INTERNATIONAL BIENNIAL PSEUDOMONAS CONFERENCE

1–5 September 2024

#Pseudomonas2024

INVITED AND OFFERED TALKS

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INTERNATIONAL BIENNIAL CONFERENCE
Pseudomonas
Copenhagen, Denmark 2024

The Scandic Copenhagen Hotel
Vester Søgade 6, DK-1601,
Copenhagen V, Denmark

MICROBIOLOGY SOCIETY

Invited talk: Conflict-management and molecular diplomacy of the TOL plasmid for happily inhabiting *P. putida*

Victor de Lorenzo

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Victor de Lorenzo

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Abstract

Environmental bacteria such as *Pseudomonas putida* often receive new metabolic genes and pathways through various HGT mechanisms, thereby expanding their biochemical network towards new substrates. However, for achieving durable implantation, the new activities need to adjust various enzymatic, regulatory and spatial parameters to the existing molecular landscape of the recipient cells. The process through which the TOL plasmid pWW0 of *P. putida* mt-2 has ended up as a stable—yet autonomous—component of the metabolic complement of the strain, exposes a number of conflicts between the incoming biochemical module for degradation of toluene/*m*-xylene and the already existing route for benzoate catabolism. To solve these, in particular the critical routing of shared catechol intermediates through either an *ortho* or *meta* cleaving pathway, diverse evolutionary patches seem to have emerged that alleviate the ensuing physiological stress. To gain further insights on how the new toluene/*m*-xylene-degrading routes can cohabit with the preceding network, the *upper* and the *lower* pathways of the TOL plasmid were excised from their natural DNA context, reassembled in a conjugative vector and reintroduced in the plasmid-cured variant of *P. putida* mt-2 named KT2440. Adaptive laboratory evolution of the resulting strain for growth on *m*-xylene delivered variants that had acquired significant changes in the regulatory devices that rule transcription of the *upper a*nd the *lower* operons. Taken together, the data exposed a complex series of tradeoffs between the new genes and the host's standing metabolism which could inform more effective approaches for designing heterologous gene expression in e.g. metabolic engineering.

Invited talk: Structural studies of Pseudomonas aeruginosa biofilm formation using electron cryotomography

Tanmay Bharat

MRC LMB, Cambridge, United Kingdom

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Abstract

Most prokaryotes including *Pseudomonas aeruginosa* form macroscopic, surface-attached, multi-cellular communities known as biofilms. Biofilms constitute the majority of bacterial biomass on earth, representing a fundamental mode of prokaryotic existence. While biofilms may prove beneficial to eukaryotes as host-associated microbiomes, the formation of bacterial biofilms by pathogens such as *P. aeruginosa* is associated with the establishment of serious chronic antibiotic-tolerant infections.

We perform structural biology of *in vitro* reconstituted specimens, coupled with *in situ* imaging of bacterial biofilms, to investigate how molecules on the surface of *P. aeruginosa* cells mediate biofilm formation. In my talk, I will present molecular structures of several biofilm related molecules from *P. aeruginosa*, focusing in particular on filamentous proteins that stabilise the biofilm matrix. I will relate these structures with high-resolution pictures of bacterial biofilms, which together provide insights into how bacterial biofilms are organised at the molecular level.

Our work on several different molecules present on the *P. aeruginosa* cell surface in biofilms, allows us to propose two distinct mechanisms by which biofilms are stabilised. Our work also provides ideas for therapeutic intervention against *P. aeruginosa* biofilms, which have allowed us to begin development of multiple biofilm inhibitors.

Invited talk: Surface-active metabolites (biosurfactants) as socially beneficial exoproducts in cooperative bacterial behaviours

Eric Déziel

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Eric Déziel

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Abstract

Microorganism can alter their physical environment, which can affect the behavior of surrounding cells. Some bacterial species produce extracellular amphiphilic molecules, but their exact function often remains unclear, as they seem to play several roles. One emerging picture is their function as «wetting agents», promoting the rapid surface migration of a group of bacteria in a phenomenon typically called «swarming motility». Such social form of motility is performed by several bacterial species, which have in common, beside at least one flagellum, the biosynthesis and release of surface-active metabolites. These biosurfactants support social behaviors like swarming motility and contribute to emerging properties of organized microbial communities, such as antibiotic tolerance.

Pseudomonas aeruginosa is very well known for the production of a mixture of surfactants called rhamnolipids and its ability to swarm, both of which requiring cell-cell communication (quorum sensing). Rhamnolipids and their associated metabolites confer complex effects to the swarming group behavior of this bacterium.

We know that interactions between different bacterial species can be detrimental, neutral or beneficial in a mixed community. There is accumulating evidence that biosurfactants can act as shared metabolites, mediators of interspecies interactions, where bacteria not producing a biosurfactant can benefit from the metabolites produced by another. A few examples have been identified, for instance between *P. aeruginosa* and *Burkholderia cenocepacia*. Surface-active exoproducts could be considered sharable «public goods» that can be exploited by neighbour cells in a community. We are just starting to uncover the ecological implications of biosurfactant production in the context of microbial interactions.

Invited talk: Characterizing bacterial behavior during human infection to guide new discoveries

Marvin Whiteley

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Abstract

Bacterial behavior and physiology during human infection is difficult to study and largely unknown, as our vast knowledge of infection microbiology is primarily derived from studies using in vitro and animal models. A key challenge to assessing bacterial physiology during human infection is the difficulty in acquiring and assessing bacterial function in human-derived samples. Here, I will discuss the use of microbial metatranscriptomics from chronic human wound, lung, and oral infections to tackle this gap in knowledge. We have leveraged these data in two primary ways: to assess and improve the accuracy of pre-clinical infection models using a quantitative framework recently developed in our lab; and identifying and functionally characterizing genes of unknown function that are highly expressed in humans but not in most pre-clinical models. I will also discuss additional approaches we are using to quantify microbial biogeography and heterogeneity within human infections, with the goal of using these data to develop accurate pre-clinical models for antimicrobial discovery.

Invited talk: *Pseudomonas* ecology in the wheat rhizosphere – microdiversity, colonization, and functional aspects

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Mette Nicolaisen

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Abstract

Plants recruit and maintain a unique rhizosphere microbiome which provides important functions for the plant like nutrient acquisition, stress alleviation as well as disease suppression. One key group of the rhizosphere microbiome is the genus *Pseudomonas*. This genus is of particular interest in relation to plant-microbe interactions owing to its intrinsic intragenus genomic diversity and assortment of secondary metabolites of importance for providing beneficial functions supporting plant growth. Despite this, we still lack a fundamental understanding of the *Pseudomonas* ecology and how this genus interacts with plant roots under complex soil conditions. Specifically, we need to understand the drivers for community assembly and functions on the plant roots to fully harness the beneficial potential of these interactions in an agricultural setting.

We are approaching aspects of the *Pseudomonas* ecology, focusing on root-associated *Pseudomonas* taxonomic and functional microdiversity of commercially available cultivars of winter wheat (*Triticum aestivum L.*) as well as the importance of *Pseudomonas* for microbe-microbe interactions for expression of plant-beneficial traits. Our work shows that while different wheat cultivars recruit similar proportions of *Pseudomonas* to its rhizosphere microbiome, they select for distinct *Pseudomonas* genotypes, resulting in pronounced differences in microdiversty across cultivars. This highlights that understanding *Pseudomonas* ecology in the rhizosphere is of paramount importance to develop strategies for manipulating plant-microbe interaction for optimized crop production.

Invited talk: Harnessing carbon-free energy sources for enhanced redox metabolism in Pseudomonas and sustainable bioproduction

Birgitta Ebert

The University of Queensland, Queensland, Australia

Abstract

Redox metabolism is essential for cellular function and the biosynthesis of industrially relevant molecules, particularly highly reduced compounds such as fatty acid derivatives used in biofuels, lubricants, and other chemicals. Efficient redox processes are vital for the bioproduction of these compounds, which demand substantial reducing power.

Pseudomonas species, known for their robustness and metabolic plasticity, exhibit significant capacity in regenerating reduced redox cofactors under increased demand. Consequently, *Pseudomonas*, in particular *Pseudomonas putida*, are often superior in producing chemicals, where the regeneration of redox cofactors like NADH and NADPH is crucial for maintaining high biocatalytic activity.

However, heterotrophic metabolism in *P. putida* generates redox equivalents through the oxidation of carbon sources, leading to significant carbon losses as CO_2 and impairing carbon efficiency. To address this limitation, our current work explores the co-utilization of carbon-free energy sources, particularly hydrogen, to reduce dependence on carbon source oxidation for redox cofactor regeneration. This approach not only decreases CO_2 emissions but also enhances the carbon efficiency of the microbial cell factory.

This presentation will explore the potential of synthetic hydrogenotrophy for sustainable biomanufacturing, highlighting the use of *Pseudomonas* as a versatile microbial cell factory for H_2-driven bioproduction. The findings underscore the promise of this strategy in the production of reduced industrial compounds.

