an inherently unpredictable phenomenon, where small variations in initial conditions can trigger disproportionately large and often unforeseen effects. This complexity extends far beyond weather systems, influencing agriculture, microbial ecosystems, and critically, the safety of our food supply. Among its many impacts, climate change alters the prevalence of microbial and chemical hazards, especially in fresh produce, posing significant challenges for public health. Given the uncertainty of climate change, how can we effectively predict and prioritize such microbiological/chemical hazards? Addressing this challenge, we present a comprehensive framework for hazard prioritization in fresh produce within the context of climate change. Our approach combines a bottom-up expert elicitation process with a top-down systematic evidence synthesis, integrated through Multi-Criteria Decision Analysis (MCDA) to unify these complementary data streams into a coherent ranking.

In the bottom-up approach, a structured questionnaire was distributed to experts across Europe to gather semi-quantitative inputs for criteria weighting within the MCDA framework. This analysis allowed for ranking hazards across various produce types, microorganisms, and biogeographical regions. Enteric pathogens, specifically Escherichia coli and Salmonella spp., and Campylobacter spp. ranked higher amongst biological hazards, while mycotoxins ranked higher amongst chemical hazards. These findings shaped the focus of the top-down analysis.

Concurrently, a top-down approach was undertaken through three PRISMA-compliant (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) systematic literature reviews and meta-analyses focused on the prevalence and concentration of E. coli, Salmonella spp., and Campylobacter spp. in fresh produce, primarily leafy greens such as spinach and lettuce. Data were stratified by four European biogeographical regions (Mediterranean, Boreal, Atlantic, Continental) at the country level, and across all seasons, capturing both spatial and temporal variability relevant to climate change.

The integration of expert-derived criteria weighting with systematically reviewed prevalence data enabled a robust prioritization of microbiological hazards in fresh produce within a climate change context. This combined approach addresses the inherent uncertainties of climate-influenced food safety, supporting risk-informed decision-making and proactive hazard management in fresh produce supply chains.

Acknowledgements

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CLIMATE CHANGE

S6_OP44 (FT)

PREVALENCE OF ESCHERICHIA COLI ON FRESH PRODUCE ACROSS EUROPE: A META-ANALYSIS WITH IMPLICATIONS FOR CLIMATE-RELATED RISK FACTORS

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The occurrence of Escherichia coli, particularly pathogenic strains, remains a persistent concern in foodborne outbreaks linked to fresh produce in Europe. Growing consumption of raw fruits and vegetables, combined with changing climatic conditions, raises concerns about the shifting dynamics of microbial contamination in the agri-food system. This meta-analysis aimed to estimate the prevalence of E. coli in fresh produce across European countries, organized by biogeographical zones, and to evaluate potential associations with climate parameters, especially temperature and precipitation. Following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, a systematic review was conducted using three scientific databases—Web of Science, Scopus, and PubMed—targeting peer-reviewed publications from 2004 to 2024. Included, studies reported empirical data on E. coli detection in fresh produce within Europe. "Fresh produce" encompassed a wide spectrum of products including leafy greens, fruits, herbs, and vegetables (e.g., spinach, lettuce, tomatoes, cucumbers, berries, melons, basil, parsley, beans). Data on sample size, isolation outcomes, detection methods, and sampling location were extracted. European countries were classified into four major biogeographical regions—Atlantic, Continental, Mediterranean, and Boreal—according to the European Environment Agency (EEA) framework. The results showed significant heterogeneity in the prevalence of E. coli across different geographic zones and produce types. This variability is likely influenced by diverse environmental conditions, agricultural practices (e.g., use of manure, irrigation sources), and handling techniques post-harvest. Notably, warmer temperatures and increased rainfall appeared to correlate with higher detection rates of E. coli, suggesting a potential role of climate in promoting bacterial survival and dissemination. The I^2 statistic confirmed high heterogeneity among studies, reflecting regional and commodity-specific differences in contamination levels. These findings emphasize the importance of incorporating climate data into microbial risk assessment and food safety monitoring frameworks. Understanding how environmental variables influence E. coli presence in fresh produce can support the development of adaptive, region-specific safety strategies. As climate change continues to reshape the European agricultural landscape, such insights are crucial for protecting public health and ensuring the resilience of the agri-food supply chain.

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S6_OP45 (FT)

EFFECTS OF DAILY TEMPERATURE FLUCTUATIONS AND HEATWAVES ON THE BENTHIC METABOLISM OF INTERTIDAL MUDFLATS IN THE SOUTH OF SPAIN.

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Coastal wetlands are particularly vulnerable to extreme climatic events due to the low capacity to buffer temperature variations. Prediction models suggest that heatwaves, defined as prolonged periods of unusually high temperatures, will be increasingly frequent and severe, with uncertain consequences for coastal ecosystems. The sediment surface of intertidal zones is commonly covered by benthic microalgae, (microphytobenthos MPB), which are one of the most relevant primary producers in shallow coastal ecosystems. MPB is strongly affected by environmental conditions, since it is exposed to the atmosphere during emersion period, being particularly vulnerable to weather and climatic alterations. We investigated how diurnal temperature fluctuations oscillations and heatwaves affect the primary production of MPB in shallow coastal environment in laboratory experiments and in situ. Sediment cores collected from saltmarshes in southern Spain were exposed for 7 days to different thermal regimes in the laboratory, being: constant (20°C), natural temperature oscillations (20-28°C) and heatwaves conditions (20-34°C). Laboratory temperature fluctuations and light regimes reflected those previously observed during in situ measurements in summer. In situ measurements were conducted in the same area before, during and after heatwaves events. O2 microprofiles and whole core incubations were used to estimate Net Community Production (NCP). Daily temperature oscillations, both in the laboratory and in situ, evidenced diurnal changes of productivity, with a general NCP peak in the first half of the day and a progressive decrease towards the end of the light period, being coupled to the light absorbed at the sediment surface and thus reflecting the impact of vertical migration rhythms. When the different temperature regimes are compared, our results show an initial increase on NCP in sediments exposed to higher temperatures, but decreasing to values below the control after prolonged exposures at heatwave simulations in the laboratory, resulting in an overall decrease in NCP. This research underlines the negative effects of extreme climatic events on the microphytobenthic community. Prolonged periods of high temperature episodes damaged the microbial community and revealed the importance of further investigating the effect of heatwaves on the MPB.

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CONSERVATION / RESTORATION

S6_OP46 (FT)

INDIGENOUS AND PLANT SPECIFIC MICROBIAL COMMUNITY CONSORTIA DERIVED FROM PHRYGANIC BIOMES IMPROVE PLANT FITNESS AND SOIL FUNCTIONS IN ECOSYSTEM RESTORATION OF QUARRY DEPOSITS IN MILOS (GREECE)

Georgios Leventis[1], Myrto Tsiknia[1], Dimitra Stathopoulou[1], Hamza Khassali[4,5], Georgios Petrakis[2], Christiana Chadjimichael[3], Johana Rodosthenous[3], Michalis Omirou[3], Constantinos Ehaliotis[1]

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Quarrying operations cause irreversible degradation to the local environment including fertile soil depletion, vegetation removal and alterations to the original landscape and topography. Restoration practitioners aim to restore ecosystem functions by reintroducing plants, focusing on recreating specific plant communities based on historical, reference, or other desirable output. However, routine restoration interventions often overlook the importance of soil microbial communities and plant-microbe interactions and underutilize microbiome-based tools. Although root-associated microbial communities play a pivotal role in plant-host performance and stress tolerance, support pioneer plant colonizers and may provide essential ecosystem services, such as disease suppression, pollutant remediation, nutrient cycling, water management, and carbon sequestration, their potential use in soil restoration and rehabilitation remains underexplored. This may be particularly important in the restoration of fragile, water-limited Mediterranean biomes, which is based on the use of indigenous flora. We investigated the impact of inoculations with indigenous whole rhizospheric bio-communities - derived from conspecifics or respective pioneer plant species - on the growth, performance and nutrition of two drought tolerant native legume shrubs (Anthyllis hermanniae & Calicotome villosa), along with their effects on soil fertility and functions. NGS analysis will further clarify how the modifications induced by the inoculations on the rhizospheric and endoroot microbial communities relate to the changes observed at the plant and soil level. We propose that this promising microbiome-based approach may be further developed and serve as a sustainable, eco-friendly and cost-effective strategy for enhancing sustainable revegetation and soil restoration in barren quarry areas.

Acknowledgements: The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 6750).

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S6_OP47

CROSS-KINGDOM RNAI INDUCED BY A BENEFICIAL FUNGUS TO ITS HOST REQUIRES TRANSITIVITY AND AMPLIFICATION OF SILENCING SIGNALS.

Loukia Maria Kellari[1], Athanasios Dalakouras[2], Olga Tsiouri[1], Panagiotis Vletsos[1], Afrodite Katsaouni[1], Veli Vural Uslu[3,4], Kalliope Papadopoulou[1]

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Cross-kingdom transfer of small RNA (sRNA) molecules has been identified as a means of communication between plants and interacting microorganisms, but the mechanistic details of this sRNA-based interaction remain elusive. We have previously shown that the beneficial root-colonizing fungus Fusarium solani strain K (FsK) translocates sRNAs to its host Nicotiana benthamiana (Nb) leading to systemic silencing of a reporter gene (Dalakouras et al., 2023; Kellari et al., 2025). Here, we investigated the mechanistic details of the endophyte-induced systemic silencing using an RNAi sensor system. We inoculated three Nb GFP expressing lines with conidia of an FsK transformant containing a transgene that targets host GFP (FsK-hpGF). The efficiency of silencing mediated by FsK-hpGF was monitored both phenotypically under ultraviolet light as well as quantitatively by RT-qPCR. sRNA sequencing was performed to evaluate the production of sRNAs targeting host GFP. Finally, bisulfite sequencing was used to assess plant GFP methylation levels. We showed that the translocated fungal sRNAs induced the production of secondary sRNAs mainly of 22-24-nt in size. Importantly, systemic silencing could not be induced in an RNA-DEPENDENT RNA POLYMERASE 6 (RDR6) CRISPR/Cas knockout background nor in an intron-containing gene (Kellari et al., 2025). Overall, our data show that endophyte-induced silencing in the host requires RDR6-mediated transitivity and amplification of silencing signals (Kellari et al., 2025). Our observations may reflect a more generalized and so far unexplored facet of cross-kingdom RNAi, with RDR6-based transitivity influencing the way symbionts and pathogens elicit systemic phenotypes in their host plants.

Dalakouras, A., Katsaouni, A., Avramidou, M., Dadami, E., Tsiouri, O., Vasileiadis, S., Makris, A., Georgopoulou, M. E., & Papadopoulou, K. K. (2023). A beneficial fungal root endophyte triggers systemic RNA silencing and DNA methylation of a host reporter gene. RNA Biology, 20(1), 20–30.

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S6_OP48

BACTERIAL MICROBIOTA OF THE INVASIVE RED SWAMP CRAYFISH PROCAMBARUS CLARKII UNDER EXPERIMENTAL CONDITIONS

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Procambarus clarkii, classified as an invasive species, is also an economically important farmed species for several European countries. The gut microbial communities associated with P. clarkii remain poorly described especially when the crayfish is grown under different conditions such as with/without plants and sediments. To provide insights into these questions, gut samples from P. clarkii grown in large (ca. 9 m3) open-air mesocosms, as well as control sediment, water and biofilm samples were collected from four different tanks at two different time points (June and October). Amplicon sequencing of the V3-V4 variable region of the 16S rRNA gene was performed for 131 samples. QIIME2 (2021.8) was employed for analysis of the resulting data and the SILVA 138.1 database for assigning OTU taxonomy. The metadata collected included the physicochemical properties and individual animal ID tags for the four mesocosms. Mesocosms clustered separately based solely on physiochemical parameters regardless of the date the samples were collected, indicating similar gut microbiomes between freshly inoculated animals vs. animals acclimated in the mesocosms for several months. Alpha diversity metrics (observed features) demonstrated diversity differences between mesocosms, with no apparent difference in alpha diversity between male and female animal samples. Beta diversity comparisons indicated that environmental and gut samples cluster separately, revealing that specific taxa are enriched in the crayfish gut. The top two most abundant taxa throughout the samples, and especially the gut samples, included Candidatus Bacilloplasma (family Mycoplasmataceae) and ZOR0006 (family Erysipelotrichaceae). Notably, Candidatus Bacilloplasma was previously shown to be highly abundant in Norway lobster samples. These results have implications for tracking P. clarkii fitness under different farming conditions and for understanding the role of its microbiome on new location colonization and survival.

ONE HEALTH

S8_OP58

DECODING BACTERIAL DYNAMICS IN BOVINE MASTITIS: A PROTEOMIC EXPLORATION OF S. AUREUS AND NON–AUREUS STAPHYLOCOCCI AND MAMMALIICOCCI IN MILK

Alicja Krysmann[1], Morten Kjos[1], Davide Porcellato[1]
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Mastitis, an inflammatory mammary gland disease, is a significant challenge in the dairy industry due to its economic impact and animal welfare implications. Staphylococcus aureus (SA) is one of the major pathogens responsible for contagious mastitis and can easily spread within the herd. Its problematic nature is related to its ability to persist in the mammary gland, and the difficulty of antibiotic treatment, as mastitis-causing strains are often highly adapted to the udder environment. In contrast, the role of non–aureus staphylococci and mammaliicocci (NASM) in bovine mastitis is less well understood. Although NASM are among the most frequently isolated bacteria from milk, they are rarely associated with clinical mastitis. They can be present in the quarter together with SA, however not much is known about their interactions.

In this study, we aim to investigate interactions between SA and NASM isolated from milk using a bottom-up proteomic approach. Bacterial strains (SA, S. chromogenes and S. epidermidis) isolated from bovine hindmilk were cultivated in milk at 37°C to mimic conditions in the udder. Bacterial cell pellets were collected at 8 and 72 hours of incubation and analyzed by LC-MS/MS with a gel-free suspension trapping (S-Trap) sample preparation method. Raw data was processed with Max-Quant and analyzed in R.

Differential enrichment analysis showed significant changes in the proteome of SA grown in monoculture compared to co-culture with S. chromogenes. The presence of S. chromogenes affected the expression of nearly 30 proteins, with 12 proteins upregulated and 17 downregulated in co-culture. Some proteins overexpressed in co-culture were found to be involved in stress response pathways including superoxide dismutase and oxygen-dependent choline dehydrogenase. In co-culture with S. epidermidis, expression of only 10 proteins in SA was altered, with some upregulated proteins, such as thioredoxin and nucleoid-associated protein, also linked to stress response.
The results show that SA is affected by the presence of NASM, particularly S. chromogenes, in the milk. While the exact role of NASM in mastitis is not clear, our results indicate that they regulate microbiome dynamics in the udder and interact with pathogens to modulate their physiology.

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S8_OP59

THE FATE OF MANURE AND RECYCLED WATER INTRODUCED MICROBIOME, RESISTOME AND MOBILOME TO SOIL: THE PERSISTING AND DISSIPATING ONES

Marios I Valmas[1], Massa Polasek[1], Domna Nikolaidou[1], Costas C Papagiannitsis[2], Dimitra Ntinouli[3], Georgios Argyris[4], Johanna Rhodosthenous[5], Christiana Hadjimichael[5], Michalis Omirou[5], Nikiforos A Alygizakis[6], Dimitrios G Karpouzas[1], Sotirios Vasileiadis[1]

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Water recycling and manuring are prerequisites of sustainable agricultural. They are constituents of circular economy and climate change mitigation. Since One Health's zero tolerance to pathogen and antimicrobial resistance (AMR) dispersal, our deep understanding of the fate of microbial inputs from such practices is necessary. Here, we attempt to model the impacts of these inputs employing soil microcosms. Urban wastewater treatment plant influent/effluent and pig manure were tested as inputs, including treatments with tetracycline and sulfamethoxazole at realistic concentrations. The microbial mass (qPCR) and diversity (16/18S rRNA gene and ITS amplicon sequencing) were monitored, alongside antibiotics (HPLC), at five timepoints (0, 0.5, 1, 4 and 25 days). Shotgun sequencing was also conducted for key samples. Out of the tested inputs, only manure-specific taxa (not detected in soil prior manure application) were detected post application in soil at significant amounts (>20% of the total community). Recycled water-specific taxa showed only sporadic appearances at near noise levels. Manure taxa comprised 6-34% of the total soil community right after amendment, with some prokaryotes (e.g. Clostridium) being persistent, whereas others (e.g. lactobacilli) dissipated relatively quickly (DT50, ≤5 days), or went below detection limits after 4 days (e.g. Pseudomonadota) according to the best fit exponential decay model. Markers genes coding for tetracycline (tetQ) and erythromycin (ermF) resistance (which increased by 2 and 6 orders of magnitude compared with the background abundance at day 0) were also exponentially decayed, while intI1 abundance was stimulated post manure addition. In nearly all exponential decay modelled responses, antibiotic application increased the tested-marker persistence according to estimated dissipation time values. Shotgun metagenomic AMR, rest biocide resistance, virulence and bacteriophage marker analysis showed a convergence of the amended soils with the controls after 25 days. Overall, our results demonstrate significant, yet, transient, perturbations for the bulk of the tested microbial biomarkers, with several introduced taxa persisting and horizontal markers like intI1 being stimulated. Finally, integron sequencing analysis is currently performed for the discovery of mobile AMR cassettes and elements, their origin and persistence. This study deepens our understanding about the impact of agricultural inputs from the One Health perspective.

ONE HEALTH

S8_OP60

PRESSURE EFFECTS ON THE DEGRADATION OF HYDROCARBONS BY DEEP-WATER MICROBIAL COMMUNITIES IN THE EASTERN MEDITERRANEAN SEA

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Microbial organic matter degradation is pressure-sensitive and negatively impacted by decompression of deep-sea water samples. Furthermore, removing hydrostatic pressure will likely alter the overall sign and strength of interspecies interactions with subsequent consequences on the microbial community structure and function. Following the Deepwater Horizon (DWH) accident in the Gulf of Mexico, the contamination of deep-sea ecosystems with hydrocarbons and the crucial role of indigenous microbes in bioremediation was acknowledged. Yet, the study of deep-sea oil biodegradation is largely carried out using decompressed microbial communities.

Here, we present the collective results from 2 research projects in which we studied how pressure affects the degradation of hydrocarbons in the deep Eastern Mediterranean Sea. Using an advanced high-pressure system, we were able to retrieve water samples from 1000 m depth and perform further experimentation in the lab without decompression at any stage of the process. In a series of experiments, we managed to emulate a deep oil plume in the Eastern Mediterranean Sea (EMS), similar to that observed in DWH. We followed the taxonomic and functional response of the mesopelagic microbial community to oil contamination and dispersant application. We identified 71 dereplicated high-quality metagenome-assembled genomes (MAGs) which were taxonomically classified and annotated to gain insight into their metabolic potential. We further performed sequential enrichments in suitable media to isolate piezotolerant hydrocarbon-degrading bacteria and studied the effect of re-pressurisation of decompressed samples in the lab, a common practice in deep-sea research. Our results showed that depressurization during sample retrieval resulted in the loss of microbial diversity despite the restoration of in situ pressure conditions during incubation. This, in turn, impacted function and specifically the degradation of recalcitrant oil compounds, especially PAHs. Finally, based on molecular analysis and enrichment experiments, we maintain that Alloalcanivorax venustensis plays a key role in the removal of hydrocarbons in the deep EMS. Overall, this work emphasizes the importance of maintaining undisturbed pressure conditions, if lab incubation experiments to study deep-water microbial diversity, metabolic rates and functional responses are to follow

ONE HEALTH

S8_OP61

RESTRECO: A METAGENOMIC APPROACH TOWARDS LAND RESTORATION TO ENSURE SOIL MICROBIAL DIVERSITY, FUNCTIONALITY, AND ECOSYSTEM RESILIENCE

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Global biodiversity is under unprecedented threat due to intensifying pressures such as climate change and land degradation. Ecological restoration is increasingly recognised as a critical strategy to counteract these losses. Traditionally, restoration targets have been guided by "indigenous reference systems" that aim to replicate historical species assemblages. However, this approach is becoming less feasible given the dynamic nature of ecosystems and environmental variability. The RestREco project (https://restreco.com/about/) proposes a shift in perspective; prioritising ecosystem complexity, multifunctionality, and resilience as core goals in restoration science.

In this study, we examined microbial community dynamics in 330 soil samples from 66 restored grassland sites across England and Scotland. These samples spanned diverse restoration contexts, capturing metadata such as soil pH, restoration age, establishment technique, and grazing regimes. Using 16S rRNA and ITS amplicon sequencing, we implemented an integrated bioinformatics pipeline This reproducible pipeline not only advances our understanding of soil microbial ecology in restoration but also offers actionable insights for land managers and conservation practitioners.

incorporating QIIME2, DADA2, PICRUSt2, FUNGuild, and R-based statistical analyses to explore taxonomic and functional patterns in both bacterial and fungal communities.

Our findings reveal that soil pH and restoration method are dominant factors shaping microbial diversity and community structure. Sites restored using seed mixtures or green hay exhibited more distinct and diverse microbial assemblages compared to those under natural regeneration. Functional predictions highlighted shifts in pathways related to nutrient cycling and organic matter breakdown, with strong associations between bacterial metabolic pathways and fungal ecological guilds; underscoring the interdependence of microbial functional roles in restoration contexts. To promote transparency and broader application, we developed an open-access, interactive HTML-based reporting tool, enabling users to visualise diversity trends, taxonomic distributions, and functional predictions across environmental gradients.

Explore the pipeline here:
https://github.com/kerrycranfield/dig-deeper-project

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AGRICULTURE

PP001

EVALUATING THE NITRIFICATION INHIBITION POTENTIAL OF TRIGONELLA FOENUM-GRAECUM UNDER VARYING AMMONIUM SOURCES AND CONCENTRATIONS

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Biological nitrification inhibitors (BNIs) are natural compounds secreted by plant roots that suppress the activity of ammonia-oxidizing microorganisms (AOM), offering a sustainable alternative to synthetic nitrification inhibitors (SNIs) for reducing nitrogen (N) losses in agroecosystems. BNIs are considered an evolutionary adaptation of certain plant species to retain N as NH_4^+ in N-limited soils. However, the specific environmental and physiological factors that promote their production and exudation remain unclear. In this study, we investigated the BNI potential of Trigonella foenum-graecum (fenugreek), a leguminous feedstock crop with a highly active secondary metabolism, by cultivating three distinct varieties — "Grevena", "Cappadocia", and "Ionia" — under controlled conditions. Plants were treated with urea and ammonium chloride (NH_4Cl) at N application rates of 1, 25, 50, and 100 mg N kg^{-1} in a sand–vermiculite mixture to assess the influence of ammonium source and concentration on BNI release. Both hydrophilic and hydrophobic root exudates were extracted from plant roots and screened for their inhibitory activity against three phylogenetically diverse, soil-relevant ammonia-oxidizing bacteria (AOB) strains (Nitrosomonas communis, Nitrosomonas ureae, and Nitrosospira multiformis) as well as one ammonia-oxidizing archaeon (AOA) strain (Candidatus Nitrosocosmicus franklandianus), using a high-throughput, fast-track bioassay. Preliminary results indicate that urea application stimulated the release of potential BNI compounds in the "Grevena" variety, leading to significant AOB inhibition at 25 mg N kg^{-1}. Hydrophobic root exudates exhibited consistently stronger BNI activity than hydrophilic exudates, while results for the other varieties are currently under evaluation. Future work will focus on

screening additional AOA strains (Nitrosotalea sinensis and Nitrososphaera viennensis), testing fenugreek varieties under varying pH and moisture regimes, and optimizing cultivation practices to enhance BNI exudation and nitrification inhibition. This work advances our understanding of the role of Trigonella foenum-graecum in biological nitrification inhibition and the environmental factors influencing BNI exudation, highlighting its potential application in sustainable nitrogen management strategies.

AGRICULTURE

PP002

EXPLORING BACILLUS-BASED ANTIFUNGAL AGENTS FOR SUSTAINABLE AGRICULTURE

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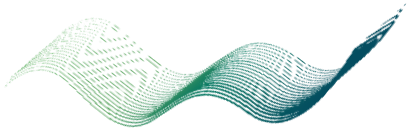
Certain filamentous fungi act as plant pathogens, leading to significant economic losses in food products and agricultural goods. Growing interest in sustainable farming practices has led to increased focus on biological methods for managing plant diseases, offering an alternative to conventional chemical fungicides. Bacillus species are used as biocontrol agents to help control a wide range of soilborne plant pathogens. This study aimed to isolate soil-derived Bacillus strains with the ability to inhibit various common field-pathogenic fungi and to evaluate their antifungal properties, including the protective role of the biosurfactants they produce in defending plants against phytopathogens. Bacillus amyloliquefaciens and Bacillus velezensis were isolated and idenified on the basis of their morphological characteristics, heat treatment and determination by MALDI-TOF MS. The isolates were tested against 13 phytopathogens using the agar well-diffusion method and showed significant antifungal activity against Penicillium expansum, Plasmopara viticola, Monilinia fructicola and Peronospora sp.. Both isolates showed the highest inhibitory effect against Peronospora sp. at 25 mm. Determination of their antibiotic resistance was performed using the Kirby Bauer test against 24 antibiotics. Antibiotic resistance testing is crucial to ensure the safe use of bacterial strains in agriculture, comply with regulatory standards, fully characterize the strains, and prevent disruption of soil microbial communities. Both isolates were resistant only to cefotaxime. Growth occurred at pH values ranging from 3.0 to 9.0, and optimum growth occurred at about pH 8.0. The optimum temperature for growth was around 44ºC. Both isolates were also tested for biosurfactant production using the oil spread method and both showed positive results. The emulsification activities of the strains using sunflower oil was 60%. Considering their antifungal and biosurfactant activities together, Bacillus

amyloliquefaciens and Bacillus velezensis show potential for use as biocontrol agents or production of antifungal preparations.



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PP003

NATURAL BIOFORMULATIONS MEDIATED SHIFTS IN RHIZOSPHERE MICROBIOME AND CROP TRAITS IN POLYCULTURE STRAWBERRY SYSTEMS

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The present study comprehands the effect of bio-organics in legume intercropped strawberry cv. Camarosa. Natural bio-organic fertilizer sources included were Jeevamrit (JV), Ghan-Jeevamrit (GJ) and Azolla. Coriander-Strawberry-Fenugreek as intercropping system was adopted. The treatments comprised were T1: GJ at 100 g/m2 +JV at 10% +Azolla at 200 g/plant, T2: GJ at 150 g/m2 +JV at 10% +Azolla at 200 g/plant, T3: GJ at 100 g/m2 +JV at 20% +Azolla at 200 g/plant, T4: GJ at 150 g/m2 +JV at 20% +Azolla at 200 g/plant, T5: GJ at 100 g/m2 +JV at 10% +Azolla at 250 g/plant, T6: GJ at 150 g/m2 +JV at 10% +Azolla at 250 g/plant, T7: GJ at 100 g/m2 +JV at 20% +Azolla at 250 g/plant, T8: GJ at 150 g/m2 +JV at 20% +Azolla at 250 g/plant, T9: GJ at 150 g/m2 +JV at 20%, T10: Farmyard manure (100% N basis) and T11: Recommended dose of N:P:K (80:40:40 kg/ha) as control. Uniform application of bio-stimulants at 50 g/plant and AM fungi @ 20 g/ plant was applied in T1 –T8. Coriander-fenugreek crop sequencing was carried between two rows of strawberry plantlets in the same plot (treatment-wise) during the cropping cycle. One month after transplanting, T3 showed positive influence on vegetative growth traits of strawberry. This treatment also observed maximum yield contributing traits. Post harvest soil chemical indicators were also significantly influenced compared to farmyard manure (100% N equivalence). Microbial biomass of total bacteria, soil fungi, actinobacterial count, phosphorous solubilizing bacteria, AM fungi, Azotobacter count and soil enzymatic activity of phosphatase and dehydrogenases showed a steady rise in rhizosphere microbiome. The positive influence NPK content in leaf and fruits were also recorded. This study inferred that application of bio-organic inputs sources which can boost up cropping behavior, post harvest soil indicators, native microbial properties and enzymatic activity in rhizosphere, and thus can have the potential to improve crop resilience and soil productivity on sustainable basis.

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PP004

NATURAL BIOFORMULATIONS MEDIATED SHIFTS IN RHIZOSPHERE MICROBIOME AND CROP TRAITS IN POLYCULTURE STRAWBERRY SYSTEMS

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Dicyandiamide (DCD) is a synthetic nitrification inhibitor (NI) widely used in agriculture to delay the microbial conversion of ammonium-N to nitrate-N, improving nitrogen use efficiency. Despite its longstanding use in the agricultural sector, the precise mode of action on target microorganisms at the cellular level is still not fully resolved. DCD is thought to impair the activity of ammonia monooxygenase, the enzyme responsible for the first and rate-limiting step of nitrification, either by reversibly binding to copper sites on the enzyme or by impairing ammonia uptake. This study aims to elucidate the physiological responses and molecular mechanisms of DCD on representative strains of ammonia-oxidizing bacteria (Nitrosospira multiformis) and archaea (Ca. Nitrosocosmicus franklandianus) by comparing their transcriptional profiles in the presence or absence of DCD. Identification of differentially expressed genes will provide insights into the cellular responses of ammonia-oxidizing strains to DCD exposure, which remains underexplored. Preliminary data suggest that DCD may primarily affect cellular aggregation in the AOA representative strain, Ca. N. franklandianus, rather than directly inhibiting ammonia oxidation, offering a potential alternative explanation for its mode of action. Data processing is ongoing and results will be presented at the conference.

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PP005

APPLICATION OF FULL-SCALE ON-FARM PILOT BIOLOGICAL SYSTEMS FOR THE MITIGATION OF POINT SOURCE CONTAMINATION OF NATURAL WATER RESOURCES OF GREECE WITH PESTICIDES

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Pesticides are significant pollutants of water systems in Greece and Europe as evidenced by their systematic detection in surface and groundwater systems. This pollution is attributed to non-point or point sources. Point sources resulting from inappropriate handling of pesticides at farm level (loading, emptying, cleaning of sprayers) contribute significantly (up to 80%) to the pollution of water resources with pesticides. A possible strategy for limiting point pollution at the farm level is the use of biobeds. These are trenches in the ground that are insulated at the bottom and filled with a bioorganic mixture consisting of soil, lignocellulosic and humified organic materials. The effectiveness of biobeds relies on the capacity of the biomixture to support microbial communities that could actively degrade pesticides, as well as to adsorb pesticides and remove them from wastewaters. Two full-scale on-farm biobeds were constructed in the premises of growers associations THESTO and SEKE in Larissa and Xanthi, Greece respectively. The two biobeds were receiving wastewaters produced by the farmers of the two associations for two consecutive cultivation seasons. The concentration of the pesticides was regularly monitored in the influent and the effluent of the biobed systems and their removal efficiency was monitored for pesticides used by the local farmers. Removal efficiencies for pesticides like flupyradifurone, chloratraniliprole, tebuconazole, difenoconazole, trifloxystrobin and abamectin ranged from 96 to 100%. Preliminary risk assessment analysis showed that the treated effluents released by the biobeds could be recirculated in the agriculture with reduced risk to non-targeted organisms. Collectively, these results demonstrate high efficiency of the biobeds as biodepuration systems for pesticide-contaminated effluents and their implementation represents an additional step towards integrating European Community directives, actions and legislation into agricultural practice.

