INTERNATIONAL BIENNIAL PSEUDOMONAS CONFERENCE

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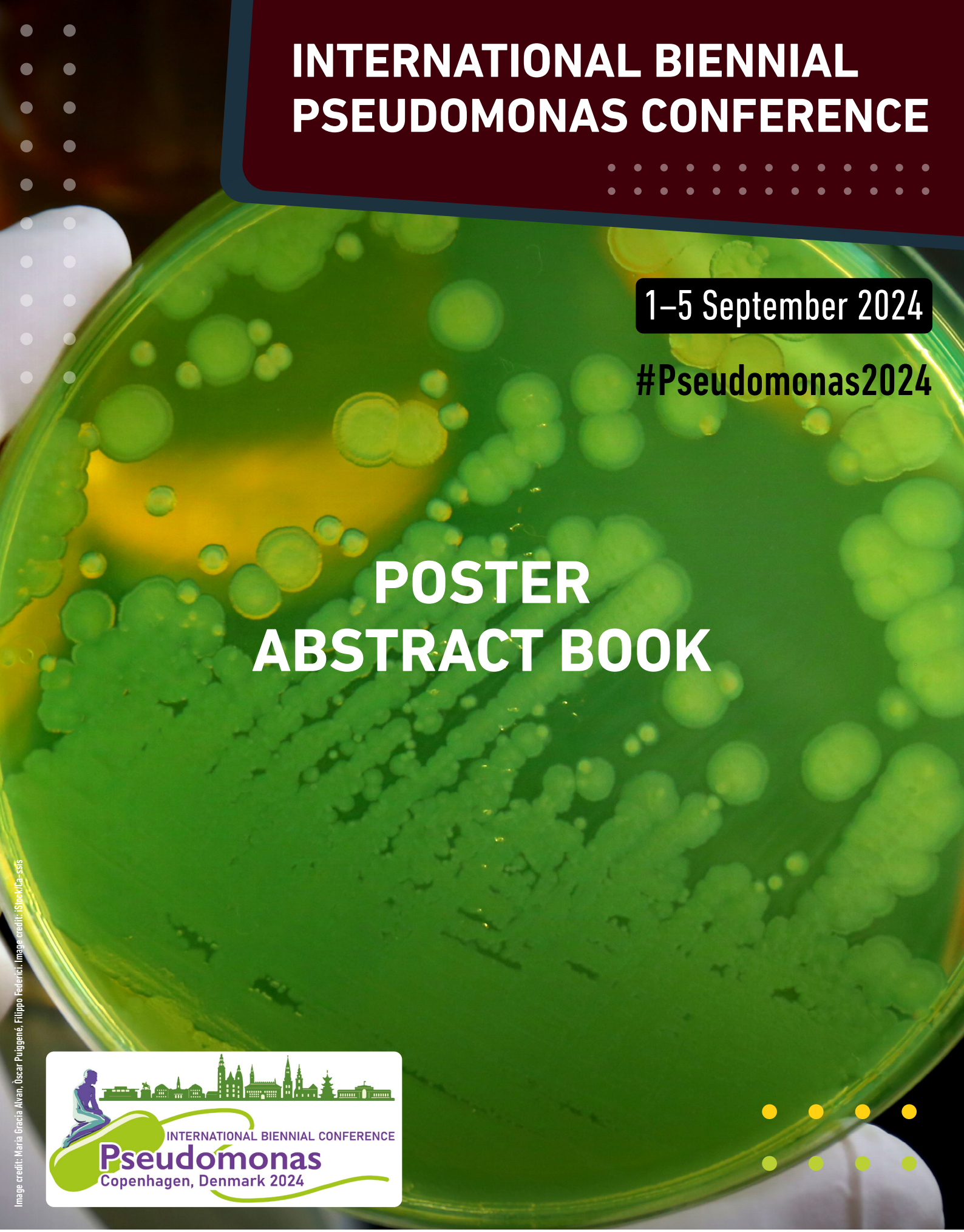
#Pseudomonas2024

POSTER ABSTRACT BOOK



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INTERNATIONAL BIENNIAL CONFERENCE
Pseudomonas
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The Scandic Copenhagen Hotel
Vester Søgade 6, DK-1601,
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MICROBIOLOGY SOCIETY

Flash Poster : 001

Antibiotic resistance alters the ability of *Pseudomonas aeruginosa* to invade the respiratory microbiome

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Abstract

The emergence and spread of antibiotic resistance in bacterial pathogens is a global health threat. One important unanswered question is how antibiotic resistance influences the ability of a pathogen to invade the host-associated microbiome. Here we investigate how antibiotic resistance impacts the ability of the opportunistic bacterial pathogen *Pseudomonas aeruginosa* to invade the respiratory microbiome, by measuring the ability of *P. aeruginosa* spontaneous antibiotic resistant mutants to invade pre-established cultures of commensal respiratory microbes. We find that commensal respiratory microbes tend to inhibit the growth of *P. aeruginosa*, and antibiotic resistance is a double-edged sword that can either help or hinder the ability of *P. aeruginosa* to overcome this inhibition. The directionality of this help or hinderance depends on both *P. aeruginosa* genotype and respiratory microbe identity. Antibiotic resistance facilitates the invasion of *P. aeruginosa* into *Staphylococcus lugdunensis,* yet impairs invasion into *Rothia mucilaginosa* and *Staphylococcus epidermidis*. *Streptococcus* species provide the strongest inhibition to *P. aeruginosa* invasion, and this is maintained regardless of antibiotic resistance genotype. Our study demonstrates how antibiotic resistance can alter the ability of a bacterial pathogen to invade the respiratory microbiome and suggests that attempts to manipulate the microbiome should focus on promoting the growth of commensals that can provide robust inhibition of both wildtype and antibiotic resistant pathogen strains.

003

Interplay between HsbA and CfcR prevents biofilm formation under nutrient starvation in *Pseudomonas putida*

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Abstract

Nutrient availability promotes biofilm formation in *Pseudomonas putida*, whereas nutrient deprivation triggers biofilm dispersal. We have previously demonstrated that starvation-induced dispersal relies on synthesis of the alarmone (p)ppGpp. Through transcriptomic analysis, we identified that (p)ppGpp induces transcription of *cfcR*, encoding a two-component system response regulator with diguanylate cyclase (DGC) activity, and the *hsbAR-hptB* operon encoding a phosphorelay pathway and an anti-σ factor antagonist.

Deletion of *hsbA* provoked resumption of biofilm formation after dispersal in late stationary phase, stimulated medium-air pellicle formation, and enhanced Congo Red adsorption. These phenotypes were attributed to increased c-di-GMP levels during stationary phase. HsbA was found to interact with CfcR in a pull-down assay, and deletion of *cfcR* suppressed all the Δ*hsbA* mutant phenotypes, suggesting that HsbA negatively regulates the DGC activity of CfcR.

Activity of the *Pseudomonas aeruginosa* HsbA ortholog is regulated by phosphorylation of the serine-56 residue. Expression of non-phosphorylatable and phospho-mimic mutant HsbA proteins showed that repression of CfcR is associated to the non-phosphorylated version and the phenotypes of Δ*hsbR* and Δ*hptB* mutants were consistent with a role of HsbR and HptB in HsbA phosphorylation. Confocal microscopy revealed that CfcA, the cognate sensor kinase of CfcR, recruits both CfcR and non-phosphorylated HsbA to discrete locations at the cell membrane.

Our findings support a model in which biofilm formation in stationary phase is actively inhibited by the interaction of unphosphorylated HsbA with CfcA and CfcR. Such inhibition may be reverted by as-of-yet-unknown signals triggering HsbA phosphorylation that leads to CfcR release.

005

The New Public Health Threat: Pseudomonas maltophilia from Ghanaian Hospital Environment

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Abstract

Background: The magnitude of antimicrobial resistance (AMR) in Gram-negative bacteria is a growing burden to public health. *Pseudomonas maltophilia*, which has been implicated in opportunistic infections, is now emerging from Ghanaian-hospital ICUs.

Methods: Fomites-resident *P. maltophilia* from Ghanaian-hospital ICUs was profiled with MALDI-TOF MS and 16S rRNA-seq. The AMR profile was determined with disc-diffusion and broth-microdilution. Hetero-resistance with E-tests and population analysis profiling. Associated AMR mechanisms and virulence were determined with *resistome* and *virulome* profiling with nanopore-seq. *In vitro* and *in vivo*, virulence and pathogenicity were assessed with bacterial survival in macrophages and the *Galleria mellonella* infection (GMI) model.

Results: The strains showed 80–100% levels of AMR, were hetero-resistant, and formed biofilms. The *resistome* and *virulome* analyses showed plasmid-mediated resistance (*oqxA, oqxB, qnrB1, qnrA*, *kpc*, *blaTEM-1B, blaCX-M-15, blaOXA-1, blaSHV-28*) markers and virulence (T4SS, T1 adhesin-fimbriae, invasive flagella, curli-fibers, salmochelin-aerobactin, siderophore) factors. Also, the strain survived the immune factors of the macrophages at low multiplicity of infection (1–10 bacterial cells/10^4–10^6 macrophages), and 10 bacterial cells/larvae were enough to kill in GMI 24-hpi, indicating virulent potential. Under antibiotic pressure, there was an increase in the bacterial loads *in vitro*, which might indicate that increased AMR increases the virulence of the strain and promotes survival within the macrophages.

Conclusion: Overall, our findings showed the emergence of superbugs from Ghanaian-hospital ICUs with the tendency to cause infections that are difficult to treat. Also, the presence of diverse infection-associated resistant markers and consequent virulence *in vitro* and *in vivo* is a threat to public health.

006

Competitive fitness of serial clonal *Pseudomonas aeruginosa* isolates from mild and severe cystic fibrosis airway infections

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Abstract

Background. Serial semiannual *Pseudomonas aeruginosa* airway isolates were collected for 10 to 40 years from CF patients regularly seen at the CF clinic Hannover who became chronically colonized between 1982 and 1991. Genome evolution of the initially acquired *P. aeruginosa* clone was examined in the six most severe and the six mildest clinical courses.

Methods. Serial clonal isolates of the same course were mixed at equal proportions and exposed to nutrient-poor and nutrient-rich liquid media, murine and human precision cut lung slices and human neutrophils. The relative abundance of clonal isolates was determined by the relative frequency of strain-specific SNVs ascertained by sequencing of multiplex amplicons.

Results. The outcome of the competitive fitness experiments was similar for all investigated habitats and was driven by the status of CF lung disease at the time when *P. aeruginosa* conquered the CF airways. The serial CF isolates from the mild courses that colonized a lung with persistent normal spirometry showed individual clone-specific patterns of growth and survival. In contrast, the strain collections from the severe courses showed a uniform outcome. The early isolates that differed least in their genomic profile from the environmental ancestor outcompeted their clonal progeny that had evolved to a state of lower general fitness to persist in an aquatic habitat or healthy lungs.

Conclusion. The disease status of the CF lung habitat and of the immune system governed the adaptation of *P. aeruginosa* more strongly than the underlying clone-type and its genetic repertoire.

008

Trace amounts of *Pseudomonas aeruginosa* DNA in healthy and cystic fibrosis airways during infancy

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Abstract

Background. *Pseudomonas aeruginosa* DNA was detected by metagenomic sequencing in airway secretions of all patients aged six years and above with cystic fibrosis (CF) and exocrine pancreatic insufficiency seen at the CF clinic Hannover. We aimed to explore the first onset of *P. aeruginosa* colonization in infancy.

Methods. Shotgun metagenomics was performed on total DNA extracted from cough swabs of 52 healthy children and 41 children with CF, aged from birth to six years, and negative controls.

Results. *P. aeruginosa* DNA was recovered from both healthy and CF airway samples, with the earliest detection in a child as young as three weeks. Detection rates of *P. aeruginosa* were similar in CF and healthy children. Neither DNA background contamination, nor age and season of sampling were linked to the presence of *P. aeruginosa* DNA in biological samples. *P. aeruginosa* was always among the 5% least abundant species and its presence did not influence the ecological diversity or the abundance of other low- and high-abundant species in the airway microbial community. Longitudinal assessment of CF infants revealed three patterns of *P. aeruginosa* DNA detection: (1) persistent DNA traces of *P. aeruginosa* in culture-negative infants, (2) absence of *P. aeruginosa* in both metagenomic sequencing and culture tests, and (3) initial absence of *P. aeruginosa* DNA until the first positive culture result.

Conclusion. Detection of trace amounts of *P. aeruginosa* DNA in infancy is not associated with disease. Children are regularly exposed to the environmental bacterium *P. aeruginosa* without requiring medical intervention.

010

A moonlighting VirB4 ATPase of the mobile accessory genome operates core genome - encoded key features of Pseudomonas aeruginosa TBCF10839

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Abstract

Background. *Pseudomonas aeruginosa* TBCF10839 is a highly virulent strain that can persist and replicate in human neutrophils.

Methods and Results. Screening of a STM TBCF10839 transposon library in phagocytosis tests identified a mutant that carried the transposon in the VirB4 homolog 5PG21 of an ICE-associated type IV secretion system of the pKLC102-subtype. 5P21 TBCF10839 insertion mutants were deficient in metabolic versatility, secretion, quorum sensing and virulence. The mutants were efficiently killed in phagocytosis tests in vitro and were avirulent in an acute murine airway infection model in vivo. The inactivation of 5PG21 silenced the *rhl, las* and *pqs* operons and the gene expression for the synthesis of hydrogen cyanide, the antimetabolite L-2-amino-4-methoxy-trans-3-butenoic acid and the H2- and H3-type VI secretion systems and their associated effectors. The mutants were impaired in the utilization of carbon sources and stored compounds that are not funneled into intermediary metabolism.

Conclusion. A single gene of the mobile accessory genome can become an essential element to operate the core genome – encoded features of metabolism and virulence.

015

The anode-driven anaerobic metabolism of Pseudomonas putida

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Abstract

Establishing an anaerobic lifestyle of the obligate aerobe *Pseudomonas putida* (*P. putida*) is of great interest for both fundamental research and industrial applications. However, research progress in this field suffers from the lack of a knowledge base that enables effective strain development. In our group, we have introduced the concept of microbial electrochemical technology to enable an active anaerobic metabolism for *P. putida*. Briefly, *P. putida* cells use an anode as an electron sink via a hydrophilic redox mediator, which was later confirmed to extract the microbial electrons from the membrane respiratory chain, especially with cytochrome c reductase as the key enzyme. The outer membrane transporter responsible for the transport of the redox mediator was also confirmed by a combined proteomics and metabolic engineering approach.

The electron balance from the anode opens up two unique opportunities for *P. putida* research. With the perspective of industrial applications, we were able to establish an electrode-driven biosynthesis process with extremely high yield that is beyond the current stoichiometric limit. Under anoxic conditions, *P. putida* cells could almost exclusively convert glucose into 2-keto-gluconate with a yield of over 90%. This process can be applied to a wide range of sugars to produce various sugar acids. With the perspective of fundamental research, this bioelectrochemical technique provides a unique cultivating solution that can continuously maintain the active metabolism of *P. putida* cells under anaerobic conditions, allowing us to gain deep quantitative insights into the anaerobic metabolism and reveal the metabolic constraints.

016

Identifying The Host-Directed Effectors in *Pseudomonas Aeruginosa*

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Abstract

Pseudomonas aeruginosa (*Pa*), is a Gram-negative bacterium with both environmental and pathogenic significance. Its diverse virulence traits, antimicrobial resistance mechanisms, and adaptive capabilities have contributed to its success as a pathogen, particularly in immunocompromised individuals.

Pa manipulates host immune cell activity to ensure longevity of infection. Phagocytic cells, which act as a first line of defence against invading pathogens, are targets for bacterial effector proteins. Several such effectors have been described, including TesG, which inhibits eukaryotic GTPases, and toxic cargo of the type III secretory system, which can prevent inflammasome and NFkB activation.

The focus of this project is to investigate the influence on *Pa*-macrophage interactions of three bacteriocin-like proteins (BLP) that are regulated by the transcriptional regulatory protein SutA. Through generation of BLP gene deletion and inducible expression strains, macrophage interaction assays, fluorescence microscopy, and *in vivo* infection models, we aim to characterise the influence of these proteins on infection outcomes.

Work to date has focused on quantifying uptake and intracellular killing of *Pa* by macrophages, using an aminoglycoside protection assay conducted with PA14 and BLP deletion or inducible expression strains. We highlight the multifactorial nature of *Pa*-macrophage interactions, with preliminary findings indicating that BLP effectors make temporally-restricted contributions to infection outcomes.

Future work will contribute to advancing our understanding of *Pa* pathogenesis and its interactions with host defences, paving the way for potential insights into therapeutic strategies.

019

Functional and Evolutionary Characterization of Carbaryl degradation pathway and enzyme 1-Naphthol 2-hydroxylase in *Pseudomonas* sp. C5pp

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Abstract

Carbaryl is a widely used broad spectrum pesticide that pollutes soil as well as water ecosystems and displays toxicity against non-target biota. The soil bacterium *Pseudomonas* sp. C5pp has evolved to metabolize Carbaryl as the sole source of carbon and energy. The genes encoding pathway enzymes are present in three distinct operons: 'upper'(Carbaryl to salicylate), 'middle' (salicylate to gentisate) and 'lower' (gentisate to TCA cycle intermediates). These operons have lower G+C content than rest of the genome and are flanked by remnants of mobile genetic elements of different ancestral origins. This suggests a multi-step 'patch-work' assembly process where strain C5pp acquired genes for Carbaryl degradation from various sources through multiple horizontal gene transfer events. A key enzyme in this pathway is 1-naphthol 2-hydroxylase (1NH) which catalyzes conversion of 1-naphthol to 1,2-dihydroxynaphthalene. Phylogenetic analysis revealed evolutionary relationship of 1NH with hydroxylases acting on phenols. Notably, it shares 50% sequence similarity to 2,4-dichlorophenol monooxygenase (2,4-DCPM) and displays 47 % activity on 2,4-dichlorophenol suggesting a common ancestral origin for 1NH and 2,4-DCPM. Comparative analysis of 1NH and 24DCPM revealed identical FAD and substrate binding sites but significant differences in the substrate entry tunnel hinting at its crucial role in determining substrate specificity. Homology model guided rationale engineering of 1NH led to identification of key residues involved in FAD binding, substrate entry and substrate binding. The aim is to reverse engineer entry tunnel of 24DCPM to trace evolution of 1NH. The study provides insights into events of evolution in microbes for degradation of man-made pollutants.

020

Exploring *Pseudomonas pluviae* MUP55 as a Biocontrol Agent for Phytopathogens

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Abstract

Numerous *Pseudomonas* strains exhibit significant potential for promoting plant growth through the synthesis of diverse substances such as phenazines and siderophores. Additionally, *Pseudomonas* spp. possess an array of antimicrobial secondary metabolites, allowing them to effectively compete with other microorganisms for plant-provided niche space and carbon resources. In this study, we employed *Pseudomonas pluviae* MUP55 to combat the growth of phytopathogens including *Pseudomonas syringae*, *Fusarium oxysporum*, and *Rhizoctonia solani*.

To assess interbacterial competition, MUP55 was co-cultured with these bacterial pathogens for 5 hours, followed by quantification of colony-forming units (CFUs) through serial dilution plating. For the fungal inhibition assay conducted in vitro, pathogenic fungi were centrally placed on potato dextrose agar (PDA) plates, while MUP55 was streaked 3cm away from the center. Inhibition efficacy was determined by calculating the percentage of fungal growth inhibition induced by MUP55.

Our findings demonstrate a remarkable reduction in the viability of *Pseudomonas syringae* by 10-fold compared to the control when co-cultured with MUP55. Furthermore, MUP55 exhibited inhibitory effects on *Fusarium oxysporum* and *Rhizoctonia solani*, suppressing their growth rates by 25% and 40%, respectively.

In conclusion, our study highlights the potential of *Pseudomonas pluviae* MUP55 in suppressing phytopathogens. Our findings demonstrate significant reductions in the viability of *Pseudomonas syringae* and notable inhibition of *Fusarium oxysporum* and *Rhizoctonia solani* by MUP55. This suggests promising applications in agriculture for disease management and improving plant health. Further research into these interactions could lead to innovative biocontrol strategies for enhancing crop productivity and sustainability.

021

Pseudomonas aeruginosa utilize a rhamnolipid-dependent mechanism to avoid competition by *Klebsiella pneumoniae* in a nutrient-limiting niche.

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Abstract

Microbial interactions are pivotal in shaping infection dynamics and consortia in natural environments. *Pseudomonas aeruginosa*, a major pathogen causing hospital-acquired pneumonia (HAP), frequently coexists with other pathogenic bacteria in the mammalian niche. Understanding these interactions is essential for developing effective infection management strategies. This study investigates how *P. aeruginosa* interact and respond to *Klebsiella pneumoniae*, another common pathogen in HAP. Using solid-surface competition assays and fluorescently tagged strains, we demonstrate that *P. aeruginosa* recognizes *K. pneumoniae* in a media-dependent manner. *P. aeruginosa* actively displaces *K. pneumoniae* biomass, forming a distinct clearance zone and toroid ring. Surprisingly, our analysis of virulence-associated genes indicates that *P. aeruginosa* does not rely on its proteases and toxins for this competition. Instead, quorum-regulated rhamnolipid production is essential for displacing *K. pneumoniae* cells. Additionally, we identify iron limitation as a key cue for this interaction, as iron supplementation inhibits both rhamnolipid synthesis and the displacement ability of *P. aeruginosa*. This study reveals how *P. aeruginosa* displaces competitors, giving us new insights into microbial interactions in nutrient-limited environments. This knowledge could improve infection control and bacterial community management. It may also influence future microbiology research and infection-fighting strategies.

028

Autoinducer-2 based signaling and spoilage properties in meat pseudomonads

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Abstract

Autoinducer-2 (AI-2) molecules represent a category of signaling molecules that play a role in regulating genes based on population density through a process called Quorum Sensing. Bacteria can utilize signal molecules to regulate the production of virulence factors and formation of biofilms. In this study production of AI-2 and the biofilm formation capabilities of *Pseudomonas* species isolated from meat. Isolates (n=83) from meat samples were characterized, identifying 15 putative *P. fragi* and 57 closely related *P. bubulae* via *rpo*D primers. All isolates exhibited robust motility. Biofilm formation assays indicated 37 isolates with significant biofilm production. Assessment of AI-2 production through luminescence via *Vibrio campbellii* (ATCC BAA-1117) biosensor revealed activity in 18 isolates. The samples, exhibiting the closest proximity to the positive control (*V. campbellii* ATCC BAA-1119) were recorded as YB91 from the *P. fragi* group demonstrated 19.10%, YK107 belonging to the *P. bubulae* group with 63.72% and a potential new species YK50 with 69.87%. In further analysis AI-2 related genes will be deleted from the isolates. This investigation illuminates AI-2 mediated spoilage activities in meat *Pseudomonas*, offering insights into microbial deterioration and strategies for mitigating foodborne risks.

030

The biofilm matrix of *Pseudomonas aeruginosa* is a distinct extracellular compartment which selectively traps key secreted proteins

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Abstract

Pseudomonas aeruginosa (PA) is both a 'professional secretor' and a prolific biofilm former. Consequently, many secreted factors need to pass through the biofilm matrix. This raises the question of whether any of these secreted factors are selectively trapped in the matrix, forming a distinct biofilm "matrixome".

To address this, we developed a method to separate biofilm cells from the matrix and from the culture supernatant, yielding clearly-differentiated "exo-compartments" for proteomic analysis. This revealed that the matrix does indeed selectively trap key secreted factors, while allowing others to pass through into the "true secretome". One of the most abundant matrix-enriched proteins was an uncharacterised protein, PA2668. Encoded immediately adjacent to PA2668 is an uncharacterised Type II Secretion (T2S) system, designated as the *hpl* cluster. Given that the substrates of many T2S systems are also encoded adjacent to their cognate secretion machinery, it seems likely that PA2668 may be exported through the Hpl system. dN/dS analyses indicate that the *hpl* cluster and PA2668 are highly conserved across clinical isolates suggesting functional importance. To investigate this further, we have purified PA2668 – which bears no similarity to any previously characterised protein - and have begun a detailed functional characterisation. Single chain Fv antibodies are being used to assess the expression and localization of PA2668, and both PA2668 and the *hpl* cluster have been cleanly-deleted for proteomic definition of the Hpl secretome and phenotypic analyses. DNA pulldowns and RNASeq are being used to assess how expression of the Hpl machinery and PA2668 are regulated.

034

Revisiting the role of the Pseudomonas aeruginosa alternative sigma factor RpoS in the quorum-sensing response

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Gloria Soberón-Chávez

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Abstract

Several *P. aeruginosa* virulence factors, such as pyocyanin (PYO), and biofilm biosynthesis, are regulated by quorum-sensing (QS). The expression of genes coding for the main QS transcriptional factors, *lasR* and *rhlR* is positively regulated by the alternative stress-related sigma factor RpoS. However, PYO production that is activated by RhlR is highly increased in a *rpoS* mutant.

To study this apparent contradiction, we constructed *P. aeruginosa* PAO1 derivatives expressing different levels of RpoS and measured by RT-qPCR the expression of QS transcriptional activators genes *lasR, rhlR* and *pqsR*, and the *pqsABCDE* operon coding for the enzymes involved in alkyl quinolone autoinducer (PQS) synthesis and the PqsE protein that interacts with RhlR and stabilize it. Our results using mutants in the RpoS *pqsA* promoter binding-site, show that RpoS directly represses its expression. Furthermore, we showed that PYO and biofilm production are increased in a *rpoS* mutant because PqsE expression is increased, resulting in RhlR stabilization.

To further determine if the main effect of RpoS in the QS response was due to its repression of the *pqsABCDE*operon we carried out a proteomic analysis of the *rpoS* mutant showing that the levels of 181 proteins are reduced while 362 are enriched, and that the proteins involved in PYO and PQS production are over expressed.

Our work represents a novel model of QS regulation by RpoS and contributes to the understanding of the modulation of virulence factors expression associated with different environmental conditions that are sensed by the alternative sigma factor RpoS.

035

Deciphering a novel Type IVB Secretion System involved in interbacterial killing

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Abstract

In most environmental niches, bacteria exist as multispecies biofilms, where cells are embedded in an extracellular matrix that protects them from external stresses, like nutrient limitation, predation and the host immune response, and also restricts the entry of biofilm invaders. Therefore, the competition for space and resources within biofilms has triggered the evolution of competitive strategies to persist in multispecies communities.

In this study, we use different experimental approaches to evaluate the competitive behaviour of the plant-associated bacterium *Pseudomonas* sp. strain IsoF in mixed species biofilms. We demonstrate that IsoF is able to outcompete various soil- and plant-associated bacterial species in a contact-dependent manner. Specifically, we show that IsoF is able to invade and displace a pre-established biofilm of *P. putida* strain KT2440, using flow-through chambers. We identified a novel type IVB secretion system (T4BSS) for contact-dependent killing in IsoF through generation and screening of a mini-Tn5 transposon insertion library. Inactivation of genes encoded by this cluster resulted in mutants that were no longer able to kill other bacteria nor were they able to invade existing biofilms, in contrast to the wild-type strain. Further examination of the cluster lead to the identification and characterisation of novel toxin proteins (effectors) of this interbacterial T4BSS, which were shown to be essential for contact-dependent killing.

These results provide evidence that the competition strategy of IsoF is based on a novel T4BSS, a defensive and offensive system used for the invasion of pre-established biofilms with potential biocontrol applications.

039

Adaptive patterns of *Pseudomonas aeruginosa* in polymicrobial infections in pediatric cystic fibrosis patients

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Abstract

Polymicrobial lung infections are frequently observed in individuals with cystic fibrosis (CF). The accumulation of nutrient-rich mucus in the airways creates a favorable environment for bacterial pathogens. Infections often follow an ecological succession with initial colonization by pathogens like *Haemophilus influenzae* and *Staphylococcus aureus*, succeeded by *Pseudomonas aeruginosa*. While the ecological succession is well described, less is known about whether the pathogens interact with and adapt to one another. Here, we examined the interactions between the three above-mentioned pathogens and investigated whether interaction patterns change over time. We first exposed clinical isolates (from 22 CF children, aged between 0-8 years) to their own supernatant and the supernatant of the other pathogens to determine the impact of secreted compounds on growth. Our findings revealed two key changes in interaction patterns involving *P. aeruginosa*. First, this pathogen strongly inhibited *S. aureus* and *H. influenzae* at early time points but showed reduced inhibition at later time points. Phenotypic assays combined with whole genome sequencing revealed that a decrease in protease production among late-stage *P. aeruginosa* strains was associated with the reduced inhibition of *S. aureus* or *H. influenzae*. Second, *P. aeruginosa* showed patterns of diversification in its interactions when exposed to either *S. aureus* or *H. influenzae* supernatants. Overall, we found that the virulent nature of *P. aeruginosa* against other bacteria was attenuated in older children. The results of our ecological study indicates that pathogens adapt to their environment and competitors already at early stages of polymicrobial colonization in CF patients.

