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MICROBIAL GENETICS

Marco Fondi- Loredana Baccigalupi

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Resistance to colistin in *Pseudomonas aeruginosa* biofilms is modulated by the *arn* operon and pH

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Colistin is a cyclic antimicrobial peptide that interacts electrostatically with the lipid A moiety of LPS leading disruption of the outer membrane (OM) and, ultimately, cell death. In *Pseudomonas aeruginosa,* remodelling of the OM through aminoarabinosylation of lipid A leads to the emergence of colistin-resistant strains. Additionally, we observed that biofilms formed by colistin-sensitive strains were resistant to this drug. Considering that *P. aeruginosa* lives in a mild acidic environment in the airways of patients with cystic fibrosis, we observed greater biofilm resistance at pH 6 compared to pH 7, with a 2- to 4-fold increase in the colistin MBIC (Minimal Biofilm Inhibition Concentration). Biofilm resistance to colistin would suggest an upregulation of the *arn* operon. Accordingly, we observed that arnT, the last enzyme involved in aminoarabinosylation, was markedly upregulated in biofilms compared to planktonic cells in both reference and clinical *P. aeruginosa* Col^S strains. Furthermore, biofilm resistance to colistin was markedly lower in in *Δarn* mutant respect to the parental wt strains, with an average 8-fold reduction in MBIC. Similarly, inhibition of ArnT activity by FDO and FDO-H (doi:10.1093/jac/dkaa200; 10.1021/acs.joc.0c01459) significantly reduced MBIC values in all tested clinical isolates. Overall, our results demonstrate that upregulation of the *arn* operon contributes to intrinsic colistin resistance in *P. aeruginosa* biofilms, irrespective of the development of resistance in planktonic cells. Targeting the ArnT enzyme appears to be a promising strategy for restoring the efficacy of colistin in clinical settings where biofilm-associated infections are prevalent, such as chronic pulmonary colonization in cystic fibrosis.

Does transcription-replication interaction impact cellular metabolism?

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Abstract

Bacterial chromosome replication is one of the main drivers of gene expression throughout the cell cycle. In species like *Escherichia coli*, high growth rates induce a switch to mero-oligoploidy, where multiple active replication forks create a gene dosage gradient along the chromosome (multiplicity), from origin to terminus. Origin-proximal genes are present in higher copy numbers and may show increased expression, whereas terminus-proximal genes may be comparatively underrepresented. While the impact of DNA replication on gene expression is well-established, it remains unclear whether this effect also extends to other cellular processes, particularly cellular metabolism. Here, we integrate genomic position, multiplicity, and expression data into the *E. coli* genome-scale metabolic model to explore the role of the multiplicity-driven unbalanced expression of *ori*- vs. *ter*-proximal genes on the metabolic phenotype of the cell. We further formalize the theoretical concept that gene expression results from both regulatory control and gene copy number, and thereby try to quantify the relative contribution of multiplicity to overall expression and its metabolic impact. To experimentally support these predictions, we are currently implementing an integrated ^{13}C-based fluxomics/RNA-seq framework in *E. coli* grown under controlled glucose-limited conditions to induce distinct growth rates and, consequently, multiplicity profiles for a direct comparison/tuning of model predictions. Our work will reveal whether the effect of transcription-replication interactions, besides impacting gene expression, also propagate to the metabolic level or, rather, cells have evolved any mechanism to buffer it.

The *Pseudomonas aeruginosa* DedA protein PA4011 functions as a C55-PP phosphatase via a PAP2-like domain

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The recycling of the lipid carrier undecaprenyl phosphate (C55-P) across the cytoplasmic membrane is a key step of the peptidoglycan biosynthetic pathway. Recent studies have proposed that proteins of the DedA family mediate the flipping of C55-P back to the cytoplasmic side. However, during the recycling process, undecaprenyl pyrophosphate (C55-PP) must first be dephosphorylated.

Pseudomonas aeruginosa has six DedA proteins, one of which, PA4029, has been demonstrated to act as a C55-P flippase. Notably, besides the DedA domain, the PA4011 protein also contains a PAP2-like domain homologous to those present in the *Escherichia coli* phosphatases YbjG, PgpB, and LpxT, which, together with UppP, are involved in C55-PP dephosphorylation. Homologs of UppP and LpxT are also present in *P. aeruginosa*.

By generating double deletion mutants and a triple conditional mutant in PA4011, *uppP* and/or *lpxT*, we confirmed that PA4011 contributes to C55-P(P) recycling by acting as a C55-PP phosphatase. Indeed, deletion of *uppP* in the PA4011 mutant increased its sensitivity to the C55-P synthesis-targeting antibiotic fosmidomycin and caused growth arrest at 25°C, a condition that strongly reduces *P. aeruginosa lpxT* expression. Accordingly, growth was completely inhibited upon LpxT depletion in ΔPA4011 Δ*uppP* cells. Moreover, expression of a PA4011 variant mutated in a conserved catalytic residue and of PAP2-like domain alone demonstrated that PA4011 activity relies on its phosphatase domain. Notably, deletion of PA4011 and/or *uppP* also reduces the emergence of colistin resistance, likely due to increased LpxT-mediated transfer of phosphate from C55-PP to lipid A, which hampers its aminoarabinosylation. Finally, although inactive in C55-PP dephosphorylation, both the DedA domain alone and the catalytically-inactive mutant of PA4011 appear to have C55-P flipping activity, as their expression restored fosmidomycin resistance in the ΔPA4029 mutant.

Large-scale analysis of genetic diversity in Patescibacteria across environments

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Patescibacteria, also referred to as the Candidate Phyla Radiation (CPR), represent a vast monophyletic division within the bacterial domain, comprising diverse lineages with reduced genomes, limited metabolic capabilities, and symbiotic lifestyles. Due to these features, CPR bacteria remain largely uncultivated and are often underdetected or misclassified in 16S rRNA gene surveys. Thus, these bacteria are commonly detected and analysed using shotgun metagenomics. Despite several reports of CPR occurrence across diverse natural and human-associated sources, their global distribution and habitat preferences remain incompletely understood.

Here, we performed a large-scale study on publicly available metagenomic datasets to investigate the environmental distribution and ecological associations of CPR lineages. We developed a machine learning–based classification approach leveraging the RecA protein as a marker for the detection and classification of CPR bacteria. We applied this approach to the MGnify protein database, which contains protein sequences from tens of thousands of metagenomic samples with associated biome information, to describe CPR diversity across environmental sources. Our results indicate that CPR bacteria are widespread in freshwater environments, with lineage-specific enrichments in wastewater and human microbiomes, alongside an expansion of human-associated lineages, consistent with potential adaptation to the human host. Overall, we provide a comprehensive overview of the global distribution patterns in CPR bacteria by applying a robust marker-based bioinformatic pipeline on an extensive metagenomic database.

Investigating the role of the protein LysX2 of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, survives within the host through sophisticated mechanisms of environmental adaptation and resistance to immune-mediated killing, largely supported by the unique properties of its cell wall. Among the factors influencing cell wall composition, MprF-like proteins play a key role by modulating surface charge through the aminoacylation of phospholipids. *Mtb* encodes two members of this family, LysX and LysX2. While LysX has been extensively characterized, the function and mechanism of LysX2 remain poorly understood.

In this study, we investigated the role of LysX2 in its natural host, *Mtb*, by generating a knock-out strain using the ORBIT technique. Our results demonstrate that LysX2 is required for rapid adaptation to mildly acidic pH. Despite this deficiency, the KO strain compensates through the overexpression of *rv1169c*, encoding the PE11 protein involved in maintaining cell wall integrity. RNA-seq analysis further revealed the upregulation of oxidative stress response genes, including *sigH*, *katG*, and *rv2466*, in the KO strain.

Functional assays showed that the LysX2-deficient strain exhibits increased sensitivity to oxidative and nitrosative stress, as well as to vancomycin, indicating enhanced cell wall permeability and altered surface properties. High-performance thin-layer chromatography (HPTLC) lipid analysis revealed the accumulation of triglycerides under acidic conditions, consistent with a stress adaptation response. Finally, infection experiments using the murine RAW264.7 macrophage cell line demonstrated that the KO strain is significantly more susceptible to macrophage-mediated killing than the wild type.

Collectively, these findings identify LysX2 as a critical virulence factor in *Mtb*, contributing to resistance against key macrophage defense mechanisms. Further studies are needed to elucidate its molecular target and mechanism of action, potentially opening new avenues for therapeutic intervention.