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PP006

GRAPEVINE WOOD MICROBIOME ANALYSIS AND ITS POTENTHIAL AS A TOOL FOR GRAPEVINE TRUNK DISEASES DETECTION

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Grapevine trunk diseases (GTDs) caused by pathogenic fungi are considered the biggest threat for viticulture worldwide with annual economic losses of 1.132 million euros. More than 70 fungal species have been associated with GTDs. The withdrawal from the market of sodium arsenate combined with the lack of efficient alternatives have exacerbated the reductions in grapevine productivity by GTDs. Early identification of GTDs fungi is a difficult task considering the fact that fungi reported to be associated with GTDs are often detected in both asymptomatic and symptomatic grapevines and symptoms appear even five years after the detection. This latency in the disease appearance suggests that other factors are also involved in GTD incidence. We aimed (a) to determine the factors shaping the grapevine wood microbiome (b) to identify potential fungal indicators in the wood microbiome for early detection of GTDs (c) to explore the grapevine wood microbiome as a pool for novel biocontrol agents against GTDs. To achieve these goals, we determined the fungal and bacterial microbiome in wood tissues of asymptomatic and symptomatic vines from Sultanina cultivar in three distinct geographical viticultural zones of Greece (Northern, Central, Southern), using amplicon sequencing. Our analysis identified biogeography as the strongest determinant of the wood fungal microbiome ($p<0.001$, 18.5%) followed by vine age (Over and Under 20 years) ($p<0.001$, 7.3%) while GTD condition showed a much weaker but still significant effect ($p<0.001$, 1.3%). Fungi previously reported as potential GTD pathogens were associated with different viticultural zones like Neofusicoccum sp., Kalmusia variispora and Phoma aloes in Northern Greece, Fomitiporia sp. in Central Greece, and Phaemoniella chlamydospora, Phaeoacremonium sicilianum, Neosetophoma rosarum in Southern Greece. Still these fungi were detected in equivalent relative abundance in asymptomatic and symptomatic grapevines. Wood bacterial microbiome showed similar but weaker signatures compared with fungi. Biogeography was the main determinant ($p<0.001$, 3.6%) of the bacterial community followed by vine age ($p<0.001$, 1.3%), while GTD condition showed no effect. Co-occurrence network analysis between GTDs pathogens and bacterial strains identified interactions that could be further pursued for the isolation and discovery of new biocontrol agents against GTD causal agents.

Acknowledgment: The present study was conducted within the framework of the project entitled: «Innovations in Plant Protection for sustainable and environmentally friendly pest control» (Acronym InnoPP, Code Act TAA: TAEDR-0535675) «Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan»

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PP007

PLANT TRITERPENOIDS AS NOVEL BIOLOGICAL NITRIFICATION INHIBITORS WITH ARCHAEAL SELECTIVITY

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Biological nitrification inhibitors (BNIs) offer a sustainable strategy to enhance nitrogen use efficiency and reduce nitrogen losses in agriculture. In this study, we evaluated the nitrification inhibition (NI) potential of 18 plant-derived triterpenoids using in vitro assays with soil ammonia-oxidizing bacteria (AOB) (Nitrosospira multiformis, Nitrosomonas ureae) and archaea (AOA) (Nitrososphaera viennensis, Nitrosotalea sinensis) at two concentration levels (5 and 20 mg L^{-1}). Our results revealed that triterpenoids were highly effective against AOA, outperforming sakuranetin, a known BNI exuded by sorghum (Sorghum bicolor) roots, while showing limited activity on AOB. Six triterpenoids achieved high levels of AOA inhibition (74–100%), with 3-O-acetyl-11-keto-β-boswellic acid emerging as the most potent (ammonia oxidation inhibition >96%), followed by 11-keto-β-boswellic acid, echinocystic acid, asiatic acid, and ursolic acid. Interestingly, the strong inhibition of AOA by certain triterpenoids may be linked to their known suppression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), an essential enzyme in archaeal membrane biosynthesis. This suggests a broader archaeal-specific mechanism that warrants further validation. Overall, this study highlights the potential of plant-derived triterpenoids as selective BNIs, contributing to the development of bio-based strategies for sustainable nitrogen management in agriculture.

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PP008

ISOLATION AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING MICROORGANISMS FOR DEVELOPING BIOINOCULANTS TARGETING SUSTAINABLE AGRICULTURE

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Climate change and global population growth are escalating pressure on food production systems, driving the intensification of agriculture. However, the extensive use of synthetic fertilizers in conventional farming has resulted in serious environmental impacts, including biodiversity loss, elevated greenhouse gas emissions, and health concerns. In response, the European Union adopted Regulation (EU) 2019/1009 to promote the use of biostimulants, which are biologically derived substances and microorganisms that boost plant growth, resilience, and environmental sustainability. Microbial inoculants, including plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), represent a key category of biostimulants. These inoculants, composed of single or mixed beneficial microbial strains, support plant development through mechanisms including phytohormone production (e.g., indole-3-acetic acid, gibberellins), nutrient solubilization (phosphate and iron), and biological nitrogen fixation. AMF, in particular, form symbiotic associations with plant roots, improving nutrient and water uptake, stress tolerance, and disease resistance, while contributing to soil structure and nutrient cycling via hyphal networks and glomalin production.

The present study aimed to isolate and characterize plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) from soil and rhizosphere samples collected in Thebes, Central Greece, for potential development as microbial inoculants. A total of 139 bacterial isolates were obtained, of which 100 were screened for key plant growth-promoting traits. Phosphate solubilization capacity varied among the isolates, with eight exhibiting high, five moderate, and sixteen low activity. Potassium solubilization was observed in thirty-six isolates, with two showing strong activity. Under aerobic conditions, nitrogen fixation was high in one isolate, intermediate in three, and low in five; under microaerophilic conditions, two isolates showed high and six moderate activity. Indole-3-acetic acid production varied, with two isolates producing high levels, thirty satisfactory, and sixteen low, highlighting functional diversity.

Three PGPR isolates were selected for subsequent pot trials based on their beneficial traits: two exhibited phosphate and potassium solubilization along with IAA production, while one showed high nitrogen fixation and IAA production. Compatibility tests were conducted to evaluate their potential use as individual or combined inoculants. In parallel, AMF spores were isolated and used to inoculate trap plants (Cechrus clandestinus and Trifolium arvense) to enhance spore populations.

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PP009

INHIBITORY EFFECT OF LAVENDER MICROCAPSULES AGAINST SOIL-BORNE PATHOGEN FUSARIUM OXYSPORUM F. SP. RADICIS-LYCOPERSICI

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The growing demand for agricultural products has intensified crop production and consequently increased the use of chemical formulations. Despite efforts by the agri-food industry to enhance yields and minimize losses, environmental degradation, along with the persistent challenge of managing soil-borne diseases, such as Fusarium wilt in tomato, poses a persistent barrier to implementing sustainable agricultural practices. Soil-borne pathogens cause significant losses in agricultural production, and their primary control method remains the application of chemicals. However, their application may have a negative impact on soil and water ecosystems, affecting ecosystem health status. The combination of biological control agents and smart delivery technologies presents a promising, environmentally friendly strategy for managing plant pathogens. Biopesticides derived from aromatic plants, including their plant extracts and essential oils, are regarded as effective alternatives for disease control. These natural, biodegradable compounds are target-specific and hold significant potential for commercialization. Additionally, encapsulation technologies for bioactive compounds can lead to stable plant protection products that enable the controlled release of bioactive substances. In this work, Lavandula dentata extract was encapsulated in sodium alginate microcapsules, and its inhibitory activity against the plant pathogenic fungus Fusarium oxysporum f. sp. radicis-lycopersici was evaluated in planta, showing promising results in plant disease control.

Keywords: botanical pesticides; plant protection; soil-borne pathogens; encapsulation; lavender

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PP010

THE EFFECT OF NITRIFICATION INHIBITOR 3,4-DIMETHYLPYRAZOLE PHOSPHATE (DMPP) ON MICROBIAL COMMUNITY ABUNDANCE AND NITROUS OXIDE EMISSIONS UNDER VARYING SOIL PH CONDITIONS IN CYPRUS

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Nitrification inhibitors such as 3,4-dimethylpyrazole phosphate (DMPP) are increasingly used in agriculture in order to reduce nitrogen losses and mitigate greenhouse gas emissions. The current study investigates the effect of DMPP, in comparison with a conventional fertiliser, on microbial community abundance and nitrous oxide (N_2O) emissions across soils with varying pH levels in Cyprus. Soil samples were collected from agricultural fields representing a range of pH conditions (acidic to near-neutral) and treated with DMPP or conventional fertiliser in controlled incubation experiments. Microbial abundance was assessed using quantitative PCR targeting key functional genes involved in the nitrogen cycle, while N_2O emissions were measured using gas chromatography over a defined period. Results showed that DMPP significantly reduced N_2O emissions under near-neutral pH conditions, but was less effective under acidic pH conditions. Shifts in the abundance of key nitrifier communities were evident, particularly a reduction in ammonia-oxidising bacteria under neutral pH, an effect that was less profound under acidic pH. In contrast, the abundance of ammonia-oxidising archaea remained largely unaffected across all pH conditions. These findings highlight the potential of DMPP as an effective mitigation strategy for N_2O emissions in diverse soil environments and underscore the importance of soil pH in determining inhibitor efficiency.

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PP011

THE EFFECT OF SIMULATED SUMMER RAINFALL ON THE ABUNDANCE OF MICROBIAL COMMUNITIES DRIVING GHG AND AMMONIA EMISSIONS FROM RUMINANT MANURE

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The surge in global meat and dairy consumption has intensified livestock farming, leading to greater manure production. Manure is a major source of ammonia (NH3) and greenhouse gas (GHG) emissions, namely nitrous oxide (N2O), carbon dioxide (CO2) and methane (CH4), all of which are significant contributors to global warming. These emissions originate from the enzymatic activity of specialised microorganisms in manure. In particular, nitrification and denitrification enzymes regulate N2O emissions, while enzymes triggering methanogenesis and methanotrophy drive CH4 emissions. The current study provides a comprehensive assessment of emissions and microbial gene abundance in dairy cow and goat manure during summer in Cyprus. In particular, the effect of a summer rainfall event was investigated by supplementing manure with water during storage. GHG and NH3 emissions were measured with automated chambers and analysed with a cavity ring-down spectrometer and the abundance of microbial genes regulating nitrification, denitrification and methanogenesis was quantified using real-time PCR. The results showed that simulation of rainfall led to a notable increase in gene abundance regulating nitrification and denitrification, accompanied by a concurrent increase in NH3, CO2, and N2O emissions. Furthermore, the two manure types displayed different GHG emission patterns, likely due to variations in feed composition and digestibility. The study highlights the significant role of moisture in regulating microbial gene abundance and subsequent GHG and NH3 emissions during manure storage, particularly under high temperatures, providing useful insights into how emissions can be mitigated in the context of global climate change.

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PP012

HERE TODAY, GONE TOMORROW? BIOLOGICAL NITRIFICATION INHIBITORS' PERSISTENCE AND DISSIPATION IN A RANGE OF AGRICULTURAL SOILS

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Biological nitrification inhibitors (BNIs) are emerging as sustainable alternatives to synthetic nitrification inhibitors, offering the potential for a more environmentally friendly nitrogen management in agricultural soils. Secreted by plant roots, BNIs have been shown to reduce nitrification activity in soil. However, their environmental fate remains insufficiently understood. Although BNIs are naturally derived, assuming that they require no monitoring may lead to underestimations of their persistence, efficacy, and behavior. We evaluated the dissipation of five BNIs—2-methoxy-1,4-naphthoquinone (MQN), sakuranetin, 1,9-decanediol, 2,7-dimethoxy-1,4-naphthoquinone (zeanone), and 6-methoxy-2(3H)-benzoxazolone (MBOA)—across ten soils selected for their different physicochemical characteristics like texture, pH and organic matter content. BNI concentrations were quantified using high-performance liquid chromatography (HPLC) methods developed and validated as part of this study. In general, BNI compounds showed low persistence in neutral to alkaline soils, while higher DT50 values were mostly observed in low-pH soils. For zeanone and MQN, DT50 values ranged from 0.29 to 88.5 days and 0.16 to 11.84 days, respectively. Sakuranetin and MBOA exhibited DT50 values ranging from 1.35 to 6.62 days and 0.31 to 7.46 days respectively. Soil fumigation performed on two representative soils—one acidic and one alkaline—significantly retarded the dissipation of sakuranetin (DT50 4.85 vs. 13.11 days in acidic, 2.68 vs. 9.23 days in alkaline soil) and MBOA (DT50 7.46 vs. 40.96 days in acidic, 1.31 vs. 3.4 days in alkaline soil), suggesting a key role for soil microorganisms in their dissipation. Ongoing work will explore correlations between soil properties and BNI dissipation, including determination of the DT50 of 1,9-decanediol which is still pending. This knowledge is essential to evaluate the true agricultural potential of BNIs and to design application regimes that ensure their efficacy in regulating nitrogen transformations in soil. Our results further highlight the need to systematically assess the fate of naturally derived compounds in soil, especially considering their reduced stability and implications for field-level performance and agronomic decision-making.

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PP013

BIOCONTROL STRATEGIES AGAINST TOMATO AND CUCUMBER PATHOGENS: A SYNERGISTIC APPROACH INVOLVING SYSTEMIN AND BENEFICIAL FUNGI

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The plant families Solanaceae and Cucurbitaceae contain some of the most important crops across the European Union, playing an important economic role and dietary relevance. These crops are facing many different biotic stresses because of pests and diseases. Notably, many pathogens are in soil and causing serious problems in tomato root and steam like Verticillium dahliae but also others like Botrytis cinerea causing foliar and fruit diseases. With the EU steadily reducing the use of chemical plant protection products, the search for effective biological alternatives has become increasingly critical. Through that study, we explore the combined action of two beneficial fungal agents and one immune defense-related peptide as a biological toolset to manage these important diseases. The synergistic interaction of Trichoderma harizianum, and Fusarium solani strain_K alone or in combination with the peptide Systemin was examined in sterile and greenhouse conditions against Verticillium dahlia and Botrytis cinerea in tomato and cucumber plants respectively. The efficiency of the treatments was evaluated through phenotypic measurements (Vascular necrosis, Disease Incidence and Disease Severity Index) and quantitative estimation of disease progression by qPCR analysis using specific primers for each microbe involved in the study. The copy numbers of the pathogen decreased in root vascular tissue in presence of beneficial fungi compared to the control, suggesting that these beneficial microbes and Systemin can significantly suppress V. dahliae and shows promising results against B. cinerea.

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PP014

APPLICATION OF SINGLE AND COMBINED INOCULA OF RHIZOBIA AND ARBUSCULAR MYCORRHIZAL FUNGI STRAINS, ISOLATED FROM STRESSED ENVIRONMENTS, TO IMPROVE LEGUME PERFORMANCE AND FITNESS UNDER NORMAL AND OSMOTIC STRESS CONDITIONS.

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Climate change is increasing xerothermic conditions and soil salinity, potentially disrupting the beneficial symbioses between legumes, rhizobia, and arbuscular mycorrhizal fungi (AMF). Such disruptions could impair plant nutrition and reduce legume crop productivity. One promising solution is harnessing stress-adapted microbes from extreme environments and applying them as tailored inoculants to enhance legume performance under adverse conditions. In this study, rhizobia and AMF strains isolated from wild legumes grown in extreme environments were applied sorely and combined as microbial consortia in Trifolium resupinatum. Preliminary experiments, in sterile- soilless substrate, demonstrated that the selected rhizobia and AMF strains benefited Trifolium resupinatum growth especially under stress. Following, plants are grown in pots filled with a 7:2:1 v/v mixture of sand, vermiculite, and soil, under normal irrigation conditions and under mild but prolonged osmotic stress, introduced by combining 50% decrease in water supply and 100mM NaCl. At harvest, we will combine plant biometric and physiological measurements and determine AMF root colonization levels, number of nodules and N fixation rate to investigate the outcome of the application of single and combined inocula of rhizobia and AMF under closer to field-like conditions. NGS analysis will further clarify how the modifications induced by the inoculations on the rhizospheric and endoroot microbial communities relate to the changes observed at the plant level. The findings are expected to advance the development of novel bioinoculants that enhance legume nutrition, growth, and productivity in challenging environments, offering sustainable solutions for climate-resilient agriculture.

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PP016

FLOODING EFFECTS ON PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SOILS FROM THE AREA OF LAKE KARLA IN THESSALY, GREECE

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Flooding may impact soil physical chemical and microbiological properties, and it is expected that as a result of climate change, flooding events will be more frequent. The region of Thessaly in Greece has been hit by unprecedented flooding, leading to the expansion of lake Karla for several months. The soil physical, chemical and microbiological properties were measured at three transects around the lake with a gradient based on flooding duration. Soil physicochemical parameters, arbuscular mycorrhizal fungal (AMF) spore number and number of morphotypes, microbial biomass N and respiration and the quantity (real time PCR) of genes related to nitrogen cycling and nitrogen fixing symbiotic capacity (nifH, nodZ, amoA) were measured. In addition, vetch was planted in pots filled with the soil samples and after a month the presence of nodules and the percentage of root length colonized with arbuscular mycorrhizal fungi were recoded. Nutrient levels, symbiotic nitrogen fixation genes, nodules, microbial biomass and respiration, AMF spore numbers and mycorrhizal root colonization were low. Overall, the results indicate the necessity of fertilizing the soils and applying rhizobial inocula for cultivation of legumes in the area. In addition, they stress the necessity of having databases of soil properties prior to flooding.

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PP017

DRIVERS OF THE NODULE MICROBIOME OF WILD LEGUMES IN THE GEOLOGICALLY DIVERSE ISLAND OF MILOS: THE EFFECTS OF SOIL CHARACTERISTICS AND HOST IDENTITY

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The root-associated microbiome plays a critical role in plant health, nutrient acquisition, and resilience to environmental stress. In legumes, symbiotic nitrogen-fixing rhizobia are well-studied, but the broader nodule microbiome—including non-rhizobial bacteria—remains poorly understood, despite its potential influence on nodulation efficiency and plant fitness. This study aims to investigate the factors shaping the nodule microbiome in two naturally co-occurring and ecologically important legume species, Lotus edulis and Onobrychis caput-galli, across different locations with contrast geology and soil characteristics on the island of Milos, Greece. To address this aim we sampled six individual plants (biological replicates) of both species from five distinct locations, with contrast geology and soil characteristics, including, a perlite soil, a bentonite soil, an undisturbed location close to sulfur mines, a salt marsh and an agricultural field under fallowing. After sampling, nodules were collected, surface-sterilized, and subjected to next-generation sequencing (NGS) of the 16S rRNA gene to characterize bacterial community composition. Statistical analyses will assess how soil properties, host species, and geographic distance influence microbiome diversity and structure, and the outcome of the analysis will be presented at the conference. These findings will advance our understanding of legume-microbe interactions in natural settings, highlighting the interplay between edaphic factors and host specificity. By elucidating the ecological drivers of nodule microbiome diversity, this study contributes to broader efforts in sustainable agriculture, where optimizing plant-microbe partnerships could reduce reliance on synthetic fertilizers.

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PP019

RESPONSE OF PURPLE NON-SULPHUR PHOTOTROPHIC BACTERIA TO MANGANESE EXPOSURE

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Environmental pollution is an important stress factor of anthropogenic origin that must be faced by all living organisms. Metal ions are known to contribute significantly to this problem. When present at high concentrations, they are toxic to animals, plants and microorganisms as they can be incorporated into cells and, for example, compete with other ions for their binding sites, effect metabolic pathways, alter the production and activity of important biological molecules, etc.

Purple non-sulphur phototrophic bacteria have been used to study the uptake and fate of some heavy and transient metals (Grattieri et al., 2021). Due to their flexible metabolic modes and high tolerance, bacteria are gaining attention as efficient agents in the removal of contaminants from wastewater of different origin (Chen et al., 2020). Here, we show the effect of manganese exposure on the growth and functionality of phototrophic bacteria and indicate their potential in remediation of manganese ions from water environment.

Anaerobic cultures of purple non-sulphur photosynthetic bacteria were treated with manganese ions in a broad range of concentrations. Spectroscopic and biological assays were carried out to specify the effects of its presence on the morphological changes of bacterial membrane and the light-harvesting properties. Qualitative and quantitative analysis of spectrometric results, pigment composition, and microscopic analyses showed diverse responses of bacteria to the applied stress factor, indicating a certain degree of flexibility of bacteria and suggesting control of their physiological adaptation also on environmental level.

Acknowledgments

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PP021

INVESTIGATING BIOMINERALIZATION OF CARBON DIOXIDE MEDIATED BY CYANOBACTERIA AND ITS POSSIBLE ECOLOGICAL ROLE

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The study of carbon dioxide biomineralization into calcium carbonates in cyanobacteria is crucial for understanding the ecological role these microorganisms play in aquatic systems and how they are contributing to the carbon biogeochemical cycle by connecting the atmospheric and non-atmospheric reservoirs of inorganic carbon. This research investigates the complex biochemical process of cyanobacterial biomineralization by characterizing its molecular bases, the evolutionary reasons that might favor this biological trait, and assessing its environmental impacts. The study applies both in vivo and in vitro analyses of selected cyanobacterial strains, conducting biochemical, functional, and structural characterizations of the proteins involved in biomineralization, along with assessing the properties of the biomineralized material. The applied methods range from cell envelope isolation and protein identification by mass spectrometry to activity assays and microscopy observation. By elucidating the mechanisms and environmental factors that regulate cyanobacterial biomineralization, this research aims at contributing to fundamental knowledge in microbial ecology and to explore sustainable CO_2-capture strategies performed by these organisms, ultimately addressing environmental challenges.

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PP022

USING SPECIES SENSITIVITY DISTRIBUTIONS TO QUANTIFY THE TOXICITY OF PESTICIDES TO SOIL AMMONIA OXIDIZING MICROBES, BACTERIAL AND FUNGAL COMMUNITIES

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Soil microbial communities are crucial for maintaining ecosystem functions and services, yet their sensitivity to pesticide toxicity remains underexplored. Traditional protocols used in regulatory terms (i.e. OECD 216 N mineralization test) could not provide an accurate assessment, necessitating a shift towards advanced high resolution methodologies available in microbial ecology. Recent benchmarking research proposed ammonia-oxidizing microorganisms (AOM) as valuable indicators of pesticide toxicity to soil microbes due to their functional significance and sensitivity to environmental stressors. Building upon prior in vitro investigations by our group on soil representative nitrifiers in liquid cultures, we evaluated pesticide toxicity on AOM, along with their effects on broad phylogenetic microbial groups, in three soils with differing physicochemical properties. We aimed to provide a more ecologically relevant assessment of the toxicity of pesticides on natural soil microbial communities using both classic and advanced omic tools within an ecotoxicological context. Soils were exposed to six dose rates of a fungicide (pyraclostrobin) and two herbicides (glyphosate, metsulfuron-methyl), all of which had demonstrated notable toxicity against various AOM strains in prior in vitro single-species tests. Key toxicity endpoints, such as nitrate levels, were monitored, and the abundance of amoA, nxrB, 16S rRNA, and ITS marker genes for AOM, nitrite-oxidizing bacteria (NOB), total prokaryotes, and fungi, respectively, were quantified by q-PCR. Amplicon sequencing was used to assess the effects of the most perturbing pesticide (metsulfuron-methyl) on the diversity of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), total prokaryotes and fungi. Species Sensitivity Distributions (SSDs) were constructed based on amplicon sequencing data for AOM, total prokaryotes, and fungi. The Hazard Concentration 5% (HC_5) values derived for metsulfuron-methyl revealed significant differences in microbial sensitivity between soil and in vitro assays. These findings confirm that in vitro assays provide a more conservative estimate of microbial sensitivity, with an HC_5 value for AOM (0.048 mg kg^{-1} dw soil) being half of that measured in soil microcosms (0.09 mg kg^{-1} dw soil). Our study contributes to the development of advanced and ecologically relevant tools for assessing pesticide toxicity in soil microorganisms.

Acknowledgments

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PP023

CAN BACILLUS BACTERIA HELP IN THE RESTORATION OF AQUATIC ECOSYSTEMS?

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Aquatic ecosystems are among the most important natural resources on Earth. They help maintain environmental balance but, most importantly, they are essential for human life and health. They serve as a primary source of drinking water, provide irrigation for crops, and are habitats for many groups of organisms. Recently, intense anthropopressure and degradation of these ecosystems have posed a serious threat to the natural environment and caused irreversible losses in biodiversity. One of the elements ensuring the ecological stability of aquatic ecosystems is bacteria that effectively participate in the decomposition of organic matter. The aim of the project is to investigate the dynamics of functional and structural diversity changes in bacterioplankton under the influence of a biopreparation based on bacteria of the Bacillus genus. Monitoring of the mentioned microbiological parameters will allow the assessment of the biopreparation's effectiveness in aquatic ecosystems and its potential future use for their restoration. To assess the integrity of the cytoplasmic membrane and cellular activity, sets of fluorescent markers will be used: the LIVE/DEAD® BacLight™ Bacterial Viability Kit and CTC (5-Cyano-2,3-di-(p-tolyl)tetrazolium chloride) as an indicator of respiratory activity. The analysis of taxonomic structure dynamics in bacterioplankton will be conducted based on the amplification of the V3-V4 region of the bacterial 16S rRNA gene. Pilot studies (without the addition of the biopreparation) carried out in 2024 showed that in the studied water reservoir, dead bacterioplankton cells dominated, constituting 71.78% (April), 65.89% (May), and 72.71% (June) of the population, respectively. The highest average abundance of metabolically active cells was recorded in May (5.72×10^4 x mL^{-1}), and the lowest in June (2.72×10^4 x mL^{-1}). Further planned analyses will be carried out from April 2025 to April 2026.

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PP025

POPULATION GENOMICS OF MICROBIAL STALACTITE BIOFILMS FORMED IN THE ACID MINE DRAINAGE

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Macroscopic biofilms from acid mine drainage (pH < 3) are known mainly as a habitat of unusual extemophilic microorganisms. They also represent a great model system for the study of the prokaryotic population genomics due to their large biomass dominated by a one (or a few) species. This provides the opportunity to assemble complete genomes from metagenomes (MAGs). Ongoing advances in the accuracy of long-read sequencing methods (Oxford Nanopore, Pacific Bioscience) are gradually increasing the efficiency of MAG recovery, even from samples where more similar genotypes were present at the same time (see related poster "The comparison of the sequencing methods and bioinformatic tools using metagenomic data from natural microbial communities"). Here we present results of our long-term research (spanning ca. two decades) of the gelatinous biostalactites formed predominantly by a single species, Ferrovum myxofaciens. We have identified distinct F. myxofaciens genotypes most of which are represented by multiple genomes from different biostalactite populations. Co-occurrence of two genotypes in a single stalactite has been recorded. We identified a specific feature, so-called "scrapyard" – ca. 200kb long variable section containing transposable elements (TEs), hypothetical proteins, and low complexity regions – which also encodes the Hfr phenotype. Different genotypes have different and probably independently acquired scrapyards with nonhomologous tra or trb operons that allow high frequency conjugal transfer of the adjacent region. Our results show that the TE integrations are the most common source of the intrapopulation variability. We hypothesize that a community-based genome maintenance by homologous recombination is the possible mechanism to overcome potentially high rates of genome erosion caused by gene disruptions by TEs. TE integrations can result in an inactivation of the disrupted gene as well as activation of the surrounding genes thus offering a possibility for gene activity modulation. We found a non-random frequency of TE integration in otherwise conserved genes for diguanylate cyclases/phosphodiesterases, enzymes regulating bacterial behavior in many important processes, including switching between formation of biofilm and swarmer cells. We propose that microdiversification of life strategies may be an adaptive outcome of random TE integration.

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PP026

MICROPLASTICS AS DRIVERS OF COMMUNITY CHANGES IN THE BALTIC SEA

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Microplastics (MPs) play a crucial role in shaping microbial community dynamics, particularly in aquatic environments. Variation in concentrations and microplastic types can significantly influence microbial abundance, respiration, and community composition. Here, we employed a genome-centric metagenomic approach to identify the response of microbial communities to the presence of bacteria-sized plastic particles from polystyrene (PS) and polyethylene (PE) with glass spheres and no particles as a control. We observed that bacterial abundance increased in the presence of PS compared to both controls. In contrast, the response in PE treatment and glass control was similar, indicating a negative effect of particle presence rather than a toxic PE effect. To understand how these responses were driven, we obtained ~1100 metagenome assembled genomes (MAGs) that were medium to high quality (>50% completeness and <10% contamination). We found that bacterial community composition may shift in response to MP exposure duration, while other microbial groups remain unchanged. Moreover, differences in metabolic pathways suggest that bacterial taxa may exhibit distinct adaptations to environments enriched in polyethylene and polystyrene. These early findings highlight that MP pollution may have a higher potential for shifts in microbial communities, with possible implications for microbial food web dynamics.

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PP027

THE COMPARISON OF THE SEQUENCING METHODS AND BIOINFORMATIC TOOLS USING METAGENOMIC DATA FROM NATURAL MICROBIAL COMMUNITIES

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The next generation sequencing revolutionized the research of the natural microbial communities. With the introduction of the long-read sequencing platforms, the assembly of complete microbial genomes from metagenomic samples (Metagenome-Assembled Genomes, MAGs) without the necessity of prior cultivation became possible. Originally, the way how to overcome the higher sequencing error rate of the long-read sequencing was to use a series of error correction (or polishing) steps either by re-analysing the raw signal data or by incorporating another short-read data with lower error rate. The latest long-read sequencing approaches by Oxford Nanopore Technologies (accompanied with gradually improving signal processing software) and by Pacific Biosciences (PacBio) substantially reduced the sequencing error rate. Over the course of more than a decade, we have sequenced samples from microbial communities inhabiting abandoned underground mines using different methods available over time (short-reads Illumina sequencing and long-read sequencing: R9 nanopore chemistry, R10 nanopore chemistry and HiFi PacBio). These unique microbial consortia forming macroscopic biofilms at acid mine drainage seepages (pH < 3) represents a great model system (see related poster: "Population genomics of microbial stalactite biofilms formed in the acid mine drainage"). Their little taxonomic diversity mostly with one dominant species (Ferrovum myxofaciens) makes the assembly of complete MAGs possible from most of our samples. Here we provide a comparison of the metagenomic sequencing methods and bioinformatic tools using the real metagenomic data from naturally occurring microbial consortia. Our results demonstrate the effect of the newest updates of the nanopore data processing software on the quality of the sequence data. We also show that the latest R10 nanopore and HiFi PacBio sequencing can produce accurate data effectively overcoming the need of the assembly polishing. While the polishing is important when dealing with the older nanopore data, it may homogenize the variability inside the assembled genome sequences in an unpredictable way (especially in repeated or highly similar regions). Also, the higher read accuracy can notably improve the automatic process of the assembly and more genotypes co-occurring in a single sample can be distinguished instead of assembling of a single consensus ("average") genome.