Invited talk: Advances in biofilm formation research in *Pseudomonas aeruginosa*: focus on genetic pathways and hypermutability

Andrea Smania

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Andrea Smania

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Abstract

Pseudomonas aeruginosa, a notorious opportunistic pathogen, poses a formidable challenge in chronic infections due to its robust antibiotic resistance and biofilm formation. This bacterium serves as a compelling model organism to elucidate the role of hypermutation in bacterial adaptation. Our research explores the intricate interplay between the second messenger bis-(3'→5')-cyclic dimeric guanosine monophosphate (c-di-GMP) signaling, biofilm development and hypermutability in *P. aeruginosa*. We observed that during cycles of biofilm formation and dispersal, compensatory mutations progressively inactivate genes regulating c-di-GMP synthesis and degradation, leading to a state of genetic constraint that hinders further biofilm formation. Intriguingly, mutator strains subjected to the same evolutionary pressures overcome this constraint, suggesting the existence of alternative biofilm formation pathways. We employed a CRISPR-based genome-editing tool to disrupt all 32 enzymes involved in c-di-GMP synthesis in *P. aeruginosa* UCBPP-PA14, resulting in a mutant strain (PA14Δ32) devoid of biofilm formation. Parallel evolution experiments using PA14Δ32 and its isogenic mutator derivative, PA14Δ32*mutS*, revealed that a significant proportion of PA14Δ32*mutS*-derived lines exhibited biofilm formation compared to the negligible biofilm production observed in the normal-mutator lines. These findings highlight the remarkable ability of hypermutability to circumvent genetic constraints and unlock alternative evolutionary routes for biofilm formation. Our research paves the way for the identification of novel mutational pathways governing biofilm development in *P. aeruginosa*, potentially leading to the development of more effective therapeutic strategies against chronic infections.

Invited talk: Maximizing bioconversion in *Pseudomonas putida* using functional genomics and systems biology approaches

Aindrila Mukhopadhyay

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Abstract

Pseudomonas putida KT2440 Is a versatile production host that provides many catabolic routes, tolerances and biosynthetic capabilities that can be optimized or engineered to convert not only sugars but other major components of engineered or sustainable feedstocks, such as aromatics from lignin. We have used Genome Scale Metabolic Models (GSMMs) to compute gene intervention designs that both increase flux, and pair substrate utilization to the desired product or precursor. The GSMM designs are further refined using functional genomics data. These multigene edit designs when implemented provide strong growth coupling and robust production metrics (TRY) across scales and production formats. Implementation of these approaches for non-canonical carbon sources such as aromatics present a challenge due to metabolic models that are less defined and missing knowledge of their catabolism by P. putida KT2440. To address this, we took an ensemble approach that combined initial GSMM-based designs with laboratory evolution, and design curation using functional genomics. We further explored the underlying challenge in implementing a complete GSMM-based design for the model aromatic para-coumarate in P. putida and gained key insights for the role of multifunctional enzymes and metabolic cross-feeding. Finally, we extrapolated the use of such GSMM- and systems biology- guided models for the production of a SAF (sustainable aviation fuel) blend stock, isoprenol, in P. putida.

Offered talk: A novel stabilization mechanism for the type VI secretion system sheath

Patricia Bernal[1], R. Christopher D. Furniss[2], Selina Fecht[3], Rhoda C.Y. Leung[3], Livia Spiga[3], Despoina A.I. Mavridou[4], Alain Filloux[5]

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Patricia Bernal

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Abstract

The type VI secretion system (T6SS) is a phage-derived contractile nanomachine primarily involved in interbacterial competition. Its pivotal component, TssA, is indispensable for the assembly of the T6SS sheath structure, the contraction of which propels a payload of effector proteins into neighboring cells. Despite their key function, TssA proteins exhibit unexpected diversity and exist in two major forms, a short ($TssA_S$) and a long ($TssA_L$) TssA. Whilst $TssA_L$ proteins interact with a partner, called TagA, to anchor the distal end of the extended sheath, the mechanism for the stabilization of $TssA_S$-containing T6SSs remains unknown. Here we discover a novel class of structural components that interact with short TssA proteins and contribute to T6SS assembly by stabilizing the polymerizing sheath from the baseplate. We demonstrate that the presence of these components is important for full sheath extension and optimal firing. Moreover, we show that the pairing of each form of TssA with a different class of sheath stabilization proteins results in T6SS apparatuses that either reside in the cell for a while or fire immediately after sheath extension. We propose that this diversity in firing dynamics could contribute to the specialization of the T6SS to suit bacterial lifestyles in diverse environmental niches.

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Low level of metabolic auxotrophies among environmental Pseudomonas isolates

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Abstract

There is increasing evidence that cross-feeding – the exchange of a metabolic product between individual cells – is common in bacteria. However, many studies on cross-feeding involve defined (and often engineered) laboratory study systems, while it is less clear how prevalent the exchange of metabolites is in natural communities.

Here, we used a collection of 320 Pseudomonas strains isolated from pond and soil habitats to quantify the frequency of amino acid auxotrophies. Auxotrophic strains are unable to produce a specific metabolite and thus become dependent on other strains to acquire the metabolite via cross-feeding. Our screen, involving defined agar minimal media in the presence or absence of specific amino acids, revealed very low frequencies of amino acid auxotrophy among pseudomonads. Specifically, we identified one strain with a histidine auxotrophy and six strains with unspecific auxotrophies, while all other strains were autonomous prototrophs. Follow-up experiments with mixed cultures revealed no clear evidence for cross-feeding. Finally, preliminary bioinformatic genome analysis point towards high metabolic versatility and amino acid prototrophy for most *Pseudomonas* strains. Taken together, our results show that environmental *Pseudomonas* strains seem to be predominantly generalists with a high level of metabolic autonomy and little engagement in cross-feeding.

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Evolution and host-specific adaptation of Pseudomonas aeruginosa

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Aaron Weimann

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Abstract

The major human bacterial pathogen, *Pseudomonas aeruginosa,* causes multidrug-resistant infections in people with underlying immunodeficiencies or structural lung diseases, such as Cystic Fibrosis (CF).

Leveraging Bayesian temporal reconstruction and graphical pan-genome inference to analyse a global strain collection, we show that a few environmental isolates, driven by horizontal gene acquisition, have become dominant epidemic clones that have sequentially emerged and spread through global transmission networks over the past 200 years.
These clones demonstrate varying intrinsic propensities for infecting CF or non-CF individuals, and clinical isolates clustered in transcriptional space based on epidemic clone host-preference. We found that high CF affinity clones were better able to survive within CF macrophages, in part mediated by expression of the stringent response modulator DksA1.
After acquisition from the environment, the epidemic clones have undergone multiple rounds of convergent and host-specific adaptation (as demonstrated by mutational burden analysis) and have eventually lost their ability to transmit between different patient groups likely due to host specialisation.

Our findings thus explain the pathogenic evolution of *P. aeruginosa* and highlight the importance of global surveillance and cross-infection prevention in averting the emergence of future epidemic clones.

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Intracellular *Pseudomonas aeruginosa* in cystic fibrosis lung tissues

Karim Malet[1], Emmanuel Faure[2], Damien Adam[3], Jannik Donner[1], Lin Liu[4], Sarah-Jeanne Pilon[5], Richard Fraser[5], Peter Jorth[6], Dianne Newman[7], Emmanuelle Brochiero[3], Simon Rousseau[1], Dao Nguyen[1]

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Abstract

Pseudomonas aeruginosa (*P.a.*) is the main bacterial pathogen colonizing the airways of adult cystic fibrosis (CF) patients and causes chronic infections that persist despite antibiotic therapy. Intracellular bacteria may represent an unrecognized reservoir of bacteria that can evade the immune system or antibiotic therapy. While the ability of *P.a.* to invade and survive within epithelial cells has been described *in vitro*, evidence of this intracellular lifestyle in human lung tissues was previously lacking. In this study, we sampled the lung explant tissues from 7 CF patients undergoing lung transplantation. We first analyzed thin lung tissue sections for the presence of intracellular *P.a.* by quantitative culture and immunohistochemistry, in parallel with histopathology and airway morphometry. Microscopy assessment revealed the presence of intracellular *P.a.* within airway epithelial cells in 3 out of the 7 lungs analyzed, at a varying but low frequency. Histomorphometric and histopathological analyses revealed that those events occurred more frequently in areas with high bacterial burden, and airways with intracellular *P.a.* were 10-fold larger in perimeter than those without intracellular *P.a*, but were not associated with greater inflammation. Confocal microscopy imaging of cleared thick tissue sections with bronchial structures revealed the presence intracellular *P.a.* within bronchial epithelial cells. This is the first study describing the presence of intracellular *P.a.* in CF lung tissues. While intracellular *P.a.* in airway epithelial cells are likely relatively rare events, our findings highlight the plausible occurrence of this intracellular bacterial reservoir in chronic CF infections.

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Metagenomics harvested genus-specific single-stranded DNA-annealing proteins improve and expand recombineering in *Pseudomonas* species

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Enrique Asin-Garcia

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Abstract

The widespread *Pseudomonas* genus comprises a collection of related species with remarkable abilities to degrade plastics and polluted wastes and to produce a broad set of valuable compounds, ranging from bulk chemicals to pharmaceuticals. *Pseudomonas* possess characteristics of tolerance and stress resistance making them valuable hosts for industrial and environmental biotechnology. However, efficient and high-throughput genetic engineering tools have limited metabolic engineering efforts and applications. To improve their genome editing capabilities, we first employed a computational biology workflow to generate a genus-specific library of potential single-stranded DNA-annealing proteins (SSAPs). Assessment of the library was performed in different *Pseudomonas* using a high-throughput pooled recombinase screen followed by Oxford Nanopore NGS analysis. Among different active variants with variable levels of allelic replacement frequency (ARF), efficient SSAPs were found and characterized for mediating recombineering in the four tested species. New variants yielded higher ARFs than existing ones in *Pseudomonas putida* and *Pseudomonas aeruginosa*, and expanded the field of recombineering in *Pseudomonas taiwanensis*and *Pseudomonas fluorescens*. These findings will enhance the mutagenesis capabilities of these members of the *Pseudomonas* genus, increasing the possibilities for biotransformation and enhancing their potential for synthetic biology applications.

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A path to more stability and efficacy: D- Peptide-conjugated antisense antibiotics as a therapeutic approach against multidrug resistant *Pseudomonas aeruginosa in vitro* and *in vivo*

Dina Moustafa[1,2], Christine Pybus[3], Sneha Banerjee[3], Rachelle Koch Koch[3], Amila Nanayakkara[3], Joanna Goldberg[1,2], David Greenberg[3]

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Dina Moustafa

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Abstract

Background: Multidrug-resistant (MDR)-*P. aeruginosa* (PA) are considered a serious threat by the CDC and WHO. Their capacity to confer resistance via multiple mechanisms has raised global concern as antibiotics are steadily losing efficacy in clinical settings thus necessitating the need for new therapeutic approaches.