047

Unearthing the Underground Metabolism Potential for Metabolic Expansion in Pseudomonas putida KT2440

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Abstract

Understanding how evolution reshape bacterial phenotypes is crucial to engineer microbes with desired properties. A starting point for the generation of new metabolic functions is the so-called underground metabolism, i.e., the subnetwork conformed by catalytically inefficient promiscuous enzymatic activities without an apparent physiological role. Here we assess the potential of this underground metabolism as source of novel phenotypes in *Pseudomonas putida* KT2440. To accomplish this, we expanded the high-quality genome-scale metabolic model *i*JN1462 of KT2440 by including the known set of promiscuous enzymatic activities deduced from its primary reactome and defined the new metabolic space driven by the underground metabolism through a system-level approach. With the aim of validate the model predictions, we performed an Adaptive Laboratory Evolution (ALE) experiment to broaden the range of available nutrients for *P. putida* KT2440. Following this technique, we obtained different strains able to degrade N-acetyl-L-alanine, an upregulated metabolite in HIV patients. Whole-genome sequencing revealed convergent evolution processes among the adapted strains that highlighted the accuracy of the underground model in estimating phenotype-genotype associations. Over a multidisciplinary approach encompassing reverse engineering, enzyme activity assays and synthetic biology, we characterized the mutations of the evolved strains and demonstrated that adaptation arose through synergistic and additive effects between modifications of enzyme and transcription factor functions. Overall, we show here how to exploit the underground metabolism to expand the metabolic versatility of *P. putida* while we shed light into the mechanisms underlying evolutionary processes driven by enhancing both, gene expression and enzyme activity.

048

Metabolic Insight into *Pseudomonas aeruginosa* Fluoroquinolone Sensitivity and Persistence

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Patricia Hare

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Abstract

Antibiotic-persistent bacteria pose a threat to modern medicine as the suspected culprits of chronic and recurrent infections. However, mechanisms governing persister survival in key pathogens such as *Pseudomonas aeruginosa* are not well understood. We found that hallmarks of fluoroquinolone (FQ) antibiotic persistence in the model organism, *Escherichia coli*, are not shared in *P. aeruginosa*. Our single-cell time-lapse microscopy experiments revealed that stationary-phase *P. aeruginosa* dies during FQ treatment by explosive cell lysis and/or loss of membrane integrity; in comparison, non-growing *E. coli* only succumbs to antibiotic treatment in the post-drug recovery phase when nutrients are replenished and cells are stimulated to divide. The observed phenotypes led us to hypothesize that *P. aeruginosa* maintains metabolic activity in the stationary phase that confers sensitivity to FQs. Utilizing molecular probes to measure reductase activity and radioactive uridine to measure *de novo* nucleic acid synthesis, we found that stationary-phase *P. aeruginosa* maintains metabolic activity and continues transcribing RNA. Comparatively, stationary-phase *E. coli* is metabolically quiescent. Inhibition of transcription or mitigation of reactive metabolites increases *P. aeruginosa* survival against FQs. This implies that combining FQs clinically with transcriptional inhibitors like rifampicin could hinder treatment of *P. aeruginosa* infection. We also found that the SOS response, which is necessary for *E. coli* FQ persister survival, is not implicated in *P. aeruginosa* FQ persistence. Overall, our findings emphasize the importance of studying antibiotic treatment failure in organisms other than *E. coli* laboratory strains because the underlying mechanisms may not be conserved.

049

Pathogen rivalry: unraveling response diversity of *Pseudomonas aeruginosa* in interactions with other opportunistic human pathogens

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Abstract

Bacterial infections often involve more than one pathogen. While it is known that polymicrobial infections can impact disease outcomes, we poorly understand how pathogens interact with each other, what molecular mechanisms are involved and whether these mechanisms are standard or tailored to specific opponents. Here, we explored how *Pseudomonas aeruginosa* reacts to six other opportunistic human pathogens that often co-occur in polymicrobial infections: *Acinetobacter baumannii*, *Burkholderia cenocepacia*, *Escherichia coli*, *Enterococcus faecium*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. We first used time-lapse fluorescence microscopy to follow interaction patterns and fitness of species in growing micro-colonies over time on agarose pads. We identified a broad spectrum of species-specific interactions including mutualism and antagonism. Second, we used a library of gene-expression reporters and deletion mutants to investigate the molecular mechanisms that *P. aeruginosa* deploys in interactions with the six other pathogens. Using a combination of flow cytometry and fluorescence microscopy, we observed both general and tailored responses of *P. aeruginosa* to specific pathogens but also environmental conditions. For example, a general response involved the upregulation of the production of the siderophore pyoverdine, an important agent for iron competition. We further observed differential and opponent-specific expression changes of various regulators including stress response, quorum sensing, and two-component systems. Overall, our insights improve our understanding of pathogen-pathogen interactions at both the ecological and molecular levels, highlighting that *P. aeruginosa* can distinguish between different opponents and mount tailored responses. This could help in predicting outcomes in polymicrobial infections.

052

The adaptive evolution of a beneficial *Pseudomonas* strain 2P24 in the wheat rhizosphere

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Abstract

The adaptive potential plays a crucial role in successful colonization of plant beneficial bacteria in the rhizosphere; however, research in this area remains limited. In this study, we utilized the model strain *Pseudomonas bijieensis* 2P24 to establish an experimental evolution system on wheat roots, aiming to track genomic modifications occurring during colonization and unveil the associated adaptive mutations. Bacteria were inoculated onto sterilized germinating wheat seeds and cultivated in a sterile substrate for 10 days, after which they were recovered from the rhizosphere and used to inoculate wheat seeds for subsequent planting cycle. Following eight cycles of artificial evolution, metagenomic sequencing and single nucleotide polymorphism (SNP) analysis were conducted on the intermediate and final evolved populations, revealing several genes with stable mutations, such as *fleN* gene regulating flagellar activity and *pvdE* gene involved in pyoverdine-type siderophore biosynthesis. These mutated sites appeared repeatedly across different evolutionary lineages, with some variants reaching frequencies as high as 50% in the final evolved population. We generated a series of FleN single amino acid mutants identical to those found in evolved strains. Compared to the ancestral strain, these FleN mutants exhibited significantly enhanced colonization ability in the wheat rhizosphere. These findings suggest that root-associated pseudomonads undergo continuous genomic alterations during colonization on wheat roots to enhance their population's adaptability. Characterization of these adaptive loci may guide selection and improvement of effective biocontrol strains.

054

Loss of T3SS function enhances long-term intracellular survival of *Pseudomonas aeruginosa* within airway epithelial cells

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Dao Nguyen

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Abstract

Intracellular *Pseudomonas aeruginosa* (*P.a.*) may represent an unrecognized bacterial reservoir protected from immune cells, extracellular host defenses and antibiotics, that contribute to the ability of *P.a* to cause persistent infections. Although *P.a* is mainly known as an extracellular pathogen, *in vitro* studies from our group and others have demonstrated the *P.a.* can invade different epithelial cell types. The long-term fate of intracellular *P.a.* and the bacterial mechanisms that promote intracellular bacterial survival in epithelial cells remain poorly understood. Using an *in vitro* model of long-term intracellular bacterial survival in human airway epithelial cells, we tested a collection of *P.a* clinical isolates from cystic fibrosis (CF) airway infections and found that a subset of non-cytotoxic type 3 secretions system (T3SS)-deficient *P.a* isolates demonstrated increased intracellular survival at 24h post-infection. We then characterized the intracellular infection kinetic in genetically engineered T3SS mutants (*exsA, popB, popB, pscD, exoSTY*) in five different ExoS(+) strain backgrounds by viable bacterial counting, flow cytometry and time lapse microscopy. We found that loss of T3SS injectisome function alone was sufficient to allow for intracellular strain survival, whereas T3SS effector mutants that retained injectisome function were unable to survive intracellularly despite being non-cytotoxic. Lastly, we determined that the increased intracellular survival of T3SS-deficient strains was driven by a sub-population of hyper-infected cells with evidence of intracellular bacterial replication. Given that T3SS-deficient *P.a* isolates are common in chronic CF infections, they may contribute to the formation of an intracellular *P.a.* reservoir that perpetuates chronic *P.a* airway infections.

058

Studying cellular solvent transport dynamics using fluorescent biosensors in *Pseudomonas taiwanensis* VLB120

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Abstract

Today, our chemical industry continues to heavily rely on the extraction and refinement of fossil resources to produce its fundamental bulk building blocks. In contrast, we target the bio-based production of hydrophobic aromatics such as styrene or ethylbenzene, addressing major challenges such as climate change and environmental pollution. However, the toxicity of these products requires highly solvent-tolerant biotechnological chassis to ensure the efficient production of hydrophobic aromatics. *Pseudomonas taiwanensis* VLB120 is a well-suited host for this approach, due to its native solvent tolerance mechanisms, genetic tractability, and the availability of streamlined production chassis.

In order to gain a deeper mechanistic insight into the tolerance mechanisms to intracellularly produced chemicals, we develop and apply fluorescent intracellular biosensors. These biosensors were developed based on transcriptional regulators of genes that encode solvent-efflux pumps, which are highly responsive to intracellular aromatic solvents. To normalize the biosensor readout to cell fitness, a second constitutively expressed fluorescent protein was integrated into the biosensor construct. Our goal with this ratiometric fluorescent biosensor is to investigate intracellular solvent concentrations and how they are affected by solvent-efflux pumps, focusing especially on externally added versus intracellularly produced hydrophobic aromatics.

Based on the data obtained, *Pseudomonas taiwanensis* chassis can be rationally developed to exhibit increased solvent production tolerance. The biosensors and improved chassis will serve as a foundation for enhancing the microbial production efficiency and achieving the *de novo* production of a second phase of hydrophobic aromatics.

059

Tracking real-time interactions of *Pseudomonas aeruginosa* with co-infecting pathogens in the host model zebrafish

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Abstract

Polymicrobial infections involving *Pseudomonas aeruginosa* are common and are often associated with poor treatment outcomes and increased host mortality. Although there is a large body of literature on *in vitro* pathogen-pathogen interactions, few study systems are available to track polymicrobial infections in real-time in living hosts. Here, we used the transparent larvae of the zebrafish (*Danio rerio*) to track polymicrobial infections involving the opportunistic human pathogens *P. aeruginosa*, *Acinetobacter baumanii* and *Klebsiella pneumoniae*. We established local infections in the otic vesicle, where infections stay contained and pathogen interactions can be tracked over time. We applied all pairwise combinations of our pathogens and observed pronounced pathogen density-dependent host mortality. The survival rate of zebrafish in mixed infections was notably lower than in *K. pneumoniae* mono-infections, while no survival difference were observed for mono- and mixed-infections involving *P. aeruginosa* and *A. baumannii*. Next, we conducted non-invasive live imaging, which revealed that pathogens first grow and then occupy a significant volume of the otic vesicle. Additionally, we found that pathogens not only co-localize but also have the opportunity to interact within this space. Finally, using transgenic fish lineages we observed the rapid recruitment of neutrophils and macrophages to the otic vesicle in infections involving *P. aeruginosa*. These insights show that our established study system is highly suitable for tracking pathogen-pathogen and pathogen-host interactions in situ.

062

Mapping the bioconversion of complex mixtures of lignin-derived compounds by *Pseudomonas putida* KT2440

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Abstract

Lignin is a complex aromatic polymer and holds great potential as a renewable resource to produce bio-based chemicals. Due to its robust metabolism, *Pseudomonas putida* KT2440 is extensively studied to transform aromatic compounds from lignin into valuable chemicals. However, few studies show a thorough assessment of the abundance and bio-convertibility of each organic compound from complex mixtures of lignin-derived streams. Here, we evaluated the efficacy of *P. putida* KT2440 to metabolize the compounds from a lignin-rich alkaline liquor from sugarcane bagasse, submitted to different hydrothermal depolymerization conditions (180-300 ºC; 30-90 min; 0-4 bar O_2). Through GC-MS and HPLC analyses, we identified and quantified up to 21 compounds present in the different liquors and accessed the individual percentage of consumption by *P. putida* KT2440. The depolymerization step released monomeric aromatic compounds and aliphatic acids, which benefited cell biomass accumulation, compared to the liquor prior to depolymerization, basically composed of macromolecular lignin and acetate. According to the depolymerization conditions, bacterial growth and metabolization of the compounds considerably varied. For instance, more severe conditions, such as higher temperature, favoured the formation of larger number and higher concentrations of phenolic compounds, some of them poorly metabolized, such as phenol and guaiacol, and others totally consumed, such as catechol. By mapping the conversion of each compound, we can adopt an integrated strategy by both selecting depolymerization conditions with higher biocompatibility and improving bacterial metabolization, through genetic engineering, designing a tailor-made strain possibly with more efficient conversion to cell biomass or to a product of interest.

065

In vitro modeling of polymicrobial airway infections in cystic fibrosis provides insights into interspecies interactions in *Pseudomonas aeruginosa*

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Abstract

Traditional *in vitro* models have focused on planktonic monocultures which do not accurately reflect the complex biofilm lifestyles of bacteria in most environments. We have developed a framework that assesses the ability of an *in vitro* model to recapitulate the gene expression of *Pseudomonas aeruginosa* (*Pa*) growing in cystic fibrosis (CF) sputum and utilized this system to evaluate three different complex *in vitro* models that examine *Pa* interactions with the host and other microbes.

Pa was cultured in synthetic CF media 2 (SCFM2) to form aggregates under several experimental conditions: *Pa* alone; *Pa* at the air-liquid interface of CF bronchial epithelial (CFBE) cells; and in an anaerobic four species polymicrobial community with *Staphylococcus aureus*, *Streptococcus sanguinis*, and *Prevotella melaninogenica*. *Pa* accuracy scores (AS_2) were calculated from normalized RNAseq reads by comparison to a *Pa* metatranscriptome from CF sputum samples.

AS_2 for the polymicrobial model was 81.1%, lower than either the monoculture (86.4%) or CFBE cell culture model (87.9%), but we observed increased accuracy of genes associated with interspecies competition. Expression of secretion system and nutrient acquisition genes more accurately reflected that of *Pa* in the CF sputum when grown in a polymicrobial biofilm model. Cell surface associated genes had lower accuracy, which could be improved by growing polymicrobial aggregates in association with CFBE cells.

Polymicrobial models are able to accurately recapitulate the gene expression of *Pa* in chronic CF airway infections. Measuring accuracy of pathways in models allows for targeted improvements to *in vitro* modeling of *Pa* infections.

067

Manipulation of resource allocation in Pseudomonas putida using the mRNA decay system

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Abstract

Pseudomonas putida KT2440 is a bacterium widely used as a chassis strain for various applications due to its versatile metabolism and genetic malleability. To enhance its utility as a cell factory chassis, a resource allocation strategy can be employed to improve its capabilities. By strategically allocating cellular resources, it is possible to increase the production of the target protein while decreasing the expression of other genes. To this end, we employed a toxin from *P. putida* and engineered its expression using an inducible system. The toxin protein “MazF” possesses endoribonuclease activity, specifically cleaving single-stranded RNA (ssRNA) at 5' UAC sequences. Interestingly, *P. putida* did not show any growth when the ribonuclease was activated with a high concentration of the inducer at the beginning of the culture. Consistent with this observation, the gradual expression of the toxin in the cell resulted in the inhibition of cell growth and viability in a dose-dependent manner. Moreover, the MazF protein of *P. putida* also acts as an endoribonuclease in Escherichia coli, indicating that the toxin is capable of decaying host mRNAs independently. This cellular mRNA decay system not only allows engineering bacteria to program resource allocation for diverse biotechnological applications but also serves as a platform for developing chassis strains.

068

Quorum sensing regulates a prophage in *Pseudomonas aeruginosa*

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Abstract

Quorum sensing (QS) is a bacterial cell-to-cell communication system that regulates various gene expressions via extracellular signals in a cell density-dependent manner. In this study, we report that QS induces phage release in *Pseudomonas aeruginosa*.

By monitoring the growth of an environmental isolate Ocean-1187, we realized that phages are released during the cultures, which number was reduced in a spontaneous QS mutant. Based on these results, we hypothesized that QS induces prophage in Ocean-1187. To examine this hypothesis, we first performed whole genome sequencing analysis to identify the prophage regions in Ocean-1187. Genome analysis showed that Ocean-1187 carries several intact prophages in its genome. To examine if these prophages can be induced, we performed qPCR analysis, and the phage structures were confirmed using TEM. The results revealed that Ocean-1187 releases at least two types of phages which one of them was induced in a QS-dependent manner. Prophages in *P. aeruginosa* are often induced by *recA* via DNA damage, however plaque assay with a *recA* mutant showed that QS induces this prophage independently to RecA. These results suggest that induction of a specific prophage in *Pseudomonas aeruginosa* is integrated into the bacterial cell-to-cell communication system, that may provide novel insights into phage-bacteria interplay.

074

Cross-regulation and cross-talk of conserved and accessory two-component regulatory systems orchestrate Pseudomonas copper resistance

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Abstract

Copper ions play a central role in the host-bacteria interaction during infection, as they are used by the host's innate immune system for their bactericidal properties. Copper resistance is therefore a critical determinant of bacterial virulence and relies on a variety of strategies and molecular machines. By combining mutagenesis, transcriptional fusion analysis and copper sensitivity plate assays, we have shown that the accessory two-component system (TCS) CusRS, present in a urinary isolate of *Pseudomonas paraeruginosa*, responds to copper and activates the expression of a nine-gene operon to confer resistance to elevated copper levels. The same operon was also found to be directly controlled by two core genome-encoded TCSs, the copper-responsive CopRS and the zinc-responsive CzcRS, with distinct transcriptional outcomes. We also observed unexpected cross-talk between the core genome-encoded CopRS system and the horizontally acquired CusRS system, although the phosphorylation state of the response regulators is differentially controlled by cognate sensory kinases: CusRS is a classical TCS whereas CopRS relies on a phosphatase-based mechanism with signal detection switching off the phosphatase activity of CopS. The locus containing *cusRS* and the copper resistance operon is part of an Integrative and Conjugative Element (ICE) found in other *Pseudomonas* strains, where its expression could confer copper resistance under appropriate conditions. Our results illustrate how acquired genetic elements can become part of endogenous regulatory networks and confer a physiological advantage. They also highlight the potential for broader effects of accessory regulatory proteins through interference with core regulatory proteins.

075

Mutational spectrum analysis of synthetically primed *Pseudomonas putida* strain evolved for catabolism of 2,4-dinitrotoluene

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Abstract

Innovations in synthetic biology have empowered scientists to methodically integrate synthetic operons within microbial platforms, enabling the exploitation of environmental microbes' catalytic pathways for biotechnological purposes. The success of such DNA heterologous integrations hinges upon the biochemical compatibility between the host metabolism and the introduced chemical activities, determining the degree of phenotypic functionality achieved. A compelling illustration of this phenomenon lies in the degradation pathway of the xenobiotic 2,4-dinitrotoluene (DNT). Numerous attempts have been made to transfer such degradative pathway to various laboratory bacterial chassis, albeit without achieving complete mineralization in the absence of supplemental carbon sources. However, a number of environmental strains able to do the job do exist. This exposes the necessity for any potential host strain to undergo genetic reconfiguration to sustain the metabolic burden imposed by xenobiotic metabolism while preserving its replicative capacity and physiological well-being.

In this work, we have adopted an upgraded Adaptive Laboratory Evolution protocol, endowed with a multi-objective selection strategy based on spectrophotometric analyses of chemical transformations of 2,4-DNT, to drive genetic diversification within a synthetically-primed parental strain *Pseudomonas putida*. Over a 420-day period, six evolutionary trajectories were followed, yielding an array of clones exhibiting varying degrees of 2,4-DNT metabolization. Through comprehensive genetic profiling coupled with phenotypic analyses, the metabolic and physiological bottlenecks that prevented utilization of 2,4-DNT as a sole source of carbon and nitrogen by the ancestral strain were sorted out and overcome. These results showcase the power of merging rationally design and evolutionary approaches to solve complex metabolic objectives.

076

G. mellonella is a suitable model for studying *P. aeruginosa* response to the host nutritional immunity strategies

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Abstract

Background

Nutritional immunity encompasses the innate defense mechanisms of mammals that restrict pathogen growth by controlling essential micronutrients, like trace metals, to starve or poison the pathogens. *Galleria mellonella* is a model widely used in *Pseudomonas aeruginosa* virulence studies, whose innate immune system shows similarities to that of mammals. However, it is yet unknown whether this organism exploits nutritional immunity mechanisms involving control of Zn concentration.

Methods

G. mellonella larvae were used for infection experiments with *P. aeruginosa* PA14, *znuA* and *znuAzrmB* mutants, and *lux* reporter strains. Promoter activities of PA14 *zrmA* (Zn-uptake) and *czcA* (Zn-efflux) were analyzed by bio-luminescence assays. Transcriptional regulation of PA14 and *G. mellonella* genes during the infections was investigated by RT-qPCR. The contribution of bacterial zinc uptake systems to virulence was evaluated by time-to-death experiments and competition assays.

Results

Infection experiments showed that P*zrmA*-driven *lux* expression is sustained and increased over time, reaching a maximum in the late stages. On the contrary, *czcA* promoter activity is slightly detectable only at earlier hours post-infection, and it decreases as the infection proceeds, suggesting that bacteria infecting *G. mellonella* face Zn shortage. Time-to-death experiments and competition assays with PA14 compared to mutant strains impaired in Zn-uptake demonstrated that the efficiency in acquiring Zn is crucial for the pathogenesis. Moreover, PA14 Zur-regulated genes, as well as *G. mellonella* effectors involved in Zn sequestration, were induced during the infection.

Conclusions

Our results indicate that *G. mellonella* exhibits Zn-mediated nutritional immunity responses to bacterial infections, thereby confirming its suitability as a model for studying *P. aeruginosa* virulence.

080

PA0630 and PA0631 are putative spanins that contribute to explosive cell lysis in *Pseudomonas aeruginosa* biofilms

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Abstract

Extracellular DNA (eDNA) is essential for *P. aeruginosa* biofilm formation. We have shown previously that eDNA is released through explosive cell lysis events that involve rapid transition of bacillary cells into round cells prior to lysis. Explosive cell lysis is mediated by the endolysin, Lys, encoded in the R-/F- tailocin (pyocin) gene cluster. Tailocins are evolutionarily related to phage tails and are released via a phage-like lysis pathway. Phage lysis involves inner-membrane holins that transport an endolysin to the periplasm where it degrades peptidoglycan. In Gram negative bacteria, phage lysis also requires spanins that promote rupture of the outer-membrane. Two-component spanins are comprised of an inner-membrane protein and an outer-membrane protein which, upon degradation of the cell wall peptidoglycan, interact and adopt a closed confirmation bringing the 2 membranes together as the final step of lysis. To date, the putative spanins of the *P. aeruginosa* R-and F-tailocin gene cluster haven't been experimentally explored. Here, we examined the role of the putative spanins PA0630 and PA0631 in explosive cell lysis by generating single and double in-frame deletion mutants in *P. aeruginosa* PAO1. Live-cell microscopy showed that PAO1*DPA0630*, PAO1*DPA0631* and PAO1*DPA0630DPA0631* produce many more round cells than PAO1 and that the round cells of these mutant strains have significantly longer survival times than wildtype. These phenotypes are consistent with the proposed functions of PA0630 and PA0631 as spanins. We are currently utilising biochemical and advanced imaging approaches to determine the sub-cellular localisation and interactions of the *P. aeruginosa* tailocin lysis components.

081

Unveiling a New Potential Target for Anti-virulence Therapies: Investigating the Role of the PqsE protein in *Pseudomonas aeruginosa* in a diverse panel of strains

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Abstract

Anti-virulence therapies have garnered interest to treat bacterial infections, by reducing virulence rather than growth, potentially mitigating resistance. *Pseudomonas aeruginosa* relies on three quorum sensing systems to control several of its virulence functions, highlighting cell-cell communication as an attractive anti-virulence target. However, further understanding is needed to identify appropriate quorum sensing targets. The transcriptional regulator of the *rhl* system, RhlR, modulates the transcription of several genes implicated in the production of virulence factors, such as pyocyanin and rhamnolipids. Importantly, in reference strains, the full activity of RhlR requires the presence of the PqsE protein to properly activate the transcription of its target genes. PqsE forms dimers with RhlR and acts as a «chaperone», engaging in a protein-protein interaction that significantly enhances the affinity of RhlR for its targets. Notably, in reference strains, a mutation in the *pqsE* gene significantly decreases the production of rhamnolipids and pyocyanin, leading to reduced virulence. Considering its distinctive role in virulence, PqsE emerges as a compelling target for anti-virulence therapies. However, its role has only been elucidated in reference strains. This study aims to assess the genomic and functional conservation of PqsE across a diverse panel of 30 strains of *P. aeruginosa*. Current results indicate that *P. aeruginosa* strains consistently carry a conserved *pqsE* gene. Additionally, deletion of the *pqsE* gene leads to decreased production of virulence factors. These results are consistent with current knowledge and highlight the potential of PqsE as a target for anti-virulence therapies due to its conservation and key regulatory function.

083

Cystic fibrosis airway-conditioned human macrophages are competent at killing *P. aeruginosa.*

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Abstract

Background: Cystic fibrosis (CF) airways are often chronically infected by *P. aeruginosa*. Prior CF infant studies indicate *P. aeruginosa* does not colonize CF airways before concomitant alterations in airway macrophages (exhaustion) and neutrophils (hyperexocytosis and active repression of bacterial killing) are detected. Here, we tested whether CF airway-conditioned macrophages derived from naïve blood monocytes would display intrinsically altered *P. aeruginosa* killing.

Methods: Blood monocytes from healthy donors were transmigrated using a biomimetic lung model using airway supernatant from CF sputum(CFASN) as an apical chemoattractant. For macrophage differentiation, cells were treated for 4 days with M-CSF post-transmigration. Leukotriene B4 combined with CCL2 were used as a chemoattractant control. Co-incubation of *P. aeruginosa* PAO1 and macrophages was performed at a MOI of 1 in different cell densities at 37°C for 1-4 hrs. Bacterial killing capacity was calculated using colony forming units, with PAO1 alone as control.

Results: Transmigrated monocyte-derived macrophages (TMDMs) produced in our model efficiently killed PAO1 at all cell densities and timepoints tested. There was no detectable defect in CFASN-conditioned TMDMs compared to control TMDMs at any cell density or timepoint tested.

Conclusions: Unlike our prior results with primary neutrophils from healthy and CF donors, transepithelial migration combined with CFASN conditioning does not induce defective *P. aeruginosa* killing by macrophages. While further validation with primary monocytes from CF donors is warranted, these data are consistent with our prior CF infant studies showing adequate intrinsic antibacterial function of CF airway macrophages prior to neutrophilic inflammation.

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084

Proteomic profiling of the stringent response in stationary phase *Pseudomonas aeruginosa*

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Abstract

The stringent response (SR) is a conserved stress response induced by accumulation of the alarmones (p)ppGpp, via synthesis from the RSH enzymes RelA and SpoT. Induction of the SR drives a pleotropic physiological shift away from active metabolic processes towards stress management and cellular quiescence.The *Pseudomonas aeruginosa* (p)ppGpp-null Δ*relA* Δ*spoT* (ΔSR) mutant displays numerous phenotypic abnormalities, particularly in stationary phase, including increased envelope permeability, decreased antibiotic tolerance and impaired antioxidant defenses. The global physiological disturbances of the ΔSR mutant have primarily been characterized at the transcriptomic level to date. To further understand the cellular processes under SR control, we performed quantitative shotgun proteomic profiling by LC-MS/MS and compared the ΔSR mutant to its isogenic PAO1 parent during exponential and stationary phase planktonic growth in rich medium.We detected an average of 1998 proteins (range 1906 to 2059) per group, with 366 SR-dependent differentially expressed proteins (DEPs) in stationary phase, representing 2.5x more than in exponential phase. Functional and pathway analyses showed enrichment of biosynthetic, metabolic and protein translation processes among up-expressed DEP in the ΔSR mutant. DEP down-expressed in the ΔSR were enriched in membrane components, efflux and transporters, signal transduction and chemotaxis. Interestingly, we observed up-expression of the PQS system and phenazines biosynthesis, and down expression of antioxidant proteins, porins, efflux pumps (including MexGHI-OpmD), virulence and biofilm pathways, which corroborated multiple phenotypes observed in the ΔSR mutant.This first characterization of the SR proteome provides insight into the processes and pathways involved in SR-mediated adaptation during stationary phase.

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Pseudomonas aeruginosa Infective Endocarditis: Developing a Novel Tool of Adhesion Under Shear Stress

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Sarah Hickson

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Abstract

Introduction

Infective endocarditis (IE) is an infection of the inner lining of the heart (endocardium) and can involve several cardiac structures. Although rare, *Pseudomonas aeruginosa* IE is associated with high incidence of mortality. Few studies have explored *P. aeruginosa* IE pathogenesis, but initial adhesion is known to be vital in the establishment of infection for other bacterial species. We have developed a novel tool to investigate *P. aeruginosa* adhesion to endothelial cells under shear stress, mimicking high blood flow in the cardiac environment.