MICROBIAL INFECTIONS AND VIRULENC

Francesco Imperi – Arianna Tavanti

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Pisa4: a potential candidate for *Mycobacterium tuberculosis* treatment

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Pisa4 is a recently identified cluster K1 mycobacteriophage that lacks integrase and encodes a partial immunity repressor. We aimed to evaluate its antibacterial activity against *Mycobacterium tuberculosis* (MTB) following complete deletion of the repressor gene, to prevent reversion to a temperate phenotype, while also exploring its host range in *Mycobacterium abscessus* (MAB) another clinically relevant species. Pisa4 was engineered using BRED, generating the Pisa4Δ_IR mutant, which was tested in time-kill assays against MTB H37Rv. Sera from TB patients in Cape Town were used to assess phage neutralization, and their MTB isolates were evaluated for susceptibility. In parallel, host range was further explored in 48 clinical isolates of MAB, collected in Pisa, Rome and Palermo. The repressor gene of Pisa4 was successfully deleted. Pisa4Δ_IR infected MTB H37Rv, showing an increase in plaque-forming units (~3.5 $\log_{10}$). From day 5 post-infection, it suppressed bacterial growth, maintaining colony-forming units close to baseline, while untreated controls increased (~2 $\log_{10}$). Overall, 30/34 (88%) clinical MTB isolates were susceptible with an EOP ≥0.01. In contrast, a limited activity was revealed against MAB, with lytic infection observed in only 8/48 isolates. No serum neutralization was detected. Deletion of the partial repressor ensures stable lytic activity. Pisa4Δ_IR effectively controls MTB growth *in vitro* and shows a broad host range among the MTB isolates, while displaying limited activity against MAB. These findings support its potential as a targeted anti-MTB therapeutic and warrant further evaluation across genetically diverse isolates, as well as in intracellular and low-metabolic conditions.

DksA–(p)ppGpp interplay is a key determinant of *Pseudomonas aeruginosa* extracellular and intracellular infection

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Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen responsible for severe acute and chronic infections. Although traditionally considered extracellular, it can persist within host cells, contributing to immune evasion and antibiotic resistance. Despite its clinical relevance, the molecular mechanisms enabling intracellular survival remain poorly understood.

The stringent response (SR), mediated by the alarmone (p)ppGpp and the transcriptional regulator DksA, is a key global regulatory system controlling bacterial adaptation and virulence. Here, the interplay between (p)ppGpp and DksA in regulating virulence and intracellular persistence of *P. aeruginosa* was investigated. Using a reverse genetics approach, mutants lacking DksA proteins (Δ*dksA*), (p)ppGpp (Δ*rs*, lacking the *relA* and *spoT* genes), or both (Δ*dksA-rs*) were generated. All SR-mutant strains showed reduced motility, impaired production of virulence factors, and attenuated pathogenicity in *Galleria mellonella*.

Importantly, SR mutants displayed decreased cytotoxicity and impaired intracellular survival in human lung epithelial cells compared to the wild type, with the Δ*dksA-rs* showing the strongest defect. This phenotype was correlated with reduced expression of the Type III Secretion System effector ExoS and an impaired ability to evade intracellular degradative pathways. Confocal microscopy revealed increased colocalization of the Δ*dksA-rs* mutant with LAMP1-positive compartments, indicating impaired escape from lysosomal degradation. Consistently, inhibition of lysosomal acidification and autophagosome-lysosome fusion by bafilomycin restored mutant survival.

Overall, (p)ppGpp and DksA act both additively to promote *P. aeruginosa* virulence and intracellular persistence, highlighting the SR as a critical determinant of host-pathogen interaction and a promising target for the development of novel antimicrobial strategies.

Targeting *de novo* L-cysteine biosynthesis (DeNoCB) in *Pseudomonas aeruginosa* for novel antimicrobial strategies

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Bacterial DeNoCB is a key metabolic hub and a promising antibiotic target due to its absence in humans, yet it remains poorly explored in *P. aeruginosa*[1,2]. Four key DeNoCB enzymes - CysK (PA2709), CysM (PA0932), CysE (PA3816), and CysH (PA1756) - were identified and physiologically/biochemically characterized. Their roles were investigated using deletion mutants grown in minimal media with different S-sources (sulfate, thiosulfate, or L-cysteine). Single deletions of *cysM* or *cysK* did not result in cysteine auxotrophy, whereas the double Δ*cysM*Δ*cysK* mutant showed no growth for up to 18 h, followed by recovery at later time points, suggesting the presence of alternative pathway(s)[3]. CysE was found to be essential for DeNoCB, by supplying the key intermediate *O*-acetylserine, as the Δ*cysE* mutant failed to grow on both sulfate and thiosulfate. Finally, the Δ*cysH* mutant was confirmed to be a cysteine auxotroph, but only when growing on sulfate.

Notably, both Δ*cysH* and Δ*cysE* exhibited reduced virulence in the *Galleria mellonella* infection model compared to the wild-type strain, thus emerging as promising antimicrobial targets.

Recombinant production and functional characterization of all four enzymes enabled compound library screening and inhibitor validation, both *in vitro* and in bacterial cultures. Preliminary results identified hit compounds targeting CysE (IC_{50} = ~30 μM, >60% growth inhibition) and CysH (IC_{50} = ~50 μM, >30% growth inhibition), while assays on CysK and CysM are ongoing.

Overall, these findings provide new insights into *P. aeruginosa* metabolic and regulatory networks, highlighting the key role of sulfur metabolism in bacterial physiology and pathogenicity.

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Drug repurposing to inhibit *Pseudomonas aeruginosa* adaptation to the cystic fibrosis lung environment

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The advent of CFTR modulators has led to remarkable improvements in lung function and overall health in many people with cystic fibrosis (CF). However, these therapies do not eradicate chronic lung infections caused by *Pseudomonas aeruginosa*, which remain a major health threat for people with CF.

Traditional antimicrobial discovery mainly relies on screening compounds in nutrient-rich media that poorly reflect *in vivo* conditions and rarely yields novel therapeutic options. Adapting screening conditions to better mimic the host environment has emerged as a promising strategy to uncover previously unrecognized antimicrobial activities in existing drugs. In particular, repurposed drugs that inhibit *P. aeruginosa* growth or biofilm formation in the synthetic cystic fibrosis medium (SCFM), which closely reproduces the chemical composition of CF sputum, may reduce bacterial burden and pathogenicity in the CF lung.

On this basis, over 3,000 FDA-approved drugs were screened in parallel in SCFM and in a standard rich medium. This approach allowed identifying drugs with potent and previously unrecognized antimicrobial or antibiofilm activity against *P. aeruginosa* specifically in SCFM. The best hits showed robust *in vitro* activity against the reference strain PAO1 and a panel of clinical CF isolates. Ongoing studies aim to identify their specific molecular target(s) and clarify their mechanism of action.

By leveraging drug repurposing in a physiologically relevant context, this study uncovered the antimicrobial potential of safe drugs that could improve the treatment of *P. aeruginosa* lung infections in people with CF.

Two AraC/XylS-family transcription factors contribute to virulence and oxidative stress tolerance in *Acinetobacter baumannii* AB5075

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Acinetobacter baumannii is a major cause of hospital-acquired infection and a critical antimicrobial resistance threat. As treatment options narrow, targeting virulence regulators may provide an alternative therapeutic strategy. We focused on on AraC/xylS-like transcriptional regulators (AFTR) in *A. baumannii* 5075 (AB5075), *virF* and *alkR.* VirF from AB5075 is conserved across diverse recent clinical isolates and shares predicted structural similarity with VirF of *Shigella flexneri,* while previous work has implicated *alkR* in *Galleria mellonella* infection. To test their contribution to virulence, we performed *G. mellonella* killing assay and found that disruption of *virF* or *alkR* significantly attenuated virulence in vivo. We then assessed antibiotic susceptibility, biofilm formation and architecture, motility and growth under multiple stress conditions. Although the mutants did not differ from the wild type in antibiotic susceptibility, biofilm, or motility, both showed impaired growth under paraquat stress, but not hydrogen peroxide stress. These findings suggest that virFA and alkR contribute to virulence through adaptation to oxidative stress, potentially in response to superoxide-generating conditions. One next step will be to test whether these regulators control oxidative-stress genes and intracellular survival in THP-1 cells. Together, these findings identify VirFA and AlkR as candidate virulence regulators in *A. baumannii* AB5075, and, given the reported inhibition of AraC/XylS-family regulators by fatty acids, suggest a potential route for anti-virulence intervention.

Rhizosphere microbiome engineering and root exudate metabolites have the potential to enhance the bioremediation of petroleum hydrocarbons

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Nature-based solutions for historically polluted sites remediation include the enhancement of the degradative potential selected in the soil microbiome over time. Biostimulation of degradative microorganisms can be achieved by adding specific compounds directly to the soil or it can be mediated by plants, that act synergistically with the soil microbiome in rhizoremediation approaches, often prompted by the addition of selected microorganisms.

Here, sunflower was employed alone or supplemented by degradative strains to optimize petroleum hydrocarbon (PHC) remediation of an industrial polluted soil. The dynamics of the rhizosphere microbiome and the abundance of PHC catabolic genes were monitored on the soil metagenome by metataxonomic and qPCR analyses, while the efficacy of microbial inoculation in boosting PHC removal was chemically assessed. Moreover, leveraging untargeted metabolomics data, the role of specific root exudate metabolites as possible biostimulants of PHC degradation was evaluated *in vitro* providing them to the microbial cells detached from the target soil.

