PhD DAY (I)

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Book of abstracts

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

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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

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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