Methods

Clinical *P. aeruginosa* IE isolates (n=6) were sequenced, genomically characterised and the proteome analysed after growth in blood. In the model of adhesion under shear stress, *P. aeruginosa* coated in serum proteins is perfused through flow chamber slides (~2.7 dynes/cm^2), seeded with endothelial cell line EA.hy926. The adhesion under flow for the clinical isolates and reference strains PAO1 and PA14 were compared to static conditions. Finally, a PA14 Δ*pilY1* mutant was compared to wildtype in this model.

Results

The genomes of IE-causing *P. aeruginosa* isolates have a full complement of virulence factors. Proteomic data found an upregulation of phenazine synthesis in IE isolates (n=5). For all strains tested, adhesion to the endothelial cells under flow was significantly higher than in static conditions. Additionally, the PA14 Δ*pilY1* strain had significantly decreased adhesion compared to wildtype under shear stress, whereas no differences could be detected in static conditions.

Conclusion

Adhesion to endothelial cells under shear stress is more biologically relevant to investigate *P. aeruginosa* IE establishment and potential antagonists.

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Engineering of *Pseudomonas taiwanensis* for efficient *de novo* production of aromatics from renewable resources

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Abstract

Aromatics are essential to modern society. Their current production mostly relies on petrochemical processes and a bio-based production is required for the transition towards a sustainable economy. Microbial biocatalysis is a promising approach to enable *de novo* biosynthesis of value-added chemicals from renewable substrates.

We deeply engineered solvent-tolerant *Pseudomonas taiwanensis* as microbial host for the bioproduction of a wide range of different aromatics applying *chassis*, tolerance and metabolic engineering. Initially, a tyrosine producer was engineered through several gene deletions (≥6) and three reverse-engineered point mutations to increase the flux into the shikimate pathway and avoid the loss of intermediates or products. The engineering of streamlined genome-reduced *chassis* (GRC) strains with improved bioprocess features further enhanced production regarding titer, rate, and yield. The tyrosine-producing GRC strain served as a basis for subsequent studies to diversify the product spectrum to multiple tyrosine-derived hydroxylated aromatics with low byproduct formation and to enable production of phenylalanine-derived chemicals such as *trans*-cinnamate and benzoate. This presentation will outline recent activities on the upscaling of a 4-hydroxyphenylacetate production process to cubic meter-scale and the engineering of a platform strain for chorismate-derived aromatics including hydroxy- and dihydroxybenzoates. Moreover, strain and process engineering (including *in situ* product removal) to enable and optimize production of structurally more complex aromatic polyketides will be highlighted. In ongoing work, we also study the tolerance towards and the production of hydrophobic and highly toxic chemicals such as styrene and aromatic aldehydes (i.e., cinnamaldehyde).

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Prophages protect *Pseudomonas putida* from several CEPEST collection phages

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Abstract

Pseudomonas putida is an environmental bacterium with a wide range of different metabolic pathways. Being metabolically highly versatile, these bacteria have great promise in bioremediation approaches to degrade various aromatic pollutants and as cell factories in biotechnological production. Different metabolic pathways of *P. putida* have been extensively studied, but surprisingly, almost no phages have been isolated for the common laboratory strain *P. putida* KT2440 or its isogenic PaW85, and no phage defence mechanisms have been characterized yet.

Here, we describe a novel Collection of Environmental Pseudomonas putida Phages from Estonia (CEPEST), consisting currently of 22 species of dsDNA phages that can be grouped into 9 phage families. The collection was isolated from soil and water samples using a predictably weakened derivative of the bacterium: a prophage-negative strain that additionally lacks 13 toxin-antitoxin systems from the genome. According to the literature, these chromosomal entities have previously been associated with the phage defence of different bacteria. However, our results demonstrate that the chromosomal toxin-antitoxin systems do not affect the phage resistance of *P. putida*. In contrast, we show that three of the four cryptic prophages in *P. putida* PaW85 chromosome strongly protect against the infection of many CEPEST collection phages. By applying deletion analysis of prophage regions, we have mapped the anti-phage loci and started deciphering the molecular mechanisms behind the phage-defence genes.

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Exploring a novel Trojan horse strategy to treat Pseudomonas aeruginosa infections

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Andrea Battistoni

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Abstract

Background: Zinc has recently emerged as a crucial element for the ability of *Pseudomonas aeruginosa* to infect animal hosts. The acquisition of zinc influences the expression of various virulence traits such as alginate production, biofilm formation, protease secretion, siderophore biosynthesis, and motility and is necessary for the ability to establish lethal infections in animals. Moreover, transcriptomic and genomic analyses have highlighted that genes regulated by zinc deficiency are highly expressed during infection and conserved across clinical isolates. The identification of a zinc import mechanism based on the metallophore pseudopaline offers opportunities to enhance antibiotic delivery in *P. aeruginosa* through a Trojan horse strategy, potentially circumventing some intrinsic mechanisms of antibiotic resistance.

Methods: Pharmaceutical chemistry strategies were developed to synthesize molecular simplified derivatives of pseudopaline, one of which was conjugated to aztreonam. The antimicrobial activity of this compound was tested on bacteria cultured in a defined medium, both in the presence and absence of zinc.

Results: We compared the antimicrobial activity of aztreonam and the conjugate between aztreonam and the simplified pseudopaline derivative. The latter exhibited significantly greater antimicrobial activity against the wild-type PA14 strain compared to the strain lacking the pseudopaline receptor, specifically under conditions of zinc deficiency. In contrast, aztreonam exhibited consistent efficacy across strains, irrespective of zinc availability.

Conclusion: These results offer proof of the principle that the pseudopaline receptor can transport metallophore-antibiotic conjugates, presenting a promising strategy to control bacterial growth in zinc-poor environments. This approach holds promise for developing novel treatments against *P. aeruginosa* infections.

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Production of long-chain rhamnolipids for leaf fertilization and plant strengthening with *Pseudomonas putida* using residual waste streams

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Abstract

Plant protection and fertilization face major challenges in fulfilling today's sustainability goals. Until now, most leaf fertilizers and plant protective agents are applied with petrochemically derived surfactants. Furthermore, the addition of trace elements needs chelators, which tend to accumulate in the environment and are suspected to be carcinogenic, necessitating sustainable solutions, which are degradable and harmless to ecosystems. Rhamnolipids have the potential to be a sustainable alternative for crop protection. Due to their amphiphilic character, these biosurfactants are good solubilizers and emulsifiers. It was already shown that rhamnolipids can be active against pest, increase the resilience against abiotic stress and can support beneficial organisms for plants, whereby the effect seems to increase with increasing chain length of the rhamnolipids.

In this study, a process was developed to produce long-chain rhamnolipids with a recombinant *Pseudomonas putida* strain to examine their role in crop protection. As the strain is able to metabolize PET monomers, an additional topic was the rhamnolipid production from waste streams which contain plastics as e.g. polycotton from the textile industry. A challenge was the mitigation of extensive foam formation caused by the rhamnolipid production, which necessitates the evaluation of different foam mitigation strategies as mechanical foam destroyers and foam fractionation columns.

In conclusion, this project contributes to the development of biological, resource conserving processes with relevance for the circular economy.

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Moonlighting is no trivial offence: How the enzyme PqsE regulates virulence of *Pseudomonas aeruginosa*

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Abstract

The opportunistic nosocomial pathogen *Pseudomonas aeruginosa* has a large arsenal of virulence factors and resistance mechanisms at its disposal, making it an imminent threat to immunocompromised patients such as those suffering from cystic fibrosis. *P. aeruginosa* orchestrates the majority of its disease-associated traits by measuring its own population density, using a cascade of the three intertwined quorum sensing systems *las*, *rhl* and *pqs*. Here, the N-butanoyl-L-homoserine lactone (C4-HSL) dependent *rhl* circuit plays a leading role in late and chronic infections.

Interestingly, most of the processes controlled by the *rhl* transcription factor RhlR not only depend on the autoinducer C4-HSL but also on the presence of the thioesterase PqsE *via* an enigmatic mechanism not involving PqsE's enzymatic activity. Here we show that PqsE and RhlR form a 2:2 protein complex that, together with C4-HSL, solubilizes RhlR and thereby renders the otherwise unstable transcription factor active. The importance of this finding is emphasized by the observation that solubility-enhancing organic molecules developed to inhibit RhlR activate rather than antagonize associated processes in *P. aeruginosa*. To gain insight into the PqsE/RhlR interaction, we determined crystal structures of the complex and identified residues essential for its formation. To further corroborate the chaperone-like moonlighting activity of PqsE, we designed stability-optimized variants of RhlR that bypass the need for C4-HSL and PqsE in activating PqsE/RhlR-controlled processes of *P. aeruginosa*. Together, our data provide insight into the unique regulatory role of PqsE and lay groundwork for developing new *P. aeruginosa*-specific pharmaceuticals.

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Profiling Pseudomonas aeruginosa interactions in an airway infection model using 3D micron-scale bioprinting.

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Abstract

Pseudomonas aeruginosa (*Pa*), a major pathogen of airway infections in people with cystic fibrosis (pwCF), interacts with other microbes, which may influence infection progression. *Pa* interactions with *Staphylococcus aureus* (*Sa*) can result in selection of aminoglycoside-resistant *Sa* variants (Barraza & Whiteley, 2021). In general, interactions can potentially modulate virulence and antibiotic resistance via intercellular signalling, as seen by co-existence of quorum sensing positive and negative *Pa* variants. Microbial interactions rely on diffusible compounds and spatial organization (micron-scale) potentially influencing ecological stability and responses to changes, yet conclusive evidence during the course of bacterial infections is lacking.

We have developed an Air-Liquid-Interphase (ALI) model to mimic human airways, implementing 3D bacterial bioprinting for precise positioning of bacterial droplets. Confocal microscopy is used for spatial insights into bacterial distribution and interactions in the ALI model. In addition, we will, we expect to use external sensors (pH and O_2) and reporter assay for real-time detection of environmental cues and microbial activities.

We have optimized 3D bacterial bioprinting of relevant fluorescently stained bacterial populations on ALI cultures. Current results include printing of uniform and reproducible bioink droplets, successful biocompatibility with *PAO1* and BCi-NS1.1 lung cells, and printing of two different *PAO1* strains at micro-scale distances of 500 μm.

Studying microbial interactions at micron-scale in ALI airway model systems can provide in-depth knowledge about *Pa* microbial dynamics with high spatial resolution, and show how such interactions affect and shape infected lungs.

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Transcriptomic response of *Pseudomonas aeruginosa* to the CFTR modulator triple therapy Kaftrio / Trikafta.

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Abstract

Pseudomonas aeruginosa is the major pulmonary pathogen of patients with Cystic Fibrosis (CF). CF is the most prevalent autosomal recessive disorder in Caucasians, caused by the inheritance of a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene from both parents. The CFTR protein facilitates the export of chloride ions across epithelial tissues, most notably within the respiratory and digestive organs. CFTR mutations lead to a loss of cilial activity and the accumulation of thick mucous in the lungs which is readily colonised by *P. aeruginosa*. Innovations in drug discovery have led to CFTR modulator drugs becoming available. Three of these drugs, elaxacaftor, tezacaftor and ivacaftor are given as a triple therapy marketed as Kaftrio in the UK and Trikafta in the USA. These drugs stabilise or assist the proper folding of the CFTR protein, leading to significant restoration of transporter function. Several ongoing studies are investigating the impact of Kaftrio on the lung microbiome of CF patients revealing that only around half of patients clear chronic *P. aeruginosa* infections. Whilst Kaftrio specifically targets the human CFTR protein, little is known about its effect on members of the CF lung microbiotia. To address this, we performed RNA-Seq on *P. aeruginosa* PA14 exposed to varying levels of kaftrio or ivacaftor alone. These experiments were performed at 1x and 100x serum concentrations of the drugs as reported by the manufacturer (Vertex Pharmaceuticals, personal communication). Our data shows significant upregulation of iron sequestration genes and components of the osmotic stress response.

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Synergistic fitness only evolves between co-isolated strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in a human chronic wound *in-vitro* model.

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Abstract

Chronic wounds are very difficult to treat and have a high recurrence rate, leading to loss of function, decreased quality of life, and significant morbidity in adults. The ongoing and recurrent nature of these wounds, despite aggressive antibiotic treatment, is in part due to the presence of bacterial biofilms. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the most common causes of biofilm infections and are often co-isolated in chronic wounds. Synergistic interactions have been demonstrated between *S. aureus* and *P. aeruginosa* in terms of colonisation, pathogenicity, and biofilm survival advantages, so it is possible that co-evolution in a chronic wound can alter gene expression and aid bacterial survival. Using a modified chronic wound biofilm (mCWB) model, two pairs of co-isolated and matched clinical isolates of *P. aeruginosa* and *S. aureus* were grown together and CFUs were conducted over an 8-day period. The clinical pairs were then mismatched, with opposite strains of each species grown together, to determine if co-evolution affects bacterial survival in this dual species biofilm model. The results showed stable CFUs of each bacterial species after 2 days in the mCWB model for the matched clinical pairs. However, with the mismatched clinical pairs the *P. aeruginosa* strains dominated, with the number of *S. aureus* colonies decreasing from day 1 onwards, and small colony variants (SCVs) appearing after 4 days incubation. This further supports the evolution of a synergistic relationship between co-isolated strains of *P. aeruginosa* and *S. aureus* in chronic wound infections.

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Identification of genes involved in aggregate associated antimicrobial tolerance of *Pseudomonas aeruginosa* using Tn-Seq guided discovery

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Lise Goltermann

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Abstract

It is estimated that about 60-70% of hospital acquired infections are caused by biofilms. Since current treatment regimens in many cases fail to cure biofilm-based infections, it is critical to identify the mechanisms which underlie biofilm-based antimicrobial tolerance. In biofilms, the bacteria grow in densely packed aggregates concealed in a self-produced matrix of exopolymers and can tolerate high doses of antibiotics. We use a super-saturated *P. aeruginosa* PA14 transposon (Tn) mutant library consisting of more than 800.000 unique mutants, to identify mutants with increased susceptibility towards a panel of antibiotics. The transposon mutants are grown in agar plates under conditions where each mutant forms an agar-embedded aggregate. Antimicrobial tolerance increases with aggregate age but even relatively young aggregates display tolerance up to 10-fold higher than their planktonic counterparts. Furthermore, the level of c-di-GMP – as measured through a c-di-GMP-monitor - is significantly higher in the aggregates compared to planktonic growth, confirming that the aggregates represent a biofilm-like growth physiology. For a single experiment, we use large agar plates that combined harbor over 150.000 aggregates. The aggregates are treated with antibiotics, washed, and then disintegrated. The identity and relative number of the mutants present in the pool of antibiotic-treated versus the non-treated bacteria are then determined by Tn-Seq analysis. Under the right selection conditions, Tn-mutants with higher susceptibility will be selectively depleted from the antibiotic-treated pool. Subsequently, all identified genes are ordered according to their potential importance for aggregate-associated antibiotic tolerance thus serving as the starting point for exploring the underlying mechanisms.

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Identification of novel host-pathogen interactions: PDIA1 and PDIA3 as novel human receptors for *Pseudomonas aeruginosa.*

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Maite Sainz

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Abstract

Pseudomonas aeruginosa is a highly antimicrobial-resistant pathogen that causes difficult-to-treat acute and chronic infections, due to its high host adaptability. New antibiotic therapies against *P. aeruginosa* are urgently needed. Elucidation of host receptors for *P. aeruginosa* is critical for the design of innovative treatments against its infections. We have identified, for the first time, the role of protein disulfide isomerases (PDI) A1 and PDIA3 in *P. aeruginosa* attachment to epithelial cells using a novel unbiased 2D proteomic approach. Treatment of human bronchial epithelial cells (16HBE14o^{-}) with the PDI inhibitor, LOC14, showed a dose-dependent decrease in *P. aeruginosa* attachment to these cell lines (p=0.0188). *P. aeruginosa* attachment to HEK293T cells overexpressing PDIA1 and PDIA3 was dramatically higher than the control (empty plasmid); (p= 0.0360, 0.0132, respectively). Bacterial attachment to CRISPR cell lines A549 *pdia3*$^{-/-}$ was lower than to A549 cells (p= 0.0344) while attachment to A549 *pdia3*$^{-/-}$ transfected with a plasmid containing *pdia3* restored the attachment levels. Confocal microscopy suggested a co-localisation of *P. aeruginosa* with the PDIs on the human cell surface. Finally, the *in-silico* superposition of human PDI structural models with *P. aeruginosa* PDIs suggested a possible *P. aeruginosa* hijacking of host PDIs. Our study enables a better understanding of *P. aeruginosa* interaction with the host, opening the possibility of understanding a critical pathway for *P. aeruginosa* interaction with the human cells that might lead to the design of novel antimicrobial therapies or the use of currently available drugs that target human disulfide isomerases.

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When food goes bad: understanding *Pseudomonas* species biofilm formation to prevent food spoilage

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Laura Nolan

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Abstract

Approximately one third of food produced for human consumption is wasted, with food spoilage by bacteria being a key contributor to this wastage. Non-pathogenic *Pseudomonas* species are common food spoilers. While it is known that spoilage *Pseudomonas* species form biofilms on food, we lack a detailed molecular mechanistic understanding of this process. A biofilm is a microbial community encased in a matrix, which is commonly composed of extracellular DNA (eDNA), polysaccharides and proteins. Our aim is to obtain a detailed understanding of how spoilage *Pseudomonas* species form biofilms on food, then use this information to develop interventions that target these biofilms to reduce food spoilage, thereby extending shelf-life and reducing food waste.

We used fluorescence microscopy to visualise *Pseudomonas* biofilm development *in situ* on a representative meat (chicken) and a leafy green (spinach). On chicken, *Pseudomonas* forms tunnel-like biofilms that extend into the tissue and are often eDNA-encased. As the chicken spoils these tunnels expand. However, at the same time, many cells are forced to disperse from the biofilm due to substratum degradation. On spinach, *Pseudomonas* forms biofilm microcolonies across the leaf surface. However, as the spinach spoils, the cells are forced to disperse from the leaf surface, with the rib being the only area in which *Pseudomonas* biofilms remain attached. Our data shows that food spoilage *Pseudomonas* species actively degrade the surface that their biofilm is forming on, and consequently appear to undergo a unique life cycle compared to biofilms that form on abiotic surfaces.

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A widespread adaptor protein family diversifies the toxin repertoire of the type VI secretion system spike

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Abstract

Type VI secretion systems (T6SS) are protein secretion machines found in Gram-negative bacteria that contribute to bacterial competition by secreting toxic effector proteins into neighbouring cells. These effectors adopt diverse structures and functions but do not contain a signal sequence that facilitates their export. Consequently, how conserved T6SS structural components export a diverse repertoire of unrelated effectors remains poorly understood. Here, we characterize a widespread family of proteins that facilitate recognition of structurally divergent toxins by closely related T6SS components in *Pseudomonas aeruginosa*. By examining two nearly identical paralogs of the T6SS spike protein, VgrG, we demonstrate that each spike exports a cognate toxin that bears no sequence or structural similarity to the other spike's toxin. The recruitment of each toxin to its respective spike protein requires a cognate adaptor protein in the DUF4123 family. Protein-protein interaction experiments show that these adaptor proteins interact with a variable surface on their cognate toxin and with a structurally conserved helix-turn-helix motif at the C-terminus of their associated VgrG spike, thus tethering the toxin to the VgrG. Using structural predictions and mutagenesis analyses, we elucidate the molecular contacts required for these interactions and discover that these bi-lobed adaptor proteins contain an N-terminal lobe that has evolved to bind VgrG helix-turn-helix motifs and a C-terminal lobe that has co-evolved with a specific family of toxins. Overall, our work provides molecular insight into a protein family that enable identification of unrelated toxins by nearly identical T6SS components, thus diversifying the toxin repertoire of this system.

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Within-host diversity and body-site translocation of nosocomial *Pseudomonas aeruginosa* infections

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Lewis Fisher

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Abstract

Background

Pseudomonas aeruginosa is an important nosocomial pathogen, however the route of infection is often unclear. Evidence is emerging that indicates *P. aeruginosa* can translocate from the gut to the lung and the gut may act as an intermediary prior to respiratory infection, yet the dynamics of this process remain elusive.

Methods

A metagenomic analysis of nasal, rectal, and respiratory samples from 257 patients was performed during April-May 2020 in San Matteo Hospital. We identified significant numbers of *P. aeruginosa* reads in 385 samples which were deconvoluted for downstream analysis. We explored within-host genomic diversity to understand evolution and body-site transmission dynamics.

Results

From 83 patients with *P. aeruginosa* positive samples, 67 were found to be infected with a single clone whereas 16 were infected with multiple clones. There were no SNPs identified in 22 (44%) of the patients with multiple samples. We observed intra-clone SNPs within the other 28 patients where we observed multiple mutations occurring within AMR genes, notably in *pmrB,* the *mexCD-OprJ* operon, and transcriptional regulators. We frequently observed the same clone infecting multiple body sites within the same patient. Two major clades were significantly more likely to be inhabiting both gut and respiratory body-sites within the same patients as opposed to the remaining patient clones which were more likely to be restricted to single body sites.

Conclusion

Our work indicates that there could be potentially important associations between *P. aeruginosa* genotype and its ability to translocate to new body sites

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Cross-fed citrate modulates *Pseudomonas aeruginosa* interactions with other microbes

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Abstract

Pseudomonads are rarely found in isolation but rather as part of complex polymicrobial communities. Studies suggest that metabolite cross-feeding is an important mechanism that contributes to community organization and the modulation of microbial interactions. For example, many bacteria and fungi secrete citrate under physiologically relevant conditions. Interestingly, cross-fed citrate can modulate *P. aeruginosa* virulence determinants. Furthermore, different common *P. aeruginosa* genotypes respond differently to citrate. In these studies, we use metabolomics and transcriptomics combined with genetic analyses of *P. aeruginosa* mutants defective in the citrate response. Experiments using these mutants emphasizes the importance of citrate cross-feeding for both intra- and inter-species interactions. We show that the *P. aeruginosa* response to citrate is controlled by the TctE-TctD two component system and citrate uptake proteins OpdH, an outer membrane porin, and TctABC, an inner membrane tripartite tricarboxylate transporter. Citrate uptake is necessary for the broad effects of cross-fed citrate including the increased production of phenazines due to the stimulation of RhlR activity in co-cultures of *P. aeruginosa* with other species including *Staphylococcus aureus* and *Candida albicans*, two species commonly found in *P. aeruginosa* polymicrobial infections. These data suggest that transport or sensing of the extracellular metabolites produced by other species activates *P. aeruginosa* quorum sensing regulation outside of the canonical autoinducing pathways that classically regulate RhlR. Broadly, studying metabolic cross-feeding interactions may lead to understanding the stability exhibited by heterogeneous polymicrobial communities. This work can guide future studies on the manipulation of human and plant microbiomes in beneficial ways.

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Pathway Characterisation for the Production of 2,5-Furandicarboxylic Acid in *Pseudomonas umsongensis* GO16

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Abstract

2,5-Furandicarboxylic acid (FDCA) is a biobased analog to terephthalic acid (TPA), a monomer of the ubiquitous plastic, polyethylene terephthalate (PET). FDCA can substitute TPA in PET, producing polyethylene 2,5-furanoate (PEF). However, the use of FDCA for plastic applications is limited by high production costs. Biotechnology offers an alternative production route by using fewer toxic reagents, milder conditions, and cheap, waste feedstocks for upcycling potential.

Pseudomonas umsongensis GO16, which can natively metabolise the PET monomers ethylene glycol (EG) and TPA as growth and energy substrates, has been used to upcycle these molecules to polyhydroxyalkanoates and hydroxyalkanoxyl-alkanoates previously. Building on this ability to convert PET monomers to valuable compounds, this bacterium is being explored as a microbial chassis for the synthesis of FDCA.

A precursor of FDCA is 5-hydroxymethylfurfural (HMF). *P. umsongensis* GO16 possesses an *hmf* operon, enabling it to utilise HMF as a sole growth substrate, with FDCA as an intermediate. FDCA decarboxylase (HmfF) was identified as the enzyme responsible for converting FDCA to further metabolism. A CRISPR/Cas9 system was used to delete it and allow GO16 Δ*hmfF* to convert 50 mM of HMF to 50 mM FDCA in 48 hours when growing on EG. FDCA production with TPA growth is poor however, at only 12 mM.

To improve HMF conversion efficiency, the minimum complement of genes required for FDCA production was heterologously expressed in *Pseudomonas putida* KT2440 for pathway characterisation. This has identified bottlenecks in FDCA production, which are being further addressed to develop a more potent biocatalyst.

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Open-endedness and exaptation as basis of metabolic versatility evolution in Pseudomonas.

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He

Abstract

Evolutionary biology investigates how adaptive traits arise and whether they consistently come from adaptive origins. Some innovations emerge non-adaptively as pre-adaptations, also called exaptations. These by-product traits are clear examples of how open-endedness works in nature and serves as a source of robustness.

Metabolic systems harbor latent potential for evolutionary innovations with non-adaptive origins, which is mainly based on enzymatic promiscuity. *Pseudomonas* is a genus well known for its robustness and adaptability. In this work, we evaluated a promiscuous pathway in charge of degrading a toxic subgroup of aromatic compounds. All this in the context of a natural open-ended process and highlighting the relevance of avoiding specialization. We identified by RNAseq the detoxification related genes and found a clear stress response and futile induction of the main aromatic compounds degradation pathway. We evaluated in vivo and in vitro if the evolution of the transport, regulators and catabolic enzymes are equally promiscuous. We concluded that promiscuity could be part of the optimal solution under changing environments. Additionally, we propose that this pathway is part of a transition from a detoxification process to a new carbon source acquisition.

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Designing Scale-Down Bioreactor Systems to Enhance Strain Optimization for Industrial Applications

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Abstract

Biotechnological processes play a crucial role in advancing sustainability within a circular, carbon-neutral economy. Central to achieving this objective is enhancing current production strains for large-scale fermentations. However, upscaling these processes often imposes additional stress on production strains as compared to laboratory-scale setups, largely due to fluctuations in dissolved oxygen, carbon, and pH, leading to the formation of starvation zones that can impact efficiency.

To better understand the effects of upscaling on growth and production, various scale-down devices have been utilized to mimic these heterologous mixing ratios. One approach employs a two-compartment scale-down reactor composed of a stirred tank reactor connected to a plug flow reactor, which facilitates the generation of starvation zone gradients (Ankenbauer A, 2020). Alternatively, a single bioreactor segmented into compartments using different disks with varying exchange areas can artificially prolong mixing times between compartments (Gaugler L, 2022). Computational fluid dynamics (CFD) simulations can provide further characterization of these systems, and tracer experiments may be used to validate these results (Mayer F, 2023).

For industrial optimization, *Pseudomonas putida* stands out due to its substrate flexibility and robust redox capacities. Employing a genome reduction strategy will further enhance its suitability for industrial applications. By leveraging scale-down bioreactor systems that mimic large scale conditions, a strain tailored for such environments can be engineered. Emphasis will be placed on optimizing these systems, particularly the single multi compartment bioreactor system, to enable more efficient screening and validation of engineered strains.

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Phage Therapy Combined with Gum Karaya Hydrogels for Treatment of multi-resistant *Pseudomonas aeruginosa* Deep Wound Infection in a Porcine Model

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Abstract

Background

Gum karaya (GK) polysaccharide-based hydrogels keep a moist environment and stimulate wound healing. Its antimicrobial potential against multi-resistant *Pseudomonas aeruginosa* strains has been established. Supplementation of GK with phage preparations can increase its antimicrobial potential.

Methods

A total of six fully immunocompetent pigs, each with twenty 5 x 5 cm skin defects, were used. The multi-resistant *P. aeruginosa* strain FF2 was applied after surgical excisions. On Days 4, 7, 11, and 14 after infection, tissue samples of wounds were taken for microbiological evaluation. The wounds of four pigs were treated using GK hydrogels supplemented with phages Pbunavirus (MB501) and Phikmvvirus (LUZ19) at concentrations of 10^9 PFU per wound. Two pigs were left untreated to monitor the progress of infection.

Results

Three days after infection, wounds showed a bacterial load of 7.8 ± 0.4 log CFU/g of tissue. During the experiment, untreated pigs exhibited a decrease in bacterial numbers in wounds to 6.9 ± 0.4 log CFU/g (1.0 log CFU/g difference). The decrease in wounds of treated pigs was to 5.1 ± 0.8 log CFU/g of tissue (2.7 log CFU/g difference). The statistical evaluation showed a significant decrease in the number of bacteria ($p < 0.01$).

Conclusion

The study shows a synergistic effect of GK and phage therapy, resulting in a significant reduction in bacterial loads within ten days of treatment. No adverse effects of the GK hydrogels of phage preparations were observed during the study. Therefore, this treatment offers a promising avenue for future research and clinical applications.