In soil, we observed that sunflower recruited a rhizosphere community diverse from that evolving over time in unplanted microcosms. Moreover, bioaugmentation with degrading strains further steered the structure of the overall rhizosphere microbiome, increasing the abundance of catabolic genes and the removal of PCHs compared to non-inoculated plants. *In vitro*, soil-detached microbiome showed improved PHC degradation when supplemented by the tested metabolites, suggesting that these plant-derived molecules trigger degradation pathways of the native bacterial populations naturally selected in the historical contaminated soil.

Our findings demonstrate that the understanding of ecological interactions in the rhizosphere, mediated by invading degrading strains and by plant via root exudation is essential for optimizing the design of rhizoremediation interventions.

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HOST- MICROBIOME INTERACTIONS

Christian Milani – Giovanni Bacci

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Fructooligosaccharide-driven metabolism of a probiotic consortium modulates hepatocyte lipid accumulation in an *in vitro* microbiota-liver interaction model

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Metabolic-dysfunction-associated fatty liver disease (MAFLD) is a metabolic disorder in which the gut-liver axis plays a central role. Microbial-derived metabolites generated from dietary substrates are key mediators linking gut microbiota activity to host metabolic regulation. Specifically, fructooligosaccharides (FOS) can modulate microbial metabolism and functional outputs.

We investigated the impact of FOS-driven microbial metabolism on hepatic lipid accumulation using a defined *in vitro* human gut microbiota model. A probiotic consortium composed of *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, and *Limosilactobacillus reuteri* was evaluated alone and in combination with a reconstructed minimal human gut microbiota core (*Clostridum symbiosum*, *Flavonifractor plautii*, *Bacteroides cellulosilyticus*, and *Escherichia coli*). Bacterial growth, strain-level dynamics, and expression of genes involved in FOS utilization were assessed. Microbial metabolites were characterized by GC-MS and tested on HepG2 cells in a palmitic acid-induced steatosis model.

All strains grew on FOS, with strain-dependent efficiency. The probiotic consortium and the overall microbial community were shaped by substrate utilization, with *L. plantarum* and *L. reuteri* dominating in both contexts. Genes involved in FOS metabolism were identified in all probiotic strains, showing differences in gene organization, and expression across different experimental conditions.

FOS fermentation resulted in the production of short-chain fatty acids and organic acids. HepG2 cells pre-treated with metabolites derived from the probiotic consortium and the reconstructed community showed reduced intracellular lipid accumulation under steatotic conditions, associated with decreased *cd36* lipid transporter gene expression.

These findings highlight the role of microbial context in shaping metabolic outputs and support the contribution of microbial interactions to the regulation of host lipid metabolism in a liver cell model.

How are microbiomes transmitted across animals? An integrative approach to quantify the ecological factors driving microbiome transfer using guppies.

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Microorganisms play an essential role in animal health, yet the mechanisms governing their colonization and transmission are poorly understood. Here, we investigated microbiome transmission using guppies (*Poecilia reticulata*), a established model in animal behavioral ecology. The aquatic environment offers the advantage to disentangle the environment from the host-driven processes of microbiome dispersal. We generated two cohorts of guppies with distinct microbial profiles and placed them in cohabitation with and without contact. Using an integrative framework combining genomics (16S rRNA, metagenomics), behavioral tracking, fluorescence microscopy, and ecological modelling we characterized microbiome dynamics across four host compartments (skin gills, gut, gonads). Our results reveal distinct transmission pathways for external versus internal microbiomes, along with pronounced sex- and microbial-specific differences. Understanding how microbiomes move between hosts, and how these processes differ across organs, is essential for elucidating microbiome function and holds great potential for optimizing microbiome-based interventions across diverse animal taxa, including humans.

Talking roots: a genotype-specific interactive dialogue between endophytic bacteria and wild and domesticated rice revealed by multi-omics

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Plant-microbe interactions are crucial for plant health and productivity, and root exudates play a central role in shaping these associations. Here, we investigated the bidirectional transcriptional and hormonal dialogue between rice and two endophytic plant growth-promoting bacteria using an integrated multi-omics approach.

We exposed *Enterobacter asburiae* RCA24 and *Kosakonia sacchari* RCA25 to root exudates from three rice genotypes: two cultivated varieties (*Oryza sativa* cv. Baldo and Vialone Nano) and the wild ancestor *Oryza rufipogon*. Bacterial RNA-seq revealed genotype-by-genotype interactions: *E. asburiae* RCA24 was able to distinguish between *O. sativa* varieties, and *K. sacchari* RCA25 responded more strongly to *O. rufipogon* exudates. Functional annotation highlighted differential expression of genes involved in central metabolism, stress response, and signal transduction among the cultivated and wild rice genotypes, suggesting that domestication has reduced the stimulatory capacity of rice exudates on beneficial microbes.

Hormonomic profiling of root exudates revealed genotype-specific phytohormone signatures. Gibberellins showed strong differentiation (PERMANOVA R^2=0.53, p=0.006), with GA_9 characterizing Baldo (956 pmol/L) and GA_{51} dominating *O. rufipogon* (577 pmol/L). Auxin profiles exhibited moderate genotypic variation (R^2=0.39, p=0.034).

To assess reciprocal effects, we also analyzed the rice transcriptome following bacterial colonization. The analysis revealed that bacterial colonization triggered tissue- and genotype-dependent responses. For *E. asburiae* RCA24, 3,813 differentially expressed genes were observed in Baldo stems, while for *O. rufipogon,* limited transcriptional responses were recorded.

Overall, these findings highlight the reciprocal and genotype-specific transcriptional crosstalk between rice and endophytic bacteria, demonstrating that *O. rufipogon* may be a reservoir of traits that could be exploited to optimize rice-microbe interactions that promote plant growth for sustainable agriculture.

Phylogenetically stratified GWAS reveals insights into *Escherichia coli* specific virulence factors in adults and neonates

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Escherichia coli is a highly diverse bacterial species capable of occupying a wide range of ecological niches, from harmless gut commensalism to severe extraintestinal infections. Identifying genetic determinants that underlie adaptation to specific host and environmental contexts remains challenging due to extensive horizontal gene transfer and strong phylogenetic structure. In this study, we performed a genome-wide association study (GWAS) on gene presence–absence across a large collection of *E. coli* genomes isolated from adult and neonatal bloodstream infections, urinary tract infections, healthy gut, hospital settings, and non-clinical environmental sources. By explicitly accounting for population structure, we identified lineage-independent, source-associated genetic signatures while minimizing confounding by vertically inherited genes.

Bloodstream and urinary isolates were significantly enriched in genes involved in metal acquisition (iron and manganese), adhesion, biofilm formation, and stress responses, highlighting shared adaptations for survival in hostile host environments. Neonatal bloodstream isolates showed a strong association with the yersiniabactin iron-scavenging system, whereas adult bloodstream isolates were enriched in acid resistance genes suggesting age-specific infection routes and virulence strategies. Urinary isolates exhibited marked metabolic flexibility, with enrichment of genes related to metal acquisition, sugar uptake, and secretion systems, consistent with adaptation to the nutrient-limited urinary tract.

Together, these findings demonstrate that *E. coli* adapts to distinct ecological and clinical niches through the acquisition and maintenance of functionally relevant genes independent of phylogenetic background. Source-specific genetic traits identified here provide valuable insights into *E. coli* pathogenesis, highlight age-dependent virulence mechanisms, and offer potential biomarkers for diagnostics, surveillance, and targeted intervention strategies, particularly in vulnerable populations such as neonates and hospitalized patients.

ENVIRONMENTAL MICROBIOLOGY

Massimiliano Marvasi - Elena Tamburini

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.

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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.

Acknowledgements

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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.

This study was carried out within the project PRIN 2022 “SUBSURFICE – Ecohydrological and environmental significance of subsurface ice in alpine catchments” (code no. 2022AL7WKC – CUP: I53D23002020008), financed by the European Union NRRP (Mission 4, Component 2, Investment 1.1 – D. D. 104 2/2/2022).