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PP028

MICROBIAL DIVERSITY AND DOMINANT FUNCTIONS UNDER HYDROTHERMAL STRESSORS

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The aim of the study was to profile the bacterial community structure and dominant functions in sediment and water samples along coastal sites in the Hellenic Volcanic Arc (HVA). Overall, 17 samples collected from areas affected by hydrothermal stressors and natural radioactivity were analyzed by means of high-throughput 16S rRNA gene amplicon sequencing (HTS) to describe bacterial diversity and composition, and 5 samples were examined by shotgun metagenomic sequencing to assess the functional potential of the microbial communities under hydrothermal stressors. Metabarcoding revealed high bacterial richness in all hydrothermal sites. The hydrothermal microbiome was dominated by Proteobacteria and Bacteroida in terms of OTU richness, while marine reference samples were characterized mainly by Alphaproteobacteria. Thermophilic and anaerobic taxa linked to the sulfur cycle, belonging to Caldisericota, Campylobacterota, Desulfuromonas, Calditrichota, Deferribacterota, Deinococcota, Gammaproteobacteria, Spirochaetota, and Thermotogota, were detected in regions with hydrothermal conditions. The sulfur-oxidizing Campylobacterota, frequently retrieved in the hydrothermal samples, plays a central role in extreme environment biogeochemistry via carbon fixation and chemosynthesis. The radon-rich natural thermal pool of Methana hosted a diverse microbiome, marked by several uncultured OTUs and a substantial presence of Candidate Phyla Radiation (e.g., Gracilibacteria, Kaiserbacteria) involved in fermentative metabolism and biogeochemical cycling. Radiation-resistant Deinococcus was also detected, likely contributing to Reactive Oxygen Species detoxification and carbon turnover. Metagenomic analysis revealed a high abundance of genes linked to methanogenesis (e.g., FmdA/FmdB), sulfur metabolism (sox, dsr), nitrogen cycling (nar, nir, nosZ), and DNA repair (recA, uvrABC). Our findings support rich microbial diversity in radon-rich and hydrothermal-associated sites, revealing distinct dominant taxa that exhibit metabolic strategies to withstand energy limitation and oxidative stress.

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PP029

TESTING A MICROBIAL DNA-BASED INDICATOR FOR GREEK EUTROPHIC LAKES' WATER QUALITY ASSESSMENT

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The water quality of four eutrophic lakes in Western Macedonia, Greece, was assessed based on phytoplankton, using the microscope-based, multi-parametric PhyCoI index. Additional eDNA-based metrics were examined for their suitability in developing a water quality indicator using metabarcoding sequencing microbial data. Water samples were collected from Lakes Kastoria, Chimaditida, Petron, and Zazari in December 2022 and July 2023, and their phytoplankton communities were examined with the inverted microscope method. Overall, 52 phytoplankton taxa were identified in all lakes with cyanobacteria dominating in Lakes Kastoria, Chimaditida and Zazari. The total phytoplankton biomass ranged from 1.49 mg L^{-1} to 27.39 mg L^{-1}, with cyanobacterial biomass reaching 8.12 mg L^{-1} in Lake Kastoria. According to the PhyCoI index, the ecological water quality was assessed as poor to moderate in all lakes. Furthermore, high-throughput amplicon sequencing was implemented on SSU rRNA genes targeting the deep prokaryotic and eukaryotic diversity and retrieving 719 prokaryotic and 759 eukaryotic OTUs overall. For the development of a multi-parametric microbial eDNA-based water quality indicator, based on regressions between the molecular datasets and the microscopy-based PhyCoI outcomes, we propose as suitable molecular metrics (i) the relative abundance of cyanobacteria in the prokaryotic partition, (ii) a molecular modified Nygaard's index that takes into consideration the relative abundances and OTUs species richness of the taxonomic groups that the traditional Nygaard's index uses, and (iii) the ratio of harmful to non–harmful taxa. A multiple linear regression model was implemented to predict the most suited eDNA indicator to describe the sampled lakes, using the above metrics. The resulting equation showed a strong coefficient of determination with PhyCoI, indicating a good fit between the predicted eDNA-based indicator and the observed ecological water quality values. Based on the proposed indicator, the ecological water quality of the lakes was assessed as poor to moderate, similarly as with the microscopy-based PhyCoI. The metrics included in the proposed indicator show promising results for the assessment of water quality in eutrophic shallow lakes stressed by anthropogenic activities. However, limitations including the lack of phytoplankton reference-site lakes in our dataset, and generally in Greece, urge for a larger scale analysis.

Acknowledgments

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PP030

EVALUATION OF A FULL-SCALE WASTEWATER TREATMENT PLANT AS A SOURCE FOR NUTRIENTS RECOVERY TOWARDS SUSTAINABLE RESOURCE MANAGEMENT IN WASTEWATER TREATMENT.

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Besides the current and future challenges the humanity is facing regarding the desirable prosperity, the sustainable development and the protection of ecosystems, the European Green Deal, where the Circular Economy Action Plan constitutes a main building block, and the United Nations Sustainable Development Goals (SDGs) are the drivers of the main goal of the transition toward sustainable resource management in wastewater treatment. More specifically the United Nations 2024 update on the progress of the treatment of wastewater declares ''By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally'' (Target 6.3 of the SDG 6). Reports on the current global wastewater production state that it lies between 330 and 360 km3/y, (Mateo-Sagasta et al., 2015; Jones et al. 2021), a fact that demonstrates the urgent need for a thorough determination of the characteristics of the produced wastewater and the discrimination among substances and nutrients of priority, that must be recovered and not wasted in receiving waters. In the present work, a detailed determination of the physicochemical characteristics of urban wastewater was performed, accompanied by the microscopic identification of protozoa and the implementation of real-time polymerase chain reaction (qPCR) to provide insight into the presence of microbial communities in a full-scale activated sludge wastewater treatment plant located in northern Greece. Results demonstrate that a more detailed investigation of activated sludge characteristics is required to highlight to a greater extent the high added value hidden in wastewater treatment systems.

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PP031

UNVEILING THE SYNERGISTIC EFFECT OF MICROPLASTICS AND ORGANIC POLLUTANTS ON SOIL MICROBIAL DIVERSITY AND FUNCTIONING

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Microplastics (MPs) are emerging contaminants in soil ecosystems, increasingly recognized for their ability to interact with co-occurring pollutants such as pesticides and veterinary medicines. While the effects of MPs on soil fauna are relatively well studied, their combined impacts with organic pollutants on soil microbial communities remain poorly understood. In this study, we evaluated the effects of three types of MPs (LDPE, PBAT, starch-based applied alone or in combination with the fungicide pyraclostrobin (PYR) and the anthelmintic albendazole (ABZ), across three European soils (Greek, Dutch, and French). We focused on (a) the abundance of ammonia-oxidizing microorganisms (AOB, AOA, and comammox bacteria) as functional indicators, and (b) the diversity and community composition of protists as representatives of broader soil microbial groups. Our results revealed marked soil-specific responses. In the Greek soil, LDPE, alone or combined with ABZ and PYR, transiently altered protist α-diversity and caused significant shifts in β-diversity. No β-diversity effects were observed in the French soil, while the Dutch soil exhibited only sporadic changes. In the Greek soil, the combined exposure significantly reduced AOB, AOA, and comammox abundance. Although AOA and comammox abundance recovered and increased after 90 days, AOB remained persistently suppressed. MPs alone reduced AOA, favored AOB, and had a limited impact on comammox. In the French soil, AOB suppression was primarily driven by the combined presence of ABZ and PYR with MPs. In the Dutch soil, ABZ—alone or with PYR—particularly in combination with PBAT, led to reduced AOB abundance. All MPs significantly reduced AOA at 30 days across the three soils. In the French soil, commamox responded similarly to AOA under MP-only exposure, while in the Dutch soil, PBAT combined with both pollutants significantly lowered their abundance. Interestingly, low MP doses alone increased comammox abundance. These findings underscore the complex and context-dependent impacts of MPs and pollutants on key microbial functions, with potential consequences for nitrogen cycling, soil health, and greenhouse gas emissions.

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PP032

POLAROMONAS SPP. NOVEL STRAINS ISOLATED FROM FRESHWATER LAKE SHOWING PHOTOTROPHIC CAPACITY.

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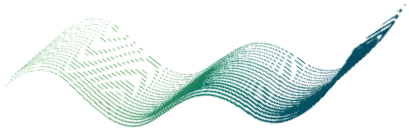
Polaromonas is a psychrotolerant and aerobic bacteria commonly found in cold environments such as glacial ice, permafrost, alpine lakes, and polar regions. Members of this genus, which belongs to the class Betaproteobacteria, are notable for their metabolic versatility with ability to adapt low temperatures and nutrient-poor conditions. They often form biofilms and associating with particulate matter to withstand environmental stress. To date, 10 species of Polaromonas have been described, none of which are known to be phototrophic.

In this study, we isolated four strains of Polaromonas (MK2, MK5, MK7, and MK13) from freshwater samples collected from the Římov Reservoir in South Bohemia, Czech Republic. All strains were rod-shaped, Gram-negative, motile, and grew optimally under aerobic conditions with a 12 h light/12 h dark cycle at 15–20 °C (growth range: 4–25 °C) and at pH 7.2. Bacteriochlorophyll a and spheroidenone were identified as the major pigment. Spectral analysis revealed an unusual absorption peak at 828 nm in strains MK5, MK7, and MK13, while MK2 exhibited a standard peak at 870 nm—suggesting the presence of a potentially novel light-harvesting complex.

The 16S rRNA gene sequences of the isolated strains showed 98.22–98.62% similarity to known type strains (Polaromonas eurypsychrophila and Polaromonas aquatic). Phylogenetic analysis suggests the presence of two novel species: one represented by MK2, and another grouping MK5, MK7, and MK13. Whole-genome analysis revealed that all strains possess intact photosynthetic gene clusters (PGCs), with strain MK7 uniquely harbouring both PGC and xanthorhodopsin genes, indicating dual phototrophy. Additionally, two strains contained genes associated with carbon fixation (RuBisco).

These findings suggest that the isolated strains represent novel species within the genus Polaromonas, exhibiting unique metabolic capabilities, including anoxygenic phototrophy, rhodopsin - based phototrophy and carbon fixation.



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PP033

COLONIZATION OF EXPERIMENTAL CONTAINERS BY AQUATIC MICROBES AND INVESTIGATION OF THE ROLE OF A NEARBY FRESHWATER SYSTEM

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Microbial dispersal and colonization of new environments are key research areas in ecology as they affect community assembly. This study experimentally examined the above processes. Duplicate containers filled with autoclaved tap water were placed in three locations, 10m, 50m, and 4km from Lake Pamvotis (Greece). Samplings were performed weekly during October and November 2023, to investigate changes in the abundance and/or diversity of bacteria, heterotrophic nanoflagellates (HNF) and ciliates. Bacterial diversity was assessed through analysis of the 16S rRNA V3-V4 region at the end of the experiment. Ciliate diversity was examined weekly using microscopy. Lake water was also sampled, considering it may be a source of microbes for the new environments. The colonization of the new aquatic systems by bacteria appeared to have started immediately after setting up the experiment. Bacterial abundance and growth rates were higher in the containers closer to the lake. Maximum rates occurred at the beginning of the experiment, followed by an increase in HNF abundance. Ciliate maximum abundances were measured at the end of the experiment. The experimental containers shared >63% of the OTUs found in each of them. Unique OTUs (detected only in one location) represented <4% of the richness in different sites. ~95% of 'lake' OTUs were found at least in one site, and ~50% were common across all locations. The Dice-Sørensen similarity index didn't show a statistically significant correlation with distance. Concerning ciliates, 15 taxa/morphotypes were identified in the lake. Ciliate colonization rate was initially high (2-5 new taxa) during the first two samplings but decreased and remained stable afterwards. Six to nine taxa were identified in the different containers, with only one not detected in the lake. Five taxa were found across all containers. Overall, our data indicated a quick colonization of water containers initially by microbial prokaryotes, followed by eukaryotes, and suggest the emergence of predator-prey relationships. The nearby lake may have served as a source of microbes for all three

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PP034

SUBSURFACE MICROBIAL HYDROGEN CYCLING: IMPLICATIONS FOR UNDERGROUND HYDROGEN STORAGE PROJECTS

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Hydrogen is considered a crucial energy carrier in energy transition roadmaps. Underground hydrogen storage (UHS) will be a critical enabler of a future hydrogen economy because it provides cost-effective large-scale and long-duration storage solution for managing supply and demand.

Microorganisms in anaerobic environments, such as underground systems, can use hydrogen for their metabolism. These hydrogen-fueled microbes, known as hydrogenotrophic microbes, can cause several problems in UHS projects including the direct loss of gas volume, decreasing H2 purity due to CH4 and H2S production, corrosion and eventually reduced storage capacity. Here, we conducted a sensitivity analysis to understand how environmental parameters affect H2 consumption by a pure culture of the hydrogenotrophic methanogen, Methanobacterium subterraneum (Archaea), isolated from deep subsurface environments. We also studied H2 consumption by an environmental microbial consortium sampled from a UHS site (aquifer) prior to H2 injection.

The optimum pH for H2 consumption by Methanobacterium subterraneum was examined by testing its kinetics at pH 7, 7.5, 8.5, and 9. Our experiments showed that this archaeon can grow -and consume H2- only at pH 7 and 7.5, with the former being the optimal one. M. subterraneum consumed H2 at low salinity (0.5M NaCl) and this ability increased with increasing temperature.

The environmental consortium was acclimatised in sequential cultures to specific conditions prior to the experiment. The microbial consortium produced H2S in the presence of H2 in the gas phase. We found a correlation between H2 and sulphate consumption and the production of H2S. The diversity of the microbial community was studied by Next Generation Sequencing (NGS) of the V3-V4 region of 16S rRNA gene. Three main bacterial genera were identified in all cultures, the sulfur-reducing Terrisporobacter and Aeromonas sp. And the acetogen Clostridium.

Microbial activity data can be used in geochemical models to evaluate hydrogen reactivity in UHS sites and improve the safety, efficiency, and longevity of the storage systems. Based on our results and the fact that hydrogenotrophic microorganisms are ubiquitous, we emphasise the need for microbial analysis at potential UHS sites before H2 injection.

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PP035

MICROBIAL DRIVERS OF VFAS AND HYDROGEN GENERATION: LINKING COMMUNITY COMPOSITION TO FERMENTATION EFFICIENCY IN MIXED WASTE BIOREACTORS

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Biomass conversion technologies, such as anaerobic digestion, are gaining interest due to the potential benefits that could offer in a circular economy framework in terms of carbon footprint decrease, resource recovery and energy production. In particular, the dark fermentation process facilitates the production of high-value products, including hydrogen and volatile fatty acids (VFAs). VFAs such as butyrate, are significant industrial products with high market price that used as precursor for various chemicals and materials. In addition, serve as common substrates for the production of polyhydroxyalkanoates (PHAs); a family of aliphatic polyesters that can be accumulated intracellularly by microorganisms under unbalanced growth conditions. The use thereof as bioplastics could provide an attractive alternative in a variety of disposable packaging goods, medicine and pharmaceutical applications. Microbial synthesis and activity drive waste conversion into VFAs, and different synthesis pathways have been associated with varying acid production. Omics and high-throughput approaches can monitor microbial complexity across multiple functional levels.

In this context, this study aims to investigate the role of bacterial communities originating from different wastes in the production of hydrogen and VFAs, and subsequently to examine the improvements achieved by combining different mixtures and conditions. The ultimate target is the development of a bioprocess that maximizes biodegradable bioplastics. Batch and continuous experiments were conducted under varying conditions, including different temperatures, hydraulic retention times, and pH.

Results revealed that under mesophilic conditions (37°C), the mixture of 80% cheese whey with 20% pig manure showed the higher VFAs concentration (acetate 7868 mg L-1), while the mixture of 40% olive mill waste, 40% cheese whey and 20% sewage sludge appeared the higher butyrate concentration with a value of 3250 mg L-1. Regarding biogas production, the mixture of cheese whey with pig manure showed the higher values. Hydrogen content in the biogas appeared higher in the mixture of 70% olive mill waste and 30% dried food waste. Metagenomics analysis demonstrated shifts in microbial communities' composition, with different species dominating in each case after the dark fermentation process. A deeper understanding of microbial communities and bioreactor performance could enhance both production efficiency and the manipulation of acid profiles.

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PP036

ASSESSMENT OF INACTIVATION OF BACTERIAL AND FUNGAL PATHOGENS IN AQUATIC MATRICES

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This study primarily investigates the role of water matrix for the disinfection of watercourses. This relies on experimental work regarding the inactivation of pathogens, such as the bacterium Acinetobacter baumannii and the fungus Candida albicans, by the conventional chlorination and UVC radiation in four matrices of varying complexity, i.e. from as simple as water to secondary treated effluent. For comparison purposes, results from recent literature are also discussed. The specific target microorganisms were selected on the basis of their significant effect on public health due to their high resistance to antibiotics and their low infectious dose.

According to the obtained results the resistance of both microorganisms was reflected on the prolonged treatment time, which was required for their complete elimination during chlorination even in simple aqueous matrices (water/surface water). For example, A. baumannii was eliminated in 180 min with 0.5-1 mg/L of free Cl2, while the respective time for C. albicans was 45 min. Furthermore, disinfection of complex matrices like wastewater samples, required considerably higher concentrations of Cl2 up to 10 mg/L. On the other hand, UVC at 15 W nominal value, proved to be highly efficient in all matrices but in a matter of few minutes. However, it was observed that after about 24 h, microorganisms exposed to UVC radiation often exhibited repair mechanisms, as a result of which complete elimination was not always achieved. The aqueous matrix mainly affected the time required for satisfactory inactivation of the selected microorganisms.

The general perception is that treatment efficiency decreases with increasing matrix complexity in terms of total concentration and/or individual components composition, irrespective of the applied treatment method and its objectives (i.e. disinfection, decontamination or mineralization). The matrix itself should be given particular emphasis for the rational design of efficient water treatment processes.

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PP037

PHAGE-BACTERIA INTERACTIONS DRIVE PHENOTYPIC DIVERSIFICATION IN ENVIRONMENTAL ESCHERICHIA COLI STRAINS

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Bacteriophages, the natural predators of bacteria, play a critical role in shaping microbial diversity through their dynamic interactions with bacterial hosts. The expanding application of lytic bacteriophages as potential antimicrobial agents in clinical and environmental settings has intensified the need to understand these interactions. Escherichia coli, a Gram-negative environmental bacterium, has long served as a model for elucidating bacteriophage biology and host–phage coevolution. In this study, we isolated and fully characterized two lytic bacteriophages targeting environmental, drug-resistant strains of E. coli. We derived multiple phage-resistant bacterial variants for each strain from liquid co-cultures and biofilm-associated environments. Adsorption assays revealed a marked reduction, up to 90%, in phage adsorption efficiency to the resistant derivatives compared to the wild-type hosts. We subsequently performed comprehensive phenotypic profiling of the phage-resistant isolates, including assessments of growth dynamics, biofilm-forming ability, and motility (swimming and swarming), alongside evaluation of susceptibility to six antibiotics. The resistant strains exhibited heterogeneous phenotypic shifts, with most demonstrating accelerated growth rates but reduced motility. Notably, isolates derived from liquid cultures also displayed significantly diminished biofilm formation at 48 hours relative to their wild-type counterparts. Antibiotic susceptibility testing revealed varied resistance profiles among the resistant strains. These findings underscore the capacity of bacteriophage pressure to drive phenotypic diversification in bacterial populations, with implications for environmental microbiology and the development of phage-based therapeutic strategies.

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PP038

ASSESSING DOSE-DEPENDENT TOXICITY OF PESTICIDES ON CILIATE AND FLAGELLATE PROTISTS

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Protists are single-celled eukaryotic microbes that play a critical role as predators of bacteria in soils, regulating bacterial community structures and driving biogeochemical cycles. However, pesticide pollution, an increasing consequence of human activities, poses a significant threat to soil biodiversity and function. Yet, the impact on protist communities remains largely overlooked. This study investigates the effects of four commonly used pesticides (glyphosate, metsulfuron-methyl, etridiazole, and pyraclostrobin) on the growth of two protist strains with distinct feeding and locomotion strategies: the ciliate Tetrahymena pyriformis and the flagellate Poterioochromonas sp. Axenic cultures (i.e., growth on dissolved nutrients only without bacteria as food source) were exposed to five concentrations of each pesticide, including a zero-concentration control, to determine strain-specific EC_{50} values. Cell densities were measure at multiple time points using Lugol's iodine fixation and microscopy. EC_{50} values were derived from dose-response models using normalized cell counts. Both strains showed resistance to the highest concentration of metsulfuron-methyl tested (100 mg/L). Among the other pesticides, etridiazole was the most toxic to T. pyriformis (EC_{50} = 1.8 mg. L-1 at 48 h), while Poterioochromonas sp. exhibited greater sensitivity to pyraclostrobin (EC_{50} = 0.598 mg.L-1). Overall, Poterioochromonas sp. exhibited higher sensitivity to pesticide exposure than T. pyriformis. These findings highlight the differential responses of protists to pesticide toxicity, suggesting potential consequences for soil microbial dynamics and ecosystem functioning.

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PP039

MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE SHAPED BY PAH-CONTAMINATED MARINE WATERS AND SEDIMENTS IN A EUTROPHIC MULTI-STRESSED COASTAL SYSTEM

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Microbial communities in marine environments are sensitive indicators of environmental stressors and play a crucial role in the biodegradation of persistent organic pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs). This study aims to examine the links between the bacterial community structure and PAHs contamination in the Inner Saronikos Gulf and Elefsis Bay. Elefsis Bay experiences seasonal hypoxia and pollutant accumulation due to limited connection with the open sea, while the Inner Saronikos Gulf receives increased contaminant pressures derived by the intense maritime activities near the port of Piraeus, and together with urban discharges create a eutrophication-driven pollution gradient. The molecular composition of PAHs was examined in seawater and sediment samples, suggesting mixed petrogenic and pyrolytic origins, with low molecular weight (LMW) PAHs dominating the water column while high molecular weight (HMW) PAHs accumulating in sediments due to their hydrophobic nature and resistance to degradation. High-throughput sequencing targeting the V3-V4 hypervariable region of the 16S rRNA gene was implemented, revealing distinct microbial communities between the water and sediment partitions. Shifts in the bacterial community composition were linked to the gradient concentrations of the priority pollutant PAHs, highlighting their potential roles as bioindicators of contamination. Furthermore, the influence of the PAHs contaminants on the microbial populations was assessed with Canonical Correspondence Analysis (CCA) on Positive Matrix Factorization (PMF) values in the water partition. According to the analysis, PAH source apportionment explained 55.3% of the observed microbial variability. Statistically significant associations between PAH-degrading or PAH-tolerant Operational Taxonomic Units (OTUs) and the PAHs sources were detected. Among these OTUs, several belonged to genera commonly associated with PAH-contaminated environments, including Microbacterium, Planktomarina and Flavobacterium. Our results suggest that specific bacterial taxa, through the expression of xenobiotic biodegradation pathways or the development of tolerance mechanisms, may serve as effective bioindicators of chronic PAH contamination and its associated ecological risks in coastal areas.

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PP040

IN VITRO TOXICITY SCREENING OF VETERINARY PHARMACEUTICALS ON AMMONIA-OXIDIZING MICROORGANISMS AND ARBUSCULAR MYCORRHIZAL FUNGUS

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Livestock production generates animal waste containing pharmaceuticals compounds that leads to soil pollution, threatens microbial diversity, and exacerbates the spread of bacterial resistance. Two microbial groups are considered key functional groups of soil ecosystems and are suitable for in vitro toxicity assays: the ammonia-oxidizing microorganisms (AOM), which include ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and the arbuscular mycorrhizal fungi (AMF).

The aim of this study was to screen the in vitro toxicity of nine veterinary pharmaceuticals—either antibiotics or anthelmintics (sulfamethazine, ofloxacin, neomycin, tetracycline, trimethoprim, tilmicosin, moxidectin, toltrazuril, and florfenicol)—and to determine their EC_{50} values for the two functional soil microbial groups: the AOM and the AMF.

We hypothesize that pharmaceutical compounds will be more toxic to Nitrospira multiformis (AOB) and Nitrosocosmicus franklandianus (AOA) than to the arbuscular mycorrhizal fungus Rhizophagus irregularis, based on the similarity between the therapeutic targets of the drugs and those present in the organisms.

A range of pharmaceutical concentrations was tested in a spore germination assay with Rhizophagus irregularis over 28 days, and in liquid cultures of either Nitrospira multiformis (AOB) or Nitrosocosmicus franklandianus (AOA), with activity monitored via NO_2^- production.

As hypothesized, AMF appeared less sensitive to the pharmaceuticals than AOM, showing higher EC_{50} values or no observable toxicity.

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PP041

EXPLORING BILBERRY - ASSOCIATED MICROBIOMES IN THE ARMENIAN HIGHLANDS

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Bilberry (Vaccinium myrtillus L.) is a nutrient-rich berry with high economic value. Wild bilberries are of growing interest in climate and environmental research, as their distribution and productivity are sensitive to soil conditions, temperature, and light availability—factors increasingly influenced by climate change. In Armenia bilberries grow at altitudes of 2300-3000 m a.s.l., at the southern edge of their global distribution, in environments highly vulnerable to climate change.

We investigate the microbiomes associated with wild bilberries, focusing on both soil microbiomes and plant-associated endophytes, with potential application in agriculture and biotechnology. Fieldwork was conducted in the alpine zone of the Hankavan community, located in the Pambak Mountain Range, where bilberry populations were surveyed. The acidic soil pH (5.1–5.4) provided favorable conditions for bilberry growth, highlighting the plant's adaptability to suboptimal nutrient availability. High-quality soil DNA extraction and effective endophyte isolation methods were developed and optimized. Soil DNA samples targeting the 16S rRNA and fungal internal transcribed spacer (ITS) regions are currently under evaluation. A total of 30 endophytic bacterial strains were isolated and preserved. Biochemical and genetic characterization of these isolates is in progress.

This research represents one of the first microbiome-based studies conducted in Armenia and contributes to both fundamental and applied science by establishing methodological frameworks that support sustainable agriculture and environmental restoration.

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PP042

EFFECT OF MICROPLASTICS ON MICROBIAL COMMUNITIES FROM THE COASTAL BALTIC SEA

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Increasing microplastic (MP) pollution is greatly affecting the aquatic environment. Thus, it is vital to know its effects on the ecosystem. The current studies focus mostly on its toxicity in marine organisms, mostly animals such as fish, but little information is available regarding microbial communities. This study was designed to find out the effects of different concentrations of Polystyrene (PS), which is of smaller size and commonly found pollutant. The natural microbial community was collected from the coastal Baltic Sea and exposed to bacteria- sized PS and glass microspheres. In this experiment, we studied the effect of PS concentrations on the abundance and activity of heterotrophic nanoflagellates (HNF) and prokaryotes over five days. We observed a consistent negative effect of PS (that was more conspicuous at higher concentrations) on HNF abundance, while for bacteria the effect was negligible or, even positive. Moreover, the presence of glass and MPs particles lowered the respiration rate of the microbial community, indicating the importance of the physical effects of increased particle concentrations in the water. The changes in microbial abundance and activity were accompanied by shifts in bacterial and HNF community composition. These results indicate the complex nature the presence of MPs may have on microbial communities. The combined effect of toxicity and the physical presence of inedible particles may alter the primary microbial consumers and cause a disturbance in microbial aquatic food webs. Further experiments are planned to understand the observed patterns.

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PP043

TOXICITY OF THE FUNGICIDES FLUDIOXONIL AND TEBUCONAZOLE ON NATURAL ASSEMBLAGES OF ARBUSCULAR MYCORRHIZAL FUNGI COLONIZING TRITICALE

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Pesticides are major environmental pollutants. To protect human health and the environment, European Commission has prescribed a rigorous regulatory framework for pesticides (Regulation 1107/2009). Arbuscular Mycorrhizal Fungi (AMF), due to their pivotal role in soil ecosystem functioning but also due to their sensitivity to pesticides, have been identified as key bioindicators for assessing the toxicity of pesticides on the soil microbiota. Monitoring pesticide effects on natural AMF assemblages is challenging due to their obligatory symbiotic nature. Hence a multi-tier experimental scheme composed of toxicity screening at in vitro or gnotobiotic systems (Tier I) an then, if toxicity identified at Tier I, moving to pot and field studies (Tier II and III). We performed a greenhouse pot experiment to determine the potential toxicity of two commonly used fungicides on cereal crops, fludioxonil and tebuconazole, applied at 1X and 10X their recommended doses. Tests were performed in two soils, an acidic (pH 4.6) and an alkaline (pH 7.7) which were planted with Triticale (× Triticosecale). Fludioxonil negatively affected both AMF colonization levels in both soils at concentrations up to 10X (5 mg Kg-1). Tebuconazole significantly affected AMF colonization and P uptake in the alkaline soil at concentrations up to 10X (10 mg Kg-1). Amplicon sequencing analysis of the soil and intraradical AMF communities was also performed and analysis is on the way to identify AM fungal species that were negatively affected or were resilient to the fungicides tested.