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Bacterial cooperation across host cell membranes enables release of intracellular vacuolar populations

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Abstract

Pseudomonas aeruginosa strains expressing ExoS are characterized by their ability to invade host cells and replicate within them. Recently, we demonstrated when *P. aeruginosa* invades epithelial cells it forms two sub-populations: one cytoplasmic (T3SS-ON), the other vacuolar (T3SS-OFF). While T3SS mutants (Δ*exsA*) are found exclusively in vacuoles it remains unclear how wild-type (WT) bacteria transitions between the two sub-populations. Here, we studied the order of events using time lapse microscopy and varying infection timing in corneal epithelial cells, comparing WT strains to mutants in various components of the T3SS, quantifying % cells infected and number of bacteria-containing vacuoles. Results showed bacteria entered vacuoles first and later disseminated into the cytoplasm, with shorter (1h) invasion times restricting WT bacteria to vacuoles. De-repression of the T3SS (Δ*exsE*) enabled vacuole escape (WT vs. Δ*exsE*: 55% decrease in vacuole numbers). Importantly, co-infecting WT with Δ*exsE* mutants rescued WT from their vacuoles, enabling a 45% decrease in WT vacuole numbers relative to WT/WT co-infection. Δ*exsE* mutants were not located inside WT infected cells suggesting the T3SS of extracellular bacteria enabled the vacuolar release. Δ*exsE* mutants with additional T3SS-component mutations implicated the T3SS needle and translocon proteins in vacuolar rescue, but not the exotoxins (co-infection WT/Δ*exsE*Δ*popBD* and WT/Δ*exsE*Δ*pscC*: no significant change in WT vacuole numbers; co-infection WT/Δ*exsE*Δ*exoSTY*: 37% decrease in WT vacuole numbers; all relative to WT/WT). Together, these results show cooperation between intracellular and extracellular bacteria, with T3SS-OFF bacteria entering vacuoles and extracellular T3SS-ON bacteria allowing their vacuolar release.

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Persistent *P. aeruginosa* co-infection of primary HBE cultures reveals novel insights into host and bacterial transcriptomic and functional response to CFTR modulator rescue

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Abstract

People with cystic fibrosis (CF) are predisposed to recurrent and chronic lung infections by *Pseudomonas aeruginosa* (*Pa*). The recent introduction of highly effective modulator therapy (HEMT) to correct the genetic defect in the CF transmembrane conductance regulator (CFTR) has greatly improved lung function and quality of life, but bacterial infections persist. Studies investigating the effect of persistent bacterial infection on airway epithelium response and CFTR rescue have not been explored. Previous studies have been limited to short, acute infection times due to bacterial overgrowth and toxicity during *Pa* infection of *in vitro* human bronchial epithelial (HBE) cultures. Using a novel primary CF HBE co-infection model with persistent *Pa* infection, we investigated changes in host pathways due to persistent bacterial infection to identify potential mitigating effects of HEMT by *Pa* and to reveal the effects of HEMT on *Pa.*

Using this model, we uncovered that persistent *Pa* infection increased CFTR mRNA, protein levels, and function. Although HEMT did not exhibit direct antimicrobial activity, we observed a reduction in bacterial burden after HEMT, similar to clinical observations, possibly due to increased CFTR function. In addition, epithelial bulk RNAseq demonstrated significant changes in epithelial expression of genes involved in ion transport, cell surface glycoprotein secretion, and hypoxia response upon *Pa* infection and CFTR rescue. RNAseq of *Pa* grown in HEMT-rescued CF mucus revealed a unique bacterial response to HEMT. Our study underscores the importance of infection and inflammation in drug pharmacology and efficacy when optimizing therapeutics for people with CF.

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Diribonuclease activity eliminates toxic diribonucleotide accumulation

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Abstract

RNA degradation is a fundamental process required for transcriptional regulation. This is a sequential process in which RNA is cleaved into fragments by endonucleolytic enzymes and these RNA fragments are degraded by exonucleolytic enzymes into mononucleotides. Of the known exoribonucleases in g-proteobacteria, Orn is the only enzyme that catalyze the conversion of the terminal diribonucleotide into mononucleotides. Orn is the only exoribonuclease that is essential in many g-proteobacteria, such as *E. coli*. But in *P. aeruginosa,* Δ*orn* mutants can be generated, but have a small colony variant (SCV) phenotype. Because *P. aeruginosa* Δ*orn* are viable, we hypothesize that *P. aeruginosa* has mechanism to cope dinucleotides accumulation. To understand these mechanisms, we carry out transposon mutagenesis of Δ*orn* and identified mutants that restored wild type growth. However, deletion of the genes disrupted by the transposon did not suppress the SCV phenotype. Genetic screening for genomic fragment from transposon mutants that restored colony morphology identified the *yciV* gene. Lysates from strains expressing *yciV* and purified YciV protein exhibited diribonuclease activity. YciV is present in all g-proteobacteria, suggesting that YciV from *P. aeruginosa* is distinct from its *E. coli* ortholog. Phylogenetic analysis revealed two key differences between *P. aeruginosa* versus ortholog from other g-proteobacteria mapped to the active site of the enzyme. The expression of *P. aeruginosa* YciV in *E. coli* permitted deletion of the chromosomally encoded *orn*. Similarly, the deletion of *yciV* from *P. aeruginosa* caused *orn* to become essential. These results show that diribonuclease activity is required in g-proteobacteria.

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An integrative Type I-F Cascade-based CRISPRi library enabled unlocking the secondary metabolites functions in *Pseudomonas aeruginosa*

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Abstract

Microbial secondary metabolites refer to low molecular mass microbial products that are characteristic of the producing organisms but are not essential for growth or reproduction in normal conditions. The extensive applications of microbial secondary metabolites in human healthcare are well documented due to their remarkable diversity in chemical structures and biological activities. However, their natural biological functions in producing organisms and their therapeutic implications remain largely unexplored.

To comprehensively investigate secondary metabolites produced by *Pseudomonas aeruginosa*, we conducted a bioinformatic search by combining COG and pathway annotation with three common secondary metabolites databases and identified 63 transcription units responsible for the biosynthesis of 30 potential secondary metabolites in PAO1. To fully unlock the physiological potentials of these secondary metabolites, we set out to develop an arrayed CRISPR interference (CRISPRi) screening library by employing the integrative type I-F CRISPR-based platform previously established in our lab. We developed a CRISPRi library encompassing 83 constructs targeting 40 transcription units, with 2 or 3 crRNAs designed to repress each unit. RT-qPCR analysis showed that the expression of 90% of the transcription units was effectively repressed with more than 50% reduction.

The antibiotic susceptibility testing of the CRISPRi library revealed that the production of four secondary metabolites: phenazine, pyochelin, spermidine, and novobiocin, enhanced the resistance of *P. aeruginosa* to ciprofloxacin and ceftazidime. Furthermore, pseudopaline production was shown to sensitize the bacterium to the two antibiotics. In conclusion, this research has unveiled the immense potential of such CRISPRi library in elucidating the novel functions of secondary metabolites and significantly propelled our comprehension of *P. aeruginosa*.

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The activity of *Pseudomonas aeruginosa*-derived rhamnolipids against other microorganisms

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Abstract

Pseudomonas aeruginosa produces rhamnolipids, which have shown potential in industrial applications. Given their increasing industrial interest as surfactants, understanding their action against other microorganisms is crucial. Therefore, the study aimed to determine microbial rhamnolipid activity.

Rhamnolipids were extracted from a 72 h *P. aeruginosa* culture and suspended in water for a final 20 mg/mL concentration. The surfactant was tested against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Briefly, twofold dilutions were performed to obtain a different rhamnolipid concentration (1.00 – 0.015 mg/mL) inoculated with the cultures prepared in twice-concentrated TSB. After 24h, the cultures' optical density, metabolic activity (in resazurin assay) and biofilm production were measured spectrophotometrically.

Microbial growth dynamics were reduced for bacteria and yeast at the highest concentration of rhamnolipids. However, for *E. coli*, an increase in metabolic activity was observed for a range of concentrations (0.5-0.125 mg/mL), and the highest biofilm biomass was observed in samples containing 0.015-0.030 mg/mL of the surfactant. In addition, for *S. aureus*, a decrease in metabolic activity and an increase in biofilm biomass were observed after exposing cells to 1 mg/mL rhamnolipid concentration. The highest metabolic activity was recorded in the range of 0.5-0.125mg/mL for *C. albicans*, and an increase in biofilm biomass was observed for concentrations of 0.031, 0.062 and 1 mg/mL.

High rhamnolipid concentrations reduce microbial growth dynamics, depending on the model microorganism. However, metabolic activity and biofilm formation can be stimulated at certain surfactant levels.

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The type VI secretion systems of longitudinally collected *P. aeruginosa* isolates from people with cystic fibrosis in Denmark

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Abstract

Longitudinally collected *Pseudomonas aeruginosa* isolates from people with cystic fibrosis (pwCF) show strong signs of adaptation by natural selection. One gene that is found mutated across pwCF is *retS*, which results in the activation of type VI secretion systems (T6SSs) and other traits. The secretion systems enable *P. aeruginosa* to kill microbial competitors, manipulate eukaryotic cells, and take up nutrients. Although such changes might result in increased T6SS activity early during chronic infection, reports on deleterious mutations in T6SS genes suggest its inactivation late during infection. Here, we use genomics to test for changes in T6SS genes in longitudinally collected *P. aeruginosa* isolates. These isolates belong to an existing collection and capture up to ten years of *P. aeruginosa* colonization per pwCF. We found differences between the three T6SSs of *P. aeruginosa* during initial colonization and later sampling timepoints. These findings suggest that the individual T6SSs of *P. aeruginosa* might be affected differently by bacterial adaptation to the CF lung and advance our molecular understanding of chronic infection.

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Pseudomonas aeruginosa secondary metabolite trigger prophage induction in *Staphylococcus aureus*

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Abstract

Bacteriophages (phages) are viruses that infect bacteria, which play an important role in shaping microbial communities by impacting their ecology, physiology, and evolution. Most of the phages found in nature and in the human body are lysogenic and they are integrated in the genome of their microbial host as prophages. Prophages can be induced upon some chemical or physical stimulus enabling them to enter the lytic cycle, leading to the production of large number of phage particles that can kill the bacterial host. However, the ecologically relevant triggers for prophage induction remain largely underexplored. The identification of novel prophage-inducing molecules plays an important role in understanding the behavior of prophages and developing strategies for controlling bacterial infections.

Microbial natural products are one of the most productive sources of bioactive compounds. The present study describes the bioassay-guided isolation of a phenolic compound from *Pseudomonas aeruginosa* species, that trigger prophage induction in *Staphylococcus aureus* strains. We have developed and adapted untargeted screening assays that allow us to detect and quantify the presence of phage particles which are then used to screen the bioactivity of bacterial samples. Fractionation of extracts was performed by semi-preparative HPLC and the compound identification by 1&2D NMR and MS. Our results demonstrate that human pathogens can produce prophage-inducing small molecules and provide an alternative to antibiotics for treating deadly bacterial infections.

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Industrial Microbiomes as a Resource for Isolating Solvent-Tolerant Pseudomonads to Enhance Product Tolerance for Sustainable Plastic Production

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Abstract

The production of plastics, predominantly driven by the petrochemical industry, relies on finite and environmentally harmful resources. Achieving global sustainability and environmental targets necessitates alternative production methods. Biotechnology offers a sustainable and renewable pathway for plastic monomer production. However, producing acrylic plastic monomers in microorganisms is challenging due to their inherent toxicity as solvents. *Pseudomonas* species, known for their resilience in industrially contaminated environments, present a potential solution. We targeted the industrial microbiome of sites historically used for plastic production isolating a Pseudomonas strain demonstrating enhanced tolerance to our acrylic monomer compared to *Pseudomonas putida* KT2440 a commonly engineered strain for the production of platform chemicals. Metagenomics and culture based approaches were used to identify strains of interest. To further characterise the tolerance mechanism of this isolate, RNA-seq will be employed to identify genes of interest. Additionally, we explored a pseudo-industrial environment, an art studio (where acrylic paints and solvents are routinely used) resulting in the isolation of two *Pseudomonas* strains with significantly improved tolerance compared to KT2440 and the industrial isolate. Notably, one strain possesses a large plasmid encoding an RND efflux pump, analogous to another pump associated with solvent tolerance. Curing this plasmid significantly decreased the strain's tolerance, underscoring the efflux pump/plasmid system's role in conferring tolerance. This plasmid offers a transferable upgrade to *KT2440's* tolerance and its ability to secrete product into culture for processing. By leveraging an Industrial Microbiome enriched for solvent tolerance, we have identified novel alternative production strains and elucidated a system that could enhance the efficacy of current production strains.

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Tsd1 and Tne3 are new T6SS effectors of clinical *Pseudomonas aeruginosa* isolates

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Abstract

The type VI secretion system (T6SS) is a widespread contractile nanomachine employed by gram-negative bacteria to deliver toxic effectors into target prokaryotic cells for interbacterial competition. *Pseudomonas aeruginosa* encodes three distinct T6SS operons (H1-, H2-, and H3-T6SS), alongside several orphan *vgrG*, *PAAR* and effector islands scattered throughout its genome encoding diverse effectors. To date, most *P. aeruginosa* T6SS investigation and characterisation has focused on the reference strains PAO1 and PA14. Here, we have performed an *in silico* analysis of the T6SSs in 52 clinical strains of *P. aeruginosa* isolated from people with Cystic Fibrosis. Employing comparative genomics and a suite of bioinformatic tools, we unveiled significant genetic diversity at T6SS loci across all strains, primarily influenced by mobile genetic elements. We identified eight new putative T6SS effectors, predicted to exert toxic activities against both prokaryotes and eukaryotes, including Tsd1 (a cytidine deaminase) and Tne3 (a NADase). *In vitro* assays confirmed the antibacterial activity of both Tsd1 and Tne3 through expression of the effector-immunity pairs in *E. coli* and biochemical assays revealed functional insights. Deletion of both T6SS effector genes, along with the negative regulator *rsmA*, and *tssB1,* encoding the sheath protein of the H1-T6SS, from the clinical strain PALA37 determined the contributions of these proteins to inter-*Pseudomonas* competition. These findings highlight the functionality of effectors encoded at the same T6SS loci within clinical isolates and their roles in competitive interactions of *P. aeruginosa* that could be occurring in the lungs of people with Cystic Fibrosis.

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Adapting a cell free protein synthesis system for use in *Pseudomonas putida kt2440* to facilitate in vitro pathway prototyping

Antonin Lenzen, This image shows Martin Siemann-Herzberg Martin Siemann-Herzberg, Ralf Takors

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Abstract

Performing transformations and testing genetic modifications are two main factors slowing down strain engineering. More data about processes in the cells can help to identify possible beneficial integrations and changes. However, testing the effect of one or multiple changes of is a major obstacle for fast strain engineering.

Cell Free Protein Synthesis (CFPS) uses active cell extracts to produce proteins in an open system. It requires only the addition of purified plasmid that contains a gene of interest, to produce a specific protein in a few hours. A CFPS system must be adapted to each organism. The protein yield of the system must also be optimized, to be used for **in vitro pathway prototyping**. As this approach benefits greatly if a larger number of parameters can be scanned, automating it using a pipetting robot is an important step as well.

Therefore, a CFPS system was developed for *Pseudomonas putida kt2440*. The system was optimized, and the cell extract was identified as a main factor determining the resulting protein titers. The cell harvest and extract generation procedures were optimized and factors limiting the protein titers were identified. Experiments showed the possibility to use a pipetting robot to automate CFPS reactions.

The established CFPS system will be useful for in vitro pathway prototyping. It will also be a useful tool for strain engineering. Additionally, it may be used to produce small amounts of proteins of interest, for characterization purposes and expression studies.

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Intracellular and aggregated Pseudomonas aeruginosa persist in vivo and evade antibiotic treatment

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Abstract

Persistent bacterial infections evade host immunity and resist antibiotic treatments through various mechanisms that are difficult to evaluate in a living host. *Pseudomonas aeruginosa* is a main cause of chronic infections in patients with cystic fibrosis (CF) and wounds. By immersing wounded zebrafish embryos in a suspension of *P. aeruginosa* isolates from CF patients, we established a model of persistent infection that mimics a murine chronic skin infection model. Live and electron microscopy revealed persisting aggregated *P. aeruginosa* inside zebrafish macrophages at unprecedented resolution. Persistent *P. aeruginosa* exhibited adaptive resistance to several antibiotics, host cell permeable drugs being the most efficient. Moreover, persistent bacteria could be partly re-sensitized to antibiotics upon addition of molecules that dispersed the bacterial aggregates *in vivo*. Collectively, this study reveals that, along with bacterial clustering, an intracellular location protects persistent *P. aeruginosa* from host innate immunity and antibiotics, and provides a powerful *in vivo* platform to assess treatments against chronic infections.

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Process optimization of formate-dependent poly(3-hydroxybutyrate) bioproduction by engineered *Pseudomonas putida*

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Abstract

CO_2-derived feedstocks, such as formate and acetate, can be readily obtained by electrocatalysis and acetogenesis, respectively. These C_1 molecules serve as renewable substrates for microbial bioproduction. Several organisms have been engineered for assimilation of C_1 substrates, yet the cognate bioprocesses need to be improved towards implementation at the industrial level. Here, we describe the optimization of a bioprocess designed for growth and polymer accumulation of a *Pseudomonas putida* engineered with the reductive glycine pathway. This strain grows on formate and acetate as carbon and energy sources, respectively. Substrate-limiting conditions highlighted the importance of adjusting the acetate concentration to support energy generation, in turn driving efficient formate utilization. By adjusting the process conditions, we improved cell densities under formatotrophic conditions by 5-fold, testing continuous, linear, and exponential fed-batch strategies in bioreactors. Bioproduction of poly(3-hydroxybutyrate) (PHB) was achieved by introducing *phaC*, *phaA* and *phaB* from *Cupriavdus necator* under control of the inducible **ChnR/P$_{chnB}$** expression system. Next, growth-coupled and uncoupled approaches were tested to increase PHB titers. To this end, the process was divided in two phases, (i) a biomass generation phase (i.e., growth) and (ii) a PHB production phase. Under optimized conditions, the engineered strain accumulated PHB at 60% (w/w), which represents 80% of the theoretical maximum. Taken together, these results expand the scope of bioproduction approaches towards implementing a C1-based bioeconomy.

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Characteristics and phylogenetic distribution of megaplasmids and prediction of a putative chromid in *Pseudomonas aeruginosa*

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Abstract

Background:

Research on megaplasmids that contribute to the spread of antimicrobial resistance (AMR) in *Pseudomonas aeruginosa* strains has grown in recent years due to the now widely used technologies allowing long-read sequencing.

Methods and results:

Here, we systematically analyzed distinct and consistent genetic characteristics of megaplasmids found in *P. aeruginosa*. Our data provide information on their phylogenetic distribution and hypotheses tracing the potential evolutionary paths of megaplasmids. Most of the megaplasmids we found belong to the IncP-2-type, with conserved and syntenic genetic backbones carrying modules of genes associated with chemotaxis apparatus, tellurite resistance and plasmid replication, segregation, and transmission. Extensively variable regions harbor abundant AMR genes, especially those encoding β-lactamases such as VIM-2, IMP-45, and KPC variants, which are high-risk elements in nosocomial infection. IncP-2 megaplasmids act as effective vehicles transmitting AMR genes to diverse regions. One evolutionary model of the origin of megaplasmids claims that chromids can develop from megaplasmids. These chromids have been characterized as an intermediate between a megaplasmid and a chromosome, also containing core genes that can be found on the chromosome but not on the megaplasmid. Using *in silico* prediction, we identified the "PABCH45 unnamed replicon" as a putative chromid in *P. aeruginosa*, which shows a much higher similarity and closer phylogenetic relationship to chromosomes than to megaplasmids while also encoding plasmid-like partition genes.

Conclusion:

We propose that such a chromid could facilitate genome expansion, allowing for more rapid adaptations to novel ecological niches or selective conditions, in comparison to megaplasmids.

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Quorum sensing in *Pseudomonas aeruginosa* as a bistable population switch

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Abstract

Quorum sensing (QS) is a widespread, cell density-dependent communication mechanism in bacteria that coordinates collective behaviors through diffusible chemical signals. Canonical QS is considered a gene expression switch that synchronizes responses at the population level, with theoretical evidence suggesting it functions as a bistable system, featuring distinct and stable on and off-states. In this presentation, we describe the experimentally testing of this foundational assumption in the *las* QS system of the opportunistic pathogen *Pseudomonas aeruginosa* under steady-state growth conditions. We investigate the responses of two *las* QS targets, the central signal synthase LasI, and the Type 6 secretion component PAAR4, by population and single-cell gene expression analysis, as well as mathematical modeling. In both cases, we demonstrate population-level bistability, defined as the synchronous, bistable state switching of the entire population. Additionally, we observed hysteresis, indicative of memory within the system, where induced cells maintain activation at considerably lower densities than previously uninduced cells. Our study provides experimental validation of a central, emergent property in bacterial QS with implications for understanding bacterial physiology, pathogenesis, and applications in synthetic biology.

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Maize root colonization patterns and competitiveness in natural soil of autochthonous *Pseudomonas* isolates

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Abstract

Seed treatment is the preferred strategy to incorporate beneficial bacteria into agricultural ecosystems for extensive crops, like maize. In this study, we evaluated root colonization patterns and root competitiveness of 7 *Pseudomonas* isolates, which performed differently during seed bacterization. For root colonization assays, bacterial suspensions (OD_{600}=1.0) were mixed at 7 ml/kg dose with surface-disinfected maize seeds (KM8701 VIP3), with the optimal additive according to the bacterization experiments: Premax® (Rizobacter, Argentina S.A., 28.6% v/v); or trehalose 1M and polyvinylpyrrolidone 1.5% w/v. Seeds were sown in Jensen's semi-solid medium (0.5 % w/v agar) and incubated for 9 days (24 °C to 13 °C; 16/8 h). Root tips were visualized using a confocal microscope (Zeiss LSM 880 with Airyscan). For competitiveness assays, non-disinfected maize seeds (KM87 VIP3) were inoculated, placed in 1L-pots containing a perlite and natural soil mixture (1:1) and incubated under greenhouse conditions for one month. Plant parameters were evaluated and the rhizospheric colonization was quantified. Confocal microscopy revealed a preference in colonizing the plant cell junctions. Different patterns were detected for each isolate: string-like arrangements (1008 and SVBP6), microcolonies (RPAN1 and SMMP3) or both (RBAN4, SVMP4 and SPAN5). In competitiveness experiments, high root colonization density was detected for strains with the highest seed bacterization levels. However, all isolates improved most plant parameters, such as shoot and root biomass. In conclusion, we demonstrated these isolates colonize maize roots after seed inoculation and can promote plant growth, even though some of them do not exhibit high competitiveness ability.

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Engineering of *Pseudomonas aeruginosa* for the production of pyoverdine analogs

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Abstract

Pyoverdine is the main siderophore produced by fluorescent *Pseudomonas*. In addition to playing an important role for the proliferation and the virulence of the bacteria, this iron-chelator holds biomedical promise in diagnosis, bioimaging and antibiotic vectorization. In this context, we are developing engineering strategies to produce pyoverdine analogs in order to extend its properties and diversify its application.

Pyoverdine in a non-ribosomal peptide synthesized by *Pseudomonas aeruginosa* by four Non Ribosomal Peptide Synthetase (NRPS). We engineered the adenylation domain of PvdD, the terminal NRPS in pyoverdine biosynthesis, to incorporate a non-canonical amino acid into the pyoverdine structure. Guided by molecular modeling, we rationally designed mutants of *P. aeruginosa* with mutations at two positions in the active site. We then monitored the growth of the mutants and analyzed the resulting PVD by mass spectrometry.

A single amino acid change in the PvdD adenylation domain resulted in the successful incorporation of an azido-amino acid, leading to the synthesis of the corresponding pyoverdine functionalized with an azide group. Using copper-free click chemistry, we conjugated the azido-pyoverdine to a dibenzocycloctine antibiotic and showed that the conjugated siderophore retains the iron chelation properties and its capacity to be recognized and transported by *P. aeruginosa*. These findings open new perspectives for the conjugation of pyoverdine with diverse antibiotics for their vectorization into the critical pathogen *P. aeruginosa*.

The production of clickable pyoverdine holds substantial biotechnological significance, paving the way for numerous downstream applications.

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Increased Transmission of *Pseudomonas aeruginosa* in ICU Settings During the COVID-19 Pandemic

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Abstract

Background: The transmission of *Pseudomonas aeruginosa* between the hospital environment and patients pose a serious threat to clinical treatment. Covid-19 pandemic potentially impact bacterial transmission in intensive care unit (ICU). We conducted the research to investigate the temporal and spatial spread of *P. aeruginosa* before and during Covid-19 pandemic.

Methods: We routinely screened for *P. aeruginosa* in both the environment and patients in a newly-opened 21-room tertiary teaching hospital ICU in Hangzhou, Zhejiang during October 2022 – April 2023. Whole genome sequencing and antibiotic susceptibility test were performed on all non-repetitive *P. aeruginosa* samples.

Results: Among 1694 environmental samples, 263 (15.53%) were positive for *P. aeruginosa*, and 40 (2.36%) were carbapenem-resistant *Pseudomonas aeruginosa* (CRPA). In 1575 oral and rectal samples (from 353 patients), 229 (14.54%) were positive for *P. aeruginosa* and 113 (7.17%) were CRPA. ST463 was highly prevalent in both patients (1st) and the environment (4th). Combined spatiotemporal distribution and genomic data, revealed no patients-related transmission before Covid-19 pandemic but detected multi-clonal patients-related transmission including high-risk ST463 clones during Covid-19 pandemic. Additionally, two independent bla_{KPC} evolution events (from bla_{KPC-2} to bla_{KPC-71}) were observed, leading in gastrointestinal and respiratory CRPA colonization in one patient.

Conclusion: Our prospective study demonstrates that Covid-19 pandemic is associated to increased *P. aeruginosa* transmission. These finding provide insights for nosocomial infection management during future public health crisis.

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Identifying pilus mutations selected for by type IV- pilus targeting phages

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Abstract

Antibiotic resistance has driven the pursuit of novel therapeutics such as bacteriophages (phages). White phages show promise as therapeutics, evolutionary pressures exerted by phages can drive spontaneous development of resistance in the host during infection, rendering the treatment ineffective. However, phage resistance can be advantageous as mutations that arise due to resistance may result in desired changes in bacterial traits such as reduced pathogenicity. These phenotypes may be selected for through the types of phages that bacteria are exposed.

The first line phage defence mechanism involves modification of phage receptors. *Pseudomonas aeruginosa* has a variety of phage receptors, including type IV- pili (T4P). To confer protection from T4P-targeting phages, bacteria may mutate the pilus to prevent phage binding. Here, we show that *P. aeruginosa* mutants resistant to PO4, a T4P-targeting phage, lose twitching motility, suggesting impaired pilus function. Using whole genome sequencing, followed by comparison of the parent strain to phage resistant isolates, we identified mutations that span all T4P structural subunits and various regulatory components. These mutations vary in their effect on pilus expression and assembly. Amongst these mutations is a 12 base-pair duplication in *pilD,* the prepilin peptidase. This mutation does not affect the pilin peptidase function of PilD. Since T4P are important virulence factors, modification to the T4P following phage exposure can affect infectivity of these resulting resistant bacteria. These data also provide us with a tool to further understand and investigate the various components of the T4P and how dispensable they are for T4P function.

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Use of Xenosiderophores by Pseudomonas aeruginosa under Iron-Deficient Conditions

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Abstract

Iron is an essential nutrient for *Pseudomonas aeruginosa* and almost all bacteria but has poor bioavailability in most biotopes. To overcome iron restriction, many microorganisms synthesize and secrete siderophores, which are low molecular weight ligands with a high affinity for ferric iron. Siderophores scavenge iron from the environment and the ferri-siderophore complexes formed are transported across the outer membrane by TonB-dependent transporters (TBDTs), powered by the proton motive force via the TonB-ExbB-ExbD complex.

P. aeruginosa produces two siderophores and can use many others produced by different microorganisms, necessitating appropriate TBDTs for each siderophore or structurally similar families of siderophores. We have shown by ^{55}Fe uptake assays ang growth assays under iron-restricted conditions that the α-carboxylate type siderophores rhizoferrin-Fe and staphyloferrin A-Fe are transported by the TBDT ActA.[1] Among mixed α-carboxylate/hydroxamate siderophores, we have shown that aerobactin-Fe is transported by ChtA, while schizokinen-Fe and arthrobactin-Fe are transported by ChtA and another unidentified TBDT.[1] Additionally, we have also demonstrated that nine hydroxamate-type siderophores from fungi and Streptomyces can be used by *P. aeruginosa*.[2] We found that iron is imported by the TBDT FpvB for coprogen, triacetylfusarinine C, fusigen, ferrirhodin and ferrirubin. Desferrioxamine G-Fe is transported by FpvB and FoxA, ferricrocin-Fe and ferrichrysin-Fe by FpvB and FiuA, and rhodotoluric acid-Fe by FpvB, FiuA, and another unidentified TBDT. These findings underscore the effectiveness of siderophores produced by other microorganisms in iron transport into *P. aeruginosa* and provide insights into the molecular mechanisms involved, which are important for understanding microbial interactions and ecological balance.