APPLIED MICROBIOLOGY (I)

Rachele Isticato – Giordano Rampioni

Book of abstracts

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Integrating GC–MS and customized MOX gas sensors for VOC-based discrimination of bacteria: towards applications in sustainable agriculture and food safety

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Abstract

Climate change is increasingly threatening agricultural productivity, particularly in Mediterranean environments where crops are exposed to recurrent drought and soil degradation. In this context, plant growth-promoting bacteria (PGPB), such as *Streptomyces violaceoruber* and *Kocuria rhizophila*, represent a promising sustainable strategy to enhance plant resilience to abiotic stresses. Microbial and plant metabolic activities are closely related to the emission of volatile organic compounds (VOCs), which can serve as non-invasive biomarkers of physiological status and stress conditions. In this study, we combined solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME/GC-MS) and custom-developed metal oxide (MOX) gas sensors to characterize and monitor VOC profiles from beneficial and pathogenic microorganisms. SPME/GC-MS analyses enabled the identification of species-specific volatilomic signatures in pure cultures of PGPB, revealing putative volatile markers associated with different growth stages. Notably, compounds such as geosmin and 2-methylisoborneol were detected in *S. violaceoruber* cultures and in soil samples, confirming the applicability of the method to real and complex environmental matrices. In parallel, MOX gas sensor arrays coupled with multivariate statistical analysis demonstrated the possibility to discriminate between different bacterial species and subspecies in a rapid and non-invasive manner. This approach was further validated on biological samples artificially contaminated with *Salmonella enterica*, highlighting distinct volatilomic fingerprints. Overall, our results demonstrate that the integration of GC-MS and MOX sensor technologies provides a robust and complementary platform for VOC-based microbial discrimination. This approach represents a promising tool for applications ranging from precision agriculture—through monitoring of PGPB activity and plant health—to food safety diagnostics, paving the way for real-time, in field monitoring systems.

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Unravelling antimicrobial resistance spread within populations trough integrated metagenomic and culturomic analyses of wastewaters.

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Antimicrobial resistance (AMR), occurring when pathogens evolve to withstand antimicrobial drugs, is making infections more difficult to treat and can be regarded as a "silent pandemic" that may cause up to 10 million deaths annually by 2050. In this scenario, wastewater-based epidemiology (WBE) may contribute to the surveillance of AMR diffusion and evolution. We implemented WBE to investigate AMR in specific settings, such as those characterized by high antibiotic usage (e.g. hospitals) and those representative of a young and healthy population (e.g. universities), in comparison with the whole community, by analysing different sewage sources from the same urban environment. ESBL-producing *Escherichia coli* prevalence was similar regardless the sewage source, coherently with the widespread use of cephalosporins. On the contrary, vancomycin resistant *Enterococci* (VRE), among major causes of healthcare-associated infections, were higher in wastewater from the hospital, where this antibiotic is manly used. Whole genome sequencing and phenotypic characterization indicated the occurrence of peculiar resistance features of hospital-derived strains. Quantification by qPCR/dPCR of selected antimicrobial resistant genes (ARGs), together with metagenomic-based high-throughput profiling of ARGs, showed population-specific resistance patterns, with a higher abundance of ARGs related to clinically relevant resistances in sewages representing the hospitalized population. Taken together these results indicate how the nosocomial environment, characterized by a large use of antibiotics, may represent a hot-spot for AMR. The application of WBE to specific settings provides high-resolution clues on the dimension of AMR within specific sub-populations, contributing to the definition of public-health interventions to combat antimicrobial resistance.

Lactococcus lactis postbiotics on human glioblastoma cell lines: _in vitro_ evaluation of antitumor activity.

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In recent years, probiotics have attracted increasing attention in the medical, pharmaceutical, and food fields due to their well-documented health benefits. This growing interest has driven scientific research toward the identification and characterization of novel probiotic strains. We established a collection of 50 lactic acid bacteria (LAB) isolated from dairy products, including *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, and *Lactobacillus helveticus*. Following a preliminary screening of probiotic-related properties, such as tolerance to acidic conditions and bile salts, antibiotic resistance profiles, cell surface hydrophobicity, autoaggregation ability, and production of antimicrobial compounds, the best-performing strains were selected for further investigation of their potential anticancer properties. In our study we focused on the potential antitumor activity of postbiotics (functional fermentation compounds) produced by three selected high-performing *Lactococcus lactis* subsp. *lactis* strains on human glioblastoma cell lines. Cell viability assays, including MTT and Trypan Blue exclusion tests, showed a significant reduction in cell proliferation following treatment. Flow cytometry analysis confirmed these findings, demonstrating cell cycle arrest in treated cells. Furthermore, postbiotic treatment significantly inhibited cancer-related processes such as wound healing and cell migration. On the other hand, primary astrocytes viability and the blood-brain barrier (BBB) integrity were not impaired, suggesting a selective effect of postbiotics on proliferating-undifferentiated cells. This preliminary study highlights, for the first time, the potential anticancer properties of postbiotics from some *L. lactis* strains on human glioblastoma cell lines.

From transcriptomic profiling to engineering target selection in *Haloferax mediterranei* for PHBV and bacterioruberin production

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Haloferax mediterranei is a promising archaeal platform for next-generation industrial biotechnology, owing to its natural ability to synthesize both poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), a promising PHA-copolymer, and bacterioruberin, a rare C-50 carotenoid [1]. Since the accumulation of these compounds is promoted by distinct cultivation conditions, understanding how carbon flux is redistributed between the two pathways is essential for rational strain engineering. In this work, transcriptomic profiling was performed on *H. mediterranei* cultivated under different conditions identified by statistical optimization as favoring either PHBV or bacterioruberin production [2]. Differential expression analysis highlighted condition-specific regulatory and metabolic responses linked to carbon flux distribution, precursor supply, and carotenoid biosynthesis.

The integration of transcriptomic data with pathway knowledge enabled the selection of candidate targets for metabolic engineering. For PHBV-oriented strategies, *korAB* and *porAB* were identified as relevant targets associated with propionyl-coA supply, while *cimA* was selected for promoter engineering to further modulate precursor flux. For bacterioruberin-oriented strategies, *lyeJ* emerged as a candidate target from transcriptomic analysis, and promoter engineering of *crtB* and *crtI* was designed to modulate flux through the carotenoid biosynthetic pathway. These results establish a transcriptomics-guided framework for target prioritization in *H. mediterranei* and support ongoing engineering efforts aimed at improving PHBV and bacterioruberin production.

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Synthesis and characterization of postbiotics from *Bacillus* spp.: study of functional properties and applications

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ABSTRACT

Despite their widespread use, the clinical efficacy of probiotics is highly variable and often inconsistent. Their functionality, in fact, is often impaired by limited survival, reduced engraftment and decreased metabolic activity during transit through the gastrointestinal tract. This hinders their capacity to exert sustained and biologically meaningful effects within the host. These limitations significantly impair their ability to colonise or transiently integrate into the resident microbiota, thereby restricting stable host–microbe interactions. Consequently, the anticipated health benefits are frequently diminished, transient, or absent, giving rise to significant concerns regarding their reliability as therapeutic interventions. In this context, postbiotics — defined as non-viable microbial cells, their components, or metabolites that confer health benefits — are emerging as a promising alternative. Unlike probiotics, postbiotics do not rely on microbial viability, thereby overcoming challenges related to stability, storage, and gastrointestinal survival. Furthermore, they offer greater safety, standardisation and mechanistic clarity, making them more suitable for precise and reproducible therapeutic applications. These features position postbiotics as a next-generation strategy for microbiome-based interventions. In this study, we focus on *Bacillus*-based postbiotic production using alternative carbon sources, particularly prebiotic substrates such as inulin and fructooligosaccharides. These are not digested by the human host, but can be metabolised by microorganisms, thereby enhancing the production of beneficial metabolites. This combined approach is a promising strategy for overcoming the current limitations of probiotics and supporting the development of applications for gut and skin microbiota.

APPLIED MICROBIOLOGY (II)

Livia Leoni – Leonardo Mancabelli

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Title

Model microbial communities for biocellulose production: dynamics, labour division and functionalities

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Presenting author: Marilisa Giavalisco

Bacterial cellulose (BC) is a biopolymer useful for several food and biomedical applications. BC production suffers from several constraints (strain-dependent, variability, high costs) and, therefore, strategies to improve BC yield is of practical relevance.

In the Project SynBioCell, 12 model microbial communities (MMC) were constructed by assembling, through a combinatorial approach, 4 acetic acid bacteria (AAB, core strains and BC-producers), 3 lactic acid bacteria (LAB, helper) and 3 yeasts (Y, helper), to boost BC yield, compared to individual AAB strains. The best MMC (including 2 AAB and 3 LAB) was select and re-combined to evaluate the microbial dynamics, cell compartmentalisation and strain functionalities during BC production (plate counting, BC yield, 16S rRNA-based qPCR, qPCR on cellulose sintasi complex, ^{1}H-NMR spectroscopy). Metatranscriptomic analyses were also performed to investigate the metabolic interaction of MMCs members.

The composition of MMCs significantly affect the BC production and, generally, LAB stimulated the BC yield. A good correlation was found for strain occurrence detected with plate counting and *16S rRNA* gene. qPCR confirmed the overexpression of CS genes in LAB-containing consortia. Metatranscriptomic data revealed that AAB functionalities significantly changed in MMC compared to the single AAB cultures, due to the shift in metabolic pathway stimulated by the presence of LAB. Our results showed that targeted MMCs could be an efficient machinery to improve BC production and provided further insights on metabolic networks and dynamics of microbial consortia.

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Salt-enhanced IAA production in *Vreelandella titanicae*: a stress-responsive for sustainable agriculture in saline soils

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Plant Growth-Promoting Rhizobacteria (PGPR) can play a crucial role in promoting plant growth under stressful conditions, such as in degraded soils. Soil salinization is becoming a rapidly expanding phenomenon, especially in Mediterranean countries, and represents a global threat to agricultural productivity. In this context, the isolation of halotolerant/halophilic PGPR and their characterization are the basis of a biotechnological and sustainable approach to addressing these problems.

We isolated the halotolerant bacterium *Vreelandella titanicae* from quinoa rhizosphere and characterized it for its PGP traits. The strain showed high indole-3-acetic acid (IAA) production, a key phytohormone involved in plant growth and development. Several studies on salt-tolerant bacteria reported the effects of different chemical-physical parameters on IAA production, however, the role of salt remains unclear. To address this gap, we characterized *V. titanicae* at genomic, metabolic and physiologic level and investigated NaCl-regulation of hormone production. Growth kinetic studies were performed under increasing salt concentrations, and IAA was quantified over time. Gene expression analysis was conducted focusing on the key genes involved in tryptophan-dependent IAA biosynthetic pathway. Increasing salinity reduced biomass but enhanced IAA production, reaching the greatest levels at 1.0 M NaCl. Expression analyses highlighted that salt positively regulated genes involved in the indole-3-acetaldehyde (IAAld) pathway, suggesting that osmotic stress activates specific metabolic ways of IAA production.

Our results demonstrate that IAA production in *V. titanicae* is a stress-responsive trait and provides further insights into its biosynthetic pathway and regulation, while, also identifying this bacterium as a promising PGPR candidate for salinized soils.

Evaluation of the anti-tubercular activity of *Kielmeyera membranacea* constituents

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Tuberculosis remains the leading cause of mortality from a single infectious agent worldwide, with 1.25 million deaths and 13.4 million new cases in 2023. The emergence of multidrug-resistant and extensive drug-resistant strains underscores the urgent need for new therapeutic alternatives. Natural products represent a valuable source of bioactive compounds, historically providing a significant contribution to antimicrobial drug discovery.

Kielmeyera membranacea (*Calophyllaceae*) has previously demonstrated antitubercular activity. This study aimed to identify the compounds responsible for this activity and to evaluate the influence of climatic factors on their production. Plant material was collected from four restinga regions across all seasons. Antitubercular activity was assessed using the Resazurin Microtiter Assay against *Mycobacterium tuberculosis* H37Rv, revealing MIC_{90} the lowest value as 125 µg/mL, with variation across regions suggesting a influence of climatic factors on bioactive compound production.

Phytochemical analysis identified the biflavonoid podocarpusflavone A (PCFA) as one of the major constituents. PCFA exhibited synergistic activity with isoniazid, significantly inhibiting intracellular bacterial growth in THP-1 macrophages. *In silico* analysis suggested protein tyrosine phosphatase B (PtpB) as a potential target. To investigate this mechanism, *ptpB*-overexpressing strain was evaluated in infection assays. Treatment with PCFA (128 µM) and isoniazid (0.2 µM) resulted in a ~10-fold increase in CFU/mL compared to H37Rv strain suggesting that PtpB overexpression reduced susceptibility to the PCFA/isoniazid combination. Additionally, IL-6 and caspase-3 levels were significantly modulated, indicating that PCFA probably modulates host immune defense mechanism.

These findings highlight PCFA as a promising antitubercular candidate with immunomodulatory properties and potential for combination therapy.

Modulation of glycolysis and homolactic fermentation of *Streptococcus thermophilus* energetically discharged cells: dissecting the role of transcription and post-translational modifications

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S. thermophilus metabolizes lactose via homolactic fermentation. Lactose enters the cell through LacS, is hydrolyzed by β-galactosidase into glucose and galactose, the galactose is exported, and the glucose is converted to lactic acid via glycolysis. To shed light on the regulatory network of glycolysis and homolactic fermentation, cells harvested in the exponential growth phase were divided into aliquots and subjected or not to an ATP and glucose depletion treatment, in presence and absence of rifamycin and chloramphenicol to block transcription and translation. The resulting ATP or glucose depleted biomasses (EdCs) were subjected to comparative metatranscriptomics and metaproteomics analysis, and quantification of (p)ppGpp. The EdCs phenotype showed the lowest levels of (p)ppGpp and an up-regulation of the S10 ribosomal protein gene cluster and purine biosynthesis genes, consistent with the regulatory scenario headed by the stringent response. The metabolic behaviour of those biomasses was then evaluated by activating them with lactose, lactose and urea, in presence or in absence of glycolytic inhibitors. EdCs activated with lactose behaved as biomasses treated with glycolytic inhibitors, displaying no production of L-lactic acid, while exhibiting a higher lactose capacity compared to their control. Data analysis revealed transcriptional regulation of genes and post-translational modification of proteins involved in glycolysis correlated with the yield of glycolysis and lactose intake capacity. Proteomic analysis showed ATP and glucose depletion significantly alters protein lactylation profiles, indicating that addition and removal of lactyl groups on lysine residues are regulated at post-translational level by acetyltransferases and deacetylases.

Display of an influenza virus multi-epitope on *B. subtilis* spores

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Bacterial spores are promising tools for the mucosal delivery for the development of new oral/nasal vaccines, offering stability, safety, and the ability to stimulate both humoral and cellular immune responses. This project explores the use of probiotic strains of intestinal origin of *B. subtilis* to display influenza epitopes and test them as mucosal vaccine on animal models. Compared to laboratory strains, probiotic strains may provide enhanced performance due to their adaptation to the gastrointestinal environment and to the positive effects of probiotics on the efficacy of mucosal immunizations. A probiotic strain of intestinal origin of *B. subtilis,* MV24*,* was selected according to the EFSA guidelines for safety. A chimeric Influenza A multi-epitope (ME-IVA) was designed, using IEDB, CALIBER and BepiPred 3.0 epitope prediction tools and a synthetic gene coding for ME-IVA produced. Specifically, the construct includes conserved T-cell epitopes from Influenza A Nucleoprotein (NP) and Matrix Protein 1 (M1), along with B-cell epitopes from Hemagglutinin (HA), Neuraminidase (NA), and the M2 ectodomain (M2e). These domains were connected with appropriate linkers to optimize processing, folding, and immunogenicity. The final sequence was codon-optimized for *E. coli* expression, allowing efficient overexpression and purification via affinity chromatography. The purified protein is being used for non-recombinant surface display through adsorption onto purified MV24 spores. In parallel, recombinant approaches are being developed by generating gene fusions between the ME-IVA sequence and spore coat proteins (CotB and CotC), enabling antigen display directly on the spore surface. Ongoing experiments are optimizing protein expression and surface display, which will allow future purification and immunological studies *in vivo*. This work focuses on developing a safe and stable probiotic spore-based platform for broad, cross-protective influenza vaccines.

PhD DAY (I)

Elena Perrin & Emanuela Frangipani

Book of abstracts

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Photobacterium halotolerans: genomic analysis and fermentative production of Polyhydroxyalkanoates (PHAs)

Alessandra Filieri[1], Chiara Morandi[1], Andrea Firrincieli[1], Vittorio Vinciguerra[1], Silvia Crognale[1]

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Environmental pollution caused by the accumulation of petrochemical-based plastics has driven the search for sustainable, biodegradable alternatives derived from renewable resources. Among these, polyhydroxyalkanoates (PHAs) are promising microbial biopolymers due to their biodegradability and physicochemical properties comparable to conventional plastics. However, their industrial-scale production remains limited by high costs and low yields compared to synthetic plastics. Halophilic and halotolerant microorganisms are valuable biotechnological resources, as high salinity may promote PHA accumulation. Here, the PHA production potential of the halotolerant bacterium *Photobacterium halotolerans*, isolated from the salt pans of Tarquinia (Viterbo, Italy), was investigated. To assess the biosynthetic potential of the strain, genomic analysis revealed the presence of the *phaBAPC* gene cluster. The genomic profile also indicated the ability to utilize various sugars, confirming a type I PHA biosynthetic pathway. Based on these findings, fermentation experiments were carried out in a bioreactor using different operational strategies, namely batch and fed-batch modes, to evaluate PHA production at different NaCl concentrations (0, 2.5, 5%), using glucose as the carbon source. Batch fermentation results showed a progressive increase in biopolymer accumulation with increasing salinity. Furthermore, a fed-batch strategy was developed as a process optimization approach, based on glucose feeding and pH control, involving the extension of the accumulation phase and nitrogen limitation. This strategy led to improved growth conditions and enhanced biopolymer production, achieving accumulation values above 60%. Overall, these results highlight the potential of *P. halotolerans* as a microbial platform for sustainable PHA production.

Microbial BioRemediation Database: a comprehensive database of genes involved in microbial bioremediation processes

Presenting author: Silvia Petraro

Silvia Petraro[1], Chiara Tarracchini[1], Leonardo Mancabelli[2,3], Gabriele Andrea Lugli[1,2], Francesca Turroni[1,2], Marco Ventura[1,2 *] and Christian Milani[1,2 *]

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Environmental pollution from a wide range of compounds poses a significant risk to both ecosystems and human health. Although bioremediation is considered a promising strategy for pollutant removal, its broader application remains limited by fragmented genomic resources and an incomplete understanding of microbial biodegradation pathways. To address this limitation, we developed the Microbial BioRemediation (MBR) database, a comprehensive and manually curated repository comprising more than 643,351 bacterial protein sequences associated with the degradation of 564 pollutant compounds across 25 chemical classes. Optimized to support both genomic and metagenomic applications, the MBR database enables high-resolution functional and taxonomic profiling of microbial communities as well as individual bacterial strains. Validation using publicly available genome and metagenome datasets from contaminated environments demonstrated the ability of the database to identify both conserved biodegradation functions and functions specifically associated with distinct environmental contexts. Furthermore, its application to host-associated microbiomes highlighted the potential of MBR for investigating how environmental exposures may shape microbial catabolic capacity across diverse ecological settings. Overall, the MBR database represents a strategic resource for the early identification and prioritization of microbial candidates for bioremediation. By enabling the *in silico* selection of relevant microbial taxa and enzymatic functions, it provides a rational strategy for targeted *in vitro* validation and experimental characterization. This integrative approach may facilitate the development of next-generation, tailored strategies for the remediation of complex polluted ecosystems.

How genetic background influence phage resistance trade-offs in three different *Pseudomonas aeruginosa* clinical isolates

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Phage steering is an approach to phage therapy leveraging the evolution of phage resistance to steer bacterial evolution toward clinically benign phenotypes. While such phage resistance trade-offs have been demonstrated in a limited number of laboratory reference strains, whether and how trade-offs vary across diverse clinical isolates is not known. Here, we explored how phage resistant trade-offs driven by a lipopolysaccharide-targeting virulent phage varied across three *Pseudomonas aeruginosa* clinical isolates. We first isolated spontaneous phage-resistant mutants from 10 independent cultures per strain and confirmed phage resistance via cross-streaking and EOP. We then compared the performance of mutants relative to their phage-susceptible ancestral strain using microplate growth curves. This revealed that phage resistant mutants from one background showed consistent performance trade-offs, while mutants from the other two strains displayed variable outcomes, with selected mutants exhibiting equal or improved growth compared to their ancestor.
Antibiotic susceptibility against three different antibiotics (ceftazidime, colistin and tobramycin) was also evaluated by standard broth microdilution assay.
Similarly to the growth curves, mutants from a genetic background showed consistent lower MIC values for all drugs compared to the ancestral strain, while the other two displayed cases of lower or higher MIC values of the wild-type bacteria.
Phage resistant mutants will be analyzed through sequencing and additional phenotypic assays.
These findings demonstrate that the outcome of phage steering is variable across clinical isolates, underscoring the importance of characterizing phage-driven trade-offs in a wider set of strains.

The *Pseudomonas aeruginosa sirB2* gene is a fitness determinant of anaerobic growth and its inactivation affects virulence and rugose small colony variant

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Pseudomonas aeruginosa (*Pa*) chronic infections in patients with cystic fibrosis (pwCF) are challenging to eradicate. Infection success relies on *Pa*'s ability to adapt to the complex CF lung environment. Transcriptional analysis of *Pa* communities from sputum samples indicates that *Pa* growth in CF airways is associated with a distinct transcriptional profile. Most of the genes modulated *in vivo* remain poorly characterized.

In this study, we characterized the gene of unknown function PA14_RS04555 (*sirB2*), whose expression is particularly stimulated in the CF lung environment and shares homology with virulence determinants in *Salmonella enterica*. Our research indicates that *sirB2* is transcriptionally controlled by the virulence regulators Vfr and AmrZ. Its deletion enhances *Pa* pathogenicity, increasing virulence in *Galleria mellonella* larvae and promoting bacterial translocation and biofilm formation in a differentiated human airway epithelial infection model. In vitro, we confirmed that *sirB2* inactivation triggers biofilm formation only when oxygen access is restricted. Under these conditions, the *sirB2* mutant leads to an increased emergence of hypervirulent rugose small-colony variants (RSCV) through the accumulation of secondary mutations in the *wsp* operon, thereby increasing the second messenger c-di-GMP levels. Our data indicate that RSCV emergence is linked to an imbalance in the NAD+/NADH ratio under oxygen-limited conditions. Indeed, the absence of the *sirB2* gene reduces fitness under anaerobic growth conditions with nitrate as the sole electron acceptor, and this phenotype is independent of the ubiquinone pool, suggesting that the *sirB2* gene is an important determinant of survival in the lungs of pwCF.

Further studies are underway to decipher the mechanism of action of the *sirB2* gene.

The *yhbB* gene contributes to the structural organization of the *Bacillus subtilis* spore coat in a temperature-dependent manner

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The spore surface of *Bacillus subtilis* is a highly organized and multilayered structure that plays a crucial role in environmental persistence and stress resistance. The correct assembly of the spore coat depends on a tightly regulated network of sporulation-specific transcription factors (1), yet the function of several coat-associated proteins remains insufficiently understood. Among these, we investigated the role of YhbB in spore coat organization. Transcriptional reporter fusions confirmed the previously reported σ^E-dependent expression of *yhbB* (2) and revealed an additional requirement for SpoIIID, with expression further modulated by temperature. YhbB-GFP fluorescence was clearly detected during sporulation at 25 °C but was strongly reduced at 37 °C, indicating temperature-dependent regulation acting at the transcriptional and/or post-transcriptional level, potentially involving the 5′UTR region. Fluorescence microscopy showed that YhbB localizes around the forespore, forming ring-like structures consistent with coat assembly intermediates. In a *cotH* mutant background, YhbB localization was altered at late sporulation stages, consistent with previous evidence of a functional interaction between YhbB and CotH, a coat protein implicated in coat stability through covalent cross-linking reactions (3). Functional assays revealed that *yhbB* mutant spores produced at 25 °C display reduced germination efficiency, particularly in response to alanine, whereas no differences were observed in spores produced at 37 °C. These results indicate that YhbB-dependent structural features established during sporulation at lower temperature influence spore germination efficiency. Overall, our findings identify YhbB as a σ^E/SpoIIID-dependent factor contributing to temperature-dependent spore coat organization, consistent with a structural role linked to CotH-mediated coat stabilization.

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Oral presentation

PhD DAY (II)

Andrea Quagliariello & Roberta Provvedi

Book of abstracts

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Host–Microbiota Crosstalk in Ovarian Cancer

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Tumor-associated microbiota is increasingly recognized as an active component of the cancer microenvironment, yet its functional contribution to ovarian cancer progression and therapeutic response remains poorly defined. In this study, we experimentally investigated bidirectional host-microbe interactions in ovarian cancer and integrated whole-genome sequencing to identify the genetic basis underlying observed phenotypes. We isolated 45 bacterial strains from ovarian cancer and non-malignant ovarian tissues and performed extensive phenotypic characterization, including morphology, motility, biofilm formation, and antibiotic susceptibility testing. Chemotherapeutic drug-response assays showed that standard treatments (carboplatin, paclitaxel, and doxorubicin) reduced viability of ovarian cancer cell lines (SKOV3, OVCAR3, Kuramochi), the non-malignant fallopian tube epithelial model (FT190), and patient-derived ovarian organoids to ≤10–20%. In contrast, the majority of patient-derived bacterial isolates, including *Escherichia coli*, *Staphylococcus hominis, Streptococcus constellatus, Klebsiella michiganensis*, *Lactobacillus paracasei*, and *Lactobacillus zeae*, remained highly tolerant to these agents under both aerobic and anaerobic conditions. To directly assess functional host-microbe crosstalk, we performed soluble-factor interaction assays using bacterial and host-derived supernatants. Cell-free supernatants from all isolates (including tumor-associated, non-malignant tissue-derived, and reference strains) collected during logarithmic and stationary growth phases, induced divergent host responses ranging from approximately 70% growth inhibition to 45% proliferation enhancement, depending on bacterial species and growth state. Together, these findings demonstrate that tumor-associated bacteria engage in dynamic, context-dependent interactions with ovarian cancer cells through drug tolerance and soluble-factor–mediated effects, highlighting the importance of incorporating microbiological assays into ovarian cancer research and therapeutic evaluation.

INVOLVEMENT OF DURA MATER TISSUE IN RODENT MODELS OF PNEUMOCOCCAL MENINGITIS

Giulia Cattabriga[1], Denis Grandgirard[2], Maria Erhardt[2], Marco Caprini[1], Stephen Leib[2], Marco R Oggioni[1], Claudia Trappetti[1].

Background: Pneumococcal meningitis remains the most common cause of bacterial meningitis in children worldwide. However, the mechanisms underlying its pathogenesis remain poorly understood.
Methods: We investigated the spatial distribution of *S. pneumoniae* during meningitis in rodents and its interactions with vascular endothelium (CD31) and macrophages (CD169) in two distinct in vivo infection models. Using microscopy-based analyses, we quantified and localised bacteria within the brains of infected animals.
Results: Quantitative image analysis showed that, in our hematogenous meningitis model, a substantial proportion of bacteria in the dura mater were extravascular, consistent with early tissue invasion. At later time points, bacterial invasion was instead observed in the pia mater and the choroid plexus. In the intracranial meningitis model, dural macrophages displayed infection-associated morphological changes, and *S. pneumoniae* localized predominantly to macrophages at 18 hpi and to vessels at 42 hpi.
Conclusions: Our findings reveal that differences between the choroid plexus, the pia and dura mater reflect distinct tissue-specific dynamics. Overall, these results highlight the central role of perivascular macrophages in pneumococcal persistence and host–pathogen interactions during meningitis, providing new insights into disease pathogenesis and potential therapeutic targets.

Elucidating key steps of *Pseudomonas aeruginosa* infection by DEV lytic phage for phage therapy.

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Phage therapy is a promising strategy to combat infections caused by antibiotic-resistant bacteria, including *Pseudomonas aeruginosa (Pa*). Understanding the biology of therapeutic phages is essential to ensure their safety and efficacy. I am investigating the adsorption mechanism of DEV, a lytic phage killing *Pa*. DEV gp53 fibers are required to infect Pa strains exposing on their outer membrane (OM) a lipopolysaccharide (LPS) capped with the O-antigen moiety, which is DEV primary receptor, but are dispensable in *galU* mutants lacking the O-antigen, suggesting a secondary receptor and a distinct receptor-binding protein.[1,2] To identify DEV secondary receptor, we isolated a DEV-resistant *galU* double mutant. The mutant carried a 33-bp deletion in *lptD*, an essential gene encoding an OM protein. Complementation assays demonstrated that *lptD* expression restored phage adsorption, suggesting that LptD may be the secondary receptor. In agreement with this hypothesis, the expression of *P. aeruginosa lptD* in *E. coli*, normally not susceptible to DEV, enabled adsorption.To identify LptD receptor binding protein, UV mutagenesis of wt DEV or DEV Δ*gp53* mutant was performed. The phages were plated on the DEV resistant *galU lptD* strain and mutant phages forming plaques were genome sequenced. In both cases, mutations were identified only in the *gp54* gene, identifying gp54 as the LptD-specific receptor-binding protein. In collaboration with G. Cingolani group (UAB, USA), we demonstrated that gp54 forms a complex with gp56 and gp55 that regulates DEV tail unplugging.[3]Given the high conservation of gp54 among *Litunaviruses*, other phages in this genus may also exploit LptD as their receptor.

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From colistin persistence to resistance in carbapenem resistant *Acinetobacter baumannii*: evidence for a novel toxin–antitoxin mRNA–asRNA regulatory mechanism

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Background: Bacterial persistence is a transient, non-heritable state of metabolic dormancy that allows susceptible sub-populations to survive lethal antimicrobial concentrations and represents a critical evolutionary reservoir facilitating the transition towards stable genetic resistance. In high-risk pathogens, such as Carbapenem-Resistant *Acinetobacter baumannii* (CRAB), understanding the molecular mechanisms underlying this transient-persistent state is essential to elucidate how bacteria survive antibiotic exposure. Here, we investigated colistin persistence subpopulations and their Toxin/Antitoxin (T/A) omics in ST2 clinical colistin-susceptible (COL-S) CRAB that subsequently developed full and stable *in-vivo* COL-R.

Methods: High-dose colistin time-kill assays were performed to detect persisters in 10 clinical CRABs. Genomics and basal transcriptomics of chromosomal/plasmid toxin–antitoxin systems (T/As) were conducted in two representative ST2 COL-S CRAB to investigate the genomics and basal T/A transcriptional profiles.

Results: All strains showed a persistent subpopulation (~1% survival at 8h) under 5X COL-MIC exposure. Genomic analysis identified ten type-II and one type-IV T/A systems. Basal transcriptomic profiling revealed active expression patterns mainly involving GNAT superfamily T/A modules, with consistently low toxin mRNA levels associated with toxin- or antitoxin-directed antisense RNAs (asRNAs) in chromosomal loci. This regulatory architecture supports novel dual-combined models in which asRNAs act as primary modulators of T/A transcript balance, potentially influencing persistence-associated dormancy transitions mainly via a translational-termination mechanism. Conversely, the plasmid-encoded BrnT/A module showed a highly balanced expression profile.

Conclusion: Our findings highlighted the type-II GNAT T/A superfamily as molecular switchers of T/A transcript-balance by a novel asRNA–mediated regulatory mechanism in high-risk developing colistin persistence and resistance CRABs.

Decoding Drug Efficacy and Host–Pathogen Interactions in Tuberculosis Using a Standardized *In Vitro* Granuloma-Like Structures Model

Enrica Campagnaro[1], Enrico Mastrostefano[2], Greta Segafreddo[1], Davide Sorze[1], Alessandro Stamilla[3], Shaiq Sultan[1], Davide Moretti[2], Michael Dal Molin[4,5], Santiago Ramón-García[6,7], Maria Rosalia Pasca[3,8] and Riccardo Manganelli[1] on behalf of the ERA4TB Consortium.

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Tuberculosis remains a major global health challenge, largely due to the ability of *Mycobacterium tuberculosis* (*Mtb*) to evade immune responses and persist within granulomatous lesions. Replicating these complex host–pathogen interactions *in vitro* remain difficult, as widely used models fail to capture the multicellular organization and dynamic microenvironment of granulomas, ultimately limiting drug development.

In this study, we developed and refined a human PBMC-derived Granuloma-Like Structure (GLS) model and directly compared it with conventional macrophage monolayers to evaluate drug activity in a more representative setting. A panel of 11 antitubercular compounds was tested across a range of concentrations and experimental replicates. We also investigated drug combinations mimicking the clinically used BPaL regimen within the GLS system.

To improve robustness, we integrated CFU-based bacterial quantification with imaging-driven analysis of GLS formation and evolution, combining brightfield and time-lapse microscopy. This approach, supported by a hybrid human- and AI-assisted pipeline, enabled quantitative assessment of GLS size and number, highlighting how similar CFU values may arise from structurally distinct granuloma populations. Consistently, drug efficacy varied with GLS size distribution, a relationship further supported by a simple mathematical framework and *in silico* modeling.

Comparison with monolayer cultures revealed distinct drug response profiles, with GLS better capturing immune-related effects. Interestingly, early GLS conditions appeared to favor bacterial expansion. Altogether, this work supports GLS as a reproducible and scalable platform for studying tuberculosis and improving preclinical drug evaluation.

Linking air and leaves: disentangling the main drivers of microbial assembly and exchange in the urban phyllosphere

Riccardo Grimoldi[1], Isabella Gandolfi[1], Maya Petricciuolo [2], Andrea Firrincieli [3], Ermanno Federici [2], Vittorio Vinciguerra [3], Maurizio Petruccioli [3], Silvia Crognale [3] , Sarah Caronni [1], Andrea Franzetti[1]

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Urban vegetation is known to support diverse microbial communities that play key roles in ecosystem functioning. However, the mechanisms governing their assembly and connectivity with the atmosphere remain incompletely understood. The present study investigates the relative contributions of different ecological drivers in shaping bacterial and fungal communities of the phyllosphere and their linkage with airborne microbiomes across an urban gradient in Milan (Italy). Leaves from four common plant species and particulate matter were sampled on a seasonal basis over one year, and microbial communities were characterised through 16S rRNA and ITS1 amplicon sequencing.

Multivariate analyses revealed that plant species, season, and sampling area (and their combinatory effects) are important drivers in shaping the microbial communities. Network-based indicator species analysis demonstrated substantial connectivity between air and phyllosphere microbiomes, with numerous taxa shared across habitats and plant species, suggesting active microbial exchange. This connectivity was particularly evident during transitional seasons, when overlap between airborne and leaf-associated communities changed actively.

Seasonal transition analyses further demonstrated dynamic shifts in microbial distribution, with alternating patterns of homogenization and diversification between habitats. Notably, bacterial communities exhibited stronger dispersal from shared to phyllosphere-specific assemblages, whereas fungal communities displayed different temporal dynamics.

The findings demonstrate that the composition of urban phyllosphere microbiomes is influenced by a complex interplay of deterministic host filtering and stochastic airborne dispersal. These have significant implications for our understanding of microbial contributions to ecosystem services in urban environments, thereby influencing the decisions of policy-makers and local authorities in the development of greener cities.

PhD DAY (III)

L. Baccigalupi & Anella Saggese

Book of abstracts

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Title: Potential Role of Treated Wastewater and Heavy Rainfall Events on Environmental Spreading of AMR into the Lambro River (Lombardy Region, Italy)

Presenting Author: Bruno Erika[a]

Authors: Lorenzini Sara[b], Lovati Giovanni[b], Pedrazzi Luca[b], Musumeci Rosario[c], Cocuzza Clementina E.[c], Franzetti Andrea[a], and Gandolfi Isabella[a]

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Background: Antibiotic-resistance indicators (ARB, ARGs, and MGEs) are frequently detected in environmental matrices, including Wastewater Treatment Plant (WWTPs) effluents. Wastewater Bypass could also contribute to their dissemination, especially during stormwater events. This project aimed to assess the potential impact of heavy rainfall on the release of ARGs and ARB into the receiving river.

Method: Water samples were collected along the Lambro River (Northern Italy), at five sampling points, in the absence and in the presence of high-intensity rainfall events. ARB were quantified using culture-based methods. DNA was then extracted for the quantification of *16S rRNA*, *intI1*, and seven ARGs. Bacterial community composition was investigated via *16S rRNA* gene Amplicon Sequencing on MiSeq – Illumina platform.

Results: In absence of rainfall, several ARGs showed significantly higher concentrations downstream of the WWTP compared to upstream, without a corresponding increase in culturable ARB. During rainfall events, both ARGs and ARB significantly increased along all the river sites, suggesting a potential contribution of wastewater bypass, stormwater runoff, and potentially additional upstream sewer overflows. PCA analysis revealed that the riverine bacterial communities collected during rainfall clustered closely with wastewater influent samples, highlighting a strong wastewater-related signature in the river during high-intensity rainfall conditions.

Conclusions: WWTP effluents may contribute to the environmental dissemination of ARGs in absence of extreme weather events, while rainfall acts as a major driver of widespread AMR dissemination, highlighting the anthropogenic pressure on the Lambro River and the importance of considering hydrological conditions when assessing environmental AMR dynamics.

Broad-spectrum analysis of beta-lactam resistant *Granulicatella adiacens*: from Penicillin-Binding Proteins mutational and antibiotic affinity profile to cell morphology and peptidoglycan composition

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Beta-lactam resistance in Gram-positive bacteria is mainly driven by penicillin-binding proteins (PBPs) alterations. They can also adapt their cell wall after antibiotic exposure. *Granulicatella adiacens* infections are typically treated with beta-lactam antibiotics but their resistance is increasing. This work aims to characterize *G. adiacens* PBPs and their mutations' role in beta-lactam resistance and evaluate peptidoglycan alteration determined by antibiotic stress.

Beta-lactam activity, synergy, and PBP binding were compared between IS48 clinical isolate and *G. adiacens* ATCC 49175. Affinity to ceftriaxone, ceftobiprole, and ampicillin was assessed with BocillinFL. PBP homology and mutations were analysed by sequence alignment and WGS. PBP1A was cloned and expressed in *E. coli* BL21(DE3) into pET24 and purified. Growth curves were performed in supplemented BHI with D-amino acids probes to analyse cell morphology. Peptidoglycan composition was studied *via* LC-MS after antibiotic exposure to ceftobiprole, ceftriaxone, ampicillin (1/2-fold MIC).

Normal diplococci morphology emerged only in BHI supplemented with blood and L-cysteine. IS48 was not susceptible to penicillin and ampicillin, resistant to ceftriaxone, with higher ceftobiprole MIC than the control strain. Ampicillin *plus* ceftobiprole/ceftriaxone were synergistic. Five PBPs were identified. Most IS48 PBPs carried mutations near catalytic motifs. PBP2B and PBP2 exhibited consistently low acylation levels, whereas PBP1A and PBP2A demonstrated significant inhibition. PBP1B reached IC_{50} only by double treatments in the ATCC strain and by ceftriaxone and ampicillin/ceftriaxone in IS48.

The mutations observed may affect the drug binding, impacting on the resistance phenotype. However, beta-lactam treatment may be effective due the inhibition of key bifunctional PBPs (PBP1A, PBP2A).

Prokaryotic diversity and ecosystem functioning in hypersaline environments: a metagenomic study of the Cagliari salterns

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Solar salterns are extreme environments of high scientific interest, hosting specialized microbial communities shaped by steep physicochemical gradients. Here we present the first comparative metagenomic analysis of two solar salterns in southern Sardinia: Conti Vecchi (SCV, active) and Molentargius (SM, inactive), located ~15 km apart and exposed to similar climatic conditions. This setting provides a unique opportunity to assess the impact of saltwork management on microbial diversity along a salinity gradient.

Water and sediment samples were collected from ponds with varying salinities in winter and summer. A total of 2786 metagenome-assembled genomes (MAGs) were reconstructed, 1537 from SCV and 1249 from SM, including 1131 MAGs from sediments and 1655 from water. Physicochemical analyses identified seasonal variation as the primary driver of environmental differences (*p*-value < 0.001), explaining 61% of total variance, although site-specific differences in thermal range and pond structure were also observed.

Benthic trophic conditions varied markedly across ponds. The medium-low salinity pond SCV-1 (57 psu) was eutrophic, with organic carbon exceeding microbial processing capacity, while the high-salinity pond SCV-17 (275 psu) showed near-complete depletion of biopolymeric carbon. Metagenomic results supported these patterns, with *Pseudomonadota* and *Bacteroidota* dominating at low salinities, and *Halobacteriota* prevailing in high-salinity ponds.

In SM, the high salinity pond SM-3 (260 psu) exhibited a striking seasonal shift, with high degradation rates and rapid turnover in winter, and reduced activity in summer, suggesting substantial changes in microbial community. Taxonomic analysis confirmed this frame, revealing an unexpected dominance of Bacteria despite the high salinity. In addition, a large fraction of MAGs could not be assigned to known taxa, suggesting unexplored novelty spanning from species to higher taxonomic levels. Overall, these findings position southern Sardinia salterns as unexplored reservoirs of prokaryotic novelty and underscore the importance of expanding metagenomic investigations to other under-characterized Mediterranean extreme environments.

Microbiome Structure and Dynamics in Novel Fixed-Bed Biofilm Systems for Medium Chain Fatty Acids Production from Organic Waste

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The microbial valorization of organic waste into medium-chain fatty acids (MCFAs) represents a promising resource recovery strategy. However, its efficiency is often limited by competition between primary fermenters and chain-elongating bacteria, with the former favored in the commonly-used fluidized-bed systems due to their higher growth rates and substrate uptake. To overcome this limitation, this study developed a novel single-stage fixed-bed biofilm reactor for the co-fermentation of food waste (FW) and waste activated sludge (WAS). Several tests were conducted under mesophilic conditions (35 °C) with a 4-day hydraulic retention time and varying organic loading rates (OLRs of 6.2, 12.4, and 18.75 $gVSL^{-1}d^{-1}$), without external pH control or the addition of external electron donors. Initially, two support-to-inoculum ratios (1:3 and 1:1 w:V) were tested using a mixture of FW and WAS at a ratio of 60:40 (on VS basis). After identifying 1:1 w:V as the best-performing ratio, additional tests were carried out using different substrates (i.e., FW:WAS at a ratio of 80:20, and FW liquid extract combined with WAS at a 60:40 ratio) combined with different feeding frequencies (i.e., 2, 3 , and 5 days per week). Control reactors without filling material were operated in parallel for each tested conditions. A comprehensive chemical and microbiological characterization of biofilm and suspended biomass was performed using complementary advanced techniques (e.g., Illumina and Nanopore sequencing, microscopy-based analysis, and real-time quantification). By promoting microbial selection and biomass retention, fixed-bed biofilm systems enhanced system stability and MCFAs production and consistently outperformed controls, representing an effective strategy for valorizing complex organic waste into high-value chemicals.

Characterization of microbial communities and functional diversity associated to native plant species *Pistacia lentiscus* L. and *Helichrysum microphyllum* subsp. *tyrrhenicum* in abandoned mining areas

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Among phytoremediation approaches, phytostabilization occurs at the root-substrate interface, where excluder-type metallophytes and associated microorganisms mitigate metal mobility and bioavailability. This study presents an integrative analysis of microbial diversity associated to two native plants of Sardinian abandoned mining areas and candidate for revegetation and restoration programmes. Spontaneous plants were studies in a Zn-Pb mine tailing dump and its surrounding areas. To deepen our understanding of the interaction among mine substrates, metals, plants, and microbes under real field conditions, a multifactorial approach was employed evaluating dehydrogenase activity, Community Level Physiological Profiling, bacterial and fungal communities through high-throughput sequencing of ribosomal genes in mine tailings, rhizosphere and roots. The studied site exhibited significant heterogeneity in environmental parameters and metal concentrations. Analysis revealed differences between the two plant species in metabolic activities and highlighted distinct abiotic drivers shaping bacterial and fungal community structures. Bacterial communities associated with the rhizosphere and roots differed between plant species, while fungal communities of both plants were dominated by the same taxa. This study represents the first comprehensive characterization of microbial communities associated with *P. lentiscus* and *H. tyrrhenicum*. Our findings demonstrate that different plant species select different microbial communities, providing critical insights into the ecological roles of root-associated microbiomes and their potential for site-specific remediation strategies. This work has been developed within the framework of the project e.INS www.einsardinia.eu (Next Generation EU- PNRR-M4 C2 I1.5 CUP F53C22000430001).