ENVIRONMENTAL MICROBIOLOGY

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Book of abstracts

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Warming Mediterranean Waters and Viral Emergence: Dissecting Betanodavirus Outbreaks through Reverse Genetics

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Global warming is drastically altering marine environments, turning the Mediterranean Sea into a climate hot spot where spikes in water temperature are directly linked to surges in viral pathogens. In September 2024, sea surface temperatures reached ~28°C, coinciding with unusual mortality events affecting dusky groupers (*Epinephelus marginatus*).

Nervous Necrosis Virus (NNV), a major viral pathogen of marine fish, was identified in affected specimens through molecular and cell culture analyses. From these events, two betanodavirus isolates were successfully recovered, providing a unique opportunity to investigate viral determinants associated with disease emergence under environmental stress.

Both isolates were fully sequenced and classified in the Redspotted grouper NNV genotype. Despite the high conservation typical of this genotype, comparative analysis revealed distinct amino acid substitutions in the capsid protein, including a mutation in the protruding domain, a key region involved in host interaction and viral entry.

To functionally characterize these differences, we established a reverse genetics system enabling the recovery of infectious viruses and, critically, the generation of homologous and reassortant strains. This approach provides a unique experimental framework to directly link viral genetic variability to phenotypic outcomes.

These findings establish a critical framework for investigating how climate-driven shifts influence viral genomics and evolution. By analyzing these shifts, we can position Betanodavirus as a primary genomic model for understanding how environmental stressors trigger genetic adaptations and the emergence of climate-sensitive viral threats.

Elemental mobilization from a Martian Regolith Simulant by *Parageobacillus thermantarcticus* M1^T

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Biomining is increasingly explored for resource recovery in space applications, but its efficiency depends on microbial physiology, substrate composition, and growth conditions[1]. We investigated the ability of the thermophilic bacterium *Parageobacillus thermantarcticus* M1^T to promote elemental mobilization from a Martian regolith simulant. Experiments were conducted at 60 °C under static conditions for five days. Culture viability, pH, and dissolved elemental concentrations were monitored, and genome mining of the predicted proteome was used to identify functions potentially associated with elemental mobilization. *P. thermantarcticus* remained viable in the presence of the simulant and shifted the medium pH from slightly acidic to mildly alkaline values. Compared with abiotic leaching, the bacterium selectively enhanced the mobilization of several elements, most clearly observed for Fe and Cr. In contrast, Ni, Zn, and Mg showed smaller or more variable responses. Genomic analysis identified functions related to urease activity and amino acid deamination, consistent with moderate alkalinization, together with FeoB-like ferrous iron transporters and Fur-family ferric uptake regulators, supporting active iron acquisition and homeostasis. Screening for chromium-associated functions did not identify a canonical ChrR-type chromate reductase, suggesting that chromium transformation may instead involve nonspecific flavin-dependent redox activity. This study highlights *P. thermantarcticus* as a promising thermophilic model for studying microbe-regolith interactions.

P. thermantarcticus M1^T is stored at the extremophilic bacterial collection (CE-ICB) of the Institute of Biomolecular Chemistry, partner of Joint Research Unit MIRRI-IT.

1. Santomartino et al. npj Microgravity (2026).

Petroleum hydrocarbon (PHC)-induced stress causes a shift in sunflower root exudation chemistry, potentially affecting recruitment and rhizocompetence of PHC-degrading bacteria

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Root exudation is a crucial mechanism of plant response to abiotic stress. In contaminated soils, plants shift root chemistry prompting a "cry-for-help" for recruiting pollutant-degrading microbial communities, thus mitigating phytotoxicity and sustaining plant growth.

Systematic studies comparing exudation patterns of plants grown in laboratory versus field-like conditions are lacking. In this study, the sunflower "cry-for-help" response to PHC was investigated through untargeted metabolomics: i) in an *in vitro* hydroponic system where plants were exposed to xylene and ii) in a historically PHC-polluted soil under outdoor conditions. The two experimental designs significantly impacted the detected exudation patterns, with a more complex profile in the soil setup, showing modified abundances of coumarins, terpenes and flavonoids, known for their role in plant stress response. DistLM analysis performed on the metabolome and 16S rRNA amplicon sequencing datasets highlighted that coumarins and pyrimidines significantly correlated with the structure of rhizosphere microbial communities in the polluted soil, with implications for degrading populations recruitment. Indeed, our results suggested that differentially exuded metabolites impacted rhizocompetence of PHC-degrading strains. Quinic acid and theophylline, upregulated under PHC stress, stimulated swimming motility and biofilm formation, important features for microbe establishment in the rhizosphere. Considering its inhibitory activity on the growth of selected strains, the decreased exudation of epicatechin gallate could instead represent a strategy to preserve degrading microorganisms in the rhizosphere.

Unravelling the "cry-for-help" to PHCs is crucial to identify molecules with biostimulant activity for degrading bacteria, facilitating their recruitment to boost plant fitness under stress and to improve bioremediation effectiveness.