Objectives: Test the efficacy and stability of peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) specifically designed to target essential genes by comparing L-isomer peptides (L-PPMOs) to D-isomer peptides (D-PPMOs).

Methods: Using different PA strains isolated from various types of infection, lead D- and L-PPMOs were compared using minimum inhibitory concentrations (MIC) and were tested against established biofilms. The concentration and stability of these PPMOs was assessed in murine and human plasma. And the therapeutic efficacy of D- and L-PPMOs were tested *in vivo* using an acute PA pneumonia model.

Results: The average MIC_{90} of the lead D- and L-PPMOs were 2 and 8 μM, respectively, when tested against 16 different PA strains. Furthermore, D-PPMOs retained activity and effectively reduced established biofilms of strains PA103 and MB580A following 7 days of treatment, compared to untreated controls. Importantly, the stability of D-PPMOs were superior to L-PPMOs in mouse and human plasma. Finally, in a murine pneumonia model, therapeutic treatment using 15 mg/kg of D-PPMOs resulted in increased survival and reduced colonization compared to untreated mice.

Conclusions: D-PPMOs demonstrate improved activity and stability compared to their L-PPMOs counterparts at the same dosage and have the potential to be effective therapeutic agents against *Pseudomonas* infections.

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Ecology and evolution of *Pseudomonas aeruginosa* antibiotic resistance in polymicrobial communities

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Ville-Petri Friman

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Abstract

Accumulating evidence suggests that the response of bacteria to antibiotics is significantly affected by the presence of other interacting microbes. To what extent this community context affects the ecology and evolution of antimicrobial resistance in polymicrobial lung communities remains unknown. We experimentally tested this using an *in vitro* Cystic Fibrosis (CF) lung infection model, focusing on *Pseudomonas aeruginosa* as the focal pathogen. Our results show that interspecies bacterial interactions can affect the responses of individual species and communities to antibiotic treatment. At the ecological scale, bacterial interactions can be facilitative, where susceptible *P. aeruginosa* persists in the presence of antibiotics when other bacteria can extracellularly break down the antibiotics, resulting in detoxification of the shared environment. Over the evolutionary timescale, these ecological interactions are significantly altered, where the evolution of chromosomal resistance in *P. aeruginosa* leads to competitive exclusion of the protective species via increased production of toxic pyocyanin. While adaptation in response to competing bacterial species typical for CF infections does not seem to lead to correlated changes in *P. aeruginosa* antibiotic resistance, exposure to phages can select for *P. aeruginosa* mutants that are more susceptible to colistin likely due to LPS mutations. Together, these results emphasize the importance of community context for *P. aeruginosa* pathogen survival and the trajectory of antibiotic resistance evolution in polymicrobial infections.

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Macrolide resistance through uL4 and uL22 ribosomal mutations in *Pseudomonas aeruginosa*

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Abstract

Macrolides are widely used antibiotics for the treatment of bacterial airway infections. Due to its elevated minimum inhibitory concentration in standardized culture media, *Pseudomonas aeruginosa* is considered intrinsically resistant and, therefore, antibiotic susceptibility testing against macrolides is not performed. Nevertheless, due to macrolides' immunomodulatory effect and suppression of virulence factors, they are used for the treatment of persistent *P. aeruginosa* infections. Here, we demonstrate that macrolides are, instead, effective antibiotics against *P. aeruginosa* airway infections in an air-liquid interface (ALI) infection model system resembling the human airways. Importantly, macrolide treatment in both people with cystic fibrosis and primary ciliary dyskinesia patients leads to the accumulation of uL4 and uL22 ribosomal protein mutations in *P. aeruginosa* which causes antibiotic resistance. Consequently, higher concentrations of antibiotics are needed to modulate the macrolide-dependent suppression of virulence. Surprisingly, even in the absence of antibiotics, these mutations also lead to a collateral reduction in growth rate, virulence and pathogenicity in airway ALI infections which are pivotal for the establishment of a persistent infection. Altogether, these results lend further support to the consideration of macrolides as *de facto* antibiotics against *P. aeruginosa* and the need for resistance monitoring upon prolonged macrolide treatment.

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Siderophore or metallophore? An investigation of Zur-regulated metal transport systems reveals an unexpected role of pyochelin in zinc homeostasis

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Andrea Battistoni

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Abstract

Background: *Pseudomonas aeruginosa* adeptly evades host zinc sequestration mechanisms, crucial for controlling bacterial proliferation, via the expression of numerous Zur-regulated genes, many of which still have unknown functions. This study aimed to explore the role of two uncharacterized Zur-regulated gene clusters, PA2911-2914 and PA4063-4066, encoding putative metal import systems.

Methods: The function of PA2911-PA2914 and PA4063-PA4066 was explored through various approaches: bioinformatics analysis of encoded proteins via SWISS-MODEL and Alphafold 2, growth assays of P. aeruginosa PA14 and deletion mutants under zinc-limiting conditions, gene expression analyses, ICP-MS for direct intracellular metal content measurements, and PzrmA-lacZ reporter strain assays for indirect analyses of intracellular zinc availability

Results: The bioinformatics analysis indicated that PA2911-PA2914 encodes a typical transport system involved in the import of metal-chelating molecules, while PA4063-PA4066 encodes a MacB transporter likely involved in exporting large molecules. Molecular genetics, biochemical experiments, feeding assays, and intracellular metal content measurements confirmed the roles of PA2911-PA2914 and PA4063-PA4066 in importing and exporting the pyochelin-cobalt complex, respectively. Notably, cobalt can reduce zinc demand and promote the growth of *P. aeruginosa* strains unable to import zinc, emphasizing the significance of pyochelin-mediated cobalt import as an innovative strategy to counteract zinc deficiency.

Conclusion: These findings unveil an unexpected role of pyochelin in zinc homeostasis and challenge the traditional view describing this molecule as a siderophore exclusively involved in iron uptake. Our results suggest that pyochelin is a metallophore with a broader role in bridging the homeostasis of different metals.

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How to fit a round peg into a square hole: Identification of *Pseudomonas aeruginosa* acyl-CoA dehydrogenases and structure-guided inversion of their substrate specificity.

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Martin Welch

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Abstract

Fatty acids are a primary source of carbon for *Pseudomonas aeruginosa* (PA) in the airways of people with cystic fibrosis, yet our understanding of fatty acid catabolism in this organism remains limited. For example, PA encodes twenty-two putative acyl-CoA dehydrogenases, yet it is not known which of these are involved in b-oxidation. We used tandem mass-tag proteomics to analyze the protein expression profile of a CF clinical isolate grown on different fatty acids. This revealed that two fatty acyl-CoA dehydrogenases (hereafter, FadE1 and FadE2) are highly-expressed during growth on long- and medium-chain fatty acids, but not on glucose. Subsequent genetic and biochemical characterization of FadE1 and FadE2 revealed that FadE1 has a strong preference for long-chain fatty acids, whereas FadE2 exclusively utilizes medium-chain fatty acyl-CoAs. To investigate the structural basis for this specificity profile, we solved the X-ray crystal structure of each protein and carried out molecular dynamic analyses of substrate binding. This revealed that certain residues in FadE2 act as a selectivity filter, preventing the binding of long-chain fatty acyl-CoA substrates. Engineering these residues to the corresponding ones in FadE1 enabled FadE2 to utilize long-chain fatty acyl-CoA substrate. Similarly, when FadE1 was engineered to become more “FadE2-like”, the mutant FadE1 lost activity towards long-chain substrates, but retained activity towards a medium-chain substrate. Finally, d_N/d_S analysis of ca. 850 *fadE1* and *fadE2* sequences in the IPCD database indicate that whereas FadE1 occupies a peak in the local adaptive fitness landscape, FadE2 is actively evolving away from its ancestral state.

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tRNA epitranscriptomic controls of Pseudomonas aeruginosa's pathogenicity through rare-codon's decoding.

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Nicolas Gomez

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Abstract

A finely regulated and coordinated gene expression is cornerstone to bacterial pathogens establishing pathogenicity. tRNA modification pathways emerge as epitranscriptomic regulators of translation, tuning tRNA decoding properties by post-transcriptional modifications. However, our understanding of the underlying molecular mechanisms of such regulation mechanisms is still incomplete.

We deciphered the role of GidA, an enzyme introducing carboxymethylaminomethyl modifications of selected tRNAs. Absence of *gidA* leads to a pleiotropic phenotype, including hypo-virulence and decreased fitness during infection. Through an extensive and integrated multi-omics approach (transcriptomic, Nano-tRNASeq, translatomic, and proteomic), we show that the absence of GidA-dependent modifications impact the decoding of selected codons in *P. aeruginosa* and drives protein output, without modifying tRNA abundance. The target codons while seldom in the genome accumulate at strategic locations in core-genes involved in virulence, suggesting a fine-tuning of codon usage driven by control of translational regulons. Expression levels of *gidA* in a large cohort of clinical isolates suggest that modulation of tRNA modification is crucial to establish pathogenicity.

Our work outlines a new emerging mechanism of post-transcriptional regulation based on a tRNA modification driving bacterial pathogenicity through a modulation of translation efficiency based on finely tuned codon usage resulting in a proteomic shift toward a pathogenic and adapted physiological state. Moreover, this work places GidA and potentially other tRNA modification

enzymes as prime targets for the development of novel alternative antimicrobial therapies allowing to tune down virulence and potentiate clinically relevant antibiotics.

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Goblet cell invasion by *Pseudomonas aeruginosa* promotes breaching of human respiratory epithelia.

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Abstract

P. aeruginosa surface colonization entails complex behaviour including adherence, virulence induction and dissemination. Infection studies are hampered by the lack of experimental models that faithfully recapitulate the physiology of human tissue and, at the same time, offer the experimental power to investigate the infection process with high temporal and spatial resolution. We established an *in vitro* 3D lung infection model from human stem cells, with air-liquid interface in a Transwell. Immunocytochemistry- and histology staining confirmed that the architecture and cellular composition of the tissue closely resembles the human bronchial epithelium. Using live cell microscopy, we demonstrate that the tissue recapitulates lung functions such as production of mucus and cilia beating. We utilize the upper airway tissues to visualize and quantify *P. aeruginosa* lung infection with unprecedented spatial and temporal resolution. These studies provide a detailed mechanistic frame for how human pathogens overcome the mucus barrier and rapidly spread on mucosal tissue and how they combine internalization into specialized cell types and collective behavior to rapidly and effectively breach the barrier function of the lung epithelium. Our results establish lung Transwells as versatile *in vitro* model to study bacterial infections and drug response in a human-like environment.

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Exploitation of an inducible hypermutator to rapidly identify mutants that bypass LasR- and RhlR-dependent quorum sensing control of gene expression in *P. aeruginosa*.

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Abstract

The opportunistic pathogen *P. aeruginosa* establishes infections by secreting an arsenal of virulence factors, many of which are controlled by quorum sensing (QS). The *P. aeruginosa* QS system is hierarchical, relying on a pair of transcription factors - LasR and RhlR - and their corresponding autoinducer molecules, OdDHL and BHL, respectively. However, *lasR* mutants are common in clinical isolates and, in many cases, retain virulence, indicating that QS "bypass" mechanisms exist. In principle, a better understanding of how this bypassing occurs will shed light on how QS interdigitates with other mechanisms controlling virulence in the organism. To investigate this further, we generated *lasR* and *rhlR* knockouts in an inducible hypermutator (HM) genetic background. Using the *P. aeruginosa* HM Δ*lasR* strain, we found that *mexT* is a hotspot for mutations that bypass *lasR* control of secreted protease production. However, we also identified derivatives in which *mexT* was not mutated. In parallel, we also developed a similar screen to look for secondary mutations that bypass RhlR-dependent gene expression. This was more challenging because protease secretion was relatively unaffected in the *rhlR* background. Instead, we introduced *lacZ* fusions downstream of RhlR-dependent promoters, enabling easy visual assessment of bypass mutants on X-Gal. Using both screens, we have identified a number of genes for further characterization.

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Pseudomonas bharatica CSV86^T: a promising host/chassis for metabolic engineering and bioremediation

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Abstract

Pseudomonas bharatica CSV86^T, a novel Indian soil isolate, preferentially degrades wide range of aromatics like naphthalene, benzoate, benzyl alcohol and phenylpropanoids over simple carbon sources like glucose or glycerol and co-metabolizes them with organic acids thus making it unique amongst *Pseudomonas* spp. The strain exhibits advantageous eco-physiological traits like indole acetic acid production, siderophore production, alginate synthesis, assimilatory nitrate reduction and organic sulphur metabolism which enhances its survivability in contaminated niches. Further, availability of genome sequence, well-characterised growth conditions and lack of plasmid make it an ideal chassis for metabolic engineering. Strain CSV86^T was engineered for Carbaryl degradation *via* 1-naphtholà1,2-dihydroxynaphthaleneààsalicylateàcatechol route by expressing Carbaryl hydrolase (CH) and 1-naphthol 2-hydroxylase (1NH) under the P*nah* promoter. The presence of transmembrane domain and signal peptide at the N-terminus of CH resulted in the compartmentalisation of Carbaryl degradation enzymes by localising CH and 1NH to the periplasm of the engineered strain, thus mitigating the toxicity of 1-naphthol. The expression of McbT (putative Carbaryl transporter) in addition to CH and 1NH enhanced the degradation efficiency suggesting its involvement in Carbaryl uptake. Enzyme activity, whole-cell oxygen uptake, biotransformation, protein analyses and quantitative-PCR studies suggest that the engineered strain preferentially utilises Carbaryl over glucose and co-metabolizes it with succinate. The engineered degradation property encoded by plasmid was stable (98%) in the absence of selection pressure for 75-90 generations. Versatile eco-physiological traits with unique preferential utilization of aromatic property make *P. bharatica* CSV86^T a promising chassis/host for metabolic engineering of aromatic degradation pathways and bioremediation.

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Unravelling the regulatory and metabolic bottlenecks of specialized metabolite production in three non-model Pseudomonads using a multi-omics approach

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Abstract

Specialized metabolites are complex chemical molecules essential for the survival of their natural producers within specific ecological niches. The *Pseudomonas* genus, renowned for its ubiquitous nature, produces a diverse range of specialized metabolites that can be repurposed as high-value chemical compounds such as antibiotics or bioherbicides. Despite their potential, their natural production titers are generally low, necessitating genetic engineering to facilitate biotechnological applications. However, engineering efforts are often complicated by the lack of knowledge concerning regulatory mechanisms that drive specialized metabolite production and the presence of shunt metabolites that reduce the metabolic flux towards the specialized metabolite of interest.

We here present a multi-omics approach to unravel the intricate dynamics of specialized metabolite production in three non-model *Pseudomonas species* (*P. syringae, P. fluorescens* and *P. chlororaphis*). Leveraging ONT-cappable-seq, our in-house developed long-read RNA-sequencing approach, we elucidated the regulatory mechanisms driving specialized metabolite production. The obtained transcriptomes revealed a complex regulatory network shedding light on transcriptional landmarks, sRNAs and riboswitches. Furthermore, combining tandem mass spectrometry with molecular networking allowed identification of shunt metabolites and elucidation of potential barriers to optimal specialized metabolite production.

In summary, our findings contribute to the identification of regulatory and metabolic bottlenecks that hamper specialized metabolite production in each of the selected *Pseudomonas* species. Moreover, these insights will aid future genetic engineering efforts to succeed at improving specialized metabolite production yields.

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Evolution of phenolic compounds-degrading bacterial populations

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Abstract

Bacteria which are evolving to degrade environmental pollutants (e.g., phenolic compounds) have to face strong selective pressure to survive in the presence of cell damaging effects of these compounds. New catabolic pathways can evolve as a result of horizontal transfer of genes and point mutations in bacterial genome. We have observed that the pollutant stress affects the frequency of homologous recombination (HR) in *P. putida*. In addition, both the frequency of HR and the occurrence of point mutations in *P. putida* genome were affected by the location of the target sequence in the chromosome. To explore mechanisms of evolution of pollutants-degrading bacterial populations in real time, we performed adaptive laboratory evolution (ALE) experiments with *P. putida* strains carrying hybrid pathway for the degradation of *m*-cresol. The constructed strains carried two plasmids – pEST1412 and TOL-plasmid pWW0. We tested hypothesis whether the survival and evolution of phenolic pollutants degrading bacteria under harsh environmental conditions could be connected with elevated mutation frequency. Characterization of populations evolved ~400 generations in the presence of pollutant (m-cresol) revealed that only strong pollutant stress facilitates the appearance of constitutive mutators in the populations of environmental bacteria. Our results also revealed an exceptional genetic plasticity of the studied bacterial strains and the preservation of genetic material. This might be one of the reasons why natural bacterial strains mostly carry several plasmids, are prone to genetic rearrangements and are highly variable in their phenotype.

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Mutations in the efflux pump regulator MexZ shift tissue colonization by *Pseudomonas aeruginosa* to a state of antibiotic tolerance

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Abstract

Mutations in *mexZ,* encoding the local negative regulator of expression of the MexXY efflux pump encoding genes, are frequently acquired in *Pseudomonas aeruginosa* at early stages of its infections. Although previously associated with resistance to tobramycin, a first-line drug for *P. aeruginosa* infections, *mexZ* mutations actually cause low-level aminoglycoside resistance when determined in the laboratory, questioning how these mutations further shape the infection process. Here we characterized the infection behavior of *mexZ*-mutated bacteria by using an *in vivo*-mimicking airway infection model. We observed that *mexZ* mutations make *P. aeruginosa* more likely to invade the airway intraepithelial compartment, where certain antibiotics have limited penetration, thus shielding the bacteria from the effects of antibiotics. The altered epithelium colonization was caused by *lecA* overexpression, a Quorum Sensing regulated gene encoding a lectin involved in *P. aeruginosa* tissue invasiveness. This upregulation of *lecA* was driven by a disrupted equilibrium between the overproduced MexXY efflux pump and the MexAB efflux pump which is responsible for extruding certain Quorum Sensing molecules. These findings suggest that antibiotic resilience can be conditional, and that standard antibiotic susceptibility tests may not always directly translate into bacterial susceptibility in the patients, highlighting the need of taking into account the subtle nuances of host-pathogen interactions affecting the infection process to improve our management of infectious diseases.

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Induction of cyclic-di-GMP in *P. fluorescens* Pf0-1 by *Pedobacter* during interspecies social spreading

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Abstract

Bacteria often reside in multi-species communities where many behaviors are the result of complex relationships between the inhabitants. In a model two-species community, a co-culture of *Pseudomonas fluorescens* Pf0-1 and *Pedobacter* sp. V48 exhibit Interspecies Social Spreading (ISS) across a hard agar surface, an environment where neither species moves alone. Because this phenomenon resembles both motility and a biofilm, we sought to determine whether cyclic-di-GMP signaling plays a role. From a collection of *P. fluorescens* Pf0-1 intracellular signaling mutants we recovered diguanylate cyclase (*Pfl01_4307*) and cAMP-regulatory protein (*Pfl01_5111*) mutants that had significantly reduced ISS and growth medium specific reductions in biofilm formation. When over-expressed in Pf0-1, *Pfl01_5111* restored normal ISS whereas overexpression of *Pfl01_4307* resulted in further reduction of ISS, suggesting nuances in timing and level of expression may be important for interactions with *Pedobacter*. Relative amounts of cyclic-di-GMP were measured using a GFP reporter construct. In an unstructured shaking co-culture, the presence of *Pedobacter* resulted in approximately 2-fold increase in cyclic-di-GMP in Pf0-1 and both mutants. In contrast, when co-cultured on a surface (structured community), in the presence of *Pedobacter,* Pf0-1 produced approximately 2.5-fold more cyclic di-GMP than a Pf0-1 monoculture while neither mutant showed any difference between mono- and co-culture. These data suggest a role for both cyclic-di-GMP and cAMP in ISS, and that the diguanylate cyclase encoded by *Pfl01_4307* is specifically important in the response of Pf0-1 to *Pedobacter* in a structured community.

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Systems biology informed engineering in *Pseudomonas putida* KT2440 improves biochemical production from lignin-derived aromatics

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Abstract

Lignin, a complex aromatic polymer abundant in plant cell walls, poses a challenge and opportunity in lignocellulosic biorefineries due to its recalcitrant yet carbon-rich nature. Strategies focusing on converting lignin into valuable products through biological funneling of the diverse lignin-related compounds generated by chemical or enzymatic deconstruction show promise for maximizing the potential of lignin streams. Here, we identify metabolic inefficiencies and bottlenecks in *Pseudomonas putida* during the production of muconate, a performance-advantaged bioproduct, from 4-hydroxybenzoate (4HB), a common component of lignin streams. After rewiring native aromatic metabolism to channel 4HB to muconate in *P. putida* CJ781 (CJ781), we found metabolic bottlenecks in the aromatic catabolic pathway and strains were optimized to improve production rates. Combining proteomics, exometabolomics, and ^{13}C-fluxomics analysis of the conversion of glucose to biomass growth and energy either in the presence or absence of 4HB further indicated that CJ781 relative to the wildtype strain had greater secretions of intracellular metabolites, higher periplasmic flux to 2-ketogluconate, and altered flux through the TCA cycle, which all contributed to changes in the cellular redox and energy balance of the production strain. Notably, CJ781 secreted pyruvate and acetate at 2.1% and 1.8% of glucose uptake, respectively, in the presence of 4HB, indicating a potential bottleneck in carbon flux entering the TCA cycle. Ultimately, this study enhances our comprehension of divided cellular metabolism between aromatic compound funneling to bioproducts and biomass production from sugars, exposing metabolic inefficiencies to target for improved bioproduction from lignin streams.

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Exploring the interaction of cherry (*Prunus avium*) and *Pseudomonas syringae* pathovars Syringae and Morsprunorum through untargeted metabolomics

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Abstract

Untargeted metabolomics was used to characterize the *Pss* and *Psm* interactions with its host woody tissue and extracts. *Pss* grew faster than *Psm* in shoots, but both experienced a distinct metabolic adjustment when interacting with the host. *Pss* faster growth rate correlated with its ability to degrade a broader range of cherry-derived glycosylated flavonoids. *Psm* accumulates a lipidic class of molecules and diacylglycerol-like metabolites related to the rhamnolipid lipidic chains, whilst *Pss* produces the phytotoxin syringolin A, though this could not be detected in the whole-tree inoculations. However, syringopeptin SP22B, syringomicin E and G, and two unknown depsipeptides were detected in woody tissue during the early stages of *Pss* colonization. A similar metabolic variation in cherry tree tissues was observed in both artificially inoculated and naturally infected trees. The main variations were observed in the flavonoid biosynthetic pathway, which was characterized by a marked accumulation of pinocembrin, naringenin, and chrysin, whose abundance was 100 times higher in diseased tissue than in healthy tissue. The alteration in the flavonoid-related metabolites was similar in *Pss* and *Psm*-infected trees, indicating that this is a conserved response to the *Pseudomonas syringae* infection. Conversely, the accumulation of some terpenoids and carboxylic acids, such as pipecolic acid and asiatic acid, was mainly observed on *Psm*-infected trees, suggesting their biosynthesis is triggered in a pathovar-dependent manner. We aim to understand the role of the accumulated flavonoids in the *P. syringae*-cherry interaction and the possible PAMPs recognized by cherry trees that trigger its response to the pathogen.

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Stress responses and tolerance mechanisms of *Pseudomonas putida* to the plastic industry monomers ethylbenzene and styrene.

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Abstract

The plastics industry is almost exclusively reliant on petrochemical feedstocks for the synthesis of large-volume commodity chemicals. Collectively, the amount used for plastic production is equivalent to approximately 1.3 billion barrels of crude oil per annum. To improve the sustainability of materials such as acrylic, the chemical industry is investing heavily in biological production methods.

Pseudomonas spp. are tolerant to a wide range of chemical stresses and we have demonstrated that this genus is significantly enriched in metagenomic experiments where complex populations of bacteria are challenged with toxic plastic monomers. In particular, *Pseudomonas putida* has gained traction as a synthetic biology chassis organism and is ideally suited to the sustainable production of plastic monomers because of its inherent solvent tolerance. To further understand and optimise *P. putida* for biosynthesis of plastic monomers we have taken a transcriptomic approach to understand its response to chemical stress associated with ethylbenzene, styrene and methacrylate esters. In addition to several efflux pumps, we identified TetR and MarR class transcriptional regulators that are strongly derepressed in the presence of all three solvents. These regulators are capable of binding diverse substrates and *in silico* docking experiments indicate the plausibility of solvent ligand binding. Deletion of each regulator facilitated assessment of their regulons by RNA-Seq revealing that the MarR type regulator may contribute to the generalised envelope stress response of *P. putida*. Applications of these regulators as biosensors for combinatorial synthetic biology will be discussed as well as recent insights gained from integrating RNA-Seq and Tn-Seq datasets.

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Unraveling mechanisms underlying biofilm-induced tolerance in *Pseudomonas aeruginosa* by applying a multi-omics approach

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Abstract

Pseudomonas aeruginosa biofilm infections pose significant challenges due to their increased tolerance to antimicrobial therapy. While biofilm-associated tolerance is well-recognized, the underlying mechanisms remain poorly understood. This study aims to gain new insights into the complex adaptation of *P. aeruginosa* resulting in increased biofilm-induced tolerance.

We screened 350 clinical *P. aeruginosa* isolates for biofilm tolerance to three common antibiotics and classified strains based on characteristic patterns of drug-specific killing. Representative strains exhibiting high- or low-level biofilm tolerance were subjected to in-depth multi-omics analyses (RNA-seq, metabolomics, Tn-seq) to uncover cellular mechanisms that drive the observed differences in biofilm tolerance.

Increased levels of the TCA intermediates succinate and fumarate, and elevated levels of pyruvate and lactate in biofilms of highly tolerant isolates indicate different rates of glucose utilization and non-oxidative fermentation compared to sensitive biofilms. RNA-seq revealed distinct regulatory patterns in genes encoding ATP synthase, suggesting reduced ATP production in high-tolerance biofilms. In addition to an altered energetic state, tolerant biofilms also appeared to have a different redox state. Elevated NAD^+ levels and GSSG (oxidized glutathione) in tolerant biofilms indicate an imbalance in the cellular redox state. This possibly triggers the induction of the oxidative stress response, thus increasing tolerance. Additionally, alterations in lipid metabolism hinted at membrane permeability as an additional determining factor for the observed differences in biofilm tolerance.

These findings provide valuable insights into the multifaceted mechanisms driving biofilm-induced tolerance in *P. aeruginosa*, paving the way for novel targeted strategies to overcome the challenges posed by chronic, biofilm-associated infections.

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Standing up to the 'bully': *Staphylococcus aureus* can sustain long-term infection in the presence of *Pseudomonas aeruginosa*, and may outcompete in the CF airway

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Abstract

Chronic biofilm-associated infections are common in people with cystic fibrosis (pwCF). We recently showed that *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureu*s (SA) co-infections are common[1], however there are disparate findings in the literature regarding their impact on clinical outcomes.

To elucidate how co-infections and their chronicity influence health, we used linear mixed models on clinical outcomes and microbiology data from 832 pwCF at the Royal Brompton Hospital (London). The Leeds criteria was used to categorise (co-)infection. We found that including PA:SA interactions significantly improved our model fit only when chronicity was considered. Unexpectedly, chronic PA/SA co-infection correlated with better health outcomes compared with chronic PA mono-infections[2].

To understand how stable co-infections were in this cohort, we used Markov chain models to study infection trajectories. For co-infected individuals 36.4% maintained this status, while 41.1% shifted to PA mono-infection, and 10.6% transitioned to SA mono-infection.PA mono-infections were mostly stable(63.6%), but 19.2% evolved to co-infection and 5.3% converted to SA mono-infections. Interestingly, for both PA and SA, whichever species established a chronic foothold first seemed to result in sustained presence or out-competition of the other species.

The complexity of polymicrobial interactions in CF is profound, and here we challenge the conventional SA-to-PA progression in pwCF. We find the role of chronicity to be crucial and are currently studying how PA/SA CF isolates interact in an *in vitro* biofilm model to understand how co-infection may impact the health of pwCF.
[1]Hughes (2021) 10.1164/rccm.202009-3639LE
[2]Mossop (2024) 10.1164/rccm.202312-2326L

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Proteomic analysis reveals that the nutrient environment shapes susceptibility in *Pseudomonas aeruginosa* through metabolic adaptations and stress response

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Abstract

The escalating global health threat of antimicrobial resistance is characterised by pathogens acquiring resistance-conferring mutations, often disseminated through horizontal gene transfer. Despite this, the mechanisms underlying the survival and resilience of "pre-resistant" cells exposed to lethal antibacterial doses remain unclear. Thus far, the role of the extracellular metabolic environment and metabolic adaptations in transiently buffering antibiotic lethality have been overlooked. This study systematically investigates the mechanisms at play in populations of "pre-resistant" cells of *Pseudomonas aeruginosa* (strain: PAO1). Using a bacterial physiology screen, we assess resilience phenotypes against major clinically applied antimicrobials under both standard laboratory and infection site-mimicking conditions. Our results reveal that susceptibility is substantially decreased in infection site-mimicking conditions compared to standard laboratory conditions. Using high-throughput proteomics to study over 400 combinations of antimicrobial treatment and culturing conditions, we further methodically explore the metabolic stress response to identify key metabolic and signalling processes supporting cell survival during sub-inhibitory antimicrobial exposure in *P. aeruginosa*. The data show increased expression of virulence mechanisms and stress response features. However, reshaping of central metabolic processes ultimately enables survival and antimicrobial protection of “pre-resistant” cells in response to antimicrobial exposure. We posit that metabolic dynamics contribute to the emergence of antimicrobial tolerant and resistant phenotypes and, in future, aim to pinpoint the crucial metabolic effectors preceding resilience-conveying adaptations that contribute to the evasion of antimicrobial treatment.

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Favorable Metabolic Processing of Lignin-Derived Aromatic Carbons in Soil *Pseudomonas* Species: Emergent Reaction Network and Physiological Characteristics

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Abstract

Valorization of lignin is an important component of a sustainable bioeconomy. Soil *Pseudomonas* strains, which natively catabolize lignin-derived aromatics (LDAs), are commonly engineered for the conversion of LDAs to value-added compounds. However, largely lacking is the quantitative characterization of the metabolic reaction networks that dictate favorable versus non-favorable conversion of LDAs. Here we employ a multi-omics approache involving kinetic ^{13}C-metabolomics and proteomics investigations of *Pseudomonas putida* KT2440 and *Pseudomonas putida* mt-2 during the conversion of four relevant LDAs as the sole carbon source: *p*-coumarate, and vanillate, or 4-hydroxybenzoate. Though kinetic isotopic flux profiling and quantitative flux analysis, we identify nodes of metabolic bottlenecks and determine confactor balance associated with the reaction network for each LDA substrate carbon fluxes. Microscopic images of the cells under different conditions highlight differences on the growth state and the morphological characteristics. The combination of metabolite profiling and protein abundance captures specific metabolic requirements that sustain the differences in the growth state of the *P. putida* strains. Our findings shed on the metabolic network underlying the favorable physiology for the processing of LDAs in soil *Peudomonas* species.

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Anti-lipopolysaccharide IgA 'cloaking antibodies' are associated with worse lung function in people with cystic fibrosis

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Abstract

Pseudomonas aeruginosa is a common cause of chronic pulmonary infections in people with cystic fibrosis (pwCF). Previously, we discovered a subset of patients with *P. aeruginosa* lung infections had high titres of O-antigen specific IgG2 and/or IgA which instead of protecting against infection, inhibited complement-mediated serum killing of infecting strains. These 'cloaking antibodies' (cAbs) were associated with worse lung function in non-CF bronchiectasis and worse outcomes in lung transplant recipients. More recently, cAbs were identified in the serum of 32% of pwCF, however no correlation to health was observed. As serum may not be reflective of the lung environment, we investigated the clinical importance of cAbs within lung secretions in pwCF.

We collected health data, serum, sputum, and isolated *P. aeruginosa* from 43 pwCF. Serum and sputum were screened for lipopolysaccharide (LPS)-specific antibodies via ELISA, and their ability to kill infecting isolates determined via serum bactericidal assays. We found 30.3% of patients had high titres of LPS-specific IgG2 and/or IgA in their serum that inhibited complement killing, corroborating previous findings. Only high titres of LPS-specific IgA were detected at relevant levels within sputum. As anti-LPS IgG2 was not relevant within lung secretions, LPS-specific IgA responses were compared to patient health. We found increased O6 LPS-specific IgA correlated to worse lung function in both serum and sputum. This correlation was validated by a USA cohort of 25 pwCF.

These findings indicate that *P. aeruginosa* LPS-specific IgA but not IgG2 may play a role in CF pathophysiology warranting further investigation.

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Innovative catabolic pathway decoupling in *Pseudomonas putida* through Toggle Switch-Induced Lifestyle Differentiation

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Abstract

Designing biological devices that orchestrate programmable metabolic heterogeneity within microbial cell factories is highly valued to address current biotechnological challenges. Throughout the spatiotemporal pattering of gene expression, this strategy allows the distribution of complex catabolic processes among isogenic bacterial populations. The toggle switch (TS), consisting of two mutually repressive systems, dynamically shapes this heterogeneity through gradients of diffusible molecules. Although extensive research has investigated dynamical properties of TS in model microorganisms like *Escherichia coli*, their practical biotechnological applications remain limited. This study extends TS application to *Pseudomonas putida*, engineering it as a synthetic regulator of dual lifestyle differentiation. This innovation splits an isogenic *P. putida* population into two functionally defined fractions—one forming biofilms and the other remaining planktonic. By taking advantages of environmental differences between both lifeforms, we expanded this implementation by coupling the degradation of the toxic dye Congo Red between them. Biofilm cells, expressing the oxygen-sensitive enzyme AzoR, catalyze the reductive cleavage of azo bonds, while planktonic cells oxidize the resultant aromatic amines. We tested three TS variants with different native sub-population splitting ratios, assessing their impact on degradation efficiency. Degradation was monitored by measuring decolorization (ABS 485 nm), and profiling of degradation products via HPLC. Results showed improved CR degradation when processes were distributed across lifeforms with a splitting ratio near 50-50. This underscores TS's potential as a versatile tool for precise spatial and functional differentiation in *P. putida populations*, crucial for enhancing biotechnological applications and employing metabolic heterogeneity as a strategy of division of labor.

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The expanding diazenium diolate signal family in bacteria

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Abstract

The diazenium diolate signal (DDS) family, which was initially identified in the opportunistic pathogen *Burkholderia cenocepacia*, has been a recent addition to the bacterial cell-cell communication systems. Bioinformatic analysis and our recent work have provided strong evidence that the production of this class of signal molecules is widespread, suggesting that DDS is a novel family of bacterial signals involved in regulating several phenotypic traits in phylogenetically diverse bacteria.

In our aim to establish this unusual class of molecule as a novel signal family in bacteria, we have been characterizing the DDS class of compounds in plant pathogens and plant beneficial strains in addition to opportunistic and human pathogens. We have structurally characterized and identified the DDS signal compounds as valdiazen and leudiazen in *B. cenocepacia* and *Pseudomonas syringae,* respectively. While valdiazen controls the expression of more than 100 genes, involving the major antifungal compound in *B. cenocepacia,* leudiazen controls the production of the major virulence factor mangotoxin in *P. syringae*. Our current study on the DDS signal in the rhizosphere isolate *Pseudomonas fluorescens* DSM 11579 revealed that it controls the production of several bioactive secondary metabolites in this strain, thus contributing to its biocontrol activity. We present the recent advances in the DDS family that confirm their role as global cellular regulators. We are currently investigating the DDS-responsive regulators to shed light on the signaling pathways that lead to target gene expression in various *Pseudomonas* strains.

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Pseudomonas-B. thuringiensis interplay: Exploring Diversification in Planktonic and Biofilm Environments

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Abstract

Pseudomonas and *Bacillus* species are widespread and have significant roles in applications such as bioremediation, agriculture, and biotechnology. Understanding the dynamics of *Pseudomonas-Bacillus* interactions is thereby essential for stable and effective consortia. This study investigated the impact of such interactions during bacterial diversification in planktonic vs. biofilm settings. *B. thuringiensis* (Bt), *P. brenneri* (PB) and *P. defluvii* (PD)-isolated from wastewater- were subjected to adaptive evolution in mono-, dual- or multispecies consortia for 8 days. Plating on congo red plates allowed species abundance quantification and identification of *Bt* morphotypes. Moreover, whole-genome sequencing revealed acquired mutations, while lectin staining and matrix proteomics detected changes in matrix glycans and proteins.

A Bt morphotypic variant emerged under all conditions but with higher frequency in the presence of *Pseudomonas* and rigid spatial structure. This variant showed mutations in the regulator *spo0A*, consistent with its lack of sporulation and significant faster doubling time, while it exhibited lower biomass in mixed-species biofilms. Matrix proteomics evidenced reduced abundance of TasA in the variants-a major biofilm matrix component in other Bacilli-while it was induced in co-culture with PB. In contrast, differential amino acid transporter abundances were observed in PB mono- versus co-culture-irrespective of Bt genotype-indicating distinct metabolic prioritization due to interspecies interactions. Furthermore, PB was favored in co-culture biofilm over planktonic conditions, suggesting that defined spatial structure facilitates co-existence.

Our results indicate that biofilm interspecies interactions promote Bt diversification and alter biofilm matrix production, factors that could directly influence applications using *Bacillus-Pseudomonas* consortia, such as growth promotion or biopesticides.

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Polyhydroxyalkanoate production by the plant beneficial rhizobacterium *Pseudomonas chlororaphis* PCL1606 influences survival and rhizospheric performance

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Abstract

Pseudomonas chlororaphis PCL1606 (PcPCL1606) is a model rhizobacterium used to study beneficial bacterial interactions with the plant rhizosphere. Many of its beneficial phenotypes depend on the production of the antifungal compound 2-hexyl, 5-propyl resorcinol (HPR). Transcriptomic analysis of PcPCL1606 and the deletional mutant in HPR production Δ*darB*, assigned an additional regulatory role to HPR, and allowed the detection of differentially expressed genes during the bacterial interaction with the avocado rhizosphere. Interestingly, the putative genes *phaG* and *phaI*, with a predicted role in polyhydroxyalkanoate biosynthesis, were detected to be regulated under HPR signalling network. Both putative genes were expressed in the HPR-producing wild-type strain, but strongly repressed in the derivative mutant Δ*darB*, impaired in HPR production.Thus, a derivative mutant in the *phaG* gene was constructed, characterized, and compared with the wild-type strain PcPCL1606 and with the derivative mutant Δ*darB*. The *phaG* mutant had strongly reduced PHA production, and displayed altered phenotypes involved in bacterial survival on the plant roots, such as tolerance to high temperature and hydrogen peroxide, and decreased root survival, in a similar way that the Δ*darB* mutant. Moreover, the *phaG* mutant does not have altered resistance to desiccation, motility, biofilm formation or adhesion phenotypes, as displayed by the HPR-defective mutant Δ*darB*. Interestingly, the mutant defective in PHA production also lacked a biocontrol phenotype against the soilborne pathogenic fungus *Rosellinia necatrix*, even when the derivative mutant still produced the antifungal HPR compound, demonstrating that the final biocontrol phenotype of PcPCL1606 first requires bacterial survival and adaptation traits to the soil and rhizosphere environment.

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Discovery of a biofilm-tropic *Pseudomonas aeruginosa* bacteriophage

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Abstract

Background: Bacteria can exist in multicellular communities called biofilms. *Pseudomonas aeruginosa* infections often involve biofilms, which contribute materially to the difficulty to treat these infections with antibiotics. Bacteriophage therapy may offer a solution to this problem.

Methods: We used microbiological techniques, Tn-Seq, and microscopy with fluorescently labeled bacteriophage to characterize phage infection. We examined biofilm infection in a static biofilm model, as well as a mouse model of keratitis.

Results: We identified a bacteriophage with the curious property that it can form plaques on a flagellar mutant but fails to plaque on wild-type *P. aeruginosa*. We discovered that the flagellar mutation results in an increase in intracellular cyclic-di-GMP, which in turn up-regulates the production of the exopolysaccharide Psl. Binding of the phage to *P. aeruginosa* is Psl-dependent. Importantly, the bacteriophage can infect and replicate on wild type *P. aeruginosa* that are in a biofilm. We further demonstrate that the bacteriophage can also reduce the bacterial burden in a mouse model of *P. aeruginosa* keratitis, which is characterized by the formation of a biofilm on the cornea.

Conclusions: We have isolated a *P. aeruginosa* bacteriophage which, surprisingly, preferentially infects bacteria in biofilms by exploiting the exopolysaccharide Psl for infection. To our knowledge, this is the first time that Psl has been implicated in infection by a bacteriophage. Moreover, we report a general strategy for uncovering biofilm-tropic phage, which will facilitate further discovery and expand their potential for therapeutic use.

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Pseudomonas aeruginosa glycolate metabolism and its role in aggregate physiology

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Abstract

The small organic acid glycolate is structurally similar to lactate, a major carbon source available to *Pseudomonas aeruginosa* during cystic fibrosis lung colonization and in chronic wounds. Although evidence suggests glycolate is also present in infection sites, little is known about its metabolism, especially in pathogenic bacteria. In *P. aeruginosa*, glycolate inhibits growth on lactate and we have shown that it inhibits expression of the lactate utilization operon. We have also demonstrated that the *P. aeruginosa glcDEFG* operon is required for glycolate consumption and that its expression is enhanced by glycolate. Intriguingly, *glcDEFG* is also expressed in the absence of added glycolate suggesting that it is produced endogenously. We speculate that the main source of this glycolate is glyoxal/methylglyoxal detoxification, a process whereby toxic metabolic byproducts are converted into either glycolate or lactate. *P. aeruginosa* contains homologs to the enzymes of this pathway, which has primarily been characterized in eukaryotes and non-pathogenic bacteria. The fact that glyoxal/methylglyoxal detoxification produces both glycolate and lactate underscores the high degree of cross-talk between the bioactivities of these two metabolites. Finally, I have demonstrated that during *P. aeruginosa* growth as multicellular aggregates, lactate- and glycolate-associated genes are induced in a spatially-segregated manner. Mutant analyses further indicate that glyoxal/methylglyoxal metabolism is the dominant source of glycolate and lactate in this environment. Because *P. aeruginosa* forms aggregates during host colonization, a definition of the integrated metabolic pathways operating in aggregates will help us understand the infection state of this common pathogen.

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Contributions of *Pseudomonas putida* to the transformation of the chemical industry – From unconventional substrates to valuable products

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Abstract

The challenges in transforming the current fossil-based linear-value-chain chemical industry into a bio-based circular industry are manifold. With its versatile metabolism, *Pseudomonas putida* is a promising workhorse for achieving the sustainable conversion of a multitude of untapped resources into valuable chemicals. On the one hand, we will report on our approaches to facilitating the use of unconventional substrates. New results on genetic engineering approaches to enhance the growth properties on ethylene glycol, a monomer from plastic waste, as well as on acetate as a fourth-generation feedstock, will be shown in detail: We increased the growth rate on EG by 140% to 0.3 h^{-1} and enhanced the tolerance to acetate, effectively halving the lag phase. Valorizing these carbon sources has been carried out by engineering the synthesis of different molecule classes in high demand (biosurfactants, biopolymers). Here, we will highlight strain engineering approaches to tailoring the molecular structures of the biosurfactant rhamnolipid and present unpublished insights into the rhamnolipid biosynthesis pathway in recombinant *P. putida*. With this panoply of research lines converging in the ultimate goal of establishing the bioeconomy, we showcase our favorite bacterium's incredible versatility and suitability for sustainable process development.

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Enhancing Iron-Sulfur Cluster Enzyme Expression for the Complete Biosynthesis of Adipic Acid in Pseudomonas putida

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Abstract

Adipic acid is a high-volume platform chemical primarily used for the synthesis of nylon-6,6. Conventional adipic acid synthesis involves the use of fossil derived benzene and results in the production of nitrous oxide (N_2O), a highly potent greenhouse gas. *Pseudomonas putida* natively produces cis, cis-muconic acid (ccma), a key precursor to adipic acid, from lignin derived aromatic monomers with catechol (1,2-dihydroxybenzene) as a central intermediate. The enoate reductase (ER) enzyme from *Bacillus coagulans* (BC) has been shown to directly reduce ccma to form adipic acid in vivo, representing a much shorter biosynthesis route for adipic acid in *P. putida* than those reported previously. The expression of ER-BC in *P. putida*, however, is challenging; as an [4Fe-4S] iron-sulfur cluster enzyme, ER-BC is sensitive to the presence of oxygen, conflicting with the aerobic lifestyle of the new host. Additionally, enzyme expression may be limited due to differences in iron-sulfur cluster biogenesis pathways. To address inactivation from oxygen, a two-step process was used with an aerobic growth stage followed by induction of ER-BC in a second, anaerobic production stage. To enhance expression of ER-BC, the *iscR* gene encoding for a transcriptional repressor of the native ISC iron sulfur cluster assembly pathway was deleted. Combined, these strategies resulted in the production of 7 mg/L of adipic acid from ccma in a whole-cell biotransformation. Further addressing the challenges identifies could result in an attractive alternative pathway for adipic acid biosynthesis.

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A balancing act: Signal sensitivity in *Pseudomonas aeruginosa* quorum sensing

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Abstract

Many bacteria communicate with kin and coordinate group behaviors through a form of cell-cell signaling called quorum sensing (QS). Moreover, bacteria typically live in polymicrobial communities where interactions between different bacterial species, such as through QS cross talk, can alter virulence and antibiotic susceptibility. Hundreds of QS systems with diverse properties have been identified, but how these systems evolved from a presumed common ancestor and how they interact in polymicrobial communities are open questions. To provide insight into these questions, we previously used a computational covariation approach to investigate the coevolution of QS signal synthases and receptors, using LasI-LasR from the pathogen *Pseudomonas aeruginosa* as a model system. Through this work, we observed that the receptor, LasR, has not evolved to maximal signal sensitivity. Taking advantage of hyper- and hypo-sensitive LasR variants, we examine the costs and benefits of altered receptor sensitivity. We find that hyper-sensitivity results in earlier and stronger expression from LasR-regulated promoters while hypo-sensitivity results in delayed and lower expression from both LasR- and RhlR-regulated promoters. Interestingly, strains expressing either the hyper or hypo-sensitivite LasR polypeptide exhibit reduced production of pyocyanin, a key antimicrobial molecule. These results highlight the complex regulatory network within which LasR is embedded. Ultimately, these changes to the *P. aeruignosa* QS regulatory network result in a defect during kin competition for the hyper-sensitive variant and during interspecies competition against the opportunistic pathogen *Burkholideria multivorans* for the hypo-sensitive variant. Our findings suggest intra- and interspecies competition work together to tune sensitivity in cell-cell signaling.

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CIFR (Clone – Integrate – Flip-out – Repeat): a transposon-based toolset for iterative genome engineering of Pseudomonas

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Abstract

Advances in genome engineering enable precise and customizable modifications of bacterial species. Toolsets that exhibit broad-host compatibility are particularly valued owing to their portability. Tn*5* transposon vectors have been widely used to establish random integrations of desired DNA sequences into bacterial genomes. However, the repeated use of the procedure remains challenging because of the limited availability of selection markers. Here, we present CIFR, a mini-Tn*5* integration system for iterative genome engineering refashioned from pBAMD vectors. The pCIFR vectors incorporate *attP* and *attB* sites flanking an antibiotic resistance marker used to select for the insertion. Subsequent removal of these antibiotic determinants is facilitated by the Bxb1 integrase, and a user-friendly counter-selection marker, both encoded in the pFNC auxiliary plasmid. Hence, this protocol delivers engineered strains harbouring stable DNA insertions and free of any antibiotic resistance cassette, allowing for the reusability of the tool. The system was validated in *Pseudomonas putida* and other Gram-negative bacteria, underscoring its portability across diverse industrially-relevant hosts. The CIFR toolbox was calibrated through combinatorial integrations of chromoprotein genes in *P. putida*, generating strains displaying a diverse colour *palette*. Next, we introduced a carotenoid biosynthesis pathway in *P. putida* in a two-step engineering process, showcasing the potential of the tool for pathway balancing. The broad utility of the toolbox expands the synthetic biology toolkit for metabolic engineering, allowing for the construction of complex phenotypes, while opening new possibilities in bacterial genetic manipulations.

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Transposon-insertion sequencing identifies common and strain-specific complement-evasion strategies for *Pseudomonas aeruginosa*

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Abstract

Clinical strains of *P. aeruginosa* show either resistance or persistence in human plasma independently of their serotype and arsenal of virulence toxins (Pont et al, PloS Pathogens, 2020). Here, we used genome-wide screens, Tn-seq, to identify strain-specific and/or common factors of *P. aeruginosa* - complement system interaction. Two resistant strains (CHA and YIK) and two persistent strains (PA14 and IHMA87) were selected on the basis of origin, clade, serotype and known exopolysaccharide (EPS) content. Libraries of transposon mutants were constructed and challenged by human plasma from healthy donors. Tn-seq analysis identified common factors that altered strain sensitivity to complement killing notably genes involved in EPS synthesis and/or regulation, such as *retS*, *rsmA*, *algU*, *pslE*, *gacA*, as previously reported by several groups. The plasma sensitivity of mutants in two other genes was significantly altered in Tn-seq of resistant strains- *crc* and *ssg*. Ssg is a putative glycosyltransferase involved in the synthesis of LPS O-antigen, and Crc is a negative regulator of global carbon metabolism. Surprisingly, targeted deletions of *ssg* and *crc* in resistant strains led to the persistent phenotype, i.e. the survival of around 1-10% of the population after prolonged exposure to plasma, while mutants in *wzy* and *wzz*, required for long OSA synthesis were readily eliminated. Together, our results show that *P. aeruginosa* strains take distinct routes to evade killing by the human complement system through a combination of common and strain-specific determinants, and suggest that the emergence of persisters may be a common strategy of complement evasion.

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Harnessing peptides and peptidomimetics against resilient Pseudomonas aeruginosa – Staphylococcus aureus co-infections

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Abstract

While antibiotics have historically been a cornerstone of infectious disease treatment, their efficacy is increasingly compromised by resistance mechanisms. This leaves clinicians with limited therapeutic options. Compounding this challenge is the prevalence of polymicrobial infections, where multiple microbial species cohabitate and interact within host environments, exacerbating the complexity of treatment strategies. Our study focuses on the potential of peptides and peptidomimetics (peptoids) as novel adjunctive therapies to enhance the activity of antibiotics against the recalcitrant, multidrug-resistant pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Using both an *in vitro* co-biofilm and *in vivo* murine skin co-infection model, we demonstrate the pharmacological efficacy of peptides in treating infections and mitigating severity of skin abscesses. We observed synergistic interactions between peptides and clinical antibiotics, suggesting promising avenues for combination therapies. Peptoids, an isomeric variant of peptides with side chains appended to the amide backbone nitrogen, emerge as particularly promising therapeutics due to their enhanced stability and cost-effectiveness. Our study revealed the anti-biofilm activity of several structurally-related peptoids to treat co-biofilms of *P. aeruginosa* and *S. aureus* under host-mimicking conditions. Ultimately, our *Pseudomonas-Staphylococcus*co-infection, skin abscess mouse model provided invaluable insights into the therapeutic efficacy of peptoids in reducing infection severity under challenging conditions. In summary, our study underscores the urgent need for innovative therapeutic approaches to address antibiotic resistance and polymicrobial infections. By harnessing the potential of peptides and peptidomimetics, we aim to expand the repertoire of treatment options and pave the way for a future where infectious diseases are more effectively managed.

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Comparison of seed bacterization levels on wheat, maize and soybean seeds inoculated with autochthonous *Pseudomonas* isolates

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Abstract

Pseudomonas play a key role in sustainable agriculture due to their broad plant-growth promoting traits. As seed inoculation is the main strategy to introduce beneficial bacteria into agricultural ecosystems, we studied the bacterization levels of 7 *Pseudomonas* isolates on wheat, maize and soybean seeds, with or without the addition of a protectant to the inoculation mix. Isolates were tagged with fluorescent proteins and antibiotic cassettes using a system based in Tn*7* transposon. Bacterial suspensions (OD_{600}=1.0) were mixed with non-disinfected wheat seeds (Baguette 550), maize seeds (KM8701 VIP3) or soybean seeds (13-146), following the recommended dose (10 ml/kg, 7 ml/kg or 0.5 ml/kg respectively). These mixtures were prepared in presence or absence of the commercial additive Premax® (Rizobacter, Argentina, 20% v/v for wheat and soybean, and 28.6% v/v for maize). Immediately after inoculation, bacteria were recovered from seeds and quantified on selective medium ("day 0"). Using this methodology, we monitored the decay kinetics of the inoculants for up to 4 days post-inoculation (dpi). Overall, the bacterial recovery (CFU/g) from wheat seeds was the highest at day 0, with the lowest values obtained for maize seeds. Additionally, all the isolates achieved good bacterization levels on wheat seeds, which were maintained during 4dpi. In contrast, on maize seeds, the recovery decayed drastically after 1dpi in all cases. On both soybean and maize seeds, Premax® showed a remarkable positive effect on the bacterial recovery. In conclusion, bacterization levels were strain-dependent and seed-dependent, being promoted to different extents by adding the commercial additive.

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Recursive implementation of synthetic C1-assimilation cycles in engineered *Pseudomonas putida* bestow methylotrophy

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Abstract

The soil bacterium *Pseudomonas putida* is a robust biomanufacturing host that assimilates a broad range of substrates while efficiently coping with adverse environmental conditions. *P. putida* is equipped with functions related to one-carbon (C1) compounds (e.g. methanol, formaldehyde, and formate) oxidation—yet pathways to assimilate these carbon sources are largely absent. In this work, we utilized modularity and growth-coupled selections to compare in a standardised manner the three known serine cycle variants to promote assimilation of methanol in *P. putida*. The serine cycle is the only known cycle which assimilates methanol and yields acetyl-CoA in nature. Nonetheless, the serine cycle has been shown particularly difficult to implement in a synthetic host. Thus, several variants, such as the modified serine cycle, the serine-threonine cycle and the homoserine cycle have been designed, for their resemblance to the central carbon metabolism architecture of heterotrophs, such as *E. coli* or *P. putida*. We hereby modularized the three serine cycle variants and constructed amino acid auxotrophic strains to test their new activities in order to identify which cycle (or module combination) has the highest potential for further synthetic methylotrophic engineering in *P. putida*. The results reported herein both expand our understanding of microbial metabolism and lay a solid foundation for engineering efforts towards valorizing C1 feedstocks using serine cycle variants.

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Release of extracellular DNA by Pseudomonas species as a major determinant for biofilm switching and an early indicator for cell population control

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Abstract

Extensive research has focused on understanding the various stages involved in biofilm formation. However, the initial cellular decision-making process that triggers the transition from a planktonic to a sessile state has yet to be fully characterized. To shed light on this, we conducted studies using *Pseudomonas putida* KT2440 and its derivatives, which exhibit different capacities for biofilm formation. In our investigation, we made an intriguing observation during the planktonic phase: a subpopulation of cells exhibited binding to extracellular DNA (eDNA), as evidenced by propidium iodide (PI) staining. Notably, the size of this subpopulation, characterized by eDNA-bound/PI-positive cells, correlated with the overall biofilm-forming ability of the bacterial population. This discovery challenges the conventional understanding of phenotypic switching and suggests that, in *Pseudomonas*, the decision to form a biofilm is collectively determined by the quantity of eDNA released into the surrounding environment. To track this process effectively, we employed automated flow cytometry, which allowed us to monitor the appearance of PI-positive cells. We identified these cells as early-warning indicators for biofilm formation. Furthermore, we successfully utilized automated glucose pulsing to interfere with the proliferation of PI-positive cells, leading to a reduction in biofilm formation. This innovative approach highlights a potential strategy for controlling biofilm development. Our study provides valuable insights into the collective factors that influence biofilm switching in *Pseudomonas* species. By understanding the role of eDNA and its correlation with biofilm formation, we open up new avenues for manipulating and managing biofilm-related processes.

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Defense mechanisms against RNA phages

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Abstract

Bacterial mechanisms that antagonize the phage lifecycle stages have been characterized as the components in antiphage defense systems that have been evolved during the arms race between phages and bacteria. The antiphage defense systems work basically at the five steps of the phage lifecycle, which include phage adsorption, genome penetration, nucleic acid and protein synthesis, virion assembly, and phage release. The arsenal of host systems to resist phages may explain the host tropism of phages, but most of the research has been done for DNA phages, rather than RNA phages. Considering the potential significance and ecological diversity of RNA phages as comparable as those of RNA viruses in higher eukaryotes, we first analyzed the host tropism of the small RNA phage, PP7 that infects *Pseudomonas aeruginosa* PAO1, but neither PAK nor PA14, among the most widely used laboratory strains. We found that a set of group II pilins are required for PP7 infection and suggested the molecular details of the phage-receptor interaction. We also revealed that other host factors might govern the host tropism of PP7 in *P. aeruginosa* clinical strains. Topics discussed will include the rational and principle to identify the defense system genes against RNA phages. This is based on our reverse genetic system of the RNA phages that can bypass their genome entry limited by receptor specificity. Genetic identification of the host factors that restrict the PP7 lifecycle stages such as genome penetration and phage release will be covered as well.

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