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Multi-mode screen of fungal extracts to identify novel antibacterial, antivirulence and antibiotic potentiator compounds

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Abstract

Background*: Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are pathogens for which novel antimicrobial drugs are urgently needed. Intrinsic antibiotic resistance in *P.a.* is mainly due to its low outer membrane permeability and expression of broad-spectrum drug efflux pumps, while its ability to form biofilms further impairs efficacy of antibiotic treatments. Alternative approaches to combat *P. aeruginosa* include anti-virulence strategies, targeting siderophore production as well as quorum sensing (QS) systems and biofilm formation.

Methods: Natural products have been extensively screened for possible antibacterial compounds, however the cultivation of microorganisms is a slow process and the frequent isolation of known antimicrobial compounds is a limitation. To circumvent these limitations a medium throughput screen on fungal extracts was set up on fungal species not known for their ability to produce antibacterial molecules.

Results: We developed a multi-mode screen and measured antibacterial activity of more than 2000 fungal extracts on an efflux-deficient, hyperpermeable mutant of *P. aeruginosa*, and on wt *S. aureus* and *E. coli*, with average hit rates of 3%, 2% and 0.2% respectively. Extracts were further tested for antibiotic potentiation against *P. aeruginosa* wild type in combination with sulfamethoxazole (3 hits), inhibition of the PQS QS system (1 hit), pyoverdin production (9 hits) as well as biofilm formation (1 hit) using luminescence, fluorescence and colorimetric assays, respectively. Extracts were analysed by UHPLC-HRMS for metabolomics and dereplication.

Conclusions: Screening of fungal extracts yielded novel chemical structures with antimicrobial and anti-virulence activities. Scale up and fractionation will allow to identify potential hit candidates.

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Bacterial valorization of the C_1-fraction from processed lignin

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Abstract

Exploring sustainable feedstocks is crucial for advancing the bioeconomy. Unlike first-generation feedstocks, second generation feedstocks such as lignocellulosic biomass do not compete with food production. While the cellulosic fraction is easily degradable, lignin is harder to process. To make lignin-based biorefineries economically viable, all lignocellulosic components must be valorized for fuel and chemicals. The C_1-fraction of the hydrolysates, methanol and formaldehyde, is often overlooked, despite its potential value.

This project aims to utilize methanol and formaldehyde as carbon sources for generation of biomass, which in turn catalyzes the production of value-added chemicals from phenylpropanoids. To this end, synthetic biology and adaptive laboratory evolution (ALE) will be employed to integrate the ribulose monophosphate (RuMP) cycle into the metabolism of *Pseudomonas putida*. By employing engineered synthetic auxotrophies and adjusting growth conditions, we will incrementally evolve *P. putida* to use methanol as sole carbon source, while maintaining strain fitness across growth phases and improving issues found in previous studies.

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Machine learning uncovers the diversity and evolution of the transcriptional regulatory networks of Pseudomonas species

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Abstract

The Pseudomonas genus is known for its diverse species that are involved in environment-specific interactions. This genus is widely studied due to the clinically relevant *Pseudomonas aeruginosa*, model plant pathogen Pseudomonas syringae, and the non-pathogenic, industrially relevant *Pseudomonas putida*. Despite a high degree of similarity in the genomes of the three species, they are functionally diverse. While the metabolic and physiological versatility of these pseudomonads is in part enabled by genomic differences, unique regulatory mechanisms contribute to their ability to survive in diverse niches. In the present study, we applied machine learning to decompose species-specific RNA-seq compendiums containing both in-house generated and public data to modularize the transcriptomes of the three species. We then mapped the independently modulated sets of genes (iModulons) identified across the three species to track the evolution of their transcriptional regulatory networks (TRNs). Through cross-species iModulon analysis, we (i) reveal the core, accessory, and rare iModulons of the three species, (ii) elucidate the implications of differential gene membership of iModulons on species-specific biology, (iii) reveal nuances in the regulation of systems prevalent in pseudomonads, and (iv) utilize the better understood TRNs of P. aeruginosa and *P. putida* to annotate the understudied TRN of P. syringae. Comparing the modularized transcriptomes of the three species provides unique insights into their evolution and diversity at the transcriptional level.

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Identification of natural substrates of the major drug efflux pumps in Pseudomonas aeruginosa

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Abstract

Background: Multidrug resistant Gram-negative bacteria are a major threat to human health and new strategies to tackle these organisms are urgently needed. In *P. aeruginosa*, four tripartite Mex efflux pumps (EPs) are major determinants for both intrinsic and acquired antibiotic resistance. Hence, inhibition of these EPs has been proposed as a promising strategy to potentiate the efficacy of existing antimicrobials.

Results: We aimed at identifying natural substrates of Mex multidrug EPs in *P. aeruginosa* to evaluate their potential use as competitive EP inhibitors. We constructed a mutant strain of PA14 genetically deleted in the four clinically relevant Mex EPs (Δ4mex) and overexpressed in this strain each Mex multidrug EP individually. Using comparative untargeted metabolomics on culture supernatants, UHPLC-HRMS analysis identified 2567 features. Of those 210 (8.2%) were significantly more abundant in the supernatant of EP overexpressors. The majority of the features were EP-specific (73.8%), while 26.2% were shared between two or more EPs. Among these we identified by-products of secondary metabolite synthesis as well as specific alkylquinolones. In comparison to the wildtype supernatant, the one of the EP deficient mutant D4mex showed decreased accumulation of fatty acids, including long chain homoserine lactone quorum sensing signalling molecules.

Conclusion: In contrast to the well-described broad-spectrum antibiotic profile, the natural substrate profile of Mex EPs seems to be rather metabolite specific. The multidrug Mex efflux pumps in *P. aeruginosa* appear to transport signalling molecules, metabolic by-products as well as fatty acids.

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Mucin promotes a planktonic lifestyle in P. aeruginosa by altering surface behavior through stimulating both twitching and swimming motility

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Abstract

The opportunistic pathogen *Pseudomonas aeruginosa* causes infections in several mucosal regions throughout the human body but how the central mucosal protein, mucin, impacts *P. aeruginosa* behavior is poorly understood. Here we provide evidence that mucin alters immediate surface colonization and surface-associated behaviors, the first steps in infection. Mucin restricts surface attachment independent of known *P. aeruginosa* adhesins (PilAY1, FliCD, LecAB, CdrA, Psl) and this inhibition was observed in commonly used *P. aeruginosa* strains (PAO1, PA14, PAK, PA103). We have determined, via widefield microscopy, that the mucosal environment reduces surface visits and residency time independent of its viscous properties and in a chemotaxis-dependent manner to promote a planktonic lifestyle. Additionally, using a fluorescent reporter construct, mucin appears to reduce secondary messengers, cAMP and c-di-GMP, again promoting the planktonic growth. In the remaining population of attached cells, we've observed mucin induces trails of the exopolysaccharide Psl through the induction of twitching motility whereby mucin-glycans alone are sufficient to induce this phenotype. Mucin not only increases the proportion of twitching surface-associated cells but also increase the range of distance traveled. Interestingly, this promotion of twitching and Psl-trail formation in mucin does not lead to an expected increase in microcolony formation, but instead, microcolony formation is completely abolished in mucin for at least 24 hours. Together, these data support the idea of *P. aeruginosa* sensing the mucosal environment to then alter its behavior to an active motility state both in planktonic and surface-associated cells.

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Two ClpP Proteases Are Involved in Cold-Adapted Survival in *Pseudomonas antarctica*

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Abstract

Clp protease is a highly conserved system to maintain protein homeostasis by degradation of misfolded or aggregated proteins in most organisms including bacteria. ClpP forms tetradecamer to build proteolytic pore. Interestingly, numerous bacteria harbors over two distinct ClpPs and regulate tetradecamer composition in accord with growth condition. Herein, we investigated gene regulation and proteolytic activity of two distinct ClpPs, $ClpP1_{Pan}$ and $ClpP2_{Pan}$, in an Antarctic isolate; *Pseudomonas antarctica*. $ClpP2_{Pan}$ exhibited several sequence variations compared to other bacterial non-Clp unfolase interacting ClpPs. In $ClpP2_{Pan}$, catalytic triad is conserved well, but no Gly residue was identified in Gly-rich heptamer dimerization domain for tetradecamer formation. Notably, $ClpP1_{Pan}$ was constitutively expressed at 5, 20, and 30℃. However, $ClpP2_{Pan}$ was 15.011-fold up-regulated at 5℃ condition compared to 20℃ condition in RNA-seq analysis. To understand the role of ClpP1, 2_{Pan} at low temperature, we analyzed its proteolytic activity at 5℃, 20℃, and 30℃ conditions. $ClpP1_{Pan}$ was identified as an essential component for proteolytic activity of Clp protease system through YFP-ssrA degradation assay at 5℃, 20℃, and 30℃ conditions. However, $ClpP2_{Pan}$ was not showed significant proteolytic activity at all conditions including 5℃. Furthermore, we tested the phenotypes under heat and freeze-thaw stress conditions. The Δ*clpP1* mutant exhibited significant lethal phenotypes under both stresses, while the Δ*clpP2* mutant did not. However, the Δ*clpP1* Δ*clpP2* double mutant showed significantly enhanced phenotypes, suggesting that $ClpP1_{Pan}$ and $ClpP2_{Pan}$ play vital and supportive roles, respectively. Further studies are needed to characterize the unknown role of $ClpP2_{Pan}$ at low temperatures and its detailed molecular mechanisms.

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Staphylococcus aureus coagulases rescue Pseudomonas aeruginosa during coinfection

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Abstract

Most infections harbor more than one microbe. Within such polymicrobial infections, microbes often produce extracellular factors that facilitate bacterial growth and virulence. While one member may produce these factors, their production can potentially benefit all constituents within a local group or population. *Staphylococcus aureus* is a prominent member of polymicrobial infections where it secretes two clotting factors, staphylocoagulase (Coa) and von Willebrand factor binding protein (vWbp), that contribute towards staphylococcal survival and persistence during infections. As is the dilemma with many extracellular factors, we find that coagulases are also exploitable by co-inhabiting bacteria that do not take part in producing them. In this study, we find that Pseudomonas aeruginosa, a Gram-negative bacteria, is able to benefit from the coagulases produced by *S. aureus*. P. aeruginosa displays enhanced survival in an in vitro clinical model and ex vivo samples of human blood where coagulases are available. Using a murine model of infection, we demonstrate that coagulases also contribute towards the hematogenous spread and persistence of *P. aeruginosa* in organ tissues. Our results provide a possible explanation as to why S. aureus and *P. aeruginosa* coinfections are recalcitrant and result in worse clinical outcomes.

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Revealing the sites of active growth and dormancy of tissue-dwelling pathogens in vitro

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Abstract

Pseudomonas aeruginosa is an important pathogen in chronic airway infections and ventilator-associated pneumonia. Monitoring of *in vitro* infections in cultured lung tissues can reveal the progression of infection and reveal important host:pathogen interactions, but spatio-temporal resolution has yet to reveal what tissue sites comprise active pathogen growth, and where dormancy is established. In this study we show in what sites pathogens are active and in which sites dormancy establish, by utilizing growth reporting strains. These strains harbour an unstable green fluorescent protein, and is studied using confocal laser microscopy. We also combine unstable growth reporting strains with pathoadaptive mutations to study how the sites change. Dormant subpopulations are clinically important, as these show higher tolerance towards antimicrobials, leading to infection recurrence. By utilizing growth-reporter strains in real-time monitoring, it will for instance be possible to study active targeting of dormant subpopulations *in vitro* by drugs and/or drug-delivery systems.

Poster : 002

Adaptation of Pseudomonas aeruginosa to long-term hypoxia is consistent with changes observed during chronic infection

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Abstract

The mechanism driving the adaptation of *Pseudomonas aeruginosa*t towards chronic colonisation is not yet fully understood. Hypoxia is one of the most important environmental pressures present in the CF lung. Thus, we hypothesise that long-term hypoxia drives adaptations in *P. aeruginosa*, which leads to the development of persistence in people with CF.

We studied the effect of long-term adaptation of an early CF isolate to 6% oxygen for 28 days. Two distinctive small colony variants (SCVs) developed, one SCV exclusively under low-oxygen pressure. Importantly, SCVs were more common in hypoxia-adapted cultures, comprising up to 98% of the population, while never exceeding 35% in normoxia-adapted cultures. Proteomic analysis showed significant changes in the abundance of >200 proteins within 28 days, including those involved in antibiotic resistance (MexA, ABC transporters, USPs, secretion systems), stress response (OsmE, OsmY, USPs) and biofilm formation (FleQ, PelC, SadB). Two independent hypoxia-adapted cultures developed higher resistance to 8 out of 13 antibiotics tested and showed increased biofilm (4.1-fold and 1.8-fold ($p<0.0001$)) and exopolysaccharide production. A third population displayed resistance to only two antibiotics and showed decreased biofilm-forming capability. Changes in virulence of the hypoxia-adapted populations were observed in the *Galleria mellonella* acute infection model. Surprisingly, no differences in the responses to either oxidative or osmotic stress, or differences in motility were observed between normoxia- and hypoxia-adapted strains.

Overall, these results confirm that hypoxia promotes the appearance of several phenotypes in *P. aeruginosa* that are associated with chronic infection and poor patient outcomes.

004

Genome-wide identification of FleQ/FleN/c-di-GMP-dependent regulatory patterns in *Pseudomonas putida*

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Abstract

Transcription factor FleQ is the master regulator in planktonic-to-biofilm lifestyle transition in the Gram-negative bacterium *Pseudomonas putida.* Its activity is allosterically regulated by direct interaction with MinD-type ATPase FleN and by the secondary messenger c-di-GMP. Our previous work suggested that FleN and c-di-GMP may act as FleQ antagonists or agonists to yield a variety of different expression patterns depending on the promoter class and the physiological function of regulated genes.

Here, we explore the genome-wide regulatory patterns generated by FleQ and its bipartite and tripartite complexes with FleN and/or c-di-GMP during lifestyle switch in *P. putida*. Using the wild-type strain and Δ*fleN* mutant as genetic backgrounds, we ectopically co-expressed the FleN protein with/without the PleD diguanylate cyclase or the YhjH phosphodiesterase. This approach led us to generate derivatives in which only one of the complexes could be formed with the components available. These strains were subjected to RNA-sequencing and differential gene expression was further analysed using the Deseq2 package. Hierarchical clustering allowed us to identify the regulatory patterns specific to each condition and results were validated by qPCR.

The findings of this study confirm the previously identified FleQ-dependent regulation patterns that lead to expression of genes involved in flagellar and type IV secretion system biosynthesis, as well as those involved in biofilm development. Moreover, our results have identified previously unidentified regulon member, revealing novel expression patterns that encourage further investigation, aiming to comprehensively understand the genome-wide transcriptional reprogramming that occurs during the lifestyle transition in *P. putida*.

007

Monitoring Cystic Fibrosis Airway Infections with Pseudomonas aeruginosa with anti-OprF Serum Antibodies

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Abstract

Background. The management of cystic fibrosis (CF) requires knowledge of the patient's microbiological status. The serology of anti-*P. aeruginosa* antibodies against exoenzymes or water-soluble antigens has gained diagnostic value, particularly to detect the onset of colonization with *P. aeruginosa.* However, the diversity and variable expression of these antigens, which was unknown when the ELISAs became common diagnostic procedures at CF clinics, prohibits the quantitative evaluation of bacterial antigen load during intermittent and chronic infection.

Methods. An ELISA was developed to measure the serum IgG antibody levels against *P. aeruginosa* porin OprF, a species-specific, conserved, immunogenic and constitutively expressed protein present in the outer membrane and extracellular vesicles.

Results. Serial serum samples were collected from 310 people with CF (pwCF) over a period of up to 15 years. Compared to a reference of *P. aeruginosa* – negative CF sera set to 1, OprF antibody titers ranged from 0.3 to 13.2 (median: 1.7) in 56 intermittently colonized patients and from 0.5 to 51.2 (median: 11.8) in 176 chronically colonized pwCF showing higher anti-OprF antibody levels during chronic than during intermittent colonization with *P. aeruginosa* (P = 0, Z = - 21.7, effect size 0.62). Inhalation with twice daily 80 mg tobramycin decreased OprF antibody titers ($P = 5 \times 10^{-5}$), particularly during the third and fourth year of chronic colonization.

Conclusion. The OprF ELISA should be an appropriate tool to monitor Pseudomonas serology at all stages of infection and disease severity and to study the impact of short- and long-term therapeutic interventions.

009

Versatile roles of rubredoxin reductase of Pseudomonas aeruginosa TBCF10839 in virulence and stress protection

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Abstract

Background. Rubredoxin RubA2 and rubredoxin reductase RubB are phylogenetically ancient iron sulfur proteins that protect anaerobic bacteria from the deleterious effect of oxygen. *Pseudomonas aeruginosa* engages this electron transfer and redox system for the degradation of alkanes as carbon source.

Methods and Results. Whereas *P. aeruginosa* TBCF10839 persisted and multiplied in human neutrophils, its isogenic plasposon mutant TBCF10839 *rubB*::Tn*5* was rapidly killed by neutrophils. TBCF10839 *rubB*::Tn*5* did not degrade alkanes, downregulated central metabolism and translation, was compromised in its versatility to degrade protein, quorum-sensing deficient, avirulent in the *Caenorhabditis elegans* fast killing infection model and was less tolerant to oxidative stress than the parental wild type. Upon exposure to hydrogen peroxide, the recombinant RubB/RubA2 electron transfer complex enabled catalase to reduce hydrogen peroxide directly to water and thus delayed the disproportionation to oxygen and water.

Conclusion. Rubredoxin reductase controls the oxidation state of iron centers in the *P. aeruginosa* cell, confers resistance against oxidative stress and facilitates bacterial growth and persistence in neutrophils, man's major antipseudomonal weapon. Rubredoxin and rubredoxin reductase are a showcase of how key elements of cellular protection and metabolism may become bacterial virulence determinants that undermine human defense in infectious disease.

011

Pseudomonas aeruginosa tunes virulence to the range of substrate rigidity found in host infection sites

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Abstract

Pseudomonas aeruginosa (PA) is a clinically important human pathogen that infects a wide range of body sites. Traditional studies targeting PA's pathogenesis have focused on how the different biochemical factors of these sites modulate virulence; however, infection sites also vary tremendously in their mechanical properties and can be as soft as mucus or as rigid as bone.

To test how mechanical factors might impact PA virulence, we use a transcriptional fluorescence reporter to monitor the activity of the Virulence Factor Regulator 'Vfr' to changes in rigidity of different stiffness tunable hydrogels. Using this assay, we establish that PA uses dynamic arm-like external appendages called type IV pili (TFP) to regulate the activity of over 100 virulence related genes to a physiologically important range of substrate rigidity between 0.1 kPa (mucus) and 1000 kPa (cartilage). Combining quantitative modeling with careful fluorescence measurements of TFP dynamics, we show that a competition between free pilin diffusing in the inner membrane and the substrate dependent depletion/gain of pilin in the membrane during TFP dynamics allows PA to distinguish substrates by rigidity. Our computational model for this mechanism is consistent with the experimental rigidity response of Vfr and correctly predicts the rigidity response in TFP motor mutants that change the dynamics of TFP. Interestingly, our data suggests that a TFP associated chemotaxis system is used to modulate the range of rigidity sensitivity to yet unknown input factors, highlighting a potential mechano-biochemical coupling between different factors.

012

Structural genome variants of Pseudomonas aeruginosa clone C and PA14 strains

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Abstract

Background. The assembly of short read genome sequence datasets typically assumes that the gross chromosomal frame is conserved although the first published *Pseudomonas aeruginosa* genome sequence already demonstrated a large inversion in the sequenced PAO1 subclone.

Methods. The genomes of *P. aeruginosa* clone C isolates from people with cystic fibrosis and clone PA14 isolates from animate and inanimate habitats were sequenced on PacBio and Nanopore platforms.

Results. Genome assembly yielded circular chromosomes with sizes from 6.762 to 6.896 Mbp for six clone C strains and from 6.515 to 7.026 Mbp for six clone PA14 strains. Compared to the NN2 and PA14 reference genomes, insertions, deletions and inversions were detected in five clone C and four clone PA14 strains. The large inversions of the five clone C strains were accompanied by two overlapping (C8) and two nested (C12, C19) inversions, copy number variations of mobile elements (C15) and 1 to 5 kbp large insertions (C12) or deletions (C8, C15, C19) flanking the inversion. Likewise, phage-like DNA, *IS* elements or transposons flanked the large inversions in the clone PA14 strains whereby a keratitis isolate from the UK and a river isolate from Germany shared the same 4.512 Mbp inversion.

Conclusion. Genome plasticity is frequent in the *P. aeruginosa* population. Recombination breakpoints are typically located in mobile genetic elements of the accessory genome.

013

Pseudomonas aeruginosa in the sputum metagenome of people with bronchiectasis

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Dr.

Abstract

Background. *Pseudomonas aeruginosa* is the most commonly isolated pathogen in people with bronchiectasis and is associated with worse outcomes, i.e. more lung lobe involvement, a higher frequency of exacerbations, a longer disease course and faster decline of lung function.

Methods. The microbial metagenome from sputa of 101 EMBARC study participants was examined by using whole genome shotgun sequencing.

Results. Our analysis of the metagenome of people with bronchiectasis revealed four clusters characterised by predominance of *Haemophilus influenzae, P. aeruginosa* or polymicrobial communities with a differential composition of non-pathogenic commensals and opportunistic pathogens. Within the low-diversity microbial communities dominated by an abundance of 80% or more of *P. aeruginosa,* the next more common species were *Staphylococcus aureus,* Rothia spp. and Streptococcus spp. Anaerobes like Prevotella spp. or Veillonella spp. and the non-pathogenic aerobes that are common in healthy airways were substantially or completely depleted.

Conclusion. If *P. aeruginosa* conquers the airways of people with bronchiectasis, it will become the primary microorganism in the lung and suppress the growth of anaerobes and non-pathogenic aerobes.

014

Adhesion of *Pseudomonas aeruginosa* cystic fibrosis isolates to airway and submaxillary mucins

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Abstract

Background. *Pseudomonas aeruginosa* resides in mucin-rich microcolonies during chronic luminal colonization of the lungs of people with cystic fibrosis (pwCF).

Methods. The adhesion of *P. aeruginosa* strains grown to midexponential phase to airway mucins of seven pwCF and to five mammalian submaxillary mucins was examined in 30 min incubations in mucin-coated microplates at 37°C taking the ratio of bound to total CFU as endpoint.

Results. The adhesion assay was performed on 57 serial clonal isolates of six lineages from five pwCF and ATCC serotype ST4, ST6, ST9, PAO1 and PA14 reference strains. The majority of strains did not bind more strongly to mucin than to the uncoated plastic of the microplate, with PAO1, ST6 and PA14 being the weakest binders. Serial clonal isolates from the same donor varied from 0.01% to 100% binding indicating substantial intraclonal temporal variation of bacterial mucin binding epitopes. Strong binders were isolated during pulmonary exacerbations requiring systemic antipseudomonal chemotherapy. Low binders did not differentiate between the 12 mucins, but the medium and strong binders showed a strain-specific gradient of the affinity to the individual mucins suggesting that strongly adhering strains recognize a set of epitopes that were present with variable frequency among the *O*-glycans of the repeat domains of the 12 mucin preparations.

Conclusion. Affinity of *P. aeruginosa* to airway and submaxillary mucin is a strain-specific feature that varies within a clonal lineage and is missed by studies with the common PAO1 and PA14 laboratory strains.

017

A novel family of uncharacterized proteins directs bacterial vesicle formation

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José Manuel Borrero de Acuña

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Abstract

Membrane encased structures serve multiple biological functions in bacteria, including the formation of intracellular compartments and extracellular vesicles, which are essential for bacterial-bacterial communication and bacterial-host interactions1. They also hold potential for biotechnological applications, especially in vaccine production, drug delivery, and valuable chemical production. Despite the tremendous advances in elucidating eukaryotic vesicle formation — endocytosis and exocytosis—and identifying key players, the proteins participating in this process in bacteria remain elusive.

Although a wide variety of bacterial processes can initiate vesicle formation, little information is available on the systems that coordinate the bacterial membrane's restructuring. We have identified a family of uncharacterized proteins, which are highly conserved across species and actively promote both intra- and extracellular vesicle generation.

This protein family is sub-divided into two groups. Overproduction of such proteins in Escherichia coli and the human pathogen Pseudomonas aeruginosa from a manifold of Gram positive and negative microbes resulted in striking membrane restructuring. Specifically, the action of a protein group led to the formation of intracellular membranous compartments either tubular or globular, whereas the other group boosted extracellular vesicle secretion. The deletion of both genes in P. aeruginosa resulted in a substantial loss of extracellular vesicle formation.

These results collectively pinpoint these two groups of proteins as active membrane re-shaping effectors, and it is to our knowledge the first report on active vesicle production in bacteria. These findings entail vast applications in synthetic biology, biomedicine and bioprocess engineering.

018

Unique carbon source utilization hierarchy in *Pseudomonas bharatica* CSV86^T

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Abstract

Aromatic pollutants are known to pose a serious threat to biota and human health. Bacterial degradation of aromatics have been well characterized. However, in the environment, presence of simple carbon sources like sugars and organic acids hinder the aromatic degradation ability of bacteria due to Carbon Catabolite Repression (CCR). *Pseudomonas bharatica* CSV86^T is a novel Indian soil isolate that degrades a variety of aromatic compounds such as naphthalene, benzoic acid, benzyl alcohol, salicylate and phenylpropanoids among many others. Spent media analysis, whole-cell oxygen uptake and enzyme induction patterns measured during growth profile ascertain the carbon source utilization hierarchy in strain CSV86^T. Results indicate that strain CSV86^T preferentially utilizes aromatics over simple carbon sources such as glucose, fructose and glycerol unlike other *Pseudomonas* species. Presence of simple carbon sources do not repress aromatic metabolism. Strain also co-metabolizes aromatics with organic acids. This unique substrate utilization pattern appears to be due to a global regulatory phenomenon that facilitates the strain to evade CCR. The strain also displays properties of ecological significance such as fusaric acid resistance, heavy metal resistance, siderophore, pyoverdine production, alginate biosynthesis and production of plant growth promoting hormone like indole acetic acid. Additionally, this strain is plasmid free, lacks virulence factors, degradation properties are stably encoded by the chromosome, has a robust central carbon metabolism and a well characterized genome. These properties make strain CSV86^T an ideal host for the bioremediation of aromatic contaminated sites and a suitable host for metabolic engineering.

022

Exploring *P. aeruginosa* Invasion and Breaching of the Human Lung Epithelial Barrier

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Abstract

Pseudomonas aeruginosa is a leading cause of severe hospital-acquired pneumonia. Using human stem cell-derived lung epithelia, our lab discovered that *P. aeruginosa* spreads across the apical tissue surface and invades the lung tissue layer using its Type-6 secretion systems (T6SS) to preferentially target mucus-secreting goblet cells. Pathogen invasion is followed by host cell killing mediated through the Type-3 secretion system (T3SS) and by tissue repair process leading to the expulsion of infected cells. Continuous infection eventually results in the formation of larger breaching sites, which serve as gateways to cross the barrier tissue and reach the unprotected basolateral side. The precise mechanisms leading to the formation of breaching sites and their link to cell invasion and tissue repair has remained unclear.

To elucidate the cellular and molecular processes governing *P. aeruginosa* invasion and goblet cell killing, and to monitor the dynamic infection events with high resolution, we have developed live fluorescence microscopy of infected lung tissues. These studies are geared towards understanding the role of specific T6SS and T3SS effectors in invasion, vacuolar escape and host cell killing. High-resolution live imaging of *P. aeruginosa* tissue infection will shed light on the temporal sequence of events, intracellular localization of the pathogen and its behavioral strategies, as well as specific virulence factors enabling tissue invasion and barrier breaching. These studies will lead to a better understanding of *P. aeruginosa* lung infections and provide the experimental basis to probe the role of immunity and antibiotic treatment in infection control.

023

Widespread fungal-bacterial competition for magnesium enhances antibiotic resistance

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Abstract

Fungi and bacteria coexist in many polymicrobial communities, yet the molecular basis of their interactions remains poorly understood. Here, we show that the fungus *Candida albicans* sequesters essential magnesium ions from the bacterium *Pseudomonas aeruginosa*. To counteract fungal Mg^{2+} sequestration, *P. aeruginosa* expresses the Mg^{2+} transporter MgtA when Mg^{2+} levels are low. Thus, loss of MgtA specifically impairs *P. aeruginosa* in co-culture with *C. albicans*, but fitness can be restored by supplementing Mg^{2+}. Using a panel of fungi and bacteria, we show that Mg^{2+} sequestration is a general mechanism of fungal antagonism against gram-negative bacteria. Mg^{2+} limitation enhances bacterial resistance to polymyxin antibiotics like colistin, which target gram-negative bacterial membranes. Indeed, experimental evolution reveals that, in co-culture with fungi, *P. aeruginosa* evolves non-canonical *C. albicans*-dependent colistin resistance; antifungal treatment renders resistant bacteria colistin-sensitive. Our work suggests that fungal-bacterial competition could profoundly impact polymicrobial infection treatment with antibiotics of last resort.