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Exploring environmental reservoirs and microbial interactions of mycobacteria in bovine tuberculosis-affected farms

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Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB), a chronic infectious disease in cattle that leads to substantial economic losses and is subject to rigorous European Union eradication programs. Transmission is believed to occur primarily through aerosol inhalation and the ingestion of contaminated environmental materials. This study aims to investigate the environmental occurrence of mycobacteria, identifying preferred ecological niches and survival microenvironments within farm settings.

Six dairy farms were selected for the study: three with a documented history of bTB outbreaks and three officially tuberculosis-free. For each farm, soil samples were collected from comparable functional areas. Key physico-chemical parameters—including temperature, redox potential (ORP), electrical conductivity, salinity, and pH—were measured *in situ*. Cultivable bacteria were isolated and subjected to competition assays with *Mycobacterium smegmatis* (a non-pathogenic surrogate) to identify antagonistic or synergistic effects. In parallel, total DNA was extracted and analyzed via Full-length 16S rRNA gene sequencing (NGS) to characterize community composition and identify taxa associated with bTB-positive environments.

Multivariate statistical analysis (PERMANOVA) identified pH and ORP as the primary environmental drivers shaping the soil microbial communities. While no antagonistic strains were found in the competition assays, several isolates were identified as strong promoters of *M. smegmatis* growth. Furthermore, a decision tree model identified low *Ornithinimicrobium* abundance combined with high ORP values as a strong predictor of TB positivity in the sampled environments.

Understanding these ecological relationships provides valuable insights into the environmental reservoirs of mycobacteria, supporting the development of improved biosecurity and prevention strategies for bovine tuberculosis.

Exploring marine microbial adaptive strategies in an atmospheric simulation chamber: implications for biogeochemical C cycle along the ocean-atmosphere continuum

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Ocean and atmosphere constantly exchange water, gases and microbes. Microbial activities in the ocean have profound influences on the atmospheric chemistry and on Earth climate. Marine microbes are important players in the biogeochemical C cycle at the planetary level, despite living in a dynamic microscale world. We are at the infancy of understanding adaptive strategies of marine microbes while ejected into the sea spray aerosol, SSA. This work is part of a project investigating the diversity of microbial communities, in the shallow hydrothermal vents of Panarea (Mediterranean Sea) across the ocean-atmosphere continuum. We isolated several microbial strains present in seawater as well as in SSA. To mimic ocean-SSA ejection, we conducted aerosolization experiments at the atmospheric simulation ChAMBRe (INFN, University of Genova) on BP1 strain, a yellow-pigmented monoderm belonging to *Microbacterium* genus, isolated from Black Point hydrothermal fluid. BP1 was exposed, at 60% RH for 1 hr, to light, dark, black carbon particles and NOx gases. Changes in cultivability, particle and cell abundance were monitored using real-time bioaerosol sensing instrument (WIBS), flow cytometry, and culture-based approaches. Overall, the stress in the atmosphere affected particle abundance and cultivability in a treatment-specific way. Unexpectedly, BP1 in the light showed an increase in cell abundance but not in the dark and black carbon conditions. I will discuss my results in the light of the importance of environmental stressors role in shaping airborne microbial communities, with implications for global biogeochemical cycles.

Resistance gene profiles in rock glacier-influenced alpine catchments: links to metal gradients and hydrogeochemistry

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Antimicrobial resistance represents a global health threat, yet its environmental drivers remain poorly understood. Heavy metals may contribute to the selection and maintenance of antimicrobial resistance genes (ARGs) alongside metal resistance genes (MRGs). Rock glaciers represent long-term water reservoirs and, in our study area, release trace metals to downslope waters. We investigated taxonomic and MRG/ARG profiles in substrates from six rock glaciers with different degrees of activity and permafrost content and nearby reference springs in the Eastern Alps across two seasons, assessing associations between MRGs and ARGs and the influence of geochemical and physical conditions. Shotgun metagenomic and geochemical data were analysed. Taxonomic composition indicated a conserved core microbiome, constant within the same location, with no seasonal variation. ARG and MRG profiles differed more by valley than by site type, with a smaller seasonal effect. Ordination analyses identified Cd, Ce, and temperature as significant correlates of ARG and MRG profiles; notably, cadmium was significantly and positively associated with the corresponding resistance genes. Across all samples, ARG abundance was positively correlated with MRG abundance (Spearman $\rho = 0.894$, $p < 0.001$). PLS-PM (GoF = 0.609) supported a dominant pathway in which trace-metal gradients were positively associated with MRGs, and MRGs strongly predicted ARGs. Overall, MRGs strongly co-varied with ARGs across samples. While both resistomes were shaped by geochemical gradients and valley-specific characteristics, MRGs were more closely associated with trace elements, suggesting that trace-metal dynamics should be considered in future monitoring and management of alpine headwater systems, particularly under ongoing climate change.

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