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PP044

HIGH-TEMPORAL RESOLUTION OF MICROBIAL FOOD WEB DYNAMICS AND STRUCTURE DURING PHYTOPLANKTON BLOOMS IN THE BALTIC SEA

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Heterotrophic nanoflagellates (HNF) are a key component of the microbial food web (MFW), facilitating nutrient recycling and energy transfer in aquatic ecosystems. They have been largely considered as typical bacterivores, but they can be also omnivorous (feeding on prokaryotes and eukaryotes) and predatory grazers (feeding on eukaryotes). This study investigates HNF community composition and dynamics in the Baltic Sea from spring to autumn, focusing on their functional roles within the MFW. To follow rapid changes in microbial communities and to match the duplication times of flagellates, we carried out two high-frequency sampling campaigns, from March to May and from September to November. We used group-specific CARD-FISH probes alongside both short and long-amplicon sequencing to track the abundance of key HNF groups and explore their diversity. By using sequencing and CARD-FISH techniques, we resolved the microbial food web structure and interactions within HNF communities at the phylotype level, providing novel insights into functioning of the MFW across seasons in the brackish environment. Omnivorous kathablepharids and predatory MAST-2 dominated the HNF community, with strong seasonal peaks in spring. Bacterivorous groups (e.g., MAST-1, CRY1) were less abundant. Long-read sequencing revealed distinct seasonal shifts in dominant phylotypes, with kathablepharis sp.1 and MAST-2D peaking in spring, while other lineages became more prominent in summer and autumn. By combining high-frequency sampling, CARD-FISH, and sequencing, we offer new insights into the seasonal dynamics and functional roles of HNF communities in the Baltic Sea, enhancing our understanding of microbial interactions in brackish ecosystems.

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PP046

HIDDEN VIRAL PLAYERS: DIVERSITY AND ECOLOGICAL ROLES OF VIRUSES IN GROUNDWATER MICROBIOMES

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The ocean contains 1010 virus-like particles (VLP) per liter, vastly outnumbering host cells. Viruses significantly affect nutrient cycling, reprogramming host cells, and promoting horizontal gene transfer. Pristine groundwater is a vital source of drinking water, yet we lack an understanding of the role of viruses in this aquatic ecosystem. We examine virus diversity, ecological importance, and functional interactions in a two-year study of seven wells within a pristine groundwater system. From ~1.3 terabases of metagenomics data, we identified >7 million virus contigs, resulting in 257,252 and 82,245 virus operational taxonomic units (vOTUs) of ≥5 kb or ≥10 kb, respectively, representing a ~22-fold increase compared to publicly available groundwater vOTUs (n=3,584, ≥10 kb). Taxonomically, 99% of the groundwater viruses were species-level unique, even when compared to a global ocean dataset. Approximately 81% could be taxonomically classified, with 99% belonging to the Caudoviricetes. Ecological analysis demonstrated site- specific endemism in virus communities, evidenced by strong grouping based on the sampled groundwater wells ($p < 0.001$). Our host prediction analysis found that 88% of viruses infect 78% of microbes from the same sample, including ecologically important groups like Patescibacteria and Proteobacteria. Additionally, about 5% of viruses may reprogram ~32% of host pathways through auxiliary metabolic genes (4,093 AMGs found), linked to ~29 host phylum. One example of AMGs is GAPDH (K00134), which breaks down glucose for energy and carbon utilization, nitrite reductase (K15876), and sulfate adenyltransferase (K00957) which help groundwater microbes to thrive in oxygen-depleted conditions. Overall, this research offers valuable insights into groundwater virus communities and the mitigation of human impacts on groundwater resources.

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FUNCTIONAL AND TAXONOMIC INSIGHTS INTO MICROBIAL COMMUNITIES IN BRATEȘ LAKE IN RELATION TO LOCAL ENVIRONMENTAL STRESSORS

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Microbial communities are essential to the ecological functioning of freshwater ecosystems, playing critical roles in nutrient cycling, organic matter decomposition, and maintaining the resilience of aquatic food webs. Brateș Lake (Galati County, Romania) has experienced increasing anthropogenic pressures, including nutrient overload (nitrogen and phosphorus), sediment accumulation, and pesticide contamination, that have led to progressive eutrophication and disrupted ecosystem balance. This study focuses on characterizing microbial community composition and functional potential using whole-genome shotgun (WGS) metagenomics, providing a baseline assessment prior to the implementation of Nature-based Solutions (NbS) aimed at ecosystem restoration. Seasonal water sampling was conducted at three ecologically distinct sites: BM (middle of the lake near agricultural land), BEVF (adjacent to the fish farm and agricultural runoff), and BC (at the river inflow near a riparian zone). Samples were collected during two key hydrological phases, autumn turnover (early November) and summer stratification (late June), with six biological replicates per site and time point. Microbial DNA extracted from filtered water samples was subjected to high-throughput shotgun sequencing using Illumina technology. Bioinformatic analyses enabled taxonomic profiling and the reconstruction of metagenome-assembled genomes (MAGs), offering high-resolution insights into community structure. Functional annotation focused on genes involved in biogeochemical processes, such as nitrogen and phosphorus metabolism, pollutant degradation, and stress-response mechanisms. Comparative analyses across seasons and sampling sites will help identify spatial and temporal variations in microbial functional profiles and community composition in response to eutrophication and chemical stress. The shotgun metagenomics approach provides a robust foundation for identifying microbial indicators of ecosystem health and supports future assessments of NbS interventions. The findings contribute to long-term ecological monitoring and inform data-driven strategies for the sustainable management and restoration of Brateș Lake under increasing environmental pressures.

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PP048

IMPACT OF BACILLUS ON THE GROWTH OF RADISH AND BEETROOT MICROGREENS

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Bacteria of the Bacillus genus are commonly found in the natural environment. Many strains of this genus exhibit endophytic properties, supporting plant growth. Their potential as biostimulants is still under investigation. For this reason, their effect on microgreens — an understudied crop — was selected for examination. Microgreens are young seedlings harvested at an early growth stage, typically after the emergence of cotyledons and the first true leaves. They are gaining popularity due to their high nutritional value and are often referred to as "superfoods". The aim of this study was to evaluate the effect of Bacillus megaterium inoculation on the growth of radish (Raphanus sativus L.) and beetroot (Beta vulgaris L.) microgreens.

The experiment was conducted in two types of growing media: garden soil and coconut substrate. Three treatments were applied: control (seeds sown without any inoculation), substrate inoculation (bacterial inoculum added directly to the growing medium), and seed inoculation (following surface sterilization, seeds were inoculated with bacteria and subjected to vacuum drying (1h/27°C/800 mBa). After 5 days of radish cultivation and 14 days of beetroot cultivation, the length of shoots and roots, chlorophyll content, and dry biomass were measured.

The results showed that Bacillus bacteria significantly stimulated the growth of radish microgreens. The most pronounced increase in shoot length (by 18%, $p \leq 0,05$) and root length (by 18% in soil and 14% in coconut substrate, $p \leq 0,05$) was observed in the seed inoculation treatment. In contrast, no significant growth improvement was observed for beetroot microgreens. Chlorophyll content did not differ significantly among treatments, regardless of plant species.

These findings confirm the potential of Bacillus spp. as biostimulants in promoting the growth of radish microgreens, while indicating a lack of similar effect in beetroot microgreens.

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PP049

EVALUATION OF THE PATHOGENIC POTENTIAL OF SELECTED ENTOMOPATHOGENIC FUNGI AGAINST TENEBRIO MOLITOR LARVAE

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Biological control in plant pest management represents one of the most effective and environmentally sustainable alternatives. Among various strategies, the use of entomopathogenic fungi (EPF) has garnered attention due to their natural origin and ecological compatibility. These fungi play a pivotal role in suppressing pest populations through infection and host mortality. Unlike synthetic pesticides, EPF do not adversely affect beneficial organisms, leave no harmful chemical residues, and support biodiversity in agroecosystems. As public concern over pesticide use, biologically based pest control solutions – particularly those involving EPF – has risen substantially.

This study aimed to assess the pathogenicity of selected entomopathogenic fungal strains from the genera Samsoniella alpina, Akanthomyces sp. and Paecilomyces sp. against Tenebrio molitor larvae, a model insect host commonly used in virulence studies.

T. molitor larvae were exposed to a single application of fungal suspension via spraying, while the control group was treated with sterile distilled water. Subsequently, the larvae were placed on moistened filter paper in Petri dishes, with proper humidity maintained throughout the 21-day observation period. Larval mortality was monitored daily.

In this study, the efficacy of three entomopathogenic fungal strains (Samsoniella alpina, Paecilomyces sp., and Akanthomyces sp.) against Tenebrio molitor larvae was evaluated. All tested strains induced significant larval mortality, although the rate of action varied. Samsoniella alpina and Akanthomyces sp. demonstrated the highest efficacy, resulting in 100% mortality within 21 days; initial signs of infection were observed from days 4 and 5, respectively. Paecilomyces sp. exhibited a slower and less pronounced effect, with mortality observed from day 7 and culminating in 96% mortality at the end of the 21-day test.

The results indicate the high efficacy of Samsoniella alpina and Akanthomyces sp. Differences in the dynamics of their action may be relevant when selecting a strain depending on application requirements, where either rapid activity (S. alpina) or a more gradual infection rate (Akanthomyces sp.) may be preferred.

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PP050

ENHANCING METABOLITE PRODUCTION IN TRIGONELLA FOENUM-GRAECUM USING MICROBIAL INOCULANTS

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Trigonella foenum-graecum, commonly known as fenugreek, is a leguminous crop cultivated for its use in food, animal feed, and medicine. Traditionally, it has been used to treat wounds or manage various ailments , including diabetes. These therapeutic properties are attributed to the specialized metabolites produced by T. foenum-graecum such as flavonoids, phytosterols, and triterpenes. Among these metabolites flavonoids such as, apigenin and kaempferol have demonstrated antioxidant, anti-inflammatory and putative anti-cancer activities. One metabolite of particular significance is diosgenin, a phytosterol saponin extensively utilized in the pharmaceutical industry as a critical intermediate for the semi-synthesis of various steroid hormones such as estradiol and progesterone. The enhanced production of these bioactive metabolites is of significant interest due to their diverse applications. A promising strategy to improve metabolite biosynthesis in plants involves the use of microbial inoculants, which are well-documented for their ability to modulate the production of secondary metabolites. Fusarium solani strain K (FsK) is a beneficial endophytic fungus that confers resistance against various abiotic and biotic stresses, including water stress, nutrient deficiencies and fungal pathogens. Due to its established effects in other plant species, FsK may be used as a biostimulant for the production of specialized metabolites in T. foenum-graecum. In this study, we investigated the ability of FsK to colonize the roots of fenugreek and examined its impact on the transcriptional regulation of genes related to the biosynthesis of specialized metabolites.

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PP051

HARNESSING SYNTHETIC MICROBIAL BIOTECHNOLOGY FOR EFFICIENT PLASTIC WASTE UPCYCLING

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Plastics have become indispensable in various industries today, but their extensive use has resulted in a severe environmental crisis—plastic waste accumulation. Traditional methods of plastic waste management are insufficient, facing persistent pitfalls, such as overall material downgrading and release of hazardous microplastics and chemicals into the environment. Plastic upcycling, on the contrary, is gaining prominence in converting plastic waste into valuable chemicals and materials in an eco-friendly manner. The current project presents an innovative approach to plastic waste upcycling: employing green bio/mechano/chemical technologies for the hydrolytic degradation of plastic polymers into oligomers and their constituent building blocks, is followed by the creation of microbial cell factories capable of producing industrially relevant compounds from these plastic-derived feedstocks. Successful microbial upcycling hinges on efficient carbon assimilation from plastic feedstocks and the presence of functional intracellular biosynthetic pathways to generate valuable bioproducts. To achieve this, a wide variety of microorganisms with natural capabilities for these processes are identified through bioprospecting, and synthetic biology is employed to engineer or enhance the other necessary functions. Alternatively, some well-established microbial hosts including Bacillus spp., Pseudomonas spp. or Yarrowia spp. serve as suitable chassis. By integrating these advanced methodologies, we aim to create sustainable and efficient microbial systems for plastic waste valorization. Our ultimate goal is to establish a research and innovation hub in Synthetic Microbial Biotechnology that would advance scientific knowledge, raise public awareness, promote environmental protection, and boost economic development, all the while minimizing humanity's reliance on finite fossil fuel resources.

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BIOTECHNOLOGICAL VALORIZATION OF PLA HYDROLYSATES VIA MICROBIAL PHA SYNTHESIS BY PSEUDOMONAS SPP.

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Plastic pollution is a growing global concern, with nearly 10 million tons of waste entering the ocean annually. Biodegradable alternatives like polylactic acid (PLA) are increasingly used, especially in packaging, yet their disposal presents challenges. The presence of PLA in polyethylene terephthalate (PET) recycling streams complicates mechanical recycling, highlighting the need for selective depolymerization and biological valorization. In this context, upcycling plastic waste into higher-value products offers a promising solution that reduces environmental impact and enhances material recovery efficiency. This study contributes to circular bioeconomy strategies by exploring the targeted hydrolysis of PLA and the microbial utilization of the resulting lactic acid, its monomer. In order to achieve this, post-consumer PLA waste was selectively hydrolyzed using water, separating it from PET. The resulting hydrolysate, primarily composed of lactic acid, was then explored for biological upcycling.

Currently, Ralstonia eutropha is the only bacterium known to produce polyhydroxyalkanoates (PHAs) directly from lactic acid [1], [2]. This study explores the potential of Pseudomonas spp. to convert PLA hydrolysates into PHAs, a class of biodegradable bioplastics with diverse material properties and promising applications as sustainable plastic alternatives. As a preliminary step, selected Pseudomonas strains were cultivated on pure lactic acid to assess their capacity for growth and PHA biosynthesis. Growth was monitored daily via optical density (OD_{600}) and dry biomass measurements, while high-performance liquid chromatography (HPLC-RI) was used to analyze lactic acid consumption. Results confirmed that Pseudomonas spp. can utilize lactic acid and initial indications of PHAs accumulation were observed. These findings provided the basis for the next phase of the study, which involved the evaluation of bacterial growth and PHA production on PLA hydrolysates.

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BIOTECHNOLOGY

PP053

OPTIMIZATION OF INTEGRATED BIOPROCESSES FOR IMPROVED PRODUCTION OF BACTERIAL NANOCELLULOSE FROM LIGNOCELLULOSIC BIOMASS

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Environmental concerns have increased interest in biodegradable alternatives to synthetic plastics [1]. Nanocellulose is a promising material due to its renewable origin, biocompatibility, and versatile applications. Bacterial nanocellulose (BNC), unlike plant-derived forms (cellulose nanofibers and cellulose nanocrystals), is synthesized through microbial fermentation of sugars and is characterized by higher purity (without lignin or hemicellulose residual compounds), making it easier to use directly in biomedical or high-performance applications without extensive purification [2,3]. In this study, a combined bioprocess was optimized for BNC production from lignocellulosic residues. Agricultural and forestry biomass (wheat straw, beechwood) underwent mild OxiOrganosolv pretreatment [4], yielding cellulose-rich solids which were subsequently hydrolyzed enzymatically to fermentable sugars. These hydrolysates were used as carbon sources by Komagataeibacter sp. strains (K. xylinus and K. medellinensis) to produce BNC, and the results were compared to those employing pure sugars (glucose, xylose) as substrates. Daily monitoring of nanocellulose production and sugar consumption was performed, while the final BNC produced was characterized by Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). Enzyme-mediated oxyfunctionalization of BNC with a lytic polysaccharide monooxygenase (LPMO) further enhanced the properties of BNC, thus increasing its value as a sustainable, high-performance material [5]. This work offers an optimized process for BNC production from renewable feedstocks, supporting scalable and sustainable material development.

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PP054

STRUCTURAL INSIGHTS INTO THE CATALYTIC MECHANISMS OF PLASTIC-DEGRADING ENZYMES

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The accumulation of plastic waste in terrestrial and marine ecosystems poses a severe environmental threat nowadays. With an estimated 450 million metric tons of plastic waste produced annually, developing effective recycling methods is critical to achieve sustainability (Ritchie et al., 2023). While current solutions for end-of-life plastics include mechanical recycling, incineration, and landfill disposal, biochemical recycling -particularly via enzymatic depolymerization- is gaining attention as a greener method that operates under milder conditions (Shalem et al., 2024; Qiu et al., 2024). The incorporation of enzymes that tackle different plastic polymers (e.g poly(ethylene terephthalate)-PET or poly(lactic acid)-PLA) in cocktails could enable selective depolymerization of plastic mixed waste, addressing an additional key challenge behind the low rates of plastic recycling.

In this study, we focus on two enzymes, an engineered leaf-branch compost cutinase (LCC_ICCG) (Tournier et al., 2020) and a glucuronoyl esterase from Sporotrichum thermophile (StGE) (Taxeidis et al., 2024). LCC_ICCG displays enhanced thermostability and has been used as a template for engineering efforts aimed at developing variants with enhanced catalytic properties. LCC_ICCG displays activity strictly on PET, while StGE is able to tackle PET as well as amorphous PLA polymers. The goal of the present study is to define the structural determinants that define hydrolytic activity towards the aforementioned substrates and propose modifications that could increase catalytic efficiency. The crystal structure of apo LCC_ICCG was determined to 1.64 Å resolution. Docking simulations with PET oligomers reveal subsites that mediate terephthalate binding as well as hydrophobic residues that form critical interactions with the substrate. Moreover, docking simulations of PET and PLA oligomers using the crystal structure of StGE (PDB ID 4G4G) highlight interactions that determine the promiscuous nature of this enzyme.

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PP055

THE FIRST CRYSTAL STRUCTURE OF A GH30_12 SUBFAMILY MEMBER IN APO AND PRODUCT BOUND FORM

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Xylan is the major constituent of hemicellulose, and the second most abundant polysaccharide of the plant cell walls after cellulose. It is found in softwoods, hardwoods, and herbaceous plants, including cereals [1]. Efficient utilization of the hemicellulosic component of lignocellulose, particularly xylan, is essential for producing high value-added products, in the frame of the lignocellulosic Biorefinery concept. Some members of the glycoside hydrolase family 30 (GH30) of CAZy database can degrade recalcitrant forms of hemicellulose, such as xylan, by cleaving the β-1,4-linked-D-xylanopyranosyl bonds in its backbone. Thus, GH30 enzymes are particularly valuable for biotechnological applications, including biomass conversion and biofuel production [2, 3].

This study focuses on a bacterial GH30 from Acetivibrio clariflavus (AcXyn30B), the first biochemically characterized member of subfamily GH30_12. It is a non-specific xylanase acting on glucuronoxylan, arabinoxylan, and aryl glycosides of linear oligosaccharides [4]. Recombinant AcXyn30B was expressed in Escherichia coli, purified and crystallized, resulting in the first crystal structure of a GH30_12 enzyme at 1.14 Å resolution (PDB code: 9QY5). In addition, crystal soaking with linear xylooligosaccharides lead to the determination of the structure of AcXyn30B in complex with the reaction product, xylotriose. Further analysis of these structures will enhance our understanding of the catalytic mechanism of AcXyn30B and the structural determinants underlying its promiscuity.

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A XYLOSE ASSIMILATING SHUTTLE VECTOR FOR C5/C6 SUGAR FERMENTATION

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Bacterial and yeast strains, engineered to co-ferment pentoses and hexoses, have been among the first microorganisms implemented to convert cellulosic materials to fuels and chemicals. In order to create stable C5/C6 sugar assimilating strains, necessary metabolic genes need to be introduced into the genome, which usually entails insertions in the chromosome. Another, more flexible, option is to construct transmissible and maintainable shuttle vectors that carry the metabolic genes of interest. These, can be horizontally transferred and can complement any platform strain – i.e., any natural fermenting strain bearing useful traits or any strain already engineered to exhibit desirable biorefinery properties. Plasmid pZB301, designed in the US Department of Energy National Renewable Energy Laboratory (NREL), has been one such plasmid offered to the biofuels community, to enable C5-sugar catabolism in the C6-consuming bioethanol producing bacterium Zymomonas mobilis. pZB301 is constructed to harbor E. coli vector and Z. mobilis plasmid replication properties (derived from strain ATCC 10988 native plasmid pZMOBP6), and carries the necessary complement of genes for xylose and arabinose assimilation [US Pat No 5,843,760]. In our laboratory, we failed to maintain pZB301 in the widely used Z. mobilis strains of interest, CP4 and ZM4. While attempting to address this, we observed a marked structural instability of pZB301 replicating inside the transformed bacteria. We therefore undertook to transfer the necessary gene cassettes for xylose catabolism – genes tal, tkt and xylA, xylB, driven from the strong native enolase and glyceraldehyde-3-phosphate dehydrogenase gene promoters – from pZB301 into pJAD1, a highly stable shuttle vector we have created for Z. mobilis, which is readily mobilizable and well-maintained in also other a-proteobacteria. The new plasmid, namely pPS22, has converted three different wild-type Z. mobilis strains – ZM4 (ATCC 31821), CP4 (NRRL B-14023) and Z6 (ATCC 29191) – into xylose-assimilating strains, a feat quite satisfactory since xylose is often the major pentose component of cellulosic biomass. Addition of arabinose operon genes (araBAD) into pPS22, as well as pentose transporter overexpression, is currently underway.

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ENGINEERING THE ACTIVITY OF A THERMOPHILIC ESTERASE FROM ZHIZHONGHEELLA CALDIFONTIS FOR MHET DEGRADATION

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Polyethylene terephthalate (PET) is the world's most extensively recycled polymer, the dominant material for beverage packaging, and valuable enough to drive sustained R&D into post-consumer recovery strategies.1 Enzymatic depolymerization can fully convert PET into its monomers, terephthalic acid (TPA) and ethylene glycol (EG), enabling separation from mixed-plastic streams, upgrading to higher-value chemicals, and re-synthesis of virgin-quality PET, in contrast to the chain-degrading nature of conventional thermomechanical recycling.2,3 Although various PET hydrolases (PETases) have been reported for this biorefinery route, their activity is inhibited by accumulation of the intermediate mono-(2-hydroxyethyl) terephthalate (MHET) and bis(2-hydroxyethyl) terephthalate (BHET).4 Integrating an MHET hydrolase (MHETase), which converts MHET to TPA and EG, alleviates this bottleneck and restores full catalytic efficiency. Here, we report the engineering and characterization of a thermotolerant esterase from Zhizhongheella caldifontis (ZcEST) and its variants, including ZcMHETase (ZcEST_D355N) and ZcBHETase (ZcEST_D355S), which display up to 21-fold and 56-fold higher activity on MHET and BHET, respectively, compared to wild-type. High-performance liquid chromatography confirmed efficient conversion of PET-derived oligomers, while thermal shift assays and kinetic methods were employed to evaluate thermal stability and catalytic performance. These analyses reveal the structural basis for enhanced activity, substrate specificity, and thermal stability of the engineered variants. Our findings establish ZcEST-derived hydrolases as robust candidates for integration into enzymatic PET recycling processes and highlight how rational enzyme design can guide the future of industrial biocatalysis, contributing to the development of efficient biocatalysts for circular plastic economies.

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Protein engineering; Substrate specificity; MHET hydrolysis; plastic depolymerization; PET recycling

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EXTRACTION OF BIOACTIVE COMPOUNDS FROM ROSA CANINA L. PSEUDOFRUIT USING ENZYME ASSISTED EXTRACTION IN NATURAL DEEP EUTECTIC SOLVENTS

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Enzyme-Assisted Extraction (EAE) in Natural Deep Eutectic Solvents (NADES) was investigated as a green approach to extract bioactive compounds from the pseudofruit of Rosa canina L.

Initially, the thermal stability of Viscoferm®, a hemicellulolytic enzyme preparation, was evaluated at different temperatures (30, 40, 50, 60, 70 and 80 °C) in the NADES Choline Chloride: Glycerol (1:2 molar ratio, pH=5.3) (ChCl: Gly) with 20% (v/v) water as a co-solvent, and also in a buffer solution of the same pH. The deactivation rate constants (kd) were calculated at each temperature. NADES-enzyme system exhibited higher stability compared to buffer–enzyme system.

The impact of enzyme loading (0.1, 0.5, 0.75, and 1% v/v) and extraction time (1, 2, 3, and 4 hours) were also studied. The extracts were evaluated based on their total phenolic content (TPC). The highest TPC yield (113.77 mg GAE/g dry weight) was achieved applying enzyme loading 0.5% v/v and 2-hour extraction time. The anti-diabetic (α-amylase, α-glucosidase inhibition), anti-aging (mushroom tyrosinase inhibition), antioxidant (DPPH radical scavenging method) and antimicrobial (inhibition of Escherichia coli growth) activities of several extracts were also assessed. The strongest antioxidant activity (IC_{50} = 0.79 μLextract/mL), α-glucosidase inhibition (IC_{50} =2.66 mg/mL), and antimicrobial activity (66.8%) was recorded applying enzyme loading 0.5% v/v, while the highest α-amylase inhibition (IC_{50}=47.77 mg/mL) and tyrosinase inhibition (IC_{50}= 29.98 mg/mL) applying 1% v/v. In all cases, EAE in NADES outperformed the control extraction samples.

Overall, the study highlighted the potential of EAE in NADES to enhance the enzyme stability along with the extraction efficiency of bioactive compounds from Rosa canina L., offering a green and effective alternative extraction process.

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PP060

FROM WASTE TO RESOURCE: BIOCONTROL POTENTIAL OF WINE LEES IN GRAPEVINE PATHOGEN MANAGEMENT

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Wine lees are the second most abundant by-product generated by wineries, consisting of the sediments that accumulate at the bottom of barrels, tanks, or bottles during winemaking. These sediments are primarily composed of dead yeast cells and contain a diverse mixture of organic and inorganic compounds, including proteins, peptides, polysaccharides, sterols, and long-chain fatty acids. Due to their complex composition, wine lees are often considered environmental pollutants. The primary objective of this study was to evaluate the potential of wine lees, derived from different yeast strains and grape musts, to inhibit grapevine pathogens. A sequential inoculation strategy was employed using one non-Saccharomyces yeast strain and two Saccharomyces cerevisiae strains in three mono-varietal grape musts from Greek cultivars. Fermentation progress was monitored daily by measuring glucose and fructose concentrations enzymatically, alongside CO_2 production. Microbial populations were also tracked throughout the fermentation process. Following fermentation, yeast biomass was collected and subjected to autolysis and enzymatic digestion to produce two biomolecule mixtures: High Molecular Weight (HMW-BM) and Low Molecular Weight (LMW-BM). These were tested in vitro for antifungal activity against grapevine pathogens, including Botrytis cinerea, Aspergillus carbonarius, Phaeomoniella chlamydospora, and Phaeoacremonium minimum. The results demonstrated that LMW-BM was the most effective in suppressing pathogen growth. More precisely wood discoloration caused by Phaeoacremonium minimum was reduced by both biomolecule mixtures while Phaeomoniella chlamydospora only by LMW-BM. To further investigate peptide content and potential mechanisms of action, an untargeted analysis was performed using reverse-phase liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOF MS), incorporating advanced techniques such as Trapped Ion Mobility Spectrometry (TIMS) and Parallel Accumulation–Serial Fragmentation (PASEF).These findings suggest that wine lees are a promising, sustainable source of bioactive compounds with potential applications in viticulture for the biocontrol of grapevine pathogens.

The research project entitled «reLees» is implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union – NextGenerationEU (H.F.R.I. Project Number:15100). undisturbed pressure conditions, if lab incubation experiments to study deep-water microbial diversity, metabolic rates and functional responses are to follow.

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PP061

BIOACTIVE PROPERTIES OF BACTERIOCINS ISOLATED FROM MICROORGANISMS WITH PROBIOTIC POTENTIAL

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Bacteriocins, the cationic mainly ribosomally synthesized peptides secreted by probiotic bacteria have attained scientific interest due to their multifaceted roles including antimicrobial and antioxidant activity, as well as their selective cytotoxic effects against cancer cells [1, 2]. In the present study, the bioactive properties of the purified bacteriocins from two bacterial strains with accredited probiotic potential, namely Lactococcus lactis ATCC 11454 and Bacillus subtilis NCIB 3610, were investigated. The cell-free culture supernatant (CFS) from a 24h culture of each strain was collected and then was subjected to a dual purification protocol, including ammonium sulfate and organic solvent precipitation, followed by size exclusion chromatography, ion exchange chromatography, solid phase extraction, and lyophilization. The two resulted seperate fractions, particularly samples L1 and L2 in case of L. lactis, and samples B1 and B2 in case of B. subtilis, were evaluated for their antimicrobial, anti-biofilm and free radical scavenging effects, as well as their cytotoxic properties against colon cancer cell lines and normal cells. The purified bacteriocin samples have shown stronger antimicrobial and anti-biofilm activity against selected pathogens, including Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Shigella flexneri and Enterococcus faecalis, at concentrations varying from 20 to 100 μg/mL. All samples exhibited great scavenging of radicals, thus indicating the bacteriocins' antioxidant potential. Furthermore, all samples resulted in significant reduction in CRC cell viability in a dose- and time-dependent manner, while cytotoxicity against normal cells was low. Only the B2 sample caused increased cytotoxicity to normal cells possibly due to the presence of the lipopeptide surfactin. In conclusion, purified bacteriocins could be exploited as next-generation bioactive agents in food and pharmaceutical industries.

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PP062

WHOLE-GENOME NANOPORE SEQUENCING OF 84 ENVIRONMENTAL BACTERIAL ISOLATES FROM THE ELTE COLLECTION

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Antimicrobial resistance is escalating into a global health crisis, attributed to multiple factors such as the inappropriate use of antibiotics, the release of antimicrobial agents into the environment and the adaptive potential of microbes to acquire resistance mechanisms. The rise of resistant pathogens highlights the urgent need for novel antimicrobial agents. Recent advances in microbial genomics have contributed to the identification of new bioactive compounds by revealing previously unknown biosynthetic gene clusters. We undertake genomic sequencing of bacterial strains isolated from various natural or polluted environments by investigators at Eötvös Loránd University (ELTE collection). A taxonomically representative set of 84 isolates, mainly streptomycetes, has been analysed so far, in four runs on the MinION platform of Oxford Nanopore Technologies, using the Native Barcoding Kit 24 V14 chemistry and R10.4.1 flow cells. The sequencing output was analysed through a rigorously validated bioinformatics pipeline. Basecalling of raw reads was performed using Guppy bioinformatics toolkit, deploying both the FAST and SUP models, with the SUP model achieving higher read quality scores, the majority of which surpassed Q15. Adapter trimming and read filtering were carried out using Porechop and Filtlong, respectively. Three different programs were utilized to assess the quality of the generated sequences, FastQC, MinIONQC and NanoPlot. A de novo assembly strategy, using the Flye assembler, was preferred over reference-based approaches to enable the incorporation of diverse genomic regions. The assembly process was followed by polishing with Medaka, resulting in high-quality consensus sequences that achieved over 90% gene completeness. Functional annotation, employing prokka, was successfully applied in 81 out of 84 genome assemblies, demonstrating an average of 7,546 coding, 17 rRNA and 88 tRNA genomic sequences per genome. Consensus sequences were further examined for potential contamination employing the ContEst16S tool. Generated genomic data and the complete analysis workflow will be made public to support researchers in advancing the understanding of microbial metabolic potential, phylogenetic relationships and biotechnological significance.

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PP063

BIOPAPER FROM TEA AND BACK: USE IN TEABAGS

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Bacterial cellulose (BC) is a glucose polymer like plant cellulose, synthesized and exported to the extracellular milieu by various bacterial strains. In their natural habitat, bacteria of the genera Agrobacterium, Acetobacter, Azotobacter, Rhizobium, Alcaligenes, Sarcina and Pseudomonas form BC biofilms to facilitate their attachment to plant hosts and shield themselves against environmental threats. However, it is members of Acetobacter (renamed to Komagataeibacter) that lead to laboratory BC yields exceeding 15 g/L. BC has been attracting interest since it is purer, of greater tensile strength and water holding capacity, and more moldable than plant cellulose. Glucose chains in BC, or else nanocellulose (BNC), form ordered crystalline microfibrils that create ultrafine porous networks with exceptional mechanical and rheological properties. These, render BC an important ingredient in the food, textile, drug, cosmetics, enzyme and biomedical industries, and an almost sole component for biopaper production. In this work, and in order to make cheap and abundant biopaper, we sought to isolate acetobacteria from a commercial kombucha tea source. In doing so, we identified a Komagataeibacter sp. as the main BC biofilm component. However, and quite surprisingly, a second organism, a Bacillus sp., was present and co-isolated. The Bacillus was in tight symbiotic relationship with the Komagataeibacter isolate and in fact their combination proved favorable in certain standard growth conditions, compared to Komagataeibacter alone. Furthermore, when the isolated Bacillus was purposefully co-cultivated with Komagataeibacter sucrofermentas DSM 15973 to make biopaper from beer waste, again the yield was higher. This relationship as well as the Komagataeibacter-Bacillus co-culture dynamics are currently under investigation in order to optimize BC yields. We also pursue to identify the isolates to a species level, using a multi-locus molecular marker approach, since 16S rDNA sequencing resulted in only genus discrimination. With the recent publication of several Komagataeibacter sequenced genomes, including that of K. sucrofermentas last year, obvious genomic targets for acetobacterial strain improvement are being proposed. Last but not least, and in an entrepreneurship-oriented direction, the application of BC biopaper for teabag manufacturing has been envisioned as an important commodity and a product-to-market business model (GreenTech Challenge).

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PP064

NOVEL LYTIC BACTERIOPHAGES AS TOOLS FOR MICROBIOTA MODULATION IN ASTHMA: FROM ISOLATION TO DEFENSE SYSTEM PROFILING

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Asthma, a chronic inflammatory disease of the airways, is increasingly linked not only to viral infections but also to disruptions in the respiratory and gut microbiome—commonly referred to as microbial dysbiosis. Certain infectious bacterial genera such as Streptococcus, Moraxella, Haemophilus, and Staphylococcus colonize and induce inflammation, with the relevant microbiome being a potential target for asthma therapy. In this work, we aim to explore the role of bacteria-infecting viruses, known as bacteriophages (phages), and the interplay of bacteriophage-bacteria interactions in respiratory tract. For that reason, we isolated and fully characterized lytic bacteriophages against species recently implicated in airway microbiota, while also we generated an in silico pipeline for studying antiphage defense systems of well-known bacteria. Two newly identified lytic phages infecting Bacillus licheniformis, have been isolated from environmental enrichment sources. Both phages exhibited lytic activity in vitro and distinct plaque morphology. Whole genome sequencing and bioinformatic analysis confirmed the absence of lysogenic or virulence-related genes, supporting their suitability for therapeutic applications. The phages displayed narrow host range specificity, an essential feature for targeted microbiota manipulation. Bacteriophage-resistant strains were also generated, monitoring microbiologically their phenotypic alterations in terms of kinetics, swarming and swimming potential as well as their capacity to form biofilm. Additionally, defense systems analysis against lytic phages of Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus influenza, and Staphylococcus aureus revealed diverse systems in terms of quality and quantity, information that will be pivotal in understanding the dynamics of the binary system bacterial host: lytic phages within the respiratory tracts and possibly putative clinical implications. These findings contribute to the expanding studies for better understanding bacterial-related asthma, as well as enriching the first Greek Bacteriophage Biobank against a clinical challenge. Future research will explore their interactions within host microbial communities and evaluate their effects in preclinical asthma models.

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PP065

TUNING MATERIAL PROPERTIES BY BLENDING BACTERIAL BIOPOLYMERS POLYHYDROXYALKANOATES AND NANOCELLULOSE

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Bacterial biopolymers offer several advantages over conventional petroleum-based plastics, especially from environmental, biomedical, and sustainability standpoints. They are biodegradable, biocompatible, produced from renewable resources, with low carbon footprint, and offering number of end-of life options. Bacterial biopolymers such as polyhydroxyalkanoates (PHAs) and bacterial nanocellulose (BNC) have unique properties allowing them to be used on their own for variety of applications. However, they can also be used for blending with other materials to tune their properties. By blending bacterial biopolymers, mechanical strength (e.g., tensile strength, elasticity), thermal stability, barrier properties (water vapor, gas permeability), biodegradability and biocompatibility, as well as processability (e.g., film-forming, molding) can be remarkably improved [1]. PHAs are biodegradable and thermoplastic, hydrophobic biopolymer while BNC is strong, flexible and hydrophilic. Combining these two biopolymers outcomes the material with improved tensile strength and flexibility, enhanced moisture resistance and wettability with the potential application in both biomedicine and packaging industry. However, they can also be blended with variety of other bio-based polymers, including poly(lactic acid) (PLA), poly(caprolactone) (PCL) or even some natural lignocellulosic materials such as straw or corn stover. From the proccesability point of view, PHAs physical properties limit its suitability for electrospinning—the most commonly used method for fabricating fibrous scaffolds. To overcome this limitation, poly(hydroxyoctanoic acid), PHO, was blended with poly(lactic acid), PLA, enabling the production of fiber biomaterials using electrospinning. The resulting blended PLA/PHO fibers had smaller diameters, increased hydrophilicity, and enhanced mechanical properties compared to native PLA fibers. Moreover, incorporating ~20% of PHA into corn stover-based materials significantly improved their flexural strength and water resistance, while maintaining full biodegradability and enabling thermoplastic processing. Using PHAs as an additive in corn stover-based materials is a novel and sustainable strategy to enhance the mechanical, thermal, and processing characteristics of lignocellulosic biomass demonstrating a pathway for high-value upcycling of agricultural waste.

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PP066

SUBSTRATE SPECIFICITY AND KINETIC CHARACTERIZATION OF BIOH, A PYRETHROID-HYDROLYZING ESTERASE ISOLATED VIA FUNCTIONAL METAGENOMICS

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Pyrethroid pesticides are an important class of insecticides widely used to improve crop yields and control mosquito-borne diseases in household settings. Their widespread and persistent use has led to the accumulation of residues in the environment, raising concerns about food safety, human health, and impacts on non-target organisms. These concerns highlight the need for effective biodegradation strategies, and microbial enzymes such as pyrethroid esterases offer a sustainable solution by hydrolyzing the ester bond central to pyrethroid structure. In previous work from our lab, functional metagenomic analysis of a biobed system exposed to pyrethroid compounds led to the identification of a fosmid clone capable of degrading α-cypermethrin. Transposon mutagenesis subsequently pinpointed the esterase-encoding gene bioH as essential for this activity. This study aims to better understand the role of BioH in pyrethroid degradation by characterizing its substrate specificity and determining its kinetic parameters (Km and Vmax) for a range of structurally diverse pyrethroids. Enzyme assays showed that BioH catalyzes the degradation of multiple Type II pyrethroids, including cypermethrin, cyfluthrin, deltamethrin, and acrinathrin. In contrast, no activity was detected against bifenthrin (Type I), etofenprox (a non-ester pyrethroid), or tau-fluvalinate (Type II). The observed selectivity suggests that BioH targets the ester bond in substrates containing specific structural motifs, such as the α-cyano group, characteristic of Type II pyrethroids, and the cyclopropane ring, which may enhance substrate recognition and catalytic efficiency. Although tau-fluvalinate contains the α-cyano group, it lacks the cyclopropane ring found in other degradable Type II pyrethroids, which likely impairs recognition by BioH and explains its resistance to degradation. Ongoing work focuses on determining the kinetic parameters for each pyrethroid substrate. The outcomes will clarify the potential of BioH as a candidate for enzymatic degradation of pyrethroid residues in food and environmental systems.

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DISCOVERY OF BIOACTIVE SIDEROPHORES IN MEDITERRANEAN DEEP-SEA ACTINOBACTERIA USING ADVANCED METABOLOMICS TOOLS AND COMPUTATIONAL MASS SPECTROMETRY

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Deep sea habitats such as marine sediments, harbor a vast and largely untapped microbial diversity, including actinobacteria (Actinomycetota), with extremophilic representatives being especially notable for their ability to biosynthesize diverse specialized metabolites exhibiting different biological activities. Among these, specialized molecules that chelate iron cations, known as siderophores, hold significant promise for drug development and biomedical uses. This work outlines a comprehensive pipeline combining microbial isolation and cultivation with advanced computational mass spectrometry approaches to accelerate the discovery of such compounds.

Strains of the genus Streptomyces (Actinomycetes, Kitasatosporales, Streptomycetaceae) used in this study originate from sediments of Mediterranean coastal waters near Spain and were cultured in liquid media under the SECRETed project. Post-cultivation, biomasses and culture supernatants were separated by centrifugation and processed independently. The supernatants underwent a liquid-liquid extraction, while the cellular component was sequentially extracted using solvents of varying polarity to yield a range of extracts. These were analyzed by LC-MS/MS, serving two key functions: (a) enabling semi-automated dereplication of siderophore-related metabolites and (b) refining purification strategies for candidate compounds.

On the computational side, LC-MS/MS data processing was conducted using mzmine, followed by comparative metabolomics to annotate chemical features that differentiate extracts tested positive for the presence of siderophores from those in which siderophores were absent. Annotation was achieved through spectral library searches and matches against open resources like GNPS2, complemented by in silico fragmentation predictions using SIRIUS. Molecular networking further facilitated the identification of potential analogs, with networks enriched by bioactivity and ferric ion (Fe+3 or Fe(III)) binding data to highlight clusters of interest.

Purification efforts, directed by these findings, were carried out with close monitoring using TLC combined with ferric chloride reagent for siderophore presence visualization. The purified substances were subsequently identified employing LC/MS alongside NMR spectroscopy. Among the key metabolites characterized were both linear and cyclic hydroxamate siderophores belonging to the ferrioxamine family, in addition to germicidin-related compounds known to be autoregulatory inhibitors of spore germination in Streptomyces. Furthermore, a variety of other bioactive molecules were uncovered, demonstrating significant potential for biotechnological exploitation.

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PP068

UNLOCKING THE BIOTECHNOLOGICAL POTENTIAL OF STREPTOMYCES VIOLACEORUBER JS520 FOR POLYMER UPCYCLING

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Plastic pollution poses a critical environmental challenge due to its persistent accumulation in terrestrial and aquatic ecosystems and its contribution to rising CO_2 emissions. Conventional recycling methods are limited by energy demands, mixed waste complexity, and product quality. In contrast, enzymatic degradation has emerged as a promising alternative, offering polymer specificity, mild reaction conditions, and recovery of high-purity monomers. PET-degrading enzymes, particularly, have demonstrated notable progress in recent years (Arnal et al., 2023). However, for a truly circular plastic economy, it is essential to integrate microbial systems capable of not only degrading synthetic polymers but also converting the resulting monomers into valuable bioproducts. Such a strategy would enable plastic waste to serve as both a carbon source and a platform for biotechnological production.

The aim of this study was to sequence and identify bacterial strain JS520 at the species level and to investigate its ability to produce secondary metabolites while utilizing a variety of polymer-derived monomers, including ethylene glycol, terephthalic acid, 3-hydroxy butyric acid, lactic acid, ε-caprolactone, etc. Genome sequencing of Streptomyces sp. JS520 revealed a 7.26 Mbp genome with a GC content of 72.34% and an N50 of 0.27 Mbp, assembled into 74 contigs. Comparative genomic analysis identified the strain as Streptomyces violaceoruber. AntiSMASH analysis uncovered 20 biosynthetic gene clusters (BGCs), including those responsible for the synthesis of undecylprodigiosin and actinorhodin—compounds with antibacterial, anticancer, UV-protective, and pH-responsive properties. Proteome analysis identified 8 homologs of known plastic-degrading enzymes, including those active against PLA, PET, and PHA, supporting the strain's potential for polymer upcycling. Experimental validation confirmed the strain's growth on various polymers, further indicating its degradative capability.

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PP069

ENGINEERING MICROBIAL PLATFORMS FOR SIMULTANEOUS BIODEGRADATION AND UPCYCLING OF PCL

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Nowadays, the accumulation of plastic waste in the environment has become a significant global concern, with long-lasting ecological and health impacts. This has driven the urgent need for novel strategies, particularly those targeting the degradation of polyesters, which are widely used but poorly degraded in natural environments [1]. Biological approaches, particularly those utilizing microorganisms, offer a sustainable alternative for addressing plastic waste [2]. In this study, we evaluated the ability of various microbial strains to metabolize polycaprolactone monomers. These included Ralstonia eutropha H16, newly isolated pigmented Streptomyces isolates, Streptomyces albus wild-type strain, as well as a strain evolved via adaptive laboratory evolution, which was selected to better utilize the PCL monomer as a sole carbon source. Initial screening revealed variable growth across the studied microorganisms, which are known to produce valuable bioproducts, such as bioplastics, biopigments, and antibiotics [3] [4]. Additionally, aiming to construct strains that achieve polymer degradation and further metabolize the monomers, plasmids harboring selected polyesterase genes, Se1JFR [5] or DmPETase [6], were introduced into R. eutropha and Streptomyces species. Transformed strains were identified and further analyzed for their polyester-degrading abilities by growth assessment as well as the determination of esterase activity in culture supernatants. Overall, this work contributes to the broader field of microbial upcycling by combining metabolic screening, genetic engineering, and synthetic biology to construct bacterial strains capable of degrading and isolating synthetic polymers.

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PP070

ENZYMATIC RECYCLING OF POLY(3-HYDROXYBUTYRATE) AND UPCYCLING OF THE DEGRADATION PRODUCTS

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Major environmental issues, caused by the excessive use of petroleum-derived plastics in modern societies have led to the need of new alternatives. Substitution of conventional plastics by bio-based and biodegradable ones produced via microbial bioconversion using either renewable feedstocks or biological/chemical recycling of biopolymers constitute crucial technologies towards the transition to a circular bio-economy. Polyhydroxyalkanoates (PHAs) is a group of biodegradable polyesters with similar properties to conventional plastic, with poly(3-hydroxybutyrate) (PHB) being the most extensively studied. PHB is produced as a secondary intracellular metabolite via bacterial fermentation, and it could be used for packaging applications. Sustainable production and commercialization of PHB should be accompanied with the development of chemical and/or biological recycling of post-consumer PHB-based bioplastics. Enzymatic PHB recycling emerges as a vital process for sustainable process circularity.

PHB depolymerases are carboxylesterases and along with lipases can decompose PHB to produce monomers, dimers, oligomers or longer MW chains depending on the type of enzyme and the producing strain. PHB depolymerases from microorganisms that thrive in extreme environmental sites were screened via high-throughput metagenomic technologies. The optimal PHB depolymerase was overproduced in Escherichia coli as a recombinant expression host. The PHB depolymerase was used for PHB hydrolysis in an electrochemical membrane reactor for integrated production and purification of 3-hydroxybutyric acid or oligomers. Electrochemical membrane extraction has been applied for integrated hydrolysis and extraction PHB monomers. The current driven flux of 3-hydroxybutyric anions from the enzyme reactor into the acid concentrate enables the purification of the hydrolysis products. The monomers and/or oligomers from PHB hydrolysis have been evaluated as carbon sources for bacterial growth and PHB accumulation by Paraburkholderia sacchari.

Keywords: poly(3-hydroxybutyrate), PHB depolymerases, heterologous expression, upcycling, microbial fermentation

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PP071

UNIQUE BIOCHEMICAL FEATURES OF A BI-FUNCTIONAL GLUCURONOXYLASE/XYLOBIOHYDROLASE: THE CASE OF TTXYN30A AS A KEY BIOCATALYST FOR NATIVE HEMICELLULOSE BIODEGRADATION

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The enzymatic valorization of residual plant biomass, within the scope of circular bioeconomy, has emerged as an environmentally friendly approach for chemicals production. Xylan is the most abundant hemicellulose, yet it is underutilized in agricultural and forestry industries [1]. Due to its complex and diverse composition, xylan could serve as raw material for the production of sugars, prebiotics, and functional polymers [2]. Therefore, novel hemicellulases demonstrating new specificities are considered key players for xylan utilization [3].

The bi-functional glycuronoxylanase/xylobiohydrolase TtXyn30A from Thermothelomyces thermophilus belongs to the glycoside hydrolase 30_7 subfamily [3]. Its bi-functional nature attracts scientific interest, as it combines an exo- and an appendage-dependent endo-mode of action. In specific, a site-directed mutagenesis study was conducted concerning the catalytic glutamates (E188 and E278) and an alternative glutamate (E233) [4]. Structural and molecular docking studies on TtXyn30A were performed to gain better insight into its catalytic activity. Finally, TtXyn30A was studied on pretreated beechwood and native lignocellulosic biomasses.

Along with the catalytic residues, E233 participates in substrate binding and is also suggested to assist the endo-mode of action of TtXyn30A. This residue was also demonstrated to undergo significant structural rearrangements upon substrate binding. When acting on lignocellulose, the enzyme releases acetylated xylobiose. More specifically, the enzyme was suggested to tolerate acetyl decorations of the xylan backbone within the -2 and +1 subsites of the catalytic cleft. This specificity falls in line with the periodic acetylation pattern of xylan in plant biomass [5]. Considering all these biochemical features, TtXyn30 bears the potential to produce acetylated and glucuronidated oligosaccharides from plant biomass, with high prebiotic value [6].

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PP072

IMPACT OF 16S RRNA GENE SEQUENCING STRATEGIES ON METATAXONOMIC PROFILES IN DAIRY PRODUCTS AND IMPLICATIONS FOR QUANTITATIVE MICROBIAL RISK ASSESSMENT (QMRA)

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The application of Whole Genome Sequencing (WGS) in food microbiology has significantly enhanced the ability to characterise complex microbial communities in dairy products. However, the choice of sequencing strategy-including platform type, read length, library preparation, and downstream bioinformatics can introduce variability that influences taxonomic profiling. This variability poses a challenge for robust Quantitative Microbial Risk Assessment (QMRA), particularly when assessing the safety of dairy products prone to contamination by pathogens.

This study investigates the metagenomic discrepancies arising from different 16S rRNA gene sequencing approaches (Illumina short-read, Oxford Nanopore long-read) on the same set of raw and processed dairy product samples from Spain and Poland. The aim was to identify and quantify the taxonomic differences resulting from various next-generation sequencing (NGS) strategies and assess their potential implications for Quantitative Microbial Risk Assessment (QMRA).

Preliminary results show notable discrepancies in microbial diversity profiles. The resulting datasets were integrated into a QMRA framework to evaluate how sequencing-induced variability affects hazard identification, exposure assessment, and risk characterisation. In scenarios involving the consumption of dairy products, such differences substantially impacted predicted illness probabilities, highlighting the public health implications of methodological choices in metataxonomic workflows.

This work underscores the need for standardised 16S rRNA sequencing protocols and validated reference databases in dairy microbiota analysis. Reliable interpretation of microbial community data is critical for informed risk assessments and regulatory decision-making in the dairy industry. Future efforts should focus on platform benchmarking and developing consensus guidelines for metataxonomic applications in food safety.

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PP074

FROM CELL TO COMMUNITY: UNLOCKING MICROBIAL DIVERSITY WITH SINGLE-CELL GENOMICS

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Single-cell DNA sequencing complements the metagenomic analysis of uncultured bacteria by revealing cell-to-cell variation, linking host genomes to extrachromosomal DNA, and providing strain-level taxonomic resolution. However, current techniques are limited to processing fewer than 1,000 cells and produce single-amplified genomes (SAGs) of low completeness due to biases in whole genome amplification (WGA).

We present an innovative, cost-effective approach to sequence up to 10,000 SAGs with superior genome recovery. Individual microbial cells are isolated into 70 µm semi-permeable capsules (SPCs), enabling compartmentalized multi-step processing - including lysis, WGA, and barcoding - of all cells simultaneously at a cost of less than $1 per cell. Using well-characterized E. coli and B. subtilis, we demonstrate >90% genome recovery per SAG at sequencing depths below 10x and <1% cross-contamination. Additionally, we processed a commercially available microbial community standard and were able to detect all species within the mixture.

The advantages of SAG sequencing are especially valuable for uncovering the diversity and unique adaptations in the context of microbial ecology. We processed soil and aquatic samples to generate matched SAG and MAG datasets, with the latter obtained through bulk metagenomics. SAG assemblies produced longer contigs and revealed detailed genomic features, most notably the linkage between viral and plasmid sequences and their hosts. The linkage information was lost in the MAG dataset.

Our high-throughput SAG sequencing workflow provides a detailed view of microbial communities, offering unmatched resolution and scalability. This capsule-based single-cell sequencing technology opens new horizons for microbial genomics research.

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ACETATE CO-FEEDING INCREASES ETHYLENE GLYCOL ASSIMILATION AND GLYCOLIC ACID PRODUCTION IN YARROWIA LIPOLYTICA

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The upcycling of plastic-derived monomers, such as ethylene glycol (EG), is a key strategy for establishing a circular economy and reducing plastic waste. In this study, we demonstrate the efficient bioconversion of EG into glycolic acid (GA), a high-value compound, by the non-conventional yeast Yarrowia lipolytica. While EG alone does not support growth in minimal medium, co-feeding with acetate -a cost-effective and renewable carbon source- enabled both biomass formation and increased GA production. Using 13C-labeled substrates and metabolic flux analysis, we show that EG-derived carbon is not only oxidized to GA but also assimilated into biomass through the glyoxylate cycle and amino acid biosynthesis, particularly via glycine and serine pathways.

Shake flask experiments revealed that co-feeding EG and acetate significantly enhanced substrate consumption and GA production compared to EG alone or EG with glucose. Under bioreactor conditions, a pulse-feeding strategy with acetate and an initial high EG concentration resulted in a GA titer of 48.4 ± 1.4 g/L, a molar yield of 73%, and a productivity of 0.73 g/(L·h). These are the highest GA production levels reported in yeast using a one-step process in minimal medium.

Our results provide the first direct evidence of EG carbon incorporation into yeast biomass and highlight the importance of co-substrate selection to overcome metabolic bottlenecks in EG assimilation. The oxidative conversion of EG likely contributes to NADPH regeneration, supporting biosynthesis without relying heavily on the pentose phosphate pathway. This co-feeding strategy also mitigates pH imbalances, enhancing acetate uptake and overall process stability.

This work establishes Y. lipolytica as a robust microbial platform for the upcycling of PET-derived monomers and underscores its potential in developing scalable, sustainable bioprocesses within a circular bioeconomy framework.

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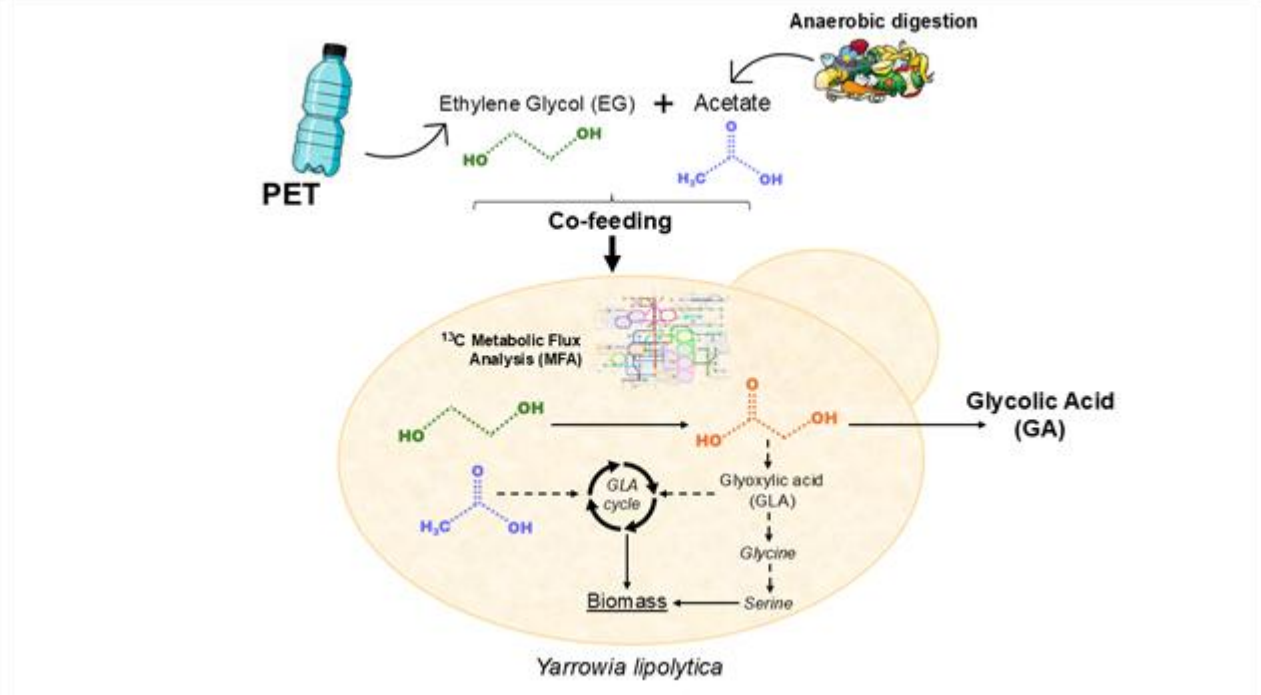

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NOVEL CONSERVED FUNCTIONAL AND STRUCTURAL MOTIFS REVEALED THROUGH AN EVOLUTIONARY STUDY OF THE GLYPHOSATE OXIDOREDUCTASE (GOX) BASED ON ORTHOLOGOUS PROTEINS.

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Glyphosate, the active ingredient in widely used broad-spectrum herbicides, is a synthetic amino acid comprised of glycine bound to a phosphonic acid. The extensive use of glyphosate-based herbicides raises concerns about its persistence and its accumulation in the environment. It is known to be toxic to aquatic life with long lasting effects, whereas studies have shown adverse effects on the growth of cultivated plants, the gut microbiome of insects, and concerns have been raised regarding human health, for its possible role as an endocrine disruptor, in neurodegenerative disorders and in endometrial cancer. Glyphosate Oxidoreductase (Gox) is a FAD-dependent enzyme known to degrade glyphosate. Despite its discovery back in 1995, the knowledge around its physiological role in bacteria, its distribution across the bacterial kingdom and its structure is limited. Such information could contribute to better understanding of the degradation pathways of glyphosate and to the discovery of new enzymes with similar activity, which could be used in bioremediation programs. Considering the lack of information about Gox, an evolutionary analysis was performed in order to identify homologous proteins within the FAD-dependent/binding oxidoreductases family. A total of 2,220 representative protein sequences from 843 species representing 10 classes of bacteria were analyzed. Following the identification of known conserved domains in biological databases and the inference of conserved motifs based on their multiple sequence alignment, four protein domains, two characteristic/functional regions and eight conserved motifs were identified. A novel updated phylogenetic tree of the Gox-related proteins was also constructed, in order to identify major protein clusters, based on their sequence and correlated to annotation information in the databases. The present work marks an initial step toward elucidating the physiological role of Gox and classifying the protein group it belongs to. These results can be further used for a more in-depth functional characterization, including the investigation of possible functional pathways and structural characteristics of Gox and its closest relatives.

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ENZYMATIC DEGRADATION OF PBAT: TOWARD A SUSTAINABLE STRATEGY FOR POLYESTER WASTE RECYCLING

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Polymers have become indispensable in modern life and the global economy due to their low cost, high performance, and ease of processing. However, poor end-of-life management has led to widespread plastic pollution and significant resource loss. Among synthetic polyesters, polyethylene terephthalate (PET) and poly(butylene adipate-co-terephthalate) (PBAT) are widely used due to their durability, lightweight nature, and cost-effectiveness. In agriculture, PBAT-based mulch films are frequently employed to improve soil quality and crop yield. While PBAT is classified as a compostable polyester and is structurally more susceptible to enzymatic degradation than polymers with carbon-carbon backbones, its natural degradation rate in the environment remains relatively slow. Bio-based degradation using renewable biological agents such as enzymes or microorganisms presents a sustainable and environmentally friendly strategy to reduce and recycle plastic waste.

In this study, we investigated the enzymatic degradation of PBAT using a series of polyester hydrolases, including HiC, LCC, DmPETase, Se1JFR, and FoCut. An initial screening was performed on powdered PBAT to assess the degradation efficiency of each enzyme, with a focus on the release of monomeric degradation products. The extent of degradation was evaluated through high-performance liquid chromatography (HPLC) and mass loss measurements. Following the screening phase, enzymatic degradation experiments were scaled up using commercial PBAT-based mulch film, applying the most promising enzymes identified in the preliminary tests. The degradation products were further characterized through a combination of thermal analysis (TGA and DSC), spectroscopic analysis (ATR-FTIR) and molecular weight distribution (GPC), in addition to monomer release and mass loss quantification. Overall, this study highlights the potential of enzymatic depolymerization as a bio-based strategy for PBAT waste valorization, offering a sustainable alternative to conventional disposal methods.

Keywords: circular economy, plastics, recycling, enzymatic degradation, sustainability

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PROTEIN ENGINEERING OF A FUNGAL FERULOYL ESTERASE ENHANCES BOTH PLASTIC AND LIGNOCELLULOSE BREAKDOWN

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The persistent accumulation of plastic waste has intensified the demand for sustainable waste management strategies. Enzymatic degradation emerged as a promising approach, particularly for polymers with hydrolysable bonds, such as polyethylene terephthalate (PET). PET-degrading enzymes (PETases) catalyze the breakdown of PET into water-soluble intermediates, primarily mono(2-hydroxyethyl) terephthalate (MHET), while further hydrolysis of MHET to terephthalic acid (TPA) is crucial for efficient polymer degradation [1]. Structural analysis of IsMHETase, the benchmark MHETase from Ideonella sakaiensis, reveals significant structural similarities to feruloyl esterases (FAEs), enzymes involved in lignocellulose deconstruction [2,3].

Building on the structural homology between FoFaeC, a FAE from Fusarium oxysporum [4], and IsMHETase, specific single-point mutations (T202E, G122S, I415F) were designed to enhance FoFaeC's MHETase activity. Single, double and triple FoFaeC variants were expressed in Pichia pastoris. Experimental results demonstrated that the FoFaeC-G122S variant exhibited a 4.4-fold increase in catalytic efficiency on MHET and a 2.0-fold increase in activity towards PET trimer, compared to the wild-type enzyme (WT). Regarding natural-substrate mimicking compounds, FoFaeC-G122S displayed 2.0- and 1.2-fold higher catalytic efficiency towards MpCA and MCA, respectively. In PET degradation, supplementation of IsPETase with FoFaeC-G122S led to a 17-fold decrease of released MHET, achieving its complete conversion to TPA, while in biomass breakdown, FoFaeC-G122S exhibited a 2.0-fold higher ferulic acid release rate from destarched wheat bran compared to the WT, when combined with a GH11 xylanase.

These findings demonstrate that a single structure-based mutation on FoFaeC can enhance its activity on a non-natural substrate (MHET), establishing its accessory role for PET degradation, while boosting its natural synergistic role in lignocellulose breakdown.

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PP078

SUSTAINABILITY OPTIMIZATION FOR SECURE FOOD SYSTEMS-SOSFOOD

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In the current environmental emergency, the food system has to become more productive, inclusive, sustainable, and resilient. SOSFood will therefore use data-exploitation and AI-based technologies to provide a holistic and comprehensive image of the EU food system and develop tailored predictive tools to support well-informed decisions of all stakeholders of the food chain, with a multi-factorial, multi-actor and multi-scale approach, thanks to its multidisciplinary consortium of experts, from private and public sectors, academia research and food system representative (consumers, producers and industries). Although several initiatives have been taken in the same line, none has fully succeeded at considering the global picture of the system or delivering a reliable and accessible message to the target audience. Hence, SOSFood will result in a consolidated food data space focused on sustainability and health and decision-making tools adapted to each level of the chain (a predictive dashboard displaying data and predictions for industries, and a mobile app for consumers comprising an eco-healthy fingerprint visualization plot, a greening indicator of industries, reformulated European recipes promoting safe and healthy, local and seasonal ingredients and general recommendations), following these steps: (1) creating a multi-actor network gathering social, political, legal, economic, technological, food, health, environmental and climatic data, promoting transparency and data-sharing, (2) mapping the food system scenario with a multidimensional strategy, exploiting the interoperability of data with advanced impact analysis and innovative AI-technologies, (3) co-designing solutions i.e., decision-making tools fitting the context and priorities of each user, validated through field case studies to ensure viability and representativeness.

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MICROBIAL ECOLOGY OF TWO TRADITIONAL CHEESES FROM GREEK ISLANDS: A STUDY OF LADOTYRI PDO AND MELICHLORO

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The microbial ecology of artisanal cheeses is a key factor in their safety, quality, and sensory properties. The traditional method of their production using unpasteurized milk has been crucial to Greek culture and gastronomy since ancient times. Ladotyri PDO and Melichloro, two such cheeses made from sheep and goat milk on the Greek islands of Lesvos and Lemnos, harbor diverse microbial communities that contribute to fermentation and preservation. This study assessed the microbial composition of these cheeses by focusing on lactic acid bacteria (LAB) and yeasts, both of which play essential roles in texture, flavor development, and bioprotection. Eleven samples were analyzed, of which five were produced using unpasteurized milk, following the traditional method by individual residents of the two islands. In contrast, small local cheese factories made the other six commercial cheeses. LAB populations exceeded 7 log CFU/g in all samples, while yeast counts reached over 3 log CFU/g, with some Ladotyri samples surpassing 7 log CFU/g. Additionally, all cheese samples were tested for the populations of important hygiene indicators including Enterobacteriaceae, coliforms, enterococci, and staphylococci, as well as for the presence of pathogens like Salmonella spp., Listeria monocytogenes, and Staphylococcus aureus. Hygiene indicator levels were elevated in some samples, highlighting the need to improve production hygiene practices. Notably, pathogens were generally absent, except for S. aureus found in some traditional Melichloro cheeses. Our findings reinforce the essential role of microbial diversity in traditional cheese production, balancing safety with the preservation of unique sensory and functional properties.

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PP080

TARGETING THE INVA GENE AS A POTENTIAL MARKER FOR DIFFERENTIATING SALMONELLA GENOTYPES

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Salmonella is a well-known pathogen found in a wide range of foods. Climate change appears to influence its prevalence in the food chain. From an epidemiological perspective, it is crucial to characterize the pathogen at both the species and serovar levels. Additionally, serovar characterization is essential for understanding the associated disease, antimicrobial resistance, foodborne transmission, intervention strategies, and for enabling regulatory agencies to impose appropriate controls. Serovars are typically discriminated using a combination of serological and molecular methods. Nowadays, advanced high-resolution molecular techniques such as MLST and WGS facilitate this goal. However, these methods are relatively costly and require specialized expertise. The aim of this work is to develop an alternative, simple, and cost-effective protocol to distinguish Salmonella species and/or serovars. In brief, an in silico digestion of the invA gene—commonly targeted to confirm the presence of Salmonella—was performed. Sequences of invA from several serovars were retrieved from the KEGG database and digested in silico using a wide range of restriction enzymes. The resulting restriction fragments from a representative set of enzymes were then compared. This comparison demonstrated that different serovars could be differentiated into distinct genotypes and that Salmonella enterica could be distinguished from Salmonella bongori. These results are very promising, as invA is a highly conserved gene, and this approach may help narrow down the range of serovars under investigation.

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PP081

CHARACTERIZATION OF INDIGENOUS YEAST STRAINS OF THE GENUS SACCHAROMYCES AS BIOCONTROL AGENTS AGAINST PHYTOPATHOGENIC AND SPOILAGE FUNGI

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Plant pathogenic and food spoilage microorganisms cause serious losses in crop production and severe damage during food processing, transportation and storage. Chemical antimicrobial agents are commonly employed to control fungal growth and activity. However, the recent trend is shifting from chemicals towards safer, sustainable and more eco-friendly options [1]. Among a wide variety of microorganisms, certain yeasts could become a promising alternative to chemical fungicides as biocontrol agents [2,3]. The objective of this work was to evaluate the inhibitory effect and technological characteristics of two Saccharomyces cerevisiae strains against filamentous fungal species associated with plant disease and food spoilage. Twenty-four fungal isolates belonging to the Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Paecilomyces, Phoma, Rhizopus, and Ulocladium genera were characterized by macro- and microscopic observation, and by Sanger sequencing at the ITS and beta-tubulin loci to confirm their taxonomic identity. In vitro essays were performed to evaluate the inhibitory effect during co-culture of the two yeast strains with the fungal isolates under controlled conditions. In addition, the fungi were screened for technological traits, such as proteolytic and amylolytic activities, as these may contribute to their interaction with yeast antagonists. Results showed that both S. cerevisiae strains demonstrated inhibitory effect, exceeding 80% in certain cases, depending on the yeast strain and fungal species. Biochemical tests showed variability in proteolytic and amylolytic activity, both between and within fungal genera. This enzymatic diversity indicates that certain fungal species exhibit higher substrate degradation capacity, potentially influencing their competitiveness. Our findings suggest that S. cerevisiae strains have promising biocontrol potential and could be employed in the development of effective in-field and food-commodity biocontrol strategies.

Keywords: Saccharomyces cerevisiae, antagonistic yeasts, biocontrol, filamentous fungi

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PP082

EXPLORING A MULTILAYER PERCEPTRON MODEL ON DUAL-SPECIES BIOFILM GROWTH DATA

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The ability of bacteria isolated from leafy salads to affect biofilm formation with Salmonella Typhimurium has been previously reported. In this study, we used previously collected data of various isolates recovered from either rocket or spinach salads and cultured together with S. Typhimurium to form dual-species biofilms on stainless steel coupons at 20°C. Three separate datasets (n=51-167) are provided, based on the growth medium used: Tryptic Soy Broth (TSB), rocket sterile extract, and spinach sterile extract. The main goal of this analysis was to test the ability of a basic machine learning (ML) algorithm to predict the final population of native biota or S. Typhimurium in dual-species biofilms. The attributes used in the ML model were the inoculum population (log CFU/ml), initial population (log CFU/cm^2), final population of mono-species biofilms (log CFU/cm^2), and final population of dual-species biofilm and S. Typhimurium in dual-species biofilms (log CFU/cm^2). For the initial exploration of model training and evaluation, we used the Java-developed WEKA software. For the development of the MLP model on broth data, a training set of 100 data points was used (Learning epoch=100, learning rate=0.3, and momentum value=0.2). After several trials, the highest efficiency was achieved with a shallow model of one hidden layer with 50 neurons for predicting both the final population of native biota or S. Typhimurium (correlation coefficient 97% and 84% respectively, and RMSE<0.25). However, the 10-fold cross-validation results showed a correlation coefficient of 0.56, which means moderate accuracy of this model in predicting dual-species biofilm growth. When we asked the model to predict the final population of S. Typhimurium within a single instance of an assessment data set of 67 points, it gave a predicted value of 5.11 log CFU/cm^2 as compared to the actual one of 5.3 log CFU/cm^2, however the correlation coefficient of the model was 0.49. Further model development and testing are needed using the rocket and spinach extract data sets. These results contribute to our knowledge related to dual-species biofilms and may be helpful in our efforts to control Salmonella diseases connected with the consumption of contaminated fresh salads

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PP083

INVESTIGATING THE IMPACT OF MULTI-LAYER BIO-ACTIVE COATINGS CONTAINING PROBIOTIC CULTURES ON THE SHELF-LIFE OF STRAWBERRIES

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The multi-layer coating technique is an emerging alternative for addressing potential limitations of the conventional dipping method usually applied in active packaging, i.e., insufficient adherence of coatings to the hydrophilic surfaces of fruits. This method uses sequential immersions in oppositely charged polyelectrolyte solutions to create chemically bonded layers. Incorporating probiotics into such coatings may extend the shelf-life of fresh produce, while providing health benefits. This study aimed to evaluate the effect of probiotic-based, multi-layer coatings on the shelf-life of strawberries.

Strawberries (var. Victory) were coated using a layer-by-layer method involving 5 sequential dipping steps, each lasting 2 min. For coatings containing probiotic cultures, the dipping sequence followed the order: 1% calcium chloride ($CaCl_2$) → 1% sodium alginate (SA) → $CaCl_2$ → SA supplemented with either Lacticaseibacillus casei Shirota (EC-LC) or Lacticaseibacillus rhamnosus GG (EC-LR) (ca. 8.0 log CFU/mL) → $CaCl_2$. Uncoated (C) or multi-layer coated with SA without probiotic cultures (EC) strawberries were also studied. Samples were stored in macro-perforated PET trays at 4°C. Various parameters were monitored throughout storage i.e., weight loss, visible decay, pH, titratable acidity, total suspended solids (TSS), ripening index (TSS/TA), color (L*,a*,b*), texture (Fmax, N; via penetration), molds, and total viable counts. Additionally, sensory evaluation was conducted, and the viability of both probiotic species was confirmed throughout storage.

TSS accumulation and visible decay was inhibited in EC-LC or EC-LR compared to controls and EC, indicating slower ripening. Color change (ΔE) in controls was significantly lower ($p \leq 0.05$) compared to EC, EC-LC, and EC-LR, however the sensory panel did not macroscopically identify color differences ($p > 0.05$) among treatments. Fmax in EC-LC was 2 to 3-fold higher than controls at all samplings. Controls were not acceptable by the sensory panel on day 9, while EC-LC or EC-LR became non-acceptable on day 14. The presence of probiotic cultures significantly ($p \leq 0.05$) inhibited fungi by 1.5–2.0 log CFU/g compared to controls, while the probiotic cultures remained viable at ca. 6.0 log CFU/g throughout storage.

Multi-layer coatings containing probiotic cultures may raise new perspectives for the shelf-life extension of perishable fruits like strawberries, while also providing them with functional properties.

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PP084

ANTIMICROBIAL EFFECT OF OREGANO ESSENTIAL OIL IN NA-ALGINATE EDIBLE FILMS FOR SHELF-LIFE EXTENSION OF FETA CHEESE

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Introduction: Natural antimicrobial compounds, including essential oils, have gained significant interest due to their potential to improve food quality and safety. By inhibiting microbial growth and postponing spoilage, they increase the shelf-life of perishable food commodities.

Purpose: This study evaluated the effect of Na-alginate edible films, with or without oregano essential oil (EO), on the shelf-life extension and inhibition of Listeria monocytogenes and Escherichia coli O157:H7, in aerobically stored Feta-cheese.

Methods: Feta-cheese slices were wrapped in Na-alginate edible films without (control) or with 0.5% (v/v) oregano EO. Samples were divided into two groups: (i) inoculated with a three-strain cocktail of L. monocytogenes (Batch 1) or E. coli (Batch 2) strains (~ 4 log CFU/g), to assess pathogen inhibition, and (ii) non-inoculated, to evaluate spoilage delay. All samples were stored under aerobic conditions at 4°C and 12°C. Microbiological counts, pH, and sensory attributes (non-inoculated group) were monitored during storage (31 days).

Results: The initial microbial population of Feta-cheese was ca. 8.3 log CFU/g, with LAB (ca. 8.2 log CFU/g) and lactic cocci/streptococci (ca. 8.1 log CFU/g) being the predominant groups, while yeast-molds were present at lower levels (ca 3.4 log CFU/g). In the inoculated group, L. monocytogenes populations decreased by approximately 3 log CFU/g in the EO-treated samples compared to control, at both 4°C and 12°C, indicating inhibition of growth. Growth of E.Coli at both 4 and 12°C was similar for both EO and control samples. Slight inhibition of yeasts/molds population was visible on EO samples at 4°C (Batch 1) and 12°C (Batch 2). Shelf-life was shorter for control samples, while EO-treated samples exhibited an extension of storage time by up to 10 days at 4°C and 7 days at 12°C. Sensory evaluation indicated that EO-treated samples were preferred over control.

Significance: The use of oregano EO in Na-alginate edible films demonstrated potential antimicrobial activity against pathogens and spoilage microorganisms, extending the shelf-life and safety of Feta-cheese.

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FOOD & NUTRITION

PP085

EVALUATION OF THE ANTIMICROBIAL EFFECT OF OREGANO ESSENTIAL OIL-ENRICHED EDIBLE FILMS ON SEA BREAM (SPARUS AURATA) FILLETS UNDER AEROBIC STORAGE USING CLASSICAL AND RAPID MICROBIOLOGICAL ASSESSMENT METHODS

Stamatina Xenou[1], Fotoula Schoina[1], Symeon Makris[1], Aggeliki Doukaki[1], Olga Papadopoulou[2], Cryssoula Tassou[2], Panagiotis Skandamis[3], George-John Nychas[1], Nikolaos Chorianopoulos[1]

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Introduction: Sea bream (Sparus aurata) is a highly perishable Mediterranean fish of great commercial and nutritional value. Thus, there is a growing need for rapid, non-invasive methods to monitor spoilage, ensure safety, extend shelf life, and preserve organoleptic quality.

Materials and Methods: Sea bream fillets were preserved at 4 different temperatures of 0, 4, 8 and 12°C under aerobic conditions. Microbiological counts and pH were monitored throughout storage, and sensory assessments were conducted. Fourier transform infrared (FTIR) and multispectral imaging (MSI) analyses were performed as rapid, non-destructive techniques to estimate the microbiological status of samples from the fish's flesh and skin. For spectral data analysis, 70% of each dataset was used for training and 30% for testing the models. Partial Least Squares Regression (PLS-R) model was used for quantitative prediction, correlating spectral data for estimating TVC, Pseudomonas spp. and H2S-producing bacteria.

Result: Results showed an initial microbial load of 3.8 (±0.2) log CFU/g (TVC), with Pseudomonas spp. and H_2S-producing bacteria as the dominant spoilage organisms. Organoleptic rejection of fresh fillets without edible films occurred at 120, 72, and 48 h at 4, 8, and 12°C, respectively, while fillets remained acceptable up to 144 h at 0°C. Fillets with edible films without essential oil were rejected at 144, 144, 72, and 48 h at 0, 4, 8, and 12°C, respectively. In contrast, edible films with essential oil extended acceptability to 144 h at 0 and 4°C, and to 96 and 54 h at 8 and 12°C, respectively. pH remained stable (6.5–6.8) under all storage conditions. PLS-R models did not yield satisfactory predictive performance, with the best results observed for H_2S-producing bacteria on skin samples (R^2 = 0.168, RMSE = 1.434) using VideometerLite data.

Discussion: The findings on the antimicrobial activity of edible films enriched with essential oils, used as active packaging for sea bream fillets, are encouraging. However, multispectral imaging and FTIR spectroscopy,

in combination with PLS-R analysis, were not effective in detecting the biochemical changes occurring during storage of the fillets.

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PP086

EVALUATION OF QUALITY AND SAFETY OF UN-GUTTED SEA BREAM (SPARUS AURATA) USING CLASSIC AND RAPID DETECTION METHODS WITH OPTICAL SENSORS TECHNOLOGY.

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Introduction: Reducing food waste and ensuring effective quality control are key challenges in the food supply chain. Since fish are perishable products characterized by short shelf-life, real-time monitoring of food freshness and detecting spoilage at early stages before advanced deterioration is considered necessary.

Purpose: The purpose of the present study was to develop a rapid, non-invasive method that can estimate the microbial quality of farmed un-gutted sea bream (Sparus aurata).

Methods: During the experimental procedure, whole un-gutted sea bream fillets were preserved at four different temperatures, 0, 4, 8 and 12 ^{0}C, in modified atmosphere conditions packaging (45% CO2, 35% N2 & 20% O2) including an optical sensor inside the packaging. Microbiological analysis, pH measurements and sensory evaluation were performed, and then spectral data of the optical sensors were obtained using portable-multispectral imaging (MSI) instrument.

Results: Results showed that the initial population for the fresh ungutted sea bream was approximately 104 CFU/g, while Pseudomonas spp. and H2S-Producing bacteria were the dominant spoilage microbiota of sea bream at all storage temperatures. No significant differences were observed in pH values during storage, which maintained from 6.0 – 7.1. A decrease in the reflectance spectra was observed, as the storage time increased, demonstrating the ability of the optical sensors to detect spoilage-associated volatile compounds through noticeable color fading during the storage of packaged sea bream.

Signature: The collected information from this study is promising, as the use of optical sensor technology provides a non-destructive method for real-time quality monitoring of packaged sea bream.

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PP087

DEVELOPMENT OF A PREDICTIVE MODEL OF LISTERIA MONOCYTOGENES GROWTH IN XINOTYRI (SOFT CHEESE) FROM UNPASTEURIZED GOAT MILK

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Listeria monocytogenes is a pathogenic microorganism of significant concern in the dairy industry, particularly in traditional cheeses made from unpasteurized milk. Xinotyri, a Greek artisanal soft cheese traditionally produced from raw goat milk without the addition of starter cultures, presents a unique challenge in ensuring microbial safety while preserving its characteristic sensory attributes. This study aimed to develop a predictive model for the growth behavior of L. monocytogenes in Xinotyri under varying environmental and processing conditions.

Experimental batches of Xinotyri were inoculated with low levels (~3 log CFU/g) of a L. monocytogenes strain and stored under different temperature (4, 10, 16 and 20°C) conditions, reflecting realistic post-processing and retail environments. Microbiological analyses were performed at regular intervals to monitor pathogen growth over a 21-day period. Concurrently, physicochemical parameters such as pH, and water activity (aw) were recorded.

A primary model (Baranyi and Roberts) was applied to describe microbial growth kinetics, and a secondary Ratkowsky model incorporating temperature as influencing factor was developed. Model performance was evaluated through statistical indicators including R^2, RMSE, and bias and accuracy factors. Validation was conducted using independent data from separate cheese production trials under dynamic temperature conditions.

Results demonstrated that L. monocytogenes growth was significantly influenced by storage temperature and initial pH, with the highest proliferation observed at 20°C and pH > 5.0. The predictive model accurately described the growth dynamics of the pathogen, with R^2 values exceeding 0.95 in most cases.

The developed model offers a valuable tool for risk assessment and microbial safety management in traditional raw milk cheese production. Future work will focus on model expansion to include additional variables such as salt concentration and competing microflora.

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PP088

FUNGAL PREVALENCE AND GENETIC DIVERSITY IN COMMERCIAL MAIZE-DERIVED PRODUCTS FROM THE GREEK MARKET

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Maize (Zea mays L.) holds a pivotal role in the agricultural sector in Europe, ranking as the second most cultivated cereal crop, with an average annual production exceeding 65 million tons over the past five years. However, maize and maize-derived products are highly vulnerable to fungal contamination, which poses a significant risk due to the potential production of hazardous mycotoxins associated with adverse effects on human health. This study aimed to evaluate the prevalence and genetic diversity of fungi in commercially available maize-based products (e.g., corn flour, corn cakes, pudding powder, etc.) in the Greek market. A total of 160 samples were collected and subjected to microbiological and molecular analyses to enumerate and identify the predominant fungal contaminants. Initial fungal isolation was conducted using culture-based techniques on three selective media: Potato Dextrose Agar, Malt Extract Agar, and Rose Bengal Chloramphenicol Agar. For molecular identification, genomic DNA from representative isolates was used to amplify the ITS region with primers ITS1 and ITS4. Amplicons were Sanger sequenced, and species identified via BLAST, using >97% similarity as the threshold. The results showed high variability in the fungal population of the examined products, ranging from below the detection limit to 5.7 log CFU/g. Accordingly, 53 isolates were sequenced and ten fungal genera were identified: Aspergillus (9), Cladosporium (4), Fusarium (8), Hamigera (1), Mucor (6), Paecilomyces (2), Penicillium (9), Phanerodontia (4), Rhizopus (7), and Talaromyces (3). Among these, Aspergillus, Fusarium, and Penicillium are of particular concern due to their known capacity to produce mycotoxins such as aflatoxins, fumonisins, ochratoxins, and trichothecenes. The findings underscore the critical need for continuous monitoring and rigorous quality control measures in the production and distribution of maize-based products. Furthermore, the application of molecular diagnostic tools in routine screening protocols can significantly enhance the accuracy of fungal identification, facilitating the early detection of mycotoxigenic species and reducing potential health risks.

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ENUMERATION OF MESOPHILIC AEROBES IN GREEK ORIGIN COW'S, SHEEP'S AND GOAT'S MILK.

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Enumeration of mesophilic aerobes (MA) is the main quality and hygiene parameter for raw and pasteurized milk. High levels of these microorganisms indicate valuable information about sanitary and hygienic conditions of milking, storage, and processing, and suggest the presence of pathogenic microorganisms. To guarantee adequate monitoring, governmental agencies are responsible for determining parameters and rules that must be followed during all steps of production. These organizations are also responsible for systematic verification of whether these parameters and rules are being met. In Greece, the responsible body for controlling the quality of the milk produced is the Hellenic Agricultural Organization – DIMITRA with its statutory laboratories.

According to the Hellenic Food Control Authority (EFET), Part B, Annex III, point 1, and to the Regulation (EC) No 853/2004 of the European Parliament and of the Council, the number of microorganisms in cow's milk must be equal to or less than 100,000 CFU per mL, while in sheep's and goat's milk it must be equal to or less than 1,500,000 CFU per mL. However, when raw sheep's or goat's milk is used for the preparation of dairy products, the process of which does not require heat treatment, then it must be ensured that the number of microbes does not exceed 500,000 CFU per mL. The sampling frequency is set at 2 times per month.

In the tested cow's milk, at the country level, the average MA value for the years 2020 to 2024 was around 80,000 CFU/mL. However, 19% of the samples, corresponding to 9.6% of the controlled quantity, were outside the limits, showing a high concentration of MA. Respectively, in both sheep and goat raw milk, the concentration of MA was below 400,000 CFU/mL and only 3.1% and 2.4% of the samples respectively (corresponding to 2.2% and 1.7% of the controlled quantities) were outside the limits.

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IN VITRO ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY OF POMEGRANATE RESIDUES EXTRACTS

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Pomegranate residues (PRs), the solid by-products remaining after juice extraction, are abundantly generated in Greece. Rich in carbon sources and bioactive compounds, such as phenolics, PRs hold significant potential for various applications including biofuel production, food preservation and sustainable packaging [1,2]. In this study, various extracts and hydrolysates from dried PRs were assessed for their antimicrobial activity against foodborne pathogenic and spoilage microorganisms.

The antimicrobial activity of the PRs extracts (aqueous and ethanolic) and hydrolysates (previously prepared and utilized for bioethanol production) was initially evaluated using the disc diffusion method, along with the corresponding positive (chloramphenicol and cycloheximide for bacteria and fungi, respectively) and negative (the solvents of each extract/hydrolysate) controls. Based on the results of the disc diffusion assays, inhibition zones (1.2–6.0 mm) were noted for almost all tested extracts/hydrolysates against the bacterium Bacillus subtilis, whereas the bacterium Salmonella enterica was inhibited by only two extracts/hydrolysates (inhibition zones of 2.5–3.5 mm). On the other hand, the bacteria Escherichia coli, Pseudomonas sp. and Enterococcus faecalis were not inhibited by none of the tested extracts/hydrolydates. Furthermore, most of the tested extracts/hydrolysates were active against the yeast species Saccharomyces cerevisiae, Rhodosporidium toruloides, Torulaspora delbrueckii and Candida tropicalis, with the recorded inhibition zones varying from 1.8 to 12.5 mm. Regarding filamentous fungi, various levels of activity of extracts/hydrolysates (inhibition zones of 3.0–17.0 mm) were demonstrated against Mucor sp., Aspergillus niger and Penicillium expansum. When ethanolic extracts were evaluated in laboratory culture media for their activity against selected fungi, a significant effect on the growth rate of the filamentous fungus Mucor sp. and the yeast R. toruloides was noted.

Overall, PRs-derived extracts and hydrolysates showed considerable antimicrobial activity, primarily against Gram-positive bacteria and fungi.

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IMPACT OF GROWTH CONDITIONS ON THE ANTIMICROBIAL ACTIVITY OF LAB STRAINS AGAINST LISTERIA MONOCYTOGENES SCOTT A AND ASSOCIATED METABOLOMIC PROFILES

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The increasing emergence of antimicrobial resistance in foodborne pathogens like Listeria monocytogenes highlights the need for natural and sustainable control strategies. Lactic acid bacteria (LAB) represent a promising group of microorganisms with inherent antimicrobial potential. This study investigated the growth kinetics of co-cultures of L. monocytogenes Scott A with selected LAB strains in different matrices under glucose-present and glucose-absent conditions. Emphasis was placed on exploring how metabolic responses may influence antimicrobial activity. LAB strains were co-cultured with L. monocytogenes in BHI:MRS (50:50 v/v) and modified TSB media, both with and without glucose supplementation. The presence of glucose significantly affected the inhibition profile of specific LAB strains. Notably, Lactococcus lactis spp. lactis strains exhibited enhanced antilisterial activity in glucose-free environments, suggesting adaptive metabolic shifts linked to antimicrobial function. To further explore this hypothesis, LC-MS/MS-based untargeted metabolomic profiling was conducted on LAB supernatants from each condition. Comparative analysis revealed that certain LAB strains secreted distinct metabolite patterns depending on glucose availability. While several overlapping metabolic features were observed, a subset of strains showed glucose-dependent secretion of unique compounds, potentially contributing to differential antimicrobial effects. These preliminary results suggest a link between environmental nutrient availability, metabolic expression, and antimicrobial function in LAB strains. Although the specific metabolites responsible for inhibition remain unidentified, the observed strain-dependent variation in secreted compounds warrants deeper investigation. This study highlights the importance of integrating metabolic profiling with antimicrobial assays to better understand the mechanistic basis of LAB functionality. Overall, these findings provide novel insight into the complexity of LAB-pathogen interactions and support the targeted application of specific LAB strains as natural biopreservatives tailored to distinct food system environments.

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THE INFANT MICROBIOTA HOPSCOTCHES BETWEEN COMMUNITY STATES TOWARD MATURATION

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The infant gut microbiome differs significantly from that of an adult. The maturation of the infant gut microbiome begins from the first days of infants' life and continues for several years. Over the past years, the different stages of maturation and the expected microbial composition in different developmental phases have been thoroughly studied. Despite those studies, the actual effect of diet and other early life conditions on microbiome maturation, as well as the longitudinal trajectories followed, are not fully understood. We performed a longitudinal study examining the 16s rRNA gene microbiota profiles and faecal parameters of 540 European infants, fed either a symbiotic or control infant formula during their first year, up to 36 months of age. Our findings reveal that the microbial diversity gradually increased until the 36 months of age, with the most significant shifts occurring between 4 and 12 months of age. By the age of 36 months, the children's gut microbiome starts resembling that of an adult, suggesting an early formation of the enterotypes. Also, we describe the gradual increase in pH values and alteration of common SCFAs. Furthermore, through longitudinal profile clustering, we provide evidence for the existence of multiple distinct microbial community states present at each stage of microbial maturation. Infants' microbiome exhibited transitions through these community states towards maturation, a phenomenon we termed as "hopscotching". Our findings suggest that although the overall trajectory of maturation may be rather uniform, the exact successions among those early community states are not deterministic but a complex and individualised process. Early life factors like mode of birth and diet did not fully explain those transitions, pointing towards yet unknown factors that may control microbiome maturation.

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PP095

DETECTION OF ADULTERATION IN SHEEP AND GOAT DAIRY PRODUCTS WITH TOUCHDOWN POLYMERASE CHAIN REACTION

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Adulteration of milk and dairy products is widespread nowadays, since they are the most consumed food products worldwide, due to their high nutritional value at a particularly low cost. The addition of cow's milk and water to higher-cost milk types are the most frequent adulteration practices. Beyond the significant impact on economic and commercial relations, the phenomenon of adulteration poses a serious threat to public health due to the potential allergic reactions resulting from undeclared ingredients on the label of the product.

The most common methods for detecting adulteration are based on the determination of milk proteins; however, these techniques are showcasing limited sensitivity and specificity due to their unsuitability for heat-treated products or for discriminating between closely related material. In this sense, DNA-based techniques can be a suitable alternative as they have good sensitivity, even after heat-treating dairy products. Consequently, several methods have been developed including DNA sequencing with RFLP, RAPD, and NGS, with PCR remaining the main method.

In the present study, two previously developed touchdown PCR (TD-PCR) assays for detection of adulteration in milk (Kourkouli et al., 2024) were applied in dairy products of sheep and goat origin. Application of this method can overcome problems related to high annealing temperatures required for some primer-template combinations and be useful for difficult-to-amplify templates, such as those with extensive secondary structures or high %GC content. Additionally, a comparative study was initially conducted to optimize DNA isolation from dairy samples using an exogenous standard and assessing the recovery. Three commercially kits were tested, and the results were evaluated in terms of recovery of the exogenous standard, cost, time, and complexity of the process.

Overall, the methods successfully detected cow's milk while identifying the declared species—sheep or goat. The sheep protocol was able to detect cow's milk concentrations of 5% in white sheep cheese, 1% in yellow cheese, and 1% in yogurt, while the goat protocol established a limit of detection (LOD) of 5% in white cheese and 1% in yogurt. Finally, applications were carried out on commercially available samples of goat and sheep dairy products, revealing high occurence of fraudulent practices.

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PP096

DISRUPTING FOODBORNE PATHOGEN BIOFILMS: THE ROLE OF LACTIC ACID BACTERIA IN MODULATING AI-2 QUORUM SENSING AND MICROBIAL INTERACTIONS

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Microbial interactions within food environments significantly impact food safety and spoilage. Quorum sensing (QS), a key mechanism for cell-to-cell communication in bacteria, regulates group behaviors such as biofilm formation, which enhances pathogen persistence in food-related and other habitats. The autoinducer-2 (AI-2) QS system facilitates both intra- and inter-species signaling, influencing microbial community dynamics. Lactic acid bacteria (LAB), commonly associated with fermented foods, produce bioactive compounds that may interfere with QS in foodborne pathogens. This study explores the potential of LAB-derived cell-free supernatants (CFSs) to modulate AI-2-mediated QS and inhibit biofilm formation by Listeria monocytogenes and Staphylococcus aureus. Using Vibrio harveyi luminescence assays, 89 foodborne LAB isolates were screened for AI-2 QS interference. Twenty CFSs with significant QS-modulating activity were further assessed for their ability to inhibit biofilm formation at a sub-minimum inhibitory concentration (sub-MIC) using a microtiter plate assay. Both pathogens' planktonic growth kinetic parameters were also analyzed upon exposure to each CFS. Results showed that 61.8% of CFSs contained AI-2-like signals, while 28.1% exhibited AI-2 QS inhibition. Most CFSs with QS interference significantly reduced L. monocytogenes biofilms, and one also decreased S. aureus biofilm biomass by 45.4%. Notably, the observed antibiofilm effects did not result from planktonic growth inhibition, suggesting a targeted disruption of bacterial interactions. These findings highlight the ecological role of LAB metabolites in shaping microbial communities within food environments and their potential as natural biofilm control agents in food safety applications.

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L-CYSTEINE AND ITS TRANSPORTER CTAP AFFECT BIOFILM FORMATION, SWIMMING AND SWARMING MOTILITY IN LISTERIA MONOCYTOGENES

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Listeria monocytogenes is of a significant concern for the food industry, largely due to its ability to form biofilms, its motility, and stress resistance. Flagellar motility and environmental factors are crucial for biofilm formation. Cysteine is an important compound affecting the behavior of this bacterium; therefore, we investigated its role in growth, biofilm formation and motility of L. monocytogenes 10403S through the use of a mutant in cysteine uptake (ΔctaP). Basal defined media (DM) and L-cysteine-supplemented DM were used. Biofilm formation was promoted by L-cysteine supplementation in both wild type (WT) and ΔctaP. Lower biofilm formation of ΔctaP compared to WT indicates the significance of the cysteine transporter and cysteine uptake. A negative correlation was found between growth and biofilm formation, especially in the presence of high L-cysteine concentrations. Motility experiments showed that as the L-cysteine concentration increased, the swarming motility of WT decreased. Furthermore, swimming motility of WT was enhanced with L-cysteine supplementation, while the swimming motility of ΔctaP remained unaffected. To evaluate the role of cysteine and CtaP in biofilm formation and motility, transcriptome analysis, comparing WT and ΔctaP in basal and L-cysteine-supplemented (1.57 and 3.67 mM) DM, was conducted at 37 °C. The investigation of biofilm-related genes explained the role of ctaP and revealed induced expression of flagella and chemotaxis genes by L-cysteine.

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PP098

ARABINOXYLANS FROM ZEA - A NATURAL XYLAN SOURCE PROMOTING STIMULATION OF PROBIOTIC BACTERIA

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Zea (Triticum dicoccum), an ancient cereal, produces underutilized bran rich in bioactive polysaccharides. This study aimed to extract and characterize arabinoxylans (AX) from defatted zea bran and assess their prebiotic potential. AX are valued in food applications for their water-holding, emulsifying, and prebiotic properties, supporting their use in health-oriented, clean-label products (Hernández-Pinto et al., 2024). Zea bran, rich in AX and nutrients, is a promising functional ingredient source (Dhanavath & Prasada Rao, 2017). Dried bran samples were sequentially defatted with hexane and enzymatically treated with amylase and protease to remove starch and proteins. A comprehensive physicochemical and nutritional analysis of the raw and lyophilized bran was conducted, including macronutrient content, sugar composition, amino acid profile, ash, and moisture. AX were extracted using alkaline treatment (0.57 M NaOH, 4 h, 60 °C, 1:17 g/mL), followed by ethanol precipitation. Three AX-containing samples yielded: one was used directly without further processing, while the other two underwent additional purification via dialysis (12–14 kDa cut-off), with one being freeze-dried and the other oven-dried. All three AX samples were evaluated for yield and molecular weight distribution by size exclusion chromatography (SEC). Monosaccharide composition and arabinose-to-xylose (A/X) ratio were determined by standardized methods (NREL). In vitro prebiotic potential was assessed by monitoring the growth of selected probiotic strains in media containing AX-samples as the sole carbon source. Additionally, microbial metabolic activity was evaluated by quantifying organic acid production via HPLC after 4, 8 and 24 h of fermentation. Alkali and enzyme mediated extraction led to AX recovery that reached a 6%wt yield based on the initial DSDP biomass, correspronding to 13.7% hemicellulose recovery. SEC revealed Mw values of 28.24 kDa (crude), 15.10 kDa (freeze-dried), and 59.64 kDa (oven-dried). The lower Mw after dialysis-freeze drying reflects efficient removal of low-Mw impurities, while the higher Mw in the oven-dried sample likely results from aggregation during drying (40 °C, 20 h). In vitro fermentation assays showed that probiotic growth of Lactobacillus reuteri, L. plantarum and L. delbrueckii was stimulated by AX samples. Bacterial proliferation was accompanied by increased organic acid production, indicating efficient fermentation and metabolic activity on AX substrates.

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FOOD & NUTRITION

PP099

NOVEL BIODEGRADABLE, ANTIMICROBIAL AND SMART PACKAGING AND COATINGS FOR INCREASED SHELF-LIFE OF MEDITERRANEAN FISH FILETS (NOVISHPAK PROJECT)

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In this poster an outline of the NOVISHPAK project, which is funded by the PRIMA 2023 call is presented. The main objective of the project is to develop innovative biodegradable, antimicrobial and smart packaging films and edible coatings based on brown seaweed polysaccharides used for the extension of shelf-life of Mediterranean fish fillets. A novel post-harvest bioprocesses will be used to enhance the content and physicochemical characteristics of the algal polysaccharides. The materials will be, then, enhanced with natural parabiotic compounds originating from probiotic microorganisms as well as various antimicrobial agents from fish fillet production waste. The materials will also be incorporated with scavengers and colour-changing dyes that respond to ammonia and pH levels to provide an extra layer of monitoring fish quality and evaluate the chill chain. Further, cutting-edge technologies such as cold atmospheric plasma (CAP) will be employed to enhance material's surface characteristics, mechanical, and thermal properties. The developed novel packaging solutions will be used in Mediterranean fish fillets shelf-life extension that is an extremely perishable product with a lot of loss and waste. The applicability and efficiency of the new film and coating materials along with the use of innovative smart packaging tools that includes ICT, will be tested in real chill chain scenarios by applying field tests.

The ambition of NOVISHPAK is to substantially contribute to the packaging sector by developing novel and sustainable packaging materials that address the limitations of conventional plastics and reduce food waste in the Mediterranean food supply chain. The expected impact of NOVISKPAK will be:

• To demonstrate the efficacy of bio-based materials for packaging to extend the shelf-life and to improve food safety of Mediterranean fish fillets.

• To empower blue-bioeconomy by applying marine feedstock and ingredients (seaweed and fish waste extracts) for the production of packaging materials.

• To introduce new environmentally friendly techniques to reduce food waste

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ONE HEALTH

PP101

COMPARATIVE GENOMIC ANALYSIS OF LISTERIA MONOCYTOGENES STRAINS: IDENTIFICATION OF PATHOGENICITY-RELATED LOCI AND SURVIVAL MECHANISMS IN FOOD ENVIRONMENTS

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Listeria monocytogenes is a major foodborne pathogen of high clinical and epidemiological relevance. This study focuses on the genomic analysis of various L. monocytogenes strains using advanced bioinformatics tools to explore genetic diversity, identify virulence factors, and assess mechanisms of environmental survival and food contamination. The methodology includes genome assembly from next-generation sequencing data, annotation using tools such as Prokka, EggNOG-mapper, and BlastKoala, and further phylogenetic and pangenome analyses to highlight core and accessory genes. MLST and cgMLST analyses, as well as genome alignment through Mauve and Circoletto were performed to evaluate evolutionary relationships and genetic variations among the strains. The results revealed the presence of genes associated with disinfectant resistance, phage-related elements, genomic islands, and mutations impacting virulence. Pangenome analysis uncovered notable variability across strains, likely reflecting distinct ecological adaptations. Furthermore, several loci involved in stress response and host interaction were identified as potential determinants of L. monocytogenes pathogenicity and persistence. This work underscores the value of genomic and computational approaches in unraveling the molecular basis of L. monocytogenes pathogenicity and survival, offering essential insights for outbreak prevention and food safety surveillance.

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CHALLENGES IN CONDUCTING A MULTICENTER CLINICAL STUDY TO UNDERSTAND THE ROLE OF THE RESPIRATORY MICROBIOME IN THE PATHOGENESIS OF SEVERE COMMUNITY-ACQUIRED PNEUMONIA (SCAP).

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Microbiome studies require extensive sample collection to balance the high variability inherent in microbial communities. Often, this translates into the need for multi-spatiotemporal data collection. The success of such studies relies on strict uniformity in the sample and metadata collection as well as detailed protocols for sample transfer and processing. Here we will present the experience from Pro-sCAP, a multicenter clinical study to evaluate, among others, the role of respiratory microbial communities of patients hospitalised with severe Community Acquired Pneumonia in the outcome of the disease. Through a combination of extensive multi-omic techniques with immunological functional profiling, the consortium of clinicians, immunologists, microbiologists and bioinformaticians will deeply profile patients and dissect the mechanisms leading to impaired immunity and susceptibility to secondary bacterial or fungal infections. We will discuss the challenges in the organisation of large consortia, enforcing transparent and traceable procedures for all stages of the sample collection, as well as specific considerations relevant to studies performed on the respiratory microbiome. Finally, we will present the preliminary data on the microbial communities from the upper and lower respiratory tract, as well as the informatics infrastructure for clinical and omic data collection and analysis.

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PP106

CLOSTRIDIUM DIFFICILE IN CARP FARMING: UNDERSTANDING RESERVOIRS AND TRANSMISSION PATHWAYS FOR EFFECTIVE CONTROL

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Clostridiodes difficile (formerly Clostridium difficile) is a Gram-positive, spore-forming, anaerobic, motile bacterium, ubiquitous in nature. It causes serious diarrheal infections in individuals with disturbed gut microbiota. While particularly linked to hospitalized elderly patients after antibiotic therapy, community-acquired infections are increasing. The primary niche for C. difficile multiplication is the gut of humans and animals, but its spores are commonly found in soil and aquatic environments, especially in areas affected by fecal pollution or manuring. C. difficile strains are distributed into more than 900 PCR ribotypes and studies that determine their presence in environmental samples greatly contribute to understanding the spread and identifying potential sources of infection.

Here we aim to isolate C. difficile form chicken manure samples used for water enrichment, and sediment samples collected form a carp fish (Cyprinus carpio) nursery ecosystem located at the aquaculture farm in central Croatia and to determine PCR ribotypes. Samples were collected in May and June 2024 during the carp fish nursery setup at the aquaculture farm as part of the MicrobMonitor research project (KP-2024): chicken manure, lake sediment samples before the addition of chicken manure; and sediment samples one month after enrichment of water with chicken manure. C. difficile was isolated from all samples, yielding total of 37 strains grouped into 8 ribotypes, with 014/020 the most prevalent in both chicken manure and sediment samples. In chicken manure samples the second most present PCR ribotype was the so-called hypervirulent 018, while in the sediment samples SLO218.

Our results indicated high diversity of PCR ribotypes present in carp fish (C. carpio) nursery ecosystem. The next step is genome sequencing of representative isolates to fully understand the ecology and dynamics of transmission and spread of this pathogenic bacterium in carp fish nursery ecosystem at the aquaculture farm. Understanding the C. difficile in carp farming ecosystem can improve fish health, welfare, and system sustainability by fostering better breeding and rearing practices.

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GENOMIC ANALYSIS OF MULTIDRUG-RESISTANT, BIOFILM-FORMING STAPHYLOCOCCUS HAEMOLYTICUS ISOLATED FROM BOVINE MILK

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Introduction: Milk is an excellent growth medium for microorganisms due to its nutritive composition. Microorganisms have been implicated in bovine mastitis (BM) in dairy cows as well as causing infections in animals and humans. Despite extensive endeavours to manage BM, this condition continues to persist on a global scale. Non-aureus staphylococci (NAS) species such as Staphylococcus haemolyticus are currently the predominant microbiological agents identified as the main cause of subclinical udder infections and are also considered opportunistic pathogens in cases of clinical mastitis in dairy cows.

Objective: The study was designed to characterise three phenotypically determined multidrug-resistant NAS environmental strains (NWU MKU1, NWU MKU2, and NWU MKS3) obtained from dairy cows.

Method: Whole-genome sequencing was explored to unravel the genomic profiles of the strains.

Findings: The results confirmed that the three isolates were S. haemolyticus with genome sizes of 2.44, 2.56, and 2.56 Mb and a G+C content of 32.8% each. The genomes contained an array of antibiotic resistance genes that may potentially confer resistance to a range of antibiotic classes, such as macrolides, fluoroquinolones, and aminoglycosides. Furthermore, all the genomes carried virulence genes, which are responsible for several functions, such as adhesion, enzyme and toxin production. The genomes of these organisms contained signatures encoding mobile genetic elements such as prophages and insertion sequences.

Conclusion: These findings indicate there is a need for diligent monitoring with improved management practices and quality control strategies on farms to safeguard milk production systems and human health.

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PREVALENCE OF SALMONELLA IN FRUITS AND VEGETABLES PRODUCED IN EUROPE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Salmonella spp. are major foodborne pathogens, responsible for millions of illnesses and thousands of deaths annually worldwide. Recently, concern has been raised regarding fresh fruits and vegetables safety as they are mainly consumed raw or minimally processed, meaning that Salmonella, if present, may not be sufficiently eliminated during handlings and cause food poisoning. The aim of this study was to evaluate and map the current prevalence of Salmonella in fresh fruits and vegetables across Europe and test for any seasonal and spatial trend of Salmonella occurrence.

A systematic review and meta-analysis were performed according to PRISMA guidelines. The search was conducted in Web of Science, Pub Med, and Scopus between January 2014 to December 2024, involving only surveys in European countries, divided into four biogeographical regions: Atlantic, Continental, Mediterranean, and Boreal. Inclusion and exclusion criteria were used for the selection of studies incorporated in review and meta-analysis. The data retrieved were then transported to R for further processing where random-effects model was selected for the estimation of overall prevalence, using "metafor" package in R, while heterogeneity among studies was measured with I2.

The combination of studies involved in this survey indicated a low overall prevalence of Salmonella spp. in fresh fruits and vegetables across Europe. A variability among different fruits and vegetables was also identified while prevalence followed a seasonal trend. The prevalence of Salmonella exhibited a variable pattern among different countries in certain fruits and vegetables. Finally, by considering the four biogeographical regions as representative of the major climatic zones, the influence of climate can be effectively demonstrated.

Although Salmonella contamination of fresh fruits and vegetables in Europe is generally low, the risk to human health still exists as even a small infectious dose is capable of causing illness. Thus, constant monitoring is essential for detecting Salmonella, preventing the distribution of contaminated products to the consumer. In future research and analyses of this kind, the impact of climate should not be underestimated.

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CREATING AN INNOVATIVE DIGITAL PLATFORM THAT COMBINES CLIMATE MODELS AND FOOD SAFETY DATA TO SUPPORT RISK MANAGEMENT IN RESPONSE TO CLIMATE CHANGE, USING A MULTI-STAKEHOLDER APPROACH

Anastasia Kapetanakou[1], Chrysoula Tassou[1], Anthoula Argyri[1], Olga Papadopoulou[1], Agapi Doulgeraki[2], Leonardos Stathas[2], George Papadopoulos[3], Spyros Fountas[3], Fady Mohareb[4], Christopher Brewster[5]

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The AMBROSIA project, titled "Modelling and Communicating Climate Impacts on Food Safety through a Food System Digital Platform," aims to introduce a comprehensive and systemic approach to food safety risk assessment across Europe's supply chain in the context of climate change. By leveraging digital technologies, including AI, the project will unfold in three key phases: (i) Developing a holistic risk assessment framework tailored to analyze the impact of climate change on specific food safety hazards, such as Fusarium mycotoxins in grains and enteric pathogens like Salmonella spp. and E. coli in fresh produce. (ii) Integrating spatio-temporal climate model projections with models related to these food safety hazards across Farm-to-Fork, enabling the creation of precise predictive models for different European biogeographical regions. (iii) Building the AMBROSIA digital platform, an innovative tool designed to integrate climate models with food safety data. This platform will provide a user-friendly environment for diverse stakeholders along the Farm-to-Fork chain, facilitating hazard monitoring and supporting mitigation and adaptation strategies.

Acknowledging the vital role of stakeholders in the food value chain, the AMBROSIA project, will adopt a multi-actor approach to establish ecosystems across five Demonstration Regions (DRs) in Europe: Atlantic, Boreal, Continental, and both East and West Mediterranean. Dedicated beneficiaries, known as Demonstration Managers (DMs), will be responsible for ecosystem development to bring together key food system actors, including representatives from the primary sector, the food industry, policymakers, technicians, and food advisors, ensuring a diverse range of expertise and perspectives. The objectives of these five ecosystems are: (i) Identifying key challenges and requirements for the AMBROSIA Platform through co-creative workshops and surveys. (ii) Contributing to the evaluation of the digital platform and its tools via interactive workshops and surveys. (iii) Sharing knowledge and best practices through interviews, covering various stages of food production. (iv) Providing critical feedback on the final version of the AMBROSIA Platform through workshops and surveys.

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MICROBIAL COMMUNITIES ASSOCIATED WITH THE SPONGE CHONDRILLA NUCULA UNDER CURRENT AND EXPECTED CLIMATE CHANGE CONDITIONS IN THE AEGEAN SEA

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Climate change is an ongoing driver of transformation in the Mediterranean Sea, with projections indicating accelerated impacts in the coming decades. These changes are expected to induce structural and functional shifts in marine ecosystems. Microbes living in association with marine animals or plants often exhibit greater genomic plasticity and faster adaptive responses than their hosts, suggesting that symbiotic communities could play a critical role in host survival under future climate conditions.

In this context, we investigated the microbial community associated with the sponge C. nucula, a sessile marine invertebrate likely to be affected by both the increasing frequency of marine heatwaves and ocean acidification—two major stressors under climate change. Despite its ecological relevance, limited information is currently available on the microbial symbionts of C. nucula, particularly concerning eukaryotic taxa such as Fungi.

Our experimental approach involved: (i) replicate sampling of two geographically isolated populations from the Aegean Sea; (ii) exposure of individuals to a common-garden experiment simulating two climate change scenarios based on IPCC projections, and (iii) targeted next-generation sequencing of microbial gene markers following a 3-month experimental period. To assess shifts in the bacterial community in response to combined temperature and acidification changes, we performed Illumina sequencing of the V3-V4 region of the 16S rRNA gene and bioinformatic identification of molecular operational taxonomic units using a custom pipeline. For the fungal symbionts, we opted for long-read sequencing of a ribosomal locus fragment harboring 3 rRNA genes (18S, ITS, 28S) using Oxford Nanopore MinION technology. This novel approach, once validated, offers enhanced taxonomic resolution essential for fungal identification.

Here, we present the key microbial associates of C. nucula and analyze their community dynamics in relation to climate-induced stressors. Given the limited mobility of the sponge, understanding its microbial flexibility is crucial for predicting holobiont resilience.

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OCCURRENCE OF MYCOTOXINS IN PLANT-BASED MEAT PRODUCTS AND ASSESSMENT OF THEIR MICROBIOLOGICAL QUALITY

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Despite the increased demand for meat alternatives, limited data exist on their quality and safety. This study investigated mycotoxin contamination and shelf life in these products.

A total of seventy (70) samples—either fresh or frozen/thawed—were analyzed, including products based on legumes, wheat, and soy. Initial microbiological analyses were conducted on all samples to determine total aerobic counts (TAC), Pseudomonas spp., Enterobacteriaceae, yeasts and molds, lactic acid bacteria (LAB), and bacteria of the Bacillus cereus group. Additionally, 25 samples were stored for seven days to assess the progression of microbial populations over time. The presence of 16 different mycotoxins was also investigated using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The targeted mycotoxins included aflatoxins (AFB1, AFB2, AFG1, AFG2), fumonisins B1 and B2 (FB1, FB2), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), ochratoxin A (OTA), zearalenone (ZEN), T-2/HT-2 toxin, deoxynivalenol (DON), enniatin B (ENNB), and beauvericin (BEA).

The initial aerobic populations varied significantly among samples, ranging from 1.0 to 6.8 log CFU/g. Among the 70 samples analyzed, 20 exhibited initial total aerobic counts (TAC) greater than 5.0 log CFU/g. Populations of Pseudomonas spp. and Bacillus cereus group bacteria ranged from 1.0 to 5.0 log CFU/g. Additionally, in 18 of the 70 samples, lactic acid bacteria (LAB) were identified as the dominant microbial group. All samples contained at least one of the investigated mycotoxins, with frequent co-occurrence observed. In legume-based meat alternatives, a high incidence of beauvericin and enniatin B was observed, with occurrence rates of 94.7% and 78.9%, respectively. In cereal-based meat alternatives, in addition to beauvericin and enniatins, fumonisin B1, fumonisin B2, and the Alternaria toxins tentoxin and alternariol monomethyl ether were frequently detected, with occurrence ranging from 67.7% to 100%.

As mycotoxins presence is expected to increase due to climate change, this study highlights the need to include plant-based meat alternatives in risk assessments to reflect potential consumer health risks.

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MICROBIAL RESPONSES TO CLIMATE-DRIVEN WATERLOGGING STRESS: IMPLICATIONS FOR PLANT-MICROBE INTERACTIONS AND RESILIENCE IN WHITE CABBAGE

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The occurrence of unfavorable environmental conditions, such as extreme temperatures and irregular water supply, has become more intense and unpredictable due to climate change, hindering plant growth and survival. Although seasonal flooding is a common phenomenon in certain ecosystems - and can positively contribute to biodiversity and productivity by replenishing soil nutrients in floodplains - temporary and, more importantly, prolonged flooding stress, exacerbated by climate change, can have significant negative impacts on plant development and viability.

The rhizosphere, the narrow zone of soil surrounding and influenced by plant roots, along with the nearby bulk soil, serves as a natural habitat for numerous beneficial microorganisms and is considered one of the most biologically complex ecosystems on Earth. This biologically active zone is crucial for fostering positive interactions between plants and associated microbes, driving essential nutrient cycling, enhancing plant growth, and increasing resistance to various abiotic and biotic stresses. Such beneficial interactions are now a critical focus of research, particularly in the context of climate change.

To investigate the potential of plant growth-promoting microorganisms (PGPB and PGPF) in mitigating climate-induced stress, we designed a study within the PERSPIRE project. The aim was to determine the effects of long-term waterlogging (lasting seven days) on structural changes and beneficial traits of bacterial and fungal isolates. Microbial samples were collected during a five-week experiment using white cabbage (Brassica oleracea) as a model plant, known for its tolerance to abiotic stressors. Additionally, amplicon sequencing was employed to track changes in the overall microbial community. The experiment featured two waterlogging scenarios: (i) in Model A, both plants and soil were subjected to two waterlogging events, and (ii) in Model B, plants and soil experienced a single waterlogging event occurring at a later stage of plant growth. The results demonstrated that waterlogging leads to significant alterations in the soil microbial community, affecting both its structural composition and the PGP functions provided by beneficial bacteria and fungi. Based on the results bacterial and fungal isolates were designating as the most promising bioinoculants i.e. strains with good potential for future applications in potential waterstress recover of the soil and plants.

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SAMPLE MATCHER: A METHOD FOR COMPARING AND CLUSTERING METAGENOMIC SAMPLES BY TAXONOMIC AND FUNCTIONAL PROFILES

Nefeli Kleopatra Venetsianou[1], Konstantinos Kalaentzis[1], Alexios Loukas[1], Christina Damianou[1], Savvas Paragamian[2,1], Vincenzo Lagani[3,4], Lars Juhl Jensen[5,6], Evangelos Pafilis[1]

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Metagenomic studies are increasingly prevalent, progressing the exploration of microorganisms in diverse environments. Each sample of these studies reveals the taxonomic composition and biochemical potential of microbial communities in a specific setting, thus leveraging the growing amount of environmental genomic data. Integrative research can combine information across studies and samples, utilizing this vast dataset. The latter requires effective large-scale methods to compare and cluster cross-metagenomic study samples. To address this, the Sample Matcher algorithm has been developed to compare and cluster metagenomic samples using their taxonomic and functional profiles. Leveraging MGnify data, it constructs a global reference space that enables sample-to-sample comparison through similarity metrics, such as euclidean and cosine distances, chosen for their robustness. Dimensionality reduction techniques enhance the analysis by improving clustering accuracy. The taxonomic profile workflow begins by organizing MGnify data into abundance tables, filtered by taxonomic depth and normalized by relative abundance. Similarity metrics are then used to compare samples, followed by clustering to group similar samples. The functional profile workflow explores three strategies based on different profile types: (1) GO Slim abundances reported by MGnify, (2) an algorithmically-defined set based on the term frequency within a study and across all studies ("CCMRI GO Subset"), and (3) dimensionality-reduced representations. Within the CCMRI framework, Sample Matcher may identify climate-change-related studies and samples based on taxonomic and functional data, rather than relying solely on text-based queries. Nevertheless, the algorithm is flexible and adjustable for a wide range of comparative metagenomic analyses. For instance, a marine sample from a North Sea plastic incubation study (MGYS00004601) was compared against the MGnify database using its taxonomic profile. Excluding samples from the same study, the algorithm retrieved the 20 most similar samples. Notably, the top matches included oil-contaminated ocean sites and cold seep environments, suggesting microbial convergence in hydrocarbon-rich or low-oxygen marine conditions. Additionally, a few similar freshwater pond sediment samples were identified, indicating either shared microbial taxa or limits in biome-based separation. This use case highlights the adaptability of Sample Matcher and its ability to uncover biologically meaningful connections across diverse and unrelated metagenomic studies.

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CCMRI: A CLASSIFICATION AND CURATED DATABASE OF CLIMATE CHANGE-RELATED METAGENOMIC STUDIES

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Climate change (CC) is transforming the functions and structures of ecosystems. Metagenomic data can offer insight into understanding how microbial communities both influence and respond to these climatic changes. Yet, identifying metagenomic studies specifically linked to climate change remains challenging due to the overwhelming volume of environmental genomic data available. The Climate Change Metagenomic Record Index (CCMRI) presents metagenomic records related to CC in the form of a curated database. The database includes studies exploring how climate change alters microbiomes, or how microbial processes impact climate change (e.g., by accelerating or mitigating its effects). For the population of the database, the aquatic and terrestrial studies in Mgnify were manually curated. Subsequently, all other MGnify studies were automatically processed. Those shortlisted as CC-related ones were forwarded for a last manual check before being included in the database. The pertinent CCMRI pipeline first retrieves study textual metadata (like title, description, PubMed abstracts) and then detects textual clues related to climate change. To this end, several classification methods have been tested, including machine learning (logistic regression, XGBoost) and a rule-based system (both supported by text-mining Named Entity Recognition modules). Large language models (LLMs) have also been explored. For the manual inspection step, it is key that the classification process does not miss CC-related studies. Thus, high recall was the primary criterion for the classification method selection. LLMs outperformed other approaches, yielding a recall of over 90% with moderate precision (35%). By adopting this approach, future curation efforts in CCMRI remain scalable and long-term sustainable. For the evaluation, beyond populating the database, a manually curated corpus based on the aquatic and terrestrial MGnify metagenomic studies was created, split into a training (60%) and an evaluation (40%) part. Finally, the CCMRI web platform, in addition to the curation module, provides access to the database contents and offers email alerts when new CC-related studies become available.

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DISTRIBUTION, POPULATION MONITORING, AND EVALUATION OF CULTIVATION PARAMETERS OF GREEK WILD STRAINS OF THE ENDANGERED EDIBLE MUSHROOM SPECIES PLEUROTUS NEBRODENSIS

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Pleurotus nebrodensis (Basidiomycota, Pleurotaceae) is a rare saprotrophic species that grows exclusively on the roots and stems of Prangos ferulacea (Apiaceae), in arid pastures and calcareous soils at altitudes ranging from 1200 to 2000 m a.s.l. It was originally described from Sicily, but recently its presence was also confirmed in Greece [1]. It is the first mushroom species recognized as Critically Endangered by the International Union for Conservation of Nature (IUCN), while later it was re-evaluated as Endangered following its discovery in Greece [2].

In the present study, known distribution areas of P. ferulacea in central and southern Greece were surveyed and mapped. A quantitative assessment of the existing subpopulations of P. nebrodensis was conducted, and pure cultures were established from mushrooms collected from all identified localities. The species presence was confirmed in most of the host plant's distribution areas. However, in several locations overharvesting for personal consumption and/or for trade purposes takes place; consequently, only a few basidiomata reach maturity every year.

Given the conservation concerns, successful cultivation of this species is considered crucial for reducing harvesting pressure on wild populations. When wild strains were evaluated under laboratory conditions, results revealed very low mushroom productivity, most likely due to genetic degeneration resulting from long-term isolation of small/fragmented populations. Therefore, the development of new hybrids through controlled matings of selected homokaryons appears to be a promising strategy for establishing a viable commercial cultivation protocol for this species.

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THE 'FUNDIVE' PROJECT – MONITORING AND MAPPING FUNGAL DIVERSITY FOR NATURE CONSERVATION THROUGH THE ACTIVE INVOLVEMENT OF CITIZEN SCIENTISTS

Elias Polemis[1], Vassileios Daskalopoulos[1], Savvas Christodoulou[1], Georgios Konstantinidis[2], Dimitrios Sofronis[2], Vasileios Kaounas[2], Linos Kottis[2], Georgios Koutrotsios[1], **Georgios Zervakis[1]**

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FunDive is an ambitious and well-organized initiative that brings together 33 partners from 22 European countries for increasing knowledge on the distribution of fungi, for monitoring and characterizing fungal communities across the continent by DNA metabarcoding, for enhancing fungal conservation through the help of citizen scientists, and for exploring fungal diversity by combining synchronized sampling and DNA sequencing. Among the first year's activities, specific groups of mushrooms were selected for pan-European sampling campaigns, e.g. the genera Geastrum and Tulostoma, 29 rare and/or poorly known epigeous gasteroid taxa, members of the genus Paxillus, and pine forest ectomycorrhizal fungi (notably Cortinarius s.l., the genus Tricholoma, and stipitate thelephoroid, hydnoid or poroid species). Three "discovery missions" were co-organized by the Agricultural University of Athens (AUA) and the Greek Mushroom Society, in Grevena, Euboea and Attica, with big success and the participation of numerous citizen scientists and mushroom enthusiasts. More than 200 specimens were collected, photographed and uploaded in the application "PlutoF GO", and then processed for further examination and ITS barcoding in AUA's facilities. The outcome so far revealed six species that constitute new records for the Greek mycobiota, namely Globaria (=Bovista) promontorii and Bovista furfuracea (Attica, in Pinus halepensis), Phellodon melilotinus and Tricholoma viridilutescens (Grevena, in Pinus nigra subsp. pallasiana), as well as Paxillus cuprinus (Mt. Pelion, in Castanea sativa). As regards the genus Inocybe, which will be among the next season's priority groups, five recently described Mediterranean species are first records for Greece, i.e., I. mecoana in Euboea, and I. dagamae, I. kusadasiensis, I. pinophila and I. tarda in Attica (all in P. halepensis forests). Moreover, phylogenetic results indicated that at least two Tulostoma collections might represent a new species to science.

Acknowledgements
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THE DIVERSITY OF AGARICUS SECTION AGARICUS IN GREECE – FIRST NATIONAL RECORDS AND NOVEL TAXA

Savvas Christodoulou[1], Elias Polemis[1], Georgios Konstantinidis[2], Vassiliki Fryssouli[1], Georgios I. Zervakis[1]

[1]Laboratory of General and Agricultural Microbiology, Department of Crop Science, Agricultural University of Athens, [2]Greek Mushroom Society

The genus Agaricus (Basidiomycota, Agaricales) is a saprotrophic group comprising approximately 500 species with a cosmopolitan distribution, including economically significant taxa such as A. bisporus, which is among the most widely cultivated mushrooms. In Europe, over 100 taxa have been described that are assigned in five subgenera and 14 sections; in particular, the monophyletic Agaricus sect. Agaricus includes more than 20 taxa [2,3]. The present work is a follow-up of the first detailed investigation on the diversity of the genus Agaricus in Greece [4]. Eighty-eight specimens of A. sect. Agaricus, deriving from dried material (deposited at fungarium ACAM) and recent field collections, were studied using both morphological and ecological features in conjunction with the outcome of phylogenetic analysis based on three markers (ITS, 28S, TEF1a). Samples derived from a variety of habitats (e.g. grasslands to forests), and from sea-level to elevations exceeding 2,000 m a.s.l. Examined basidiomata varied in size and color (from white to brown), with flesh that remained white or turned pinkish to reddish upon cutting. Results revealed the presence of 11 known taxa, i.e., A. argenteus subsp. annetteae, A. campestris, A. cupreobrunneus, A. langei, A. moellerianus, as well as A. fabrianensis, A. occidualis, A. porphyrocephallus subsp. pallidus, A. porphyrocephallus subsp. porphyrocephallus, A. quercetorum, and A. sanctuarii; the last six represent first records for Greece. In addition, the existence of novel phylogenetic taxa was detected, highlighting the considerable species richness of the genus Agaricus in Greece.

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RECOVERY OF SOIL FUNCTIONS AND FERTILITY IN A POST-QUARRY REHABILITATION CHRONOSEQUENCE IN MILOS ISLAND (GREECE)

Georgios Leventis[1], Myrto Tsiknia[1], Antonios Apostolakis[2], Dimitra Stathopoulou[1], Cleopatra Garezou[3], Georgios Petrakis[3], Constantinos Ehaliotis[1]

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Quarrying leaves behind degraded soils with characteristics that inhibit plant establishment and hinder ecological restoration. These limitations are further intensified by the strong seasonal climatic variations of the Mediterranean basin. Reintroducing native plant species is a promising strategy, as they possess traits shaped by long-term natural selection that support their establishment and persistence in local conditions. However, the dynamics of soil functions and properties during restoration remains elusive, especially whether restoration initiated from quarry deposits can ultimately lead to a state comparable to that of the natural ecosystem.

This study examined rhizospheric soil from three native phryganic species—Calicotome villosa, Anthyllis hermanniae, and Sarcopoterium spinosum—growing in new (>15 years) and old (>30 years) restoration sites, undisturbed natural areas and bare quarry deposits in Milos Island, Greece. We assessed soil organic carbon, nutrient availability, and enzymatic activities related to carbon, nitrogen, sulfur, and phosphorus cycling to evaluate the influence of restoration age, season and plant taxon on soil properties and functions.

Overall, the results demonstrate a clear restoration gradient, with soil properties and functions improving significantly from quarry deposits to natural ecosystems. Natural sites consistently exhibited the highest SOM and nutrient values, followed by old and new restoration sites, while bare quarry deposits showed the lowest. Alkaline phosphatase and β-galactosidase activities exhibited the fastest recovery along the restoration gradient, with activity levels in older restoration sites approaching those of natural ecosystems. Type II MANOVA reveals that within each restoration stage, both season and plant taxon significantly influenced soil properties, with their effects increasing from the new restoration to the natural site. In contrast, their effect was weaker on soil enzymes than on soil properties, likely due to the transient nature of enzymatic activity compared to the longer-term accumulation of soil nutrients. Further analysis of SOC fractions (POM and MAOM) will reveal whether, and at what rate, quarry restoration promotes the stabilization of carbon in persistent pools.

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ROOT-ASSOCIATED FUNGAL AND BACTERIAL COMMUNITIES IN DIFFERENT OAK FOREST STANDS

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[1]*Slovenian Forestry Institute*

Soil fungal and bacterial species represent a fundamental component of the biotic community in natural oak forests. They play a crucial role in ecosystem functioning by facilitating nutrient cycling, enhancing water uptake in plants, improving soil fertility, and contributing to plant defense mechanisms. Additionally, soil bacteria support the establishment of mycorrhizal symbiosis. Functional compatibility between ectomycorrhizal fungal species and strains, and host plant species and populations, is also of key importance. The presence and abundance of ectomycorrhizal species are critical belowground attributes associated with fine root density and seedling establishment in deciduous and semi-deciduous oak forests. Soil bacterial and fungal communities in these forests are shaped by multiple biotic and abiotic factors, including plant species composition and soil properties, highlighting the importance of identifying locally adapted and species-specific soil microbiomes.

The aim of this study was to identify the species-specific local microbiomes associated with oak trees (Quercus spp.). Soil samples were collected from Q. robur and Q. petraea forest stands across Slovenia to analyze differences in root-associated microbial communities. High-throughput Illumina sequencing was used to characterize the total fungal and bacterial communities associated with oak roots.

Community composition analyses revealed a clear clustering of both fungal and bacterial communities according to oak species, distinguishing between Q. robur and Q. petraea. Despite variations in geographic location and soil properties, the microbial communities within individual forest stands showed similar overall compositions. However, some differences in the relative abundances of specific fungal and bacterial taxa were observed between individual Q. robur and Q. petraea forest stands, suggesting on the importance of local biotic and abiotic factors in shaping the root-associated microbial community.

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FUNCTIONAL DISSECTION OF THE RNAI MACHINERY IN A PLANT-ASSOCIATED BENEFICIAL FUNGUS

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Endophytic filamentous fungi play a pivotal role in agriculture, affecting in various ways the plants they colonize. Fusarium solani strain K (FsK) is a root endophyte, known to colonize diverse plant species and exert beneficial effects. While the mechanisms underlying these interactions remain unclear, translocation of RNAi signals to its host plants has been recently reported and could be associated with its beneficial properties. To investigate this cross-kingdom RNAi-mediated communication between FsK and its host plants, we generated a targeted mutant of the Dicer-like 2 (DCL2) gene, a key component of the fungal RNAi machinery involved in small interfering RNA (siRNA) biogenesis. Using GoldenBraid cloning technology and Agrobacterium tumefaciens-mediated transformation, we engineered FsK mutants through homologous recombination. Growth assays confirmed that hygromycin-resistant mutants exhibited similar growth rates to the wild-type FsK strain.

To evaluate the role of the DCL-2 mutation on the phenotype of improved tolerance against drought stress, exhibited by FsK-colonized plants, a comparative assay was performed using mutant and wild-type strains. This workflow supports the functional analysis of FsK genes related to RNAi-mediated communication, facilitating the unraveling of RNAi-mediated plant-microbe interactions.

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EFFECT OF A BENEFICIAL FUNGAL ENDOPHYTE ON THE EXPRESSION OF STRESS-RELATED GENES IN TOMATO PLANTS GROWN UNDER REDUCED IRRIGATION.

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Fusarium solani strain K (FsK) is a non-pathogenic endophytic fungus, previously isolated from the roots of tomato plants, that confers resistance to biotic and abiotic stressors (Kavroulakis et al., 2007; Kavroulakis et al., 2018). FsK also colonizes the root system of Nicotiana benthamiana promoting plant growth (Dalakouras et al., 2023), as well as the whole body of Lotus japonicus, increasing iron uptake under low nutrient conditions (Avramidou et al., 2024). The goal of the current study is to investigate the effect of the fungal inoculum on the expression of stress-related genes in tomato plants grown under reduced water availability. Tomato seedlings at the cotyledon stage were inoculated with 105 conidia of FsK and reduced water availability started 12 days after inoculation. Sampling was performed after 4 and 8 days of reduced irrigation. The effect of drought was assessed both morphologically, as well as molecularly with RT-qPCR using drought-stress marker genes. Fungal root colonization was assessed with qPCR. Finally, the expression of stress-related genes of interest were studied with RT-qPCR at both sampling points. Our results indicate that inoculating plants with FsK, aids in lowering the expression of stress-related genes under reduce irrigation conditions. This study helps to better understand how FsK produces its positive effects in its hosts.

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PP123

INVESTIGATING THE ROLE OF LOTUS JAPONICUS B-AMYRIN SYNTHASE IN NODULATION

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The model legume Lotus japonicus has been explored for its ability to participate in beneficial symbiotic relationships with soil microorganisms and synthesise a plethora of specialised metabolites. In particular, the plant-rhizobia interaction is responsible for the development of root nodules and the symbiotic nitrogen fixation, supplying the plant with valuable nitrogen (N) in N-poor environments (Handberg & Stougaard, 1992). The establishment of the symbiotic interaction and the different stages of nodulation are tightly regulated by many plant mechanisms, such as the triterpene metabolism (Krokida et al., 2013; Delis et al., 2011). As yet, the mode of action of this class of specialized metabolites has not been well-characterized. In this work, we investigated the role of AMY1, which encodes for a b-amyrin synthase, in nodulation. We studied its transcriptomic response at different stages of the host-rhizobia interaction. Next, we targeted AMY1 using CRISPR/Cas9 in hairy roots via Rhizobium rhizogenes-mediated transformation and assessed the nodulation phenotype between control and amy1 mutants. Finally, we investigated the transcriptomic changes of key-nodulation genes in the same mutants. Taken together, our data suggest that AMY1 modulates different stages of nodulation.

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SYMBIOSIS

PP124

ENHANCING LEGUME RESILIENCE TO ABIOTIC STRESS USING NATIVE RHIZOBIA: IN PLANTA VALIDATION AND GENOMIC INSIGHTS

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Soil salinization and prolonged drought events, intensified by ongoing climate shifts, are jeopardizing the stability and efficiency of symbiotic interactions between legumes and nitrogen-fixing rhizobia. To address these challenges, the use of native beneficial microbes, specifically adapted to local harsh environmental conditions, offers promising potential as effective inoculants for legume crops. The present study focuses on the isolation and characterization of rhizobia (339 strains) obtained from wild legumes in extreme environments across Greece and Cyprus. These isolates were phenotypically and molecularly characterized, and tested in vitro for their tolerance to multiple abiotic stresses, with a focus on salinity, elevated temperature and drought resilience. The most tolerant rhizobial strains (able to grow at 600 mM NaCl, 42 °C, and 10% PEG) were further evaluated for their ability to establish effective symbioses with Trifolium resupinatum and Medicago sativa under both normal and stress conditions. Specifically, plants inoculated with either stress-tolerant or stress-sensitive strains were subjected to three irrigation regimes: (i) standard nutrient solution, (ii) nutrient solution supplemented with 150 mM NaCl to simulate salinity stress, and (iii) nutrient solution containing 20% polyethylene glycol (PEG) to mimic drought-induced osmotic stress. Control and stressed plants were harvested once phenotypic differences became evident and evaluated for symbiotic performances. Biometric and physiological parameters, including biomass accumulation, root system architecture, and nodule functionality, were measured to assess plant fitness and adaptive responses under the induced stress conditions. The most valuable strains capable of efficiently nodulating Trifolium resupinatum and Medicago sativa plants and to promote plant growth under salinity and drought conditions, were selected for further co-inoculation experiments combining rhizobia and AMF to explore synergistic effects on plant growth and resilience. Whole genome sequencing (WGS) performed on both the most tolerant and sensitive isolates was used to identify molecular markers associated with stress tolerance. Ultimately, integrative and comparative analyses will highlight the potential of selected microbes to enhance legume performance under adverse environmental conditions leading to the development of an eco-friendly solution that supports sustainable pasture legume cultivation in the face of the developing environmental crisis.

Keywords: Legume–microbe symbiosis, beneficial microbes, rhizobia, abiotic stress, climate-resilient agriculture

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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SUPERNATANTS FROM SUBMERGED CULTURES OF BASIDIOMYCETES AGAINST FOODBORNE PATHOGENS

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The main spoilage mechanisms affecting a wide range of food products during preparation, storage and distribution typically involve microbial growth, affecting both the organoleptic characteristics and nutritional quality of the food [1]. Synthetic antioxidants have been widely used to prevent food spoilage; however, they have been associated with potential health risks, making it necessary to seek safer, more sustainable alternatives [2]. Many mushroom fungi (Basidiomycota), in addition to their high nutritional value, possess a wide range of beneficial effects on human health, including antioxidant and antimicrobial activities. Moreover, such compounds have been suggested as natural preservatives in various food products [3,4]. Therefore, this work aims to explore whether basidiomycetes could serve as sources of natural antibacterial substances, which can offer an alternative approach to the prevention of food spoilage. To unveil promising species/strains, we have evaluated the antimicrobial potential of supernatants from submerged cultures of 52 basidiomycetes originating mainly from Greek habitats. Supernatants were collected and evaluated for their ability to inhibit and/or prevent the growth of specific food spoilage bacteria, including Bacillus subtilis, Brochothrix thermosphacta, Escherichia coli, and Pseudomonas spp., using the agar well diffusion method. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Our results showed that supernatants from Fomitopsis pinicola, Laetiporus sulfureus, Trametes hirsuta, and Lentinula edodes were able to inhibit the growth of all eight bacterial strains tested, including Gram-negative bacteria, which are generally considered more resistant (e.g. Pseudomonas fluorescens). This work highlights the significant potential of basidiomycetes as sources of natural bioactive agents that could serve as alternatives to chemical preservatives in the food industry.

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SINGLE-CELL ANALYSIS OF SALMONELLA ENTERICA REVEALS HETEROGENEOUS RESPONSES TO ANTIBIOTICS AND FOOD-RELATED STRESSES

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Antibiotic resistance is an escalating global concern, significantly impacting both public health and food safety. A major driver of this issue is the extensive use—and often misuse—of antibiotics in medical treatment and food production. Resistant bacteria originating from food-producing animals can enter the human food chain through contaminated meat, produce, or water. Traditional methods for determining the Minimum Inhibitory Concentration (MIC) of antibiotics typically rely on assessing large bacterial populations. These methods overlook phenotypic variability at the single-cell level, despite evidence that even genetically identical bacteria can respond differently to antibiotics. Such heterogeneity leads to variable MIC values and challenges the assumption of a fixed threshold for bacterial inhibition. Additionally, antibiotics can act as environmental stressors, potentially triggering adaptive resistance mechanisms that enhance tolerance to subsequent food-related stresses. This study focuses on the behavior of Salmonella enterica at the single-cell level following exposure to sub-lethal concentrations of antibiotics. To explore the interplay between antibiotic adaptation and tolerance to food-relevant stresses, specific genes potentially involved in this relationship were identified, and functional reporter strains were constructed to monitor gene expression. A direct time-lapse microscopy method was developed using viability stains, allowing real-time observation of individual cell behavior. Cells were first exposed to antibiotics and then subjected to acidic stress to simulate food processing conditions. The actual inactivation times of individual cells were recorded and analyzed. Results revealed significant heterogeneity in inactivation times, and these data were fitted to various continuous probability distributions to determine the best fit. Furthermore, key stress-response genes that may confer cross-protection were highlighted based on observed expression patterns. By investigating the post-antibiotic behavior of single cells under food-related stresses, this study contributes to a more accurate understanding of MIC estimation and antimicrobial resistance. These findings offer valuable insights for refining antimicrobial strategies, improving food safety, and addressing the growing threat of resistant bacteria.

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EVALUATION OF ANTIMICROBIAL AND ANTIBIOFILM PROPERTIES OF A NEW SCHIFF BASE COMPOUND Cu (II) COMPLEX

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The increasing prevalence of antimicrobial resistance highlights the urgent need for the discovery and development of novel antimicrobial agents. Schiff base class compounds (imine compounds) are a research topic of high interest, especially in chemistry and health, due to their wide range of applications.

The aim of the study was to investigate the antimicrobial and antibiofilm properties of a new Schiff base Cu(II) complex (SBCu) compound, which was found to be non-toxic against CCD-1072-SK cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method

Antimicrobial activities of SBCu were tested against, Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC 12228, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 4352, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 14153, Candida albicans ATCC 10231, Candida tropicalis ATCC 750, Candida parapsilosis ATCC 22019, and minimum inhibitory concentration (MIC) values were determined. Antiviral activity was tested against Parainfluenza Type-2 virus. Different concentrations of SBCu antibiofilm activities and effects of coating the wells of plate were measured against sensitive microorganisms.

The MIC values of SBCu were found to vary according to the microorganism, with a range of 19.5- >5000 µg/mL. The most effective activity was observed against Candida species and S. epidermidis. It was also found that SBCu inhibited viruses by 55%. It has been demonstrated that the substance was effective against mature biofilms of S. aureus and S. epidermidis, and inhibited the adhesion of S. aureus, S. epidermidis and C. tropicalis after coating the wells even at MIC values.

In conclusion, the findings of this study demonstrate the promising antimicrobial and antibiofilm properties of SBCu and highlight its potential as multifaceted agent. These findings also suggest that SBCu may serve as a promising candidate not only for clinical antimicrobial applications but also for environmental disinfection strategies. Its ability to inhibit biofilm formation and microbial adhesion on surfaces indicates potential utility in reducing biofilm-associated contamination in healthcare and public settings.

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STANDARDIZED ONE HEALTH SURVEILLANCE OF ANTIBIOTIC RESIDUES AND ANTIBIOTIC AND HEAVY METAL RESISTANCE IN BALTIC WATER ENVIRONMENTS AND WILD BIRDS (BALTIC-AMR) - PROJECT INTRODUCTION AND PRELIMINARY RESULTS FROM POLAND

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Considering the serious threat of increasing antimicrobial resistance, the WHO has introduced the concept of "One Health," which recognizes the interconnectedness of human health, animal health and the environment. In the BALTIC-AMR project, we plan to establish a rational surveillance strategy that includes different member states around the Baltic Sea to investigate antimicrobial resistance in the coastal region. The research includes samples of wastewater, seawater, and feces of wild waterfowl analysed for selected antibacterial compounds and heavy metals, and isolation and detailed characterisation of bacteria resistant to these compounds, including Escherichia coli, Klebsiella pneumoniae, Enterobacter spp., Acinetobacter spp. and Pseudomonas spp. Detection of waterborne pathogenic microorganisms (Vibrio and Aeromonas) and molecular analysis of resistance genes will allow the development of standardized and reliable surveillance of the occurrence of resistance to antibacterial compounds in the Baltic countries in the context of One Health.

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PUBLIC HEALTH RISK ASSESSMENT OF THE PRESENCE OF ESKAPE PATHOGENS IN ENVIRONMENTAL SAMPLES

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The growing threat of antimicrobial resistance represents a critical challenge for global public health. This study focuses on the risk assessment posed by the presence of ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter sp.) in environmental samples. These opportunistic pathogens are known for their capacity to resist multiple classes of antibiotics and are responsible for a significant portion of healthcare-associated infections (HAIs). Their increasing detection in environmental matrices raises serious concerns about their potential dissemination from environmental reservoirs to human populations.

Environmental water samples were collected from coastal, freshwater, and wastewater treatment sites in the region of Chania, Crete, between November 2023 and April 2024. Isolation of bacterial strains was achieved using filtration methods, while antimicrobial susceptibility was assessed through Minimum Inhibitory Concentration (MIC) testing against a panel of commonly used antibiotics. Additionally, the efficacy of UVC irradiation was evaluated as a disinfection method to reduce microbial load and potentially deactivate resistant strains.

Results revealed widespread occurrence of the targeted pathogens across all sampling points, with several isolates demonstrating high resistance levels to critical antibiotics such as methicillin, vancomycin, and ciprofloxacin. Notably, both methicillin-resistant S. aureus (MRSA) and carbapenem-resistant K. pneumoniae were detected, underlining the environmental presence of clinically significant multidrug-resistant organisms. Although UVC treatment significantly reduced bacterial counts, complete inactivation was not always achieved, especially in samples with higher turbidity or microbial loads.

The findings confirm the role of aquatic environments as reservoirs and potential transmission routes for antimicrobial resistance. They also highlight the limitations of current wastewater treatment technologies in eliminating resistant bacteria and support the need for integrated monitoring strategies and advanced disinfection approaches.

This study contributes valuable insights into the environmental dimension of antimicrobial resistance and supports the development of targeted public health strategies. The integration of innovative technologies such as UVC irradiation and routine environmental surveillance may help mitigate the dissemination of resistant pathogens, thereby enhancing overall public health preparedness and resilience.

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PP131

A NEW WHOLE GENOME SEQUENCING ANALYSIS TOOL FOR RISK ASSESSMENT OF MICROBIAL PESTICIDES

Stavroula Makri[1], Joao Pedro Saraiva[2], Sotirios Vasileiadis[1], Alexandre Bartholomäus[6], Francisco Cerqueira[4], Valérian Lupo[7], Antonis Chatzinotas[2,9], Kostas Papagiannitsis[3], Brader Gunter[4], Stephan Declerck[5], Luc Cornet[8], Kwnstantina Rousidou[1], Dimitrios Karpouzas[1]

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An innovative risk assessment (RA) approach is required to facilitate the safe, and efficient placement in the market of microbial pesticides including bacteria, fungi, phages, and microbiome-based solutions. Despite microbial pesticides offering significant environmental benefits and alignment with the EU's Green Deal objectives, they currently go through the same stringent regulatory hurdles as synthetic pesticides. This has hindered their uptake in the European market compared to other cases like USA, India, and China. To overcome these limitations, the EU-funded project RATION is developing a tailored RA framework specifically for microbial potential low-risk active substances. One of the main aims of RATION is to incorporate genomics in the current RA process for microbial pesticides to assess key concerns including virulence and pathogenicity, antimicrobial resistance (AMR), gene transferability, and toxic secondary metabolite production. By optimizing regulatory assessments and eliminating redundant testing requirements, the project ensures a scientifically robust yet efficient pathway for microbial pesticide evaluation. Currently WGS analysis for microbial pesticides is performed through an EFSA tool called MOPS which is a closed system, only available to regulatory bodies, and has a lot of limitations. In this frame, RATION has developed an intuitive, fully automated, and openly accessible pipeline that seamlessly integrates state-of-the-art bioinformatics tools across the entire WGS workflow. Designed with scientific robustness, the platform enables comprehensive screening for virulence factors to determine potential pathogenicity, AMR presence and transferability through the detection of mobile genetic elements, and toxic metabolite biosynthesis pathways. On-going efforts will expand capacities to detect biosynthetic pathways for mycotoxins. By bridging cutting-edge science with practical regulatory needs, this tool empowers stakeholders to navigate the risk assessment of microbial pesticides and is intended to accelerate the approval process, support sustainable agriculture, and ultimately promote wider adoption of microbial LRPs across Europe.

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DIRECT COLD ATMOSPHERIC PLASMA TREATMENT REDUCES ESCHERICHIA COLI AND LISTERIA MONOCYTOGENES BIOFILM VIABILITY ON CATHETER SURFACES VIA STRESS RESPONSES

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Urinary tract infections are among the most common infections in both community and healthcare settings. Despite established protocols, catheters remain critical entry points for bacterial colonisation, contributing significantly to nosocomial infections globally. Catheter-associated urinary tract infections are challenging to manage due to persistent biofilms and bacterial stress resilience, which limit antimicrobial efficacy. This study evaluated the effectiveness of direct cold atmospheric plasma treatment in reducing bacterial biofilm viability on catheter-relevant surfaces. To investigate the contribution of microbial stress response pathways to cold plasma, targeted mutants of Escherichia coli K-12 and Listeria monocytogenes EGDe were analysed. Their wild-type and mutants deficient in acid resistance (ΔgadA, ΔgadB, ΔgadX, ΔgadR, ΔgadD1, ΔgadD2, ΔgadD3), general stress regulators (ΔsigB, ΔrpoS), heat shock protein (ΔdnaK), and oxidative stress response (ΔsoxS, ΔoxyR, ΔsoxR, ΔmogR, ΔrsbV, ΔrecA, ΔhrcA) were exposed to cold atmospheric plasma and assessed for their survival responses. The composition of the reactive oxygen and nitrogen species under the optimal cold atmospheric plasma treatment time and power was estimated to be as follows: nitrates at 781 ppm, hydrogen peroxide at 20.8 ppm, and pH at 2.29. Under this condition, E. coli ΔgadA and ΔgadB mutants showed significantly reduced biofilm viability, resulting in 4.5 and 3.5 log CFU/mL, respectively, indicating a major role of acid and oxidative stress pathways in plasma sensitivity. In contrast, mutants ΔdnaK, ΔrpoS, and ΔsoxS exhibited similar reductions to those of the wild-type, i.e., approximately 3.5 log CFU/mL, suggesting limited involvement of oxidative stress and heat shock pathways in biofilm protection against direct plasma treatment. In L. monocytogenes, ΔgadD1 and ΔgadD3 showed enhanced susceptibility, with 3.5 and 2.5 log CFU/mL reductions. Deletions in ΔsigB, ΔdnaK, and ΔmogR didn't significantly affected by the plasma sensitivity. The impact of this process on the morphological properties of cells was demonstrated with microscopic images via Scanning Electron Microscopy. These results demonstrate that direct cold atmospheric plasma treatment effectively reduces bacterial viability on catheter surfaces. Acid and oxidative stress defence systems appear central to cold atmospheric plasma microbial tolerance. This approach may offer a promising alternative to reduce catheter-associated infections, particularly in cases where conventional antimicrobials are ineffective.

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N-CHLOROTAURINE AS A MICROBICIDAL AGENT AGAINST MONO AND MULTISPECIES BIOFILMS OF STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA

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N-chlorotaurine (NCT), the N-chloro derivative of the amino acid taurine, is a long-lived oxidant produced by activated human granulocytes and monocytes. Beyond its immunomodulatory effects, NCT has bactericidal, fungicidal, virucidal and protozoocidal activities. NCT is a mild, long-lived oxidant that can be used as a disinfectant and can also be applied to sensitive areas of the body as an endogenous antiseptic. Biofilms are highly organized microbial communities that exhibit increased resistance to antimicrobials, disinfectants and host immune responses, making eradication difficult. Therefore, this study aimed to investigate the activities of NCT against Staphylococcus aureus and Pseudomonas aeruginosa mono and multispecies biofilms in vitro.

A collagen wound biofilm (CWB) model for mimicking conditions in chronic wound and 96-well plate biofilm (WPB) model were used to determine activities of NCT (1%, 55 mM) against S. aureus ATCC 25923 and P. aeruginosa PA01 biofilms. Furthermore, in CWB model the suspensions on top of the matrices were used to analyze the effect of NCT on planktonic cells growing in a wound-like environment.

NCT reduced the number of S. aureus cells in the CWB model by 8 log and 4 log in mono- and multispecies biofilms, respectively. Against P. aeruginosa mono and multispecies biofilms, NCT reduced viability by 7 log and 4 log, respectively. NCT showed similar efficacy rates against planktonic cells resembling the wound environment. In the WPB model, NCT was also found to be effective against biofilms, but not as effective as in the CWB model. According to this method, the number of S. aureus cells was reduced by 5 log in both mono and multispecies biofilms, whereas the viability of P. aeruginosa biofilms was reduced by 4 log and 3 log in mono and multispecies biofilms, respectively.

In conclusion, NCT exhibited strong antimicrobial activity against S. aureus and P. aeruginosa biofilms across different environments. Its higher efficacy in the CWB model may relate with better activity in the presence of organic matter. These findings support that NCT can be used as an effective microbicidal agent against S. aureus and P. aeruginosa biofilms.

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FROM THE DEAD TO THE SOIL: EXPLORING ANTIBIOTIC RESISTANCE IN BURIAL GROUNDS

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Cemeteries, while primarily sites of remembrance, may also contribute to environmental contamination, including the spread of antimicrobial resistance (AMR). However, their role in this context remains poorly understood. This study investigated whether cemetery soils can serve as reservoirs for antibiotic-resistant bacteria and resistance genes.

Soil samples were collected from cemeteries located on hills in northern Poland, including surface soils, subcoffin soils obtained during exhumations, samples from outside cemetery boundaries (controls), and groundwater-influenced samples from piezometers. Initial analyses, based on culture methods and multiplex PCR, revealed the presence of bacteria resistant to amoxicillin, cefuroxime, doxycycline, and tetracycline, along with selected antibiotic resistance genes (ARGs).

To further characterize the resistome and microbial communities, shotgun metagenomic sequencing was performed on selected samples. The results confirmed the presence of diverse ARGs, mobile genetic elements, and a broad taxonomic range of microorganisms. While some evidence of contamination was detected—particularly in subcoffin and piezometer samples—cemeteries under current conditions do not appear to pose a significant AMR risk.

Nonetheless, further research is warranted, especially focusing on newer burials, to better understand the persistence, mobility, and potential public health implications of resistance genes in burial-impacted soils