024

New insights into the regulation of the virulence factor PlcH in Pseudomonas aeruginosa

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Abstract

PlcH is an important *Pseudomonas aeruginosa* virulence factor. PlcH is a secreted hemolytic phospholipase C/sphingomyelinase that can potently stimulate inflammation, suppress neutrophil respiratory burst, damage lung surfactant, and lead to direct cell damage and lysis. PlcH is known to be regulated transcriptionally by the activators GbdR, sensing glycine betaine, PhoB, sensing phosphate starvation, and SphR, sensing sphingosine, as well as being repressed by Anr and the HNS-family proteins MvaT and MvaU. Functional secretion was previously known to require PlcR, the TAT secretion system, and the Xcp/Gsp Type II secretion system. Here we present the results of our screen of the PA14 transposon mutant library for alterations in glycine betaine-dependent induction of PlcH by measuring resultant enzyme activity using the colorimetric substrate nitrophenylphosphorylcholine (NPPC). In addition to confirming the known players in PlcH expression, we identified new players in regulation of *plcH* transcription, including *clpA*, *rho*, *dksA2*, and *PA3489*, as well as novel contributors to PlcH post-transcriptional regulation, including *lepA*, *wapH*, and *PA5078*. In addition to these genes conserved in all *P. aeruginosa* strains, we also noted a surprising number of transposon insertions into genes in the PA14 pathogenicity islands, some of which lead to decreased PlcH production while some enhance PlcH production. Alternate gene carriage or different alleles/mutations in these and other components might explain the very wide range of PlcH expression in clinical isolates. We will present the complete findings from the screen and the follow-up data showing the stage at which expression is impacted.

025

The role of NahK in the HptB/RsmA Multi-Kinase Network

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is a biofilm-forming, opportunistic pathogen that is commonly associated with illnesses affecting immunocompromised individuals, such as lung infections of cystic fibrosis patients. In P. aeruginosa, the hybrid-histidine NahK (PA1976; PA14_38970) has been shown to regulate the production of the redox-active phenazine molecule, pyocyanin, through the phz2 biosynthesis operon in an RsmA dependent manner. NahK is one of many kinases involved in a much larger multi-kinase network (MKN) we refer to as the HptB/RsmA MKN. In order to determine the role of NahK in the HptB/RsmA MKN, genomic deletions for each individual signal transduction protein in the HptB/RsmA MKN were constructed/obtained and were subjected to various phenotypic screens (flow cell biofilm formation, EPS visualization with Congo Red staining, swarming motility, and pyocyanin quantification), and various other biochemical techniques (RNA-seq, quantification of intracellular c-di-GMP, and LacZ promoter activity assays). Our data suggests that NahK is the primary histidine kinase responsible for phosphorylation of HptB, leading to a predominant regulatory control over RsmA activity. RNA-sequencing validate this as both *nahK* and *rsmA* deletion strains have strong overlap in their transcriptional regulon. However, disruption of the known connection between the HptB and RsmA signaling systems, the HsbR/A/D system, in both *nahK* and *hptB* deletion strains, does not completely abolish the RsmA dependent phenotypes suggesting the existence of an additional signaling network downstream of HptB that could connect the HptB and RsmA signaling systems.

026

ExoS Effector in *Pseudomonas aeruginosa* Hyperactive Type III Secretion System Mutant Promotes Enhanced Plasma Membrane Rupture in Neutrophils

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen responsible for many airway infections that cause morbidity and mortality in immunocompromised patients. One important *P. aeruginosa* virulence factor is a type III secretion system (T3SS) that translocates effectors into host cells. ExoS is a T3SS effector with ADP ribosyltransferase (ADPRT) activity. The ADPRT activity of ExoS promotes *P. aeruginosa* virulence by inhibiting phagocytosis and limiting the oxidative burst in neutrophils. The *P. aeruginosa* T3SS also translocates flagellin, which can activate the NLRC4 inflammasome. However, recent studies with the *P. aeruginosa* laboratory strain PAO1 indicate that ExoS inhibits activation of NLRC4 in neutrophils. An ExoS^{+} CF clinical isolate of *P. aeruginosa* with a hyperactive T3SS was identified. Variants of the hyperactive T3SS mutant or PAO1 were used to infect neutrophils from C57BL/6 mice or mice engineered to have a CF genotype or a defect in inflammasome assembly. Responses to NLRC4 inflammasome assembly or ExoS ADPRT activity were assayed, results of which were found to be similar for C57BL/6 or CF neutrophils. The hyperactive T3SS mutant had enhanced resistance to neutrophil killing, like previously identified hypervirulent *P. aeruginosa* isolates. ExoS ADPRT activity in the hyperactive T3SS mutant regulated inflammasome and nuclear DNA decondensation responses like PAO1 but promoted enhanced CitH3 and plasma membrane rupture (PMR). Glycine supplementation inhibited PMR caused by the hyperactive T3SS mutant, suggesting ninjurin-1 is required for this process. These results identify enhanced inflammasome-independent PMR in neutrophils as a pathogenic activity of ExoS ADPRT in a hypervirulent *P. aeruginosa* isolate.

027

Examining ligand binding and activation mechanism of the sphingosine-binding transcription regulator SphR

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Abstract

The ability of *Pseudomonas aeruginosa* to regulate gene expression supports its success in a variety of environments and during infection, where it detects a large repertoire of host-derived compounds to tailor its survival and virulence. Sphingosine is a host-derived sphingolipid with antimicrobial properties that is enriched at epithelial surfaces. *P. aeruginosa* detects sphingosine via its direct binding to the AraC/XylS-family transcription regulator, SphR. Activation of SphR by sphingosine leads to induction of a small set of transcripts including those for sphingosine detoxification, the neutral ceramidase, *cerN*, and the hemolytic phospholipase C/sphingomyelinase, *plcH*. Given its contribution to *P. aeruginosa* interaction with sphingosine and expression of PlcH, an important virulence factor, we were interested in how SphR binds sphingosine and how sphingosine binding leads to transcriptional activation. Structural predictions of SphR and docking of sphingosine into this structure highlighted a potential hydrophobic pocket located between the N- and C-terminal domains. This prediction is supported by radiolabeled sphingosine binding to purified SphR, where full-length SphR binds sphingosine, but neither the N-terminal nor C-terminal domains are sufficient for sphingosine binding. Our screening of sphingosine analogs and those of Okino & Ito (2016) show that while SphR can also detect sphinganine and phytosphingosine, other close analogs do not lead to SphR activation of transcription. In contrast, one sphingosine analog, 1-deoxysphingosine appears to function as a competitive inhibitor of sphingosine binding to SphR. We are currently testing site directed mutants of SphR for their ability to bind sphingosine and sphingosine analogs and drive transcriptional activation.

029

Phylogenetic Analysis and Characterization of *Pseudomonas* Isolates from Refrigerated Meat

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Abstract

The genus *Pseudomonas*, comprising 250 species, showcases metabolic diversity, facilitating colonization across diverse ecological niches. *Pseudomonas* species such as *P. fragi, P. lundensis*, and *P. fluorescens* thrive as the predominant microbiota on refrigerated meat due to their competitive growth behavior. Our study delved into the analysis of complete *rpo*D gene sequences, revealing a distinct branch within the *P. fragi* and *P. bubulae* group. This study aimed to characterize isolates phylogenetically and biochemically to understand the diversity of prevalent pseudomonads on refrigerated meats. Seven *Pseudomonas* isolates were independently isolated from raw refrigerated meat, displaying unique fingerprinting patterns compared to reference type strains: *P. bubulae* DSM 107390, *P. psychrophila* DSM 17535T and *P. fragi* DSM 3456T. The samples YK24, YB92, YB55, YB144, and YK16 were the closest relative of 96.99-97.99% to *P. fragi* type strain, YK56 and YK50, followed by *P. psychrophila* with 95.81-96.99% similarities. Phenotypic and chemotaxonomic characterizations, including oxidase and catalase tests, and growth under varying conditions of NaCl concentration (0–8%), pH (4-10), and temperature (4-42°C), API 20NE and API 50CH tests were conducted. All isolates exhibited positive oxidase and catalase reactions and growth characteristics resembling reference strains in temperature and NaCl tolerance. However, differences in metabolic behavior, particularly in carbohydrate assimilation, were observed. Whole-genome sequencing was conducted to clarify the taxonomic position of the novel strains. Future analyses will include fatty acid and polar lipid profiling and examination via transmission electron microscopy. Our findings contribute to the taxonomic status of *Pseudomonas* species to understand their role as spoilage microorganisms.

031

Random mutagenesis revealed a novel gene involved in pyocin S3 uptake

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Abstract

Pseudomonas aeruginosa is a life-threatening antimicrobial resistance pathogen often associated with hospital-acquired infections. Treating *P. aeruginosa* infection has been challenging particularly with the limitations of the current antibiotics and its ability to develop further resistance which leads to an urgent need for novel antibiotics or therapeutic alternatives. This study set out to investigate pyocin S3 (PyoS3) to unravel novel treatments for *Pseudomonas* infection. PyoS3 is a potent protein produced by *P. aeruginosa* that kills closely related sensitive *P. aeruginosa* strains, however, the mechanism is poorly demonstrated. To expand the understanding of the mechanism of PyoS3 activity and uptake, a random transposon mutagenesis library was generated from the sensitive clinical strain PSA892 and examined for its response to the purified PyoS3 to identify resistant mutants with a defective gene involved in PyoS3 uptake. Over seven thousand mutants were screened for their sensitivity to PyoS3, and variable responses were observed. Mutants were generated that were either no longer killed by or showed increased sensitivity to PyoS3. DNA sequencing revealed that one of them had a transposon inserted into the *pvdE* gene which is involved in pyoverdine production. A defined mutant deficient in *pvdE* confirmed its significant role in *P. aeruginosa's* resistance to PyoS3. A complemented mutant of *pvdE* is being generated to restore sensitivity to pyocin S3 and validate the role of *pvdE* in the pyocin S3 uptake. Identifying potential genes involved in importing PyoS3 is indeed a critical step toward understanding the killing mechanism and consequently developing it as a novel therapeutic.

032

Pseudomonas syringae lytic transglycosylase HrpH interacts with the ubiquitin ligase ATL2 to modulate plant immunity

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Abstract

The bacterial plant pathogen *Pseudomonas syringae* deploys a type III secretion system (T3SS) to deliver effector proteins into plant cells to facilitate infection. But little is known about the direct interactions between T3SS components and plants. Here, we show that the specialized lytic transglycosylase (SLT) domain of *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) T3SS component HrpH is necessary for translocation. Purified HrpH or the SLT domain induces host cell death and suppresses pattern-triggered immunity. *Arabidopsis* plants bearing an *hrpH* transgene exhibit decreased PTI responses to flg22 and elf18, and enhanced susceptibility to *Pst* DC3000*ΔhrcQ-U*. Transcriptome analysis reveals that HrpH protein suppresses salicylic acid (SA) signaling. Yeast two-hybrid and bimolecular fluorescence complementation assays reveal that HrpH interacts with a putative E3 ubiquitin ligase ATL2 via its SLT domain on the plant membrane and in the nucleus, and that SLT binding activity is independent of its catalytic site. *ATL2* silencing implies that *ATL2* is essential for HrpH-triggered cell death, and suppression of MAPK and SA signaling. Our work highlights that in addition to acting as a lytic transglycosylase for effector delivery, the HrpH protein targets an E3 ubiquitin ligase to manipulate plant immunity.

033

Pseudomonas prophages regulate interactions among cystic fibrosis bacterial isolates through self-induction

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Abstract

Cystic fibrosis (CF) patients typically suffer from chronic lung infections caused by a diverse pathogen community. *Pseudomonas aeruginosa* is one of the main pathogens associated with chronic infections and deterioration of lung functions. While virulence and within-host evolution have been extensively studied for this pathogen, the role of prophages in shaping bacterial communities and CF disease progression has received little attention. Here, we work with 20 clinical *P. aeruginosa* isolates from CF children (aged 0-8 years) to explore whether prophages are present and to what extent they regulate interactions among *P. aeruginosa* isolates. We first sequenced the genomes of our isolates and found prophages to be present. Next, we used the dilution drop test to verify that phages are released into the bacterial supernatant and to distinguish prophage from toxin-induced inhibition. We found that many prophages were produced by self-induction. We isolated 37 prophage strains using double-layer agar plates and conducted a host-phage interaction network. We observed that prophages from *P. aeruginosa* isolates of older patients (later-stage) were able to infect *P. aeruginosa* isolates from younger patients (early-stage) but not vice versa. Finally, competition assays of fluorescent reporter strains showed that later-stage bacterial isolates defeated early-stage bacterial isolates in competition by releasing prophages. To sum up, we found that prophages play an important role in mediating competition between *P. aeruginosa* CF isolates during disease progression, highlighting the possibility that prophages influence both bacterial pathogen evolution and the transitions from acute to chronic infections.

037

Isolation and Identification of Antibiotic-Producing Bacteria in New Jersey Soil Samples

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Abstract

Antimicrobial resistance is a growing concern that poses a threat to food security, societal development, and global health. A new generation of effective antibiotics is urgently needed to combat the emergence of novel and stronger pathogenic microbe variants. The Tiny Earth project is an attempt to contribute to this initiative through the collection, isolation, and identification of antibiotic-producing bacteria from different soil samples. The soil samples were obtained from Somerset County, New Jersey, and a serial dilution method was used to plate and isolate distinct bacteria specimens. From the obtained sample, bacteria #13 displayed antibiotic properties against *E. Coli*, *Staphylococcus epidermidis*, and others through the observed halo formed after patch plating. After an array of metabolic tests such as MR/VP, the bacteria of interest was identified to be a gram-negative facultative anaerobe that can ferment both glucose and lactose and produce H_2S. Further identification was facilitated with the use of a dichotomous key and PCR sequencing. Based on a dichotomous key, the antibiotic-producing bacteria of interest was identified as Citrobacter. However, the PCR testing and BLAST database evidenced that the unknown might be a variation of Pseudomonas.

Further research would focus on the extraction of chemicals from bacteria with possible antibiotic activity. The program would also involve the introduction of novel antibiotics to the market to alleviate a dwindling effective antibiotic supply.

038

Involvement of the three component system CfcA/CfcF/CfcR in the synthesis of c-di-GMP by *Pseudomonas stutzeri* MJL19: Functional analysis and role on sociomicrobiology.

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he

Abstract

Two-component systems (TCS) typically consist of a membrane-bound histidine kinase (HK) that senses a specific environmental stimulus and a corresponding response regulator. CfcA/CfcR is a fundamental TCS in the regulation mediated by the second messenger c-di-GMP in *Pseudomonas putida* (*P.p.*) being a central node in a complex regulatory network connecting salts with biofilm formation. *Pseudomonas stutzeri* MJL19 (*Stuzerimonas stutzeri* (*S.s.*) now) is a rhizosphere-colonizing bacterium with plant-growth promoting activity under highly saline conditions. The CfcR$^{S.s}$ homolog shares 54% identical residues with its *P. putida* counterpart and presents GGDEF and EAL domains with diguanylate cyclase (DGC) and phosphodiesterase (PDE) activities, respectively. In MJL19, two proteins show similarities with the HK CfcA, one being 60% identical and containing an extracellular CHASE3 sensor domain, and the other with lower identity and a CHASE sensor domain. This latter HK named CfcF is encoded by the gene immediately upstream of *cfcR*$^{S.s}$. CfcF is absent from *P. putida*, leading to the question of comparing the molecular functioning of CfcA/CfcR in both bacteria. We have generated null derivatives in the genes *cfcA*$^{S.s.}$, *cfcF*$^{S.s.}$ and *cfcR*$^{S.s.}$ individually, and in double and triple combinations and observed differences in the distribution of c-di-GMP in the bacterial colonies of these mutants. They were also analyzed for colony morphology, biofilm formation on biotic surface and exopolysaccharides production on Congo red and calcofluor. Our studies reveal that CfcR$^{S.s}$ exhibits a prominent PDE activity, whereas CfcR$^{P.p.}$ presents DGC, and negative regulation by CfcF. The implication of CfcA$^{S.s}$ in this system is still under investigation.

040

Role of R5 Pyocin in Predominance of High-Risk Sequence Types of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a Gram-negative, opportunistic, human pathogen that has developed multi- or even pan-drug resistance against most frontline antibiotics. *P. aeruginosa* infections are associated with certain sequence types (STs), such as ST235, ST111, ST175, that dominate clinical settings. Previous research from several labs, including ours, identified significant enrichment of ST111 amongst strains isolated from hematologic malignancy patients and hematopoietic cell transplant recipients at OHSU.

Although ST111 is recognized as one of the most prevalent high-risk clones worldwide and frequently exhibits drug resistance, the basis for its clinical dominance has been unclear. In this study, we demonstrate that ST111 strains outcompete multiple non-ST111 strains by releasing a rapidly-acting, high-molecular-weight bactericidal factor. Using a genome-wide screen of a transposon insertion library, we identified this factor as R pyocin. Deletion of pyocin from ST111 strain abrogated its bactericidal abilities.

Specifically, ST111 strains encoded an R5 pyocin that showed broad killing activity against multidrug-resistant strains of *P. aeruginosa* (56/68 strains were inhibited, compared to 48/68 for R1, and 30/68 for R2/3/4 pyocins). Moreover, deletion of a specific enzyme from the *Pseudomonas* LPS biosynthetic pathway rendered additional strains sensitive, specifically to R5 pyocins.

An analysis of 5,135 typed *P. aeruginosa* strains revealed that top international, high-risk sequence types are enriched for R5 pyocin production, despite distinct phylogenetic lineages. Our results indicate a mechanism for the dominance of ST111 strains, show correlation between R5 pyocin production and clinical dominance, and suggest a novel approach for evaluating risk from emerging prevalent *P. aeruginosa* strains.

041

Deciphering the tRNA Epitranscriptome: Implications for *P. aeruginosa* Pathogenicity

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Abstract

tRNA anticodon modifications display a great chemical diversity and tremendously affect translation accuracy and protein homeostasis. The modification landscape – collectively known as the tRNA epitranscriptome – was demonstrated to influence bacterial virulence phenotypes on a global scale. However, the exploration of the tRNA epitranscriptomic landscape in *P. aeruginosa* remains incomplete, yet its comprehensive understanding promises to unravel novel molecular mechanisms shaping bacterial behaviour and adaptation to host niches.

The combination of LC-MS techniques with nanopore-based tRNA sequencing represents a powerful approach for systematically investigating the tRNA epitranscriptome. Furthermore, leveraging functional genomics through multi-omics approaches and phenotypic screening of genetically engineered mutant strains further expands our understanding of how the tRNA epitranscriptome collectively modulates bacterial pathogenicity.

By employing targeted LC-MS analysis and nano-tRNAseq, we were able to verify specific modification profiles on single nucleotide resolution in individual tRNAs. Moreover, we screened mutant strains lacking important tRNA modification systems for relevant phenotypes and observed global rewiring of metabolic pathways and severe reduction of virulence in the absence of these modifications. Additionally, by applying functional proteomics and transcriptomics analyses, we unraveled the molecular basis for tRNA hypomodification-induced pleiotropy.

In summary, our approach represents an unprecedented paradigm for detecting tRNA modifications. New insights into bacterial tRNA modification systems hold the potential for the development of pathoblockers, novel therapeutic strategies specifically targeting bacterial virulence mechanisms.

042

MEMBRANE VESICLES PROTECT PSEUDOMONAS AERUGINOSA AGAINST HOST DEFENCE PEPTIDES

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Abstract

Background: With the increasing bacterial resistance towards antibiotics, new innovative and effective treatment strategies are required to combat infections. Antimicrobial host defence peptides (HDPs) are regarded as promising alternatives to antibiotics as they kill a broad spectrum of microbes while exerting immunomodulatory effects. However, the antimicrobial activity of many HDPs is compromised under physiological conditions. As bacteria release membrane vesicles (MVs) for protection against antibiotics, we hypothesized that MVs may bind and incapacitate HDPs as well.

Methods: MVs were isolated from 24 h cultures by ultracentrifugation and characterized by dynamic light scattering, nanoparticle tracking analysis, cryo-electron microscopy, and SDS-PAGE. The effect of MVs on HDP function was investigated using *in vitro* killing assays and cell culture studies.

Results: *P. aeruginosa* naturally released negatively charged MVs ranging from 35-50 nm in size. We determined the effect of MVs on the activity of three HDPs and indeed found MVs to dose-dependently reduce the antibacterial effects of these peptides. MVs induced NF-κB/AP-1 release in monocytes as expected, whereas the addition of HDPs resulted in a peptide- and dose-dependent suppression of cell activation.

Conclusion: Taken together, our results reveal a previously undisclosed mechanism used by *P. aeruginosa* to modulate and circumvent host responses.

043

Metabolic engineering of *Pseudomonas taiwanensis* VLB120 as chassis for the production of chorismate-derived bulk and fine chemicals

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Abstract

Increasing depletion of fossil resources requires a shift of industry towards a more sustainable production. This led to a focus on bio-based production of both bulk and fine chemicals.

Due to the robustness of *Pseudomonas taiwanensis* VBL120 as well as its highly versatile metabolism, this bacterium displays a suitable candidate for the production of a broad spectrum of compounds, especially aromatics. Chorismate is a key precursor for many aromatics, and we therefore modified *P. taiwanensis* to increase chorismate availability in the cell. This was achieved by targeting the bifunctional enzyme *pheA*, which catalyzes the first two steps from chorismate to the aromatic amino acids phenylalanine and tyrosine. Overall, metabolic engineering approaches could be applied to increase productivity up to 20.3±0.1% (Cmol/Cmol) from glucose and up to 25.4%±2.1 (Cmol/Cmol) from glycerol, depending on the respective aromatic compound.

To demonstrate its applicability, production of different chorismate-derived hydroxybenzoates was shown. These compounds can serve either as plastic building blocks, food additives, or as precursor for more complex secondary metabolites. In the latter context, we enabled the conversion of 2,3-dihydroxybenzoate (DHB) into myxochelin. In this metal-chelating siderophore, two DHB molecules are coupled via a lysine molecule. Like many other non-ribosomal peptides, native myxochelin biosynthesis results in low titers with hosts that are difficult to handle. Transfer of its synthesis to *P. taiwanensis* enables more efficient metabolic engineering, while also opening up process engineering options like supplementation of different carbon sources or addition of a second phase extractant.

044

Pseudomonas Quinolone Signal (PQS) induces single-cell to aggregate transition in a co-infecting pathogen

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Abstract

Microorganisms commonly exist in multispecies communities where their fitness and behavior are altered by interspecies interactions. *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* are both opportunistic human pathogens that are frequently found together in polymicrobial environments including the soil, as well as chronic human wound and lung infections, but interactions between these species are understudied. Here, we show that *P. aeruginosa* secreted factors cause a lifestyle transition in *S. maltophilia* by inducing cellular aggregation. Experimental evolution of two independent *S. maltophilia* populations by repeated cycles of exposure to *P. aeruginosa* cell-free supernatant and selection of planktonic cells, resulted in non-aggregating populations. These populations had mutations in the *smf-1* gene encoding a fimbrial subunit. Additionally, exposure of *S. maltophilia* to *P. aeruginosa* supernatant led to increased expression of the *smf-1* operon, including a putative adhesin SMLT_RS03370. Clean deletion mutants showed that both Smf-1 and SMLT_RS03370 are required for *P. aeruginosa*-induced aggregation, and that Smf-1 mutants are unable to aggregate with wild-type cells. A high-throughput microscopy screen of an ordered *P. aeruginosa* transposon mutant library, in conjunction with exogenous supplementation, showed that the *Pseudomonas* Quinolone Signal (PQS) directly induced *S. maltophilia* aggregation, and PQS localized to *S. maltophilia* aggregates in an Smf-1 and SMLT_RS03370 dependent manner. Finally, we found that *S. maltophilia* aggregation is protective, and increases its survival in co-culture with *P. aeruginosa*. Thus, this study identifies a novel interspecies interaction between two co-infecting pathogens, delineates underlying genetic determinants in each species, and demonstrates the fitness consequences of this interaction.

045

LasR controls the thermoregulation of protease IV in *Pseudomonas aeruginosa*

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Abstract

Temperature regulates the expression of many virulence factors in *Pseudomonas aeruginosa*, which is thought to enable it to adapt to various environments, including a mammalian host. Expression of the gene encoding the virulence factor protease IV (PIV) is upregulated at ambient temperatures (22-28°C) compared to human body temperature (37°C), but how it was controlled by temperature was unknown. To better characterize *piv* thermoregulation, we grew *P. aeruginosa* PAO1 at 25°C, 30°C, 37°C, and 42°C, extracted RNA at exponential and stationary phases, and measured *piv* expression by RT-qPCR. We found that *piv* was only thermoregulated at stationary phase, and its expression was significantly higher at 25°C and 30°C and lower at 42°C compared to 37°C. Using a transcriptional reporter for *piv*, we found that *piv* promoter activity is thermoregulated. By assessing reporter activity in mutants of known regulators of *piv*—the quorum sensing regulator LasR and negative regulators MvaT/MvaU—we demonstrated that LasR activates *piv* expression at stationary phase at 25°C but not 37°C, while MvaT/MvaU are not required for *piv* thermoregulation. We identified a novel *las* box in the *piv* promoter, which is important for driving high *piv* promoter activity at stationary phase at 25°C. We propose that at stationary phase, LasR directly upregulates *piv* highly at 25°C but not at 37°C. This is the first gene identified to be regulated by LasR in a temperature-dependent manner and suggests that quorum sensing may function differently in the diverse environments that *P. aeruginosa* can encounter.

046

Identifying the swarm-essential genes in *Pseudomonas aeruginosa* PA14 through extensive screening of mutants in four different swarm-media

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Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that harbours several unique traits to support its survival in complex ecosystems. Swarming motility is one such trait driven by flagella, pili and a major biosurfactant, rhamnolipid. It is a quorum-dependent coordinated movement of bacterial cells over a semi-solid surface that likely mimics the mucous layer found on host epithelial surfaces. The current study aims to identify the swarm-essential genes of *P. aeruginosa* PA14 by thorough screening of 5800 T7-transposon insertion mutants. Initially, the mutants we screened in two well-defined swarm-supportive nutrient formulations, minimal medium (M9) and peptone growth medium (PGM), solidified with 0.6% agar. Out of the 5800 transposon mutants, 181 were identified to be non-swarmers in both M9 and PGM. These non-swarmers were further screened with slightly different nutrient formulations (M8 and BM2 minimal media). Finally, 107 mutants could not swarm in all four tested media. This indicates at least 107 out of 4596 predicted genes in *P. aeruginosa* PA14 to be the core regulators of swarming motility. This list includes the genes involved in bacterial motility (flagella-, pili- and chemotaxis-related); transmembrane transportation of major nutrients; biosynthesis of amino acids, fatty acids, vitamins, cell wall, siderophores and nucleic acids; carbon and nitrogen metabolism; virulence and pathogenicity apart from the transcriptional and post-transcriptional regulators. Further studies are required to analyse if these genes can impair all kinds of motilities in *P. aeruginosa*.

050

Characterizing LPS distribution on Pseudomonas aeruginosa at the single cell level

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Abstract

Pseudomonas aeruginosa is a Gram-negative bacterium and an opportunistic human pathogen, posing a wide range of life-threatening infections on immunodeficient patients. The increasing challenge in treating the infections of P. aeruginosa can be attributed to its identified capacity to resist multiple antibiotics. Colistin, a polymyxin antibiotic serves as the last-resort therapeutic antibiotics against P. aeruginosa infections, targets lipopolysaccharides (LPS) on the outer leaflet of bacterial outer membrane. The negatively charged lipid A component of LPS interacts with the positively charged colistin, resulting in the destruction of membrane permeability. The acting efficacy of colistin can be sabotaged by LPS molecular modification. A comprehensive understanding of LPS on bacterial surfaces can provide insight to develop new antimicrobial strategies against infections. Stochastic optical reconstruction microscopy (STORM) is a super-resolution microscopy, breaking the resolution limitation of optical microscopy by stochastic localising and mathematical reconstructing individual fluorophores. The aim was to investigate LPS dynamics on P. aeruginosa at the single cell level using STORM. P. aeruginosa was subjected to stain with fluorophore-conjugated polymyxin in compared to stain with a lipophilic fluorescent dye. Results by from STORM and statistical analysis, indicated that the intensity of lipophilic stained cellular membrane was uniformly distributed, suggesting that it is an integral component of the entire cell membrane. LPS strained by fluorescent-polymyxin exhibited an intermittent distribution pattern, which implies an unequal distribution of LPS across the cell membrane. The current observation lay the groundwork for future investigations in the LPS distribution in potential contribution to colistin resistance.

051

The H2 type VI secretion system of *P. aeruginosa* in interaction with the host and other bacteria

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Abstract

Pseudomonas aeruginosa has three different types of type VI secretion systems (referred to as H1-T6SS, H2-T6SS and H3-T6SS), each with a specific set of effectors. The H2-T6SS stands out by harboring a diverse array of effectors that target eukaryotic and prokaryotic cells and facilitate nutrient uptake. Some of these effectors are known to contribute to the virulence of *P. aeruginosa* in infection experiments *in vivo*. The mechanism underlying this phenotype is not yet fully understood. Here, we focus on the H2-T6SS and one of its effectors, which acts on eukaryotic and prokaryotic cells alike, to test their activity inside and outside of a host. We found that the H2-T6SS is inactive at body temperature outside of a host but active at human body temperature in infection experiments with *Galleria mellonella* insects. In western blot and gene expression analysis, we observed downregulation of H2-T6SS genes and reduced expression levels of Hcp2 (structural protein of the H2-T6SS apparatus) when bacteria were grown at 37°C outside of a host. *In vivo* at 37°C, the H2-T6SS mediated an advantage during defined times of the infection trajectory in *G. mellonella*. Our data suggests that the H2-T6SS responds to environmental cues and is activated during infection. These findings advance our mechanistic understanding of host-microbe interactions with relevance for patients infected with *P. aeruginosa*.

053

Heterogenous *recA* induction in *Pseudomonas aeruginosa* biofilms

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Abstract

Pseudomonas aeruginosa form biofilms which consist of cells and extracellular matrices. The heterogenous environment results in given rise to phenotypic variants in biofilms. Such variants are also associated with antibiotic-resistance. *recA* which is known as a recombinase and a regulator of the double-strand DNA repair system (SOS response) is indicated to be involved in the emergence of antibiotic resistant cells. However, there have been few studies on the spatiotemporal analysis of *recA* expression in biofilms and its relationship with the emergence of variants.

In this research, we introduced a *recA* promoter-reporter plasmid into *P. aeruginosa* PAO1 to detect the expression of *recA* in biofilms. The ratio of cells inducing *recA* in biofilms was approximately 8-fold higher than in planktonic conditions. We then separated the *recA*-expressing cells, using a flow cytometry.

To characterize the *recA*-expressing cells we measured their antibiotic resistance and compared it with *recA*-low expressing cells. The results showed that *recA*-expressing cells are more persistent to ciprofloxacin than low-expressing cells.

This study may provide new insights into the regulatory pathways of antibiotic resistance in *P. aeruginosa* biofilm. We would also discuss the relationship between *recA* induction and oxidative stress in biofilms. In future work, we will continue to uncover the detailed mechanisms of *recA* induction.

055

Anr-mediated heterogeneity of Pseudomonas aeruginosa in infection-like conditions

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Abstract

Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen that can infect wounds and the respiratory airways, and the leading cause of respiratory failure in cystic fibrosis (CF) patients. Phenotypic heterogeneity, the ability for individual cells to have unique gene expression profiles, was shown to be important for infection in many pathogens, and also occurs in *P. aeruginosa* in laboratory conditions. In this project, we developed a modular and customizable collection of >70 functional single cell transcriptional fluorescent reporters that allow us to learn which pathways contribute to *P. aeruginosa*'s phenotypic heterogeneity in infection-like conditions by flow cytometry and fluorescence microscopy. Based on the *norC* reporter that displays a heterogeneous behaviour in infection models, we characterized two different subpopulations by proteomics, that have different activity levels of the Anr transcriptional regulator. This was used to genetically steer the population towards one or the other subpopulation. By characterizing the role of these subpopulations in the infection process, we assessed whether *P. aeruginosa* phenotypic heterogeneity plays a role in infection progression. In the future, this knowledge could be used to devise new antimicrobial strategies that target specific subpopulations of *P. aeruginosa*, contributing to the eradication of this pathogen in chronically-infected CF patients.

056

Targeting the moonlighting activity of PQS Response Protein PqsE with small molecule inhibitors

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Abstract

The gram-negative pathogen *Pseudomonas aeruginosa* stands out as one of the most well-known multidrug-resistant bacteria. Therefore urgent explorations for alternatives to traditional antibiotics in its treatment are needed. Pathoblockers offer a promising alternative to conventional antibiotics.

The interaction between PqsExRhlR presents an attractive target for the development of pathoblockers. The transcription factor RhlR plays a leading role in the progression of late and chronic infections. However, numerous RhlR-dependent virulence traits require the presence of PqsE, a dispensable thioesterase. Notably, the absence of PqsE halts the production of the prominent virulence factor, pyocyanin. Recently, we have shown that PqsE stabilizes RhlR by forming a 2:2 complex.

This unique moonlighting activity of PqsE provides a new target for development of *P. aeruginosa*-specific pharmaceuticals. To this end, we have developed a FRET-based high-throughput screening system that detects the PqsExRhlR interaction with high reliability. With this assay in hand, over 30000 compounds were screened for inhibition of the interaction, leading to the identification of 532 potential inhibitors. Subsequent dose-response experiments with the same assay confirmed 167 compounds. A counter screen based on competition microscale thermophoresis (MST) experiments led to the identification of 13 potent inhibitors, which interestingly exhibited binding to both PqsE and RhlR. These promising lead compounds were subsequently tested in vivo in *P. aeruginosa* and six demonstrated a significant reduction in the production of pyocyanin. The inhibitors offer a starting point for new anti-infectives that act as pathoblockers, which may ultimately aid to fight infections with multiresistant *P. aeruginosa*

057

Molecular probes to diagnose pathoadaptations in bacterial infections

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that causes severe respiratory tract infections and is one of the most important nosocomial pathogens in mechanically ventilated patients at intensive care units. *P. aeruginosa* also causes high mortality in cystic fibrosis (CF) patients during chronic bacterial infections. In these patients, it rapidly evolves and diversifies during a process called “pathoadaptation”, leading to phenotypic changes in protein expression, increased antibiotic resistance and both gain and loss of virulence traits. Reliable identification of pathoadaptations is crucial for the successful treatment of bacterial infections in the patient’s lungs, especially for pathoadapted isolates which are much harder to eradicate.

In this project, we focus on developing novel molecular probe-based strategy to identify *P. aeruginosa* pathotypes based on bacterial surface properties associated with virulence. We have demonstrated that using an array of fluorescent glycopolymers, we can reliably differentiate single knockout mutants and chronically and acutely evolved CF isolates from each other, demonstrating the high potential of this system. With the help of a new extended array, I will present data on validating a wider collection of knockout mutants and clinical isolates, which will be phenotypically characterized to correlate array discrimination with differences in virulence and other phenotypic traits. Overall, this project will obtain a new technology to discriminate bacterial pathotypes providing an efficient and quick diagnosis which will help develop effective bacterial treatments.

060

Mechanisms affecting fluoride tolerance in soil bacterium Pseudomonas putida

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Abstract

P. putida can tolerate high fluoride concentrations due to a fluoride ion transporter called CrcB. We observed that spontaneous NaF tolerant mutants arise from the *ΔcrcB* background of *P. putida* KT2440. This indicated that in addition to fluoride ion transporter CrcB, some other molecular mechanisms could also contribute to the fluoride tolerance of *P. putida*.

To reveal the factors affecting the fluoride tolerance in *P. putida*, the genomes of the four natural NaF-tolerant mutants that emerged in *the ΔcrcB* background were sequenced. We discovered that NaF tolerance was acquired by extensive genomic deletions (17 598 – 340 046 bp). In addition, transposon mutagenesis was carried out in the *ΔcrcB* strain to identify genes whose inactivation increases NaF tolerance. Accordingly, we found transposon insertions in a gene encoding Cro/CI type transcriptional regulator PP_3125. Further deleting this regulator enabled *the ΔcrcB* strain to grow at ten times higher NaF concentrations than *the ΔcrcB* strain. Importantly, the same gene was also included in all spontaneous NaF-tolerant mutant deletions.

Using a complete proteome analysis of *P. putida* strains, we observed that the genes PP_2037 and *PP_2036* were upregulated almost 2000 times in the ΔPP_3125 strain. Further studies are currently in progress to describe the roles of these genes in *P. putida* NaF tolerance.

061

The panC-encoded pantothenate synthetase to tackle carbapenem-resistant OprD Pseudomonas aeruginosa mutant.

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Abstract

Background: The loss of the OprD porin in *Pseudomonas aeruginosa* (PA) is the main determinant for carbapenem resistance and is known to enhance *in vivo* fitness and virulence. We identified *panC,* which encodes pantothenate synthetase, as essential in PA OprD mutant. We aimed to determine whether targeting *panC* could tackle PA virulence.

Methods: PA14 and PA14Δ*oprD* strains were transfected with CRISPRi spacers specific to *panC* (sg*panC*) or a control spacer (sgCTL). PanC silencing was assessed by colony counts and maximum growth rates upon 1% arabinose exposure. 16HBE, Calu3, and A549 cell lines were infected and monitored using time-lapse microscopy. Dead cell times (DCT50) were determined and compared using Mann-Whitney tests.

Results: Upon 1% arabinose exposure, we observed a weaker growth of PA14Δ*oprD* sg*panC* than PA14Δ*oprD* sgCTL (colony count: 55 *vs* 157 CFU/mL; p <0.01 and MGR : 3.6 *vs* 5.0 $.10^{-4}s^{-1}$; p <0.001). PA14Δ*oprD* sg*panC* killed all cell lines between 2 and 3 times less than PA14Δ*oprD* sgCTL. DCT50 was significantly extended for PA14Δ*oprD* sg*panC* for 16HBE (9.8h *vs* 15.7h; $p < 0.001$; n=9) and A549 (10.3h *vs* 11.5h; $p < 0.05$; n=3) cell lines. The same trend was observed for the Calu3 cell line (19.3h *vs* 29.6h; p=0.076; n=6).

Conclusion: Our results demonstrate that silencing *panC* extends the time of killing of various airway cells, highlighting the potential of *panC* inhibition to limit PA14Δ*oprD* virulence. We anticipate our results as a starting point to consider *panC* as a novel target for treating carbapenem-resistant OprD-defective PA lung infections.

063

Regulation of quorum sensing architecture in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa (Pa) lives in many different environments, where it can interact with other bacteria. To modulate bacterial interactions, *Pa* uses quorum sensing (QS), a cell-cell communication system that enables it to sense cell density and to alter gene expression. *Pa* has three complete QS circuits controlled by the transcriptional regulators LasR, RhlR, and PqsR (MvfR), that together control hundreds of genes, including virulence factors. In the laboratory strain PAO1, QS is organized hierarchically, with PqsR and RhlR activity dependent on LasR. In PAO1, this hierarchy is driven by the non-QS transcription factor MexT, but the mechanism is unknown.

We aimed to identify how regulators, including MexT, modulate QS architecture. We performed an RNAseq analysis on wild-type PAO1 and PAO1Δ*mexT* and identified 152 significant differentially expressed genes. MexT does not appear to regulate *rhlR* or *pqsR* directly. We identified four genes that MexT regulates that may have a role in maintenance of the hierarchy in PAO1 and are testing how these gene products might affect the regulation of QS transcription factors. We have also discovered that there are alternate QS architectures in clinical isolates, where RhlR and PqsR activity is not dependent on LasR. In these isolates, surprisingly, MexT does not necessarily impact the relationship between LasR and the other QS regulators. We are using an experimental evolution approach to identify regulators responsible for this QS architecture. Our work reveals a new suite of factors that regulate QS in *Pa*, with implications for a variety of *Pa* behaviors.

064

Insights into persistence and adaptation of *Pseudomonas aeruginosa* following initiation of highly effective modulator therapy in cystic fibrosis using RNA expression profiling

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Abstract

Pseudomonas aeruginosa is a predominant pathogen in people with cystic fibrosis (pwCF) causing chronic respiratory infections highly resistant to host defenses and antimicrobial treatments. CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) protein and affects mostly the lungs, leading to inflammation, the accumulation of mucus in the airways and a predisposition to pulmonary infections. The latest triple-combination modulator therapy targeting the CFTR protein, Elexakaftor/Tezacaftor/Ivacaftor (ETI), has significantly improved CF symptoms for about 90% of pwCF. Despite the many clinical benefits, studies have demonstrated that while *P. aeruginosa* sputum density decreases, chronic infection persists. To investigate how the ETI-modulated lung environment impacts *P. aeruginosa* physiology, we conducted longitudinal transcriptomic studies on *P. aeruginosa* in CF sputum samples from a cohort of 10 participants. Samples were collected before ETI initiation and at 2-days, 1-week, 1-year, and 2-years post-initiation. Interestingly, initial analyses revealed that there are minimal transcriptional changes in *P. aeruginosa* post-ETI. In addition, *P. aeruginosa* maintains a distinctive human-infection gene expression signature previously identified in the Whiteley lab using machine learning approaches. While *P. aeruginosa* chronic infections in pwCF may be just as difficult to manage in the future, current *P. aeruginosa* pre-clinical infection models are still suitable to perform studies on its physiology post-ETI. Ongoing investigations aim to examine transcriptional changes at later timepoints and define an ETI-specific *P. aeruginosa* transcriptional signature as *P. aeruginosa* adapts to the post-ETI CF lung environment.

066

Type VI Secretion in Pseudomonas aeruginosa promotes persistence and competition during viral co-infections

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Abstract

Respiratory viral co-infections are linked to pulmonary disease exacerbations and declining lung function in cystic fibrosis (CF). We previously found that respiratory viral co-infection alters *Pseudomonas aeruginosa (Pa)* pathogenicity by enhancing biofilm growth to promote persistence. To elucidate *Pa*'s response to viral co-infection, we conducted RNA-sequencing of *Pa* co-cultured on respiratory syncytial virus (RSV)-infected and uninfected CF airway epithelial cells (AECs). Expression of genes involved in the H2-type VI secretion system (H2-T6SS) and the associated TseT toxin locus of *Pa* were increased during RSV co-infection. T6SS has been implicated in CF respiratory disease previously, as *Pa* T6SS proteins and antibodies have been detected in CF patient sputum. We observed that *Pa* biofilms grown in association with AECs specifically express the TseT locus and structural proteins of the H2-T6SS. Deletion of this toxin locus resulted in significantly decreased biofilm on AECs. Attachment and biofilm formation on AECs was specifically mediated by the TseT-specific immunity protein TsiT, suggesting this immunity protein plays a dual role in *Pa* pathogenicity. In addition, we found the TseT locus regulates inter-bacterial competition with multiple Gram-negative pathogens, with competition further increased during viral co-infection. The TseT locus is expressed in CF sputum and is positively correlated with loss of microbial diversity in the CF respiratory tract (Shannon Index). Taken together, our data are consistent with the conclusion that the TseT locus and T6SS aid in *Pa* colonization of the respiratory tract and dominance over other pathogens in CF, and suggest a new therapeutic target for antimicrobial development.

069

Encapsulation of extracellular molecules in MVs through explosive cell lysis

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Abstract

Most bacteria can form membrane vesicles (MVs) which are formed through different pathways. *Pseudomonas aeruginosa* has been shown to form MVs through either blebbing of the outer membrane or explosive cell lysis (ECL). It has been suggested that the MV formation pathway impact the content of MVs. Given that MVs are formed from shattered membrane during explosive cell lysis, it is likely that extracellular molecules can be encapsulated in MVs during this process, which has not yet been examined in detail. In this study we examined whether extracellular molecules can be encapsulated in MVs.

Calcein was used as one of the cargo molecule, and was added to ECL-induced and non-induced strains, which fluorescence was measured in MVs. Melanin was used for transmission electron microscopy (TEM) analysis. Antibiotics were also used, which activity in MVs was examined by monitoring cell growth.

MVs given rise from explosive cell lysis encapsulated extracellular molecules from the medium while MVs formed without inducing explosive cell lysis did not. TEM observation confirmed the presence of melanin in MVs. Furthermore, growth inhibition of PAO1 was observed with antibiotics-encapsuled MVs.

These results demonstrate that extracellular molecules can be encapsulated in MVs through explosive cell lysis. This was not observed with MVs presumably form through blebbing, which results demonstrate that the MV formation pathway largely impact the content of MVs. This demonstrate that MVs can carry molecules that are not produced by the cell itself, depending on the formation pathway, and will also open new avenues for MV applications.

070

Two Lon proteases contribute to the regulation of motility and antibiotic tolerance in *Pseudomonas aeruginosa*

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Abstract

Proteases are important cellular machines which degrade specific proteins in response to external signals or cellular demand. Lon, a highly conserved ATP-dependent cytoplasmic protease, has been associated with multiple phenotypes in *P. aeruginosa*, including motility, virulence, antibiotic tolerance and biofilm formation. However, very few Lon substrates are known to date. Additionally, a Lon-like protein named AsrA has been implicated in aminoglycoside resistance, but remains largely uncharacterized with no known substrates. In this study, we used an MS-based quantitative proteomics approach to search for proteins with significantly reduced abundance and protein stability when either protease was overexpressed. *In vitro* degradation assays of these candidates revealed several motility-related proteins as novel substrates of Lon. Among these, FliA, RpoN and AmrZ are transcription factors for motility genes while FliG, FliS and FlgE are structural components of the flagellum. This indicates that Lon plays a role in regulating the levels of flagella- and pilus-associated proteins. We also purified and confirmed that AsrA is an ATP-dependent protease. Interestingly, it does not degrade any of the Lon substrates tested so far, indicating a separate substrate pool and distinct regulatory roles in the cell. In our proteomics experiments, we identified proteins including regulators of multidrug efflux pumps as potential AsrA substrates. Other candidates are involved in metabolism and transcriptional regulation, indicating new and previously unknown roles of AsrA. Our work demonstrates distinct roles of the two Lon proteases and sheds light on how cellular proteases are involved in regulating virulence, motility and antibiotic tolerance in bacteria.

071

AmgRS is implicated in the adaptative response of *Pseudomonas aeruginosa* to cinnamaldehyde

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Abstract

Background. Cinnamaldehyde (CNA) is a phytoaldehyde able to kill *Pseudomonas aeruginosa*. At subinhibitory doses, this electrophilic molecule triggers a complex stress response, which in part results in the overproduction of efflux system MexAB-OprM and transient multidrug resistance. Induction of the pump activity is dependent on ArmR, the anti-repressor of operon *mexAB-oprM*. However, recent transcriptomic analyses of strain PA14 exposed to CNA revealed that genes regulated by the two-component system (TCS) AmgRS are also overexpressed. Since AmgRS is known to increase the activity of MexAB-OprM and membrane-bound proteases following cell envelope alterations, we investigated its protective role in the survival of *P. aeruginosa* to CNA.

Methods. Relative expression of target genes was determined by RT-qPCR. Genes *amgRS* and/or *armR* were inactivated in strain PA14 by allelic exchange. Time-kill and MIC experiments were performed to assess the susceptibility of the mutants to CNA and antibiotics.

Results. As expected, induction of *mexAB-oprM* expression (6-fold) by CNA was abolished in mutant PA14Δ*armR,* consistent with the loss of transient antibiotic resistance. Under the same conditions, PA14Δ*amgRS* overexpressed *mexAB-oprM* 2-fold, suggesting that along with ArmR, AmgRS also contributes to the regulation of the pump in CNA treated bacteria. However, this low induction was not sufficient to confer a multidrug resistance phenotype. Conversely, the deletion of *amgRS* had a higher impact on the susceptibility of PA14 to CNA than that of *armR*.

Conclusion. Beyond its role in the modulation of MexAB-OprM activity, AmgRS protects *P. aeruginosa* from electrophilic stressors by other mechanisms that likely prevent protein aggregation.

072

Engineering novel metabolic pathways for the production of aromatic compounds in *Pseudomonas taiwanensis* VLB120

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Abstract

Aromatic compounds play an indispensable role in many of our everyday products such as fuels, plastics, dyes, pharmaceuticals, or flavoring agents. Moreover, simple benzene derivatives like ethylbenzene hold great importance as strong organic solvents in industrial applications. However, the traditional production of aromatic hydrocarbons relies on depleting petroleum, energy-intensive refining, and environmental issues. To access alternative and sustainable ways to produce these challenging chemicals, we employ microbial catalysis with highly solvent-tolerant *Pseudomonas taiwanensis*. We use a streamlined chassis strains with improved bioprocess features which can withstand aromatic solvents at high concentrations, even tolerating a second phase of hydrophobic aromatics like ethylbenzene. We introduce heterologous production modules to establish biosynthetic routes for hydrophobic aromatic compounds such as 4-ethylphenol, anisole, 4-vinyl anisole, and 4-ethyl anisole. Furthermore, we are working on the production of the industrially important solvent ethylbenzene by employing cyanobacterial aldehyde-deformylating oxygenases, which have already been used for the biosynthesis of alkanes. We are pursuing pathway optimization by adaptive laboratory evolution and enzyme engineering to approach the solubility limit of our hydrophobic products. The final goal of our work is to achieve the formation of a second phase of the aromatic compound, which would allow one to harvest the pure product from the bacterial culture.

073

Role and regulation of the type VI secretion system (T6SS) of *Pseudomonas aeruginosa* in host cell internalization

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Abstract

Pseudomonas aeruginosa is an opportunistic human pathogen with the ability to breach the airway epithelium of the respiratory tract, making it a leading cause of hospital-acquired lung infections and mortality in patients with defective pulmonary function. Although widely considered an extracellular pathogen, *P. aeruginosa* can internalize into the airway epithelium by manipulating the host using toxins injected by the type VI secretion system (T6SS). Recent evidence suggests that such internalization is a key step during infection, primarily mediated by two T6SS gene clusters of *P. aeruginosa* — called H2- and H3-T6SS. However, the real-time dynamics and regulation of these systems, as well as the effectors they employ during infection, remain largely unclear. To this end, we use genetic methods, in vitro assays, and live imaging to characterize the role of T6SS during infection. Our preliminary analysis with various mutants revealed that T6SS activity indeed influences the internalization efficiency. We expand on this observation by employing live imaging during infection of lung epithelial cell lines to visualize the real-time activity and regulation of the T6SSs and characterize their function during internalization. Further, we are establishing genetic screens to identify potential regulators and effectors of the H2- and H3-T6SS clusters, which will highlight the molecular mechanism behind successful internalization. Together, our findings will shed light on a crucial step of *P. aeruginosa* pathogenesis and advance our understanding of its survival and propagation in the host.

077

Within – patient evolution of Pseudomonas aeruginosa isolated from burn wounds

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Abstract

Hospital acquired *Pseudomonas aeruginosa* (PA) infections are life–threatening, especially in immunocompromised patients. PA has been documented to cause severe infections in burn wounds after successful colonization. While the detrimental effect of PA in infections has been well documented, it is less clear whether it evolves and adapts to the burn wound environment. To address this open question, we sampled PA isolates longitudinally from the burn wounds of patients hospitalized at the University Hospital Zurich. These patients were treated with different antibiotics owing to presenting different durations of infections, stays in the hospital, and the presence of other pathogens. We profiled our isolates for their resistances toward clinically relevant antibiotics used to treat patients. We further quantified the expression of virulence factors including the production of extracellular proteases, biofilms, siderophores and the ability to perform hemolysis. We observe patient–specific variation in both phenotype and resistance profiles. Across the 48 different isolates, we see a wide range of antibiotic susceptibilities and the expression of virulence factors. While our data reveal little evidence for evolutionary change in resistance and virulence factor expression over sampling time, we find consistent negative correlations between the antibiotic resistance and virulence factor profiles across isolates. Our data thus highlight that there is a trade-off, whereby isolates are either virulent or antibiotic resistant but not both. This result suggests the maintenance of complex communities with both strain types being important for infections.

078

Functional characterisation of H3-T6SS and its effectors in Pseudomonas aeruginosa

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Abstract

The Type VI secretion system (T6SS) is a Gram-negative bacterial apparatus that facilitates extracellular translocation of effector proteins, crucial for interbacterial interactions and host pathogenesis. *Pseudomonas aeruginosa*, an opportunistic pathogen, possesses three T6SS loci (H1, H2, and H3) with distinct functions. While H1 and H2 regulation is understood, H3-T6SS remains challenging to decipher. Here we hypothesized that the H3-T6SS has a unique and distinct role and mechanism as compared to H1 and H2 and we use a multipronged approach to identify its effectors and understand regulation of its activity. Firstly, bioinformatics analysis identified that while core components are conserved, inter-strain variability exists in the *clpV3-vgrG3* region, potentially harbouring H3-T6SS-dependent effector genes. We cloned putative effector genes from this region and assess their secretion/toxicity against prokaryotic and eukaryotic cells. Secondly, although H3-T6SS genes are expressed in diverse conditions, H3-T6SS activity, monitored by Hcp3 secretion, remains poor. Screening for conditions that maximize H3-T6SS gene expression, did not result in significant impact on Hcp3 secretion. We are in the process of monitoring H3 activity by detecting TssB3C3-GFP sheath assembly-contraction and using an effector translocation assay based on the split GFP system. Thirdly, we identified a clinical isolate from bronchiectasis patient that exhibited robust Hcp3 secretion suggesting that genetic modifications can activate H3-T6SS, possibly correlating chronic infection. Mechanisms activating H3-T6SS-dependent secretion in this strain are under study. Elucidating these mechanisms and identifying H3-T6SS-specific effectors will inform the various roles of T6SS and mechanisms fine tuning activity of each system.

079

Deployment of the type six secretion system arsenal by a sigma factor and enhancer binding protein in *Pseudomonas aeruginosa*

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Abstract

The type VI secretion system is a molecular nanomachine which contributes to infection by delivering potent toxic effector proteins directly into host cells or competing microorganisms. *Pseudomonas aeruginosa* encodes three distinct T6SS machines encoded in separate gene clusters. In addition, multiple *vgrG* gene islands that encode different tips of the T6SS as well as known or putative effector protein of a T6SSs are spread throughout the genome. How the three systems are deployed and orphan gene clusters engaged to facilitate expression and loading of the widest variety of effectors is unknown. Due to its association with flagella, virulence factor and cell surface control we hypothesised that the major alternative sigma factor RpoN could facilitate this role. Using a combination of RNAseq, ChIPseq and molecular biology approaches we demonstrate that RpoN coordinates the T6SSs of *P. aeruginosa* by activating the H2-T6SS but repressing the H1- and H3-T6SS. Through the action of a designated sigma factor activator protein, Sfa2, working in conjunction with RpoN specific control of the H2-T6SS tip and effector arsenal is coordinated enabling both expression and interbacterial killing. We also show that the posttranscriptional regulator RsmA imposes a truly global coordinated repression of almost all T6SS genes. This study further delineates the mechanism which modulate the deployment of an arsenal of T6SS effectors.

082

Temperature-responsive control of Pseudomonas aeruginosa virulence determinants through the stabilization of RhlR

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Abstract

Pseudomonas aeruginosa produces various exoproducts crucial for survival and virulence, several of which regulated through quorum sensing (QS). These factors are finely regulated in response to environmental cues, such as temperature changes. As an opportunistic pathogen, *P. aeruginosa* is thought to activate its virulence factors at temperatures akin to warm-blooded hosts rather than environmental temperatures. Recent studies elucidated the functional structure of the QS transcriptional regulator RhlR, which depends on the stabilizing effects of its cognate autoinducer, C_4-HSL, and of the moonlighting “chaperone” PqsE. Given temperature's impact on biomolecular dynamics, we investigated how it affects RhlR activity using the RhlR-regulated *phzA1* promoter as proxy. Unexpectedly, we found that RhlR activity is higher at 25°C than at 37°C. This temperature-dependent regulation likely stems from altered RhlR turnover, with the presence of PqsE extending RhlR activity by tenfold from its basal level at 37 °C to that observed at 25 °C. Without PqsE, the lower, environmental-like temperature promotes increased affinity between RhlR and C_4-HSL, a trait significantly compromised in the absence of this “chaperone”. These results suggest that this response depends on the structural integrity of the complex, indicating that temperature functions as an additional regulating and stabilizing factor of RhlR function. Accordingly, the lower growth temperature fails to increase the activity of a structurally stabilized version of RhlR. The thermoregulation aspect of RhlR activity and signalling impacts the virulence profile of a mutant unable to produce C_4-HSL, underscoring its significance in bacterial behaviours and potentially conferring an evolutionary advantage.

085

Antibiotic tolerance in *Pseudomonas aeruginosa* clinical isolates from chronic cystic fibrosis infections

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Abstract

Pseudomonas aeruginosa (*Pa*) causes chronic infections, such as respiratory infections in individuals with cystic fibrosis (CF). Antibiotic tolerance, namely the ability for bacteria without genotypic resistance (i.e without high minimal inhibitory concentration MIC) to survive at high concentrations of antibiotics, likely contributes to the antibiotic refractory nature of chronic infections. Although tolerance has been implicated in antibiotic failure *in vivo*, it has primarily been studied in lab-generated strains and few studies have examined its association with chronic human infections. In this study, we hypothesized that *Pa* strains highly tolerant to fluoroquinolones arise during chronic CF infections.

We investigated a collection of 206 *Pa* isolates from 26 CF patients, with 2 to 16 longitudinal isolates per patient collected over 2 to 21 years. After excluding isolates that overproduce exopolysaccharides and those with an ofloxacin MIC >4ug/mL, we analyzed 84 *Pa* isolates by whole genome sequencing to establish phylogenetic relatedness. Preliminary analysis using ofloxacin time kill assays (4x MIC) paired longitudinal *Pa* isolates identified the emergence of ofloxacin tolerant isolates in several patients during the course of their chronic infection (range 2.5 – 17 years). Ofloxacin internalization assays suggest that decreased envelope permeability likely contributes to the ofloxacin tolerance phenotype in some of the isolates but not all. Other possible mechanisms of ofloxacin tolerance are currently under investigation.

This project provides evidence for the emergence of high ofloxacin tolerance in CF-adapted *Pa* clinical isolates and the tolerance mechanisms that may contribute to the antibiotic refractory nature of chronic CF infections.

087

Role of the Antiterminator AmiR on *Pseudomonas aeruginosa* Biofilm Dispersion

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Abstract

Background

In previous studies, we showed that human natriuretic peptide (NP) hormones bind *in vitro* to the AmiC sensor, a member of the *ami* pathway of *Pseudomonas aeruginosa*, leading to decreased biofilm formation and enhanced biofilm dispersion. Moreover, the amidase AmiE, which is the final product of the *ami* pathway, is involved in virulence regulation and seems to support the anti-biofilm effect of NP. Since the binding of NP to AmiC triggers AmiR release allowing the over-transcription of the whole *ami* operon, we sought to decipher the key role of the antiterminator AmiR on *P. aeruginosa* PA14 biofilm regulation.

Methods

To assess the role of AmiR we constructed an AmiR over-expressing strain (AmiR+) and an *amiR*-deletion mutant (*ΔamiR*). Then, confocal microscopy and omics approaches were used in the present study.

Results

The AmiR+ strain showed a reduced ability to form a biofilm, whereas the *ΔamiR* strain exhibited weakly enhanced biofilm formation. The biofilm matrix composition was also altered in both AmiR+ and *ΔamiR* strains. Data analyses of whole transcriptomic and proteomic profiling showed that AmiR triggers some metabolic pathways which may explain the observed physiological phenotypes. Interestingly, exposure of *P. aeruginosa* pre-formed biofilms to metabolites of the arginine metabolism pathway led to differentially dispersed PA14 biofilm.

Conclusion

These results suggest a new role of the *ami* pathway on *P. aeruginosa* biofilm dispersion through a transcriptional antitermination regulatory mechanism involving the AmiR protein. Therefore, understanding this complex relationship between metabolism and biofilm dispersion is essential for developing strategies to tackle chronic infectious diseases.

089

Functional analysis of multiple nitrate transporters in *Pseudomonas aeruginosa*

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Hiroaki OKAMOTO

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Abstract

Pseudomonas aeruginosa assimilates nitrate (NO_3^-) as a nitrogen source and also uses it as a terminal electron acceptor for anaerobic respiration. Both the assimilatory and dissimilatory metabolisms require NO_3^- transport across cell membrane and three homologous transporters NasA, NarK1, and NarK2 are encoded on the genome of *P. aeruginosa*.

We constructed a quadruple mutant Δ*nasA*Δ*narK1K2*Δ*nap*Δ*PA1854* (Δ4), which lacks genes of the putative NO_3^- transporters, periplasmic nitrate reductase, and a putative nitrite (NO_2^-) transporter. The complemented strains Δ4+*nasA*, Δ4+*narK1*, and Δ4+*narK2*, which express only one of the transporters were also constructed. Growth profiles of the strains were determined. For nitrate assimilation condition, NO_3^- was the sole nitrogen source and NO_3^- import was necessary for cells to grow. For denitrification condition, NO_3^- was the electron acceptor and both NO_3^- import and NO_2^- export were necessary for cells to grow.

We found that NasA and NarK1 were able to import NO_3^- and export NO_2^- since Δ4+*nasA* and Δ4+*narK1* showed growth under denitrification condition. Under either condition Δ4+*narK2* was unable to grow but coexpression of inactivated NarK1 with wild-type NarK2 led to growth under denitrification condition, indicating that NarK2 functioned as a NO_3^-/NO_2^- antiporter in the presence of NarK1. We also found that a point mutation at a highly conserved residue of NarK2 led to growth of the complementary strain under nitrate assimilation condition. The results suggested that a relatively small residue at that site was important for NarK2 function in the absence of NarK1.

091

PQS and HQNO play a major role in *Pseudomonas aeruginosa* membrane vesicle-mediated growth inhibition of Gram-positive bacteria

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Abstract

Bacteria compete with other species in nature as well as our body through contact-dependent and -independent mechanisms. *Pseudomonas aeruginosa* produces membrane vesicles (MVs), which can kill Gram-positive bacteria in a contact-independent manner, as demonstrated by Kadurugamuwa and Beveridge in 1996. In this study, the authors identified a 26 kDa zymolytic band by zymography, and suggested that the toxic effect of MVs is due to cell envelope degradation caused by the 26 kDa peptidoglycan hydrolase. However, we showed that the enzyme responsible for the zymolytic band is AmpDh3 and that it is involved in cell wall turnover and repair but not lysis. None of our peptidoglycan hydrolase deletion mutants tested showed reduced activity. Thus, the mechanism through which MVs cause cell death remained unclear.

We noticed that MVs from *P. aeruginosa* strain PA14 are much more toxic than those from strain PAO1 for Gram-positive bacteria. High bactericidal activity was also discovered in the ethyl acetate extracted MV preparations. In-line with a previous study (Mashburn and Whiteley, 2005), we found that MVs contained high amounts of quinolones. Growth inhibition of *Staphylococcus aureus* by quinolones was then examined using pure quinolone compounds and MVs from different mutants. The strongest activity was observed using PQS and HQNO, while MVs from *pqsL*, *pqsA*, and *pqsH* mutants showed highly reduced activity. Our results demonstrate that instead of hydrolytic enzymes, small bioactive compounds are the major factors involved in MV-mediated killing of Gram-positive bacteria by *P. aeruginosa*.

092

Investigating the role of the ExoY effector in *Pseudomonas aeruginosa* virulence

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Abstract

Using its Type 3 Secretion System (T3SS), *Pseudomonas aeruginosa* injects ExoS, ExoT, ExoU, and ExoY effectors, into the cytosol of host cells. Unlike other T3SS toxins that are known to contribute directly to cell death, the role of ExoY in inducing cytotoxicity is still unknown. Once injected into cells, ExoY produces various cyclic nucleotide monophosphates (cNMPs), and in particular, supraphysiological levels of cGMP and, to a lesser extent, cAMP. We therefore investigated whether cNMPs production by ExoY could modify the cytotoxic effects of the other T3SS effectors.

Based on the structure of ExoY, substitutions were performed in its nucleotide binding pocket to narrow the substrate specificity of the toxin from GTP to ATP. These modifications were then incorporated into the genome of *P. aeruginosa* strains and the cytotoxic effects of ExoS, ExoT and ExoU were measured in real time in infected cells when co-injected with ExoY mutant variants.

We discovered that depending on the type of cell infected and its production of cNMPs, ExoY modulated cytotoxicity of T3SS effectors. We are now genetically modifying bacterial strains and human cells to better understand this synergy between T3SS effectors.

Our discovery could explain why the toxin is so widespread among strains. Indeed, ExoY now appears to play a central role in *P. aeruginosa* virulence, whereas for many years its role remained unclear and controversial.

093

Pseudomonas aeruginosa lasR is a keystone gene in polymicrobial cultures

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Abstract

Pseudomonas aeruginosa (PA) is a WHO-designated critical priority pathogen, that frequently colonizes the airways of people with cystic fibrosis (CF). PA often shares this airway niche with a "zoo" of microbes; however, the dynamics of these communities, and influence of co-habiting species on PA, remain inadequately explored.

Various PA mutants frequently arise in CF-associated polymicrobial infections, indicative of likely selection pressures. However, the molecular basis for this is unclear. One such gene is *lasR*, which often correlates with a worsened patient prognosis. LasR is a master regulator of quorum sensing, and has also been linked with biofilm formation. To investigate the role(s) of LasR in CF further, we developed an *in vitro* experimental setup allowing stable co-culture of PA alongside other common airway pathogens (*Staphylococcus aureus, Candida albicans*) in artificial sputum medium.

When co-cultured with wild-type, *lasR* mutants reached 10% of the overall PA titre, correlating with the relative titres seen in CF airways. Titres of other species in steady-state were not affected by the presence of the mutant. However, when only the *lasR* mutant was present, the polymicrobial community was destabilized, especially following antibiotic challenge. Furthermore, transcriptomic analyses indicated that, while the wild-type appears to be relatively agnostic to the presence of other species, the *lasR* mutant displays substantial transcriptomic changes (cf. when grown alone). These data suggest *lasR* is a "keystone gene" that disproportionately impacts inter-species interactions. Through judicious mutation (or induced over-expression) of key *lasR*-dependent genes, we identify the likely molecular basis for these behaviour(s).

095

Environmental factors influence biofilm formation through exopolysaccharide biosynthesis in *Pseudomonas syringae* pv. syringae.

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Abstract

Pseudomonas syringae is a phytopathogenic model bacterium that is used worldwide to study plant-bacteria interactions and biofilm formation in association with a plant host. Within this species, the syringae pathovar is the most studied due to its wide host range, affecting both, woody and herbaceous plants. *Pseudomonas syringae* pv. syringae (Pss). the causal agent of the bacterial apical necrosis (BAN) disease of mango trees, exhibits major epiphytic traits and virulence factors that improve its epiphytic survival and pathogenicity. Cellulose and the Psl-like polysaccharides have been described to constitute the basic scaffold for biofilm architecture in this bacterium. Previous studies have revealed a connection between weather conditions and the incidence and severity of BAN disease symptoms. Thus, in this study, the role of some environmental factors, such as temperature and light, in biofilm formation of different Pss strains have been assessed. The results have shown that temperature and particularly light influence biofilm formation through exopolysaccharide biosynthesis. The different Pss strains have shown an oscillating pattern in biofilm formation and exopolysaccharide production depending on temperature. Noticeably, white light increases biofilm formation through exopolysaccharide biosynthesis in Pss. Furthermore, cellulose production phenotypes are congruent with the phylogenetic distribution of the Pss strains of this study regarding their cellulose gene clusters, which suggests this polysaccharide could be very important for the ecology of Pss over the mango plant surface. Finally, as previously described in *P. syringae* pv. syringae UMAF0158 strain, lower levels of exopolysaccharide production could be associated with higher virulence phenotypes of Pss.

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Lack of *sirB2* gene stimulates virulence, invasion, and small colony variants emergence in *Pseudomonas aeruginosa*

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Abstract

Background: *Pseudomonas aeruginosa* is the leading cause of death in cystic fibrosis (CF) patients. Virulence factors production and complex behaviours play a crucial role in *P. aeruginosa* pathogenesis. However, knowledge of the genetic determinants driving *P. aeruginosa* persistence in the host is limited. Based on previously collected transcriptional data from human samples, we studied the role of *sirB2*, a *P. aeruginosa* PA14 gene whose expression is enhanced in the CF lung.

Methods: *In silico* promoter analysis, molecular genetics, and biochemical approaches were used to decipher the *sirB2* gene regulation. The generation of PA14 mutants and whole-genome sequencing analysis were used to evaluate the gene's impact on phenotypes known to be important for *P. aeruginosa* pathogenicity (*i.e.*, biofilm formation, *in vitro* and *in vivo* virulence assays on different infection models).

Results: We show that *sirB2* gene belongs to the Vfr and AmrZ regulons, and its inactivation increases *P. aeruginosa* pathogenic potential in *Galleria melonella*. Similarly, gene deletion stimulates transepithelial migration and biofilm formation in an infection model based on air-liquid interface cultures of the airway epithelium. In this context, the lack of *sirB2* promotes the production of virulence determinants and the emergence of rugose small colony variants (RSCVs). RSCVs appearance depends on an increased rate of mutations in the *wsp* regulatory circuit, leading to increased c-di-GMP levels and Pel polysaccharide production.

Conclusions: Our data identified *sirB2* as a novel genetic determinant underlying the appearance of heterogenous phenotypes typical of *P. aeruginosa* infections, through a mechanism involving specific genetic rearrangement in the PA14 genome.

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Limiting the phosphate starvation response of *Pseudomonas aeruginosa* to tackle the airway epithelium infection

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Leon Jahdauti

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Abstract

Background :

Pseudomonas aeruginosa (PA) is mainly responsible for respiratory tract infections. Phosphate (Pi) homeostasis is essential for bacterial metabolism and is regulated by the PhoB-PhoR system. In gastrointestinal infection mice models, PA has been found to increase its virulence upon Pi starvation, leading to the overexpression of PhoB-PhoR-regulated virulence factors (VF). This study aims to investigate whether Pi starvation can also modulate bacterial virulence during human pulmonary infection.

Methods :

Using a PA infection 3D model of fully differentiated human bronchial epithelium, we assessed the evolution and impact of Pi starvation : Pi concentrations, Pi-transporters and PA VF-encoded genes expression, epithelium destruction monitoring by time-lapse videomicroscopy.

Results :

We found that consumption of Pi during epithelium infection resulted in the activation of Pi starvation response genes (threshold = 0.25 mM; *phnC-P* transporter x18 fold-change). This also led to increased bacterial virulence, which was evidenced by a reduction in epithelial destruction time and an overexpression of VF genes (*plcN*: x10 ; l*ecB*: x3 ; *mvfR*: x2,7 ; *phzS*: x2,3 fold-change). Pi supplementation limited PA virulence during epithelium infection (47 *vs* 56 hours).

Conclusion :

Our study demonstrate that upon low level of Pi, VF genes are overexpressed, which leads to the destruction of airway epithelium. Supplementing epithelia with Pi extends the time it takes for PA to cause destruction, which could be a useful strategy to fight against PA infections. We anticipate that our results could serve as a starting point for developing new treatments for PA lung infections.

098

Towards development of *Pseudomonas putida* as cell factory for remediation and upcycling of recalcitrant plastics: cases for polyurethane, polystyrene and polyamides.

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Abstract

Due to its tolerance to compounds generally viewed as toxic to bacteria, and its flexibility and variety of metabolic pathways, *Pseudomonas putida* is a promising candidate for efficient biological remediation and upcycling of various plastics. We discuss progress on designing cell factory remediation and upcycling approaches with *P. putida* for various plastics; polyurethanes, polystyrene and polyamides.

P. putida S12 and *P. putida* KT2440 can degrade various polyurethanes, in combination with biofilm formation on polyurethane thin films. Characterization of specific enzymes and molecular pathways involved paves the way for recycling and upcycling of degradation products, for either aliphatic and aromatic polyurethanes.

Pseudomonas furukawaii has been described to efficiently degrade biphenyl and other polyaromatic compounds. We have engineered *P. putida* with the *P. furukawaii* gene cluster encoding the biphenyl degrading enzymes. Degradation of biphenyl and other short-chain polyaromatic compounds serves as a basis for polystyrene degradation via *P. putida* S12 which can degrade styrene via specific styrene oxidation.

We have developed an efficient growth medium emulsification method for various polyamides, including nylon 6 and nylon 66. Several *Pseudomonads* were tested for their intrinsic potential to degrade polyamides, as well as to metabolize polyamide degradation compounds. Whereas metabolism and upcycling of nylon-constituting monomers has been described, efficient enzymatic degradation of polymeric nylon awaits development of a suitable cell factory.

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A tight adherence (*tad*) like gene cluster of *Pseudomonas chlororaphis* PCL1606 exhibits a crucial role in avocado roots colonization, fostering its biological control activity.

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Abstract

Pseudomonas chlororaphis PCL1606 (PcPCL1606), displays strong antagonistic and biological control abilities against several soil-borne fungal pathogens. These relevant features are mainly due to the production of the antifungal molecule 2-hexyl, 5-propyl resorcinol (HPR). HPR governs other important beneficial phenotypes, suggesting its additional regulatory activity. Published transcriptomic data identifying HPR-regulated genes involved in the interaction of PcPCL1606 with the avocado rhizosphere were used as a target database to identify putative genes involved in avocado roots colonization. The search for putative candidates revealed the induction of several consecutive genes that showed homology with genes encoding a putative type IV Flp/Tad (tight adherence) pilus. To study the role of the *tad*-like gene cluster in the biology of PcPCL1606, a chromosomal deletion mutant was constructed, and the molecular characterization of the *tad*-like gene cluster by RT-PCR was performed. Different *in vitro* and *in vivo* phenotypes related to colonization were assessed in the mutant strain respect to wild type strain. RT-PCR confirmed that the *tad*-like gene cluster was composed of three independent transcriptional units. Furthermore, the *tad*-like deletion mutant was affected in early attachment, early biofilm formation and in bacterial autoggregation. Finally, root competitiveness in avocado plants and biocontrol activity against *R. necatrix* were impaired in the *tad*-like deletion mutant. This study expands our knowledge regarding the role of HPR as signalling molecule and revealed the importance of the *tad*-like gene cluster of PcPCL1606 in the avocado roots colonization, confirming that initial attachment to roots is a fundamental mechanism for the PcPCL1606 rhizospheric performance.

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The pathoadaptive gene regulatory network of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a multi-drug resistant pathogen that causes both acute and chronic lung infections. We have previously shown that host adaptation in *P. aeruginosa* is driven by convergent mutations in 224 genes, many of which are known to affect gene expression. However, it is unclear how variants in these genes alter transcriptional control. In this study, we inferred the pathoadaptive gene regulatory network of *P. aeruginosa* using large-scale genetic perturbations and expression profiling, combined with Bayesian structural modelling. We integrated these data with measures of antibiotic resistance for a panel of over 1,000 isolates showing broad genetic diversity.

We systematically mapped the associations between genetic variation and changes in gene expression and demonstrate how the regulatory network is re-wired along discrete evolutionary pathways. We also showcase the application and extension of our approach by dissecting our genotype-transcriptome map with resistance determinants to 14 clinically relevant drugs, identifying novel associations between specific genes and the development of antibiotic resistance. We further dissect the growth condition-specific transcriptomes of *P. aeruginosa* in planktonic and biofilm states, identifying key interactions between genotype and environment. Overall, our genotype-transcriptome map points towards convergent transcriptional changes and provides a blueprint to dissect the mechanisms of adaptation in pathogenic bacteria more generally.

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Molecular architecture of native Pseudomonas aeruginosa biofilm

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Abstract

Biofilms are a ubiquitous multicellular lifestyle adopted by many bacterial species including the important human pathogen, *Pseudomonas aeruginosa.* Infections caused by *P. aeruginosa* biofilms are challenging to treat as the bacterial cells are protected against antibiotics by an extracellular matrix (ECM), composed of a complex mix of extracellular polymeric substances. Despite its importance, direct visualisation of the ECM at high-resolution has remained elusive, resulting in a gap in our knowledge regarding the molecular architecture of the biofilm ECM and how it links to antibiotic tolerance. Here, we develop a workflow to image native biofilms at high-resolution using the latest techniques in electron microscopy. We employ cryo focused-ion-beam (cryo-FIB) milling and electron cryo-tomography (cryo-ET) to visualise the *in situ* molecular architecture of *P. aeruginosa* biofilms formed under flow. We also correlate high-resolution cryo-ET data with scanning electron microscopy and optical imaging to illustrate the architecture of bacterial biofilm at multiple scales. Using this pipeline, we have obtained the first high-resolution pictures of the bacterial biofilm, revealing molecular details of the extracellular polymeric substances comprising the ECM. Our structural data depict a complicated network of filamentous proteins. This network provides cohesion between bacterial cells and potentially shields the cells from antibiotics. Our work establishes a high-resolution imaging platform for bacterial biofilms and will elucidate the molecular mechanism of antibiotic tolerance provided by a biofilm lifestyle.

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Construction of Functional Bacterial Chassis and Synthetic Consortia for the Production of Bio-Based Platform Chemicals from Lignin-Derived Aromatics

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Abstract

In the context of global environmental crisis, bio-based alternatives to petrochemical processes are essential for ensuring sustainable production of materials and chemicals. Lignocellulose is the most economical and abundant renewable carbon-based feedstock in the world,[1] [2] and its lignin fraction represents the largest potential source of aromatic compounds of biological origin.[3] [4] The bioaromatic platform is an emerging biorefinery pathway to convert lignin to functionalized monoaromatics as intermediate feedstock chemicals for subsequent conversion into high-value added products.[5] This will lead to an integrated, sustainable biorefinery, achieving full economic efficiency and optimal conversion of both holocellulose and lignin.

This study aims at valorizing lignin from lignocellulosic waste by relying on Reductive Catalytic Fractionation (RCF) and the construction of functional bacterial chassis based on *Pseudomonas putida* KT2440.

RCF is first used to fractionate the biomass and depolymerize and stabilize the lignin fraction[6]. The lignin oil obtained after RCF is a heterogeneous mixture of lignin-derived monoaromatics. The construction of bacterial chassis with *P. putida* KT2440 is explored for the metabolic funneling of the lignin-derived monoaromatics into three central metabolites: phenylacetate, protocatechuate and 3-*O*-methylgallate. Further microbial upgrading with *P. putida* KT2440 is investigated to produce tropolone (TPL) and 2-pyrone-4,6-dicarboxylic acid (PDC). Puberulic acid, derived from TPL, possesses antimalarial activities[7] while PDC is a precursor to synthetize nylon 66.[8] The final step of this study consists in the development of a fed-batch bioreactor for the conversion of a lignin oil feed catalyzed by a synthetic consortium combining the relevant bacterial chassis.

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Pseudomonas aeruginosa transcriptional reprogramming during zinc starvation reveals a shift toward anaerobic respiration

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Abstract

Background
The capability of pathogens to evade the host nutritional immunity relies on their ability to import essential micronutrients. Among these, zinc is an important cofactor of many proteins and plays a crucial role in several bacterial virulence traits. *Pseudomonas aeruginosa* employs multiple mechanisms to acquire zinc and thrive at host infection sites. However, bacterial broad metabolic adaptation to severe zinc deficiency conditions requires further investigation.

Methods
P. aeruginosa PA14 wild-type and *znuAzrmB* mutant strains were grown in chemically defined zinc-depleted medium (VB-MM). Total RNA was extracted in the early-logarithmic growth phase and analysed through RNA-seq to examine differential gene expression.

Results
The transcriptional profile of the *znuAzrmB* mutant strain showed the overexpression of the Zur-regulated genes associated with zinc import and adaptation to zinc starvation, as expected. Moreover, it revealed the downregulation of genes involved in central carbon metabolism, motility, and virulence traits. Notably, there is evidence of a shift toward anaerobic/micro-aerophilic respiration, indicated by the significant overexpression of genes involved in the denitrification pathway, the induction of the high-oxygen affinity terminal oxidases and the downregulation of low-oxygen affinity terminal oxidases.

Conclusions
Our study reveals that the transcriptional profile of the zinc-starved *znuAzrmB* mutant closely resembles that of *P. aeruginosa* in cystic fibrosis sputum samples, where the induction of anaerobic metabolism has been correlated to restricted oxygen availability. Observing the same pathways upregulated in *P. aeruginosa* grown aerobically in VB-MM suggests an unexpected direct link between zinc starvation and anaerobic respiration, potentially contributing to bacterial survival within the host tissue.

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Expression of the Omp85-phospholipase PlpD shows strain-dependent variation and is regulated by temperature

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Abstract

Omp85 phospholipases (Omp85-PLs) are a family of outer membrane embedded proteins with phospholipase activity, with the prototypical member being PlpD of *Pseudomonas aeruginosa*. Until recently, these proteins were thought to represent a class of autotransporter that exports its PL domain to the bacterial cell surface. However, more recent evidence suggests that the PL is located in the periplasm. To gain insight into the potential function of these proteins, we investigated under which conditions *plpD* would be expressed using a fluorescent reporter construct. We introduced this construct into two standard laboratory strains of *P. aeruginosa*, PAO1 and PA14, and tested a variety of conditions for changes in fluorescence. Interestingly, PAO1 did not display any difference in fluorescence compared with the control under any of the conditions, suggesting that *plpD* is not expressed in this strain. By contrast, PA14 with the reporter construct displayed consistently higher fluorescence than the control, and the highest fluorescence was observed at 37 °C, suggesting temperature regulation of the gene. Fluorescence also increased when the bacteria entered stationary phase. However, the fluorescence was never very strong under any of the tested conditions, which suggest low expression levels in PA14 as well. We further tested clinical strains of *P. aeruginosa*, all of which displayed fluorescence at levels comparable to PA14. Taken together, PlpD appears to be expressed in pathogenic strains but at low levels, suggesting a housekeeping function that may be linked to virulence.

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Enhancing therapy of *Pseudomonas aeruginosa* in urinary tract infections in human bladder microtissues

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Abstract

Urinary tract infections (UTI) are one of the most common human acquired and recurring infections worldwide. With recurrence rates that can exceed 30% within the first 6 months, UTIs are among the most frequent causes of antibiotic prescription, which in turn exacerbates the current antimicrobial resistance crisis.

Our knowledge about UTIs stems mainly from mice or cancer cell line studies, using uropathogenic *E. coli.* However, these models do not accurately reflect many of the human bladder's features including physiology, immunity and ultrastructure, hindering the development of adequate therapies and the comprehensive understanding of human UTIs.

Here, we employed the newly developed bladder microtissue model (PMID:37939183), recapitulating key human features, including full stratification, differentiation and urine tolerance, to understand and fight bladder infection caused by *Pseudomonas aeruginosa* (PA). We observed that PA preferentially forms biofilms in this human-like microenvironment, even among eight genetically distinct clinical isolates received from the University Hospital Basel and despite heterogeneity in volume and number of aggregates this strategy seems to be conserved. Currently, we are investigating the effectiveness of three novel anti-biofilm compounds, targeting different components of the biofilm matrix, in combination with standard-of-care antibiotics to clear PA infection. Preliminary tests at high concentrations of the anti-biofilm agents (100µM) have proven a strong effect inhibiting biofilm formation.

Overall, this combinatorial approach will pave the way for a better understanding of *P. aeruginosa* pathophysiology in UTIs, as well as for the development of a more effective course of treatment.

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Analysis of antibiotic-essential gene interactions in *Pseudomonas aeruginosa* using CRISPRi-seq

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Adam Kawałek

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Abstract

Pseudomonas aeruginosa (*Pae*), an opportunistic ESKAPE group pathogen, is known for its ability to evade antibiotic treatments, underscoring the need for identification of novel therapeutic approaches. The essential gene products are the targets of existing antibiotics, and represent promising targets for new therapeutics. The development of CRISPR interference (CRISPRi) offered a method for high-throughput analysis of the cell fitness upon essential gene silencing, allowing identification of pathways particularly sensitive to inhibition, and possibly pinpointing novel drug targets. In this work we optimized CRISPRi approach to probe the sensitivity of essential genes to depletion in *Pae*.

In CRISPRi, transcription inhibition is obtained by binding of inactive nuclease dCas9, targeted by a ~20 bp spacer, included in single-guide RNA (sgRNA). This spacer is used as a barcode for assessment of strain abundance in pooled libraries. Here we present a set of *Pae* CRISPRi integrative plasmids, allowing tightly controlled silencing of reporter and essential genes by up to ~12-fold. Moreover, we report optimized protocols for selection of guide sequences, efficient sgRNA library integration in PAO1 genome, and we show that introduction of mismatches into spacers allows to predictably titrate gene silencing strength in *Pae*. Finally, we report preliminary results of CRISPRi-seq screening, aiming at identification of essential genes, which silencing sensitizes *Pae* cells to the action of tobramycin. Overall these efforts pave the way for pooled CRISPRi screenings, which may re-prioritize the current drug-discovery efforts for *Pae*.

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Characterizing the plant-beneficial bacterium *Pseudomonas protegens* Pf-5 as a chassis for environmental synthetic biology

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Juan Rico

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Abstract

Pseudomonas protegens Pf-5, a soil bacterium renowned for its unique environmental functions (i.a. biocontrol and plant-growth promotion) is an emerging host for synthetic biology applications. The engineering of genetic circuits to perform predefined computations is central to the field since these synthetic constructs empower living cells with new functionalities, applicable across various domains. However, a significant challenge in expanding the scope of these circuits is that they are often tailored to the model organism *Escherichia coli*, and their intended functions may not translate well to other organisms. Understanding the performance of less familiar organisms is crucial, especially for niche-specific applications. In this study, we address this issue by using a library of NOT logic gates, also known as genetic inverters, to evaluate the performance of the root-colonizing bacterium *P. protegens* Pf-5; a newcomer to genetic circuitry. We characterized each inverter abstracting four key parameters in circuit functionality, namely output production, repressor accumulation, repression efficiency and cooperativity, and conducted a comparative analysis with the established chassis *Pseudomonas putida* KT2440. By fitting experimental results into mathematical models, we assessed the impact of the host context on circuit performance. Building on this, the mathematical models enabled the prediction of circuit outputs between these two hosts, effectively bridging circuit functionalities between them. Our results offer a new collection of characterized genetic constructs to assist the efficient engineering of *P. protegens* Pf-5 and establish a methodology towards translating circuit performance between different *Pseudomonas*.

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Integrating experimental approaches into genome-reduced strain development for large-scale bioprocesses

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Abstract

Numerous critical considerations must be addressed to transfer production processes from laboratory to industrial scale successfully. In large-scale fermentations, the inevitable occurrence of heterogeneous mixing exposes producer strains to potential starvation zones. Hence, strain robustness is fundamental to mitigate performance loss.

Several experimental approaches have been developed to mimic upscale starvation zones. For instance, the successful implementation of nutritional limitation conditions, carbon and nitrogen gradients, along with oxygen limitation, in a two-compartment scale-down reactor (STR-PFR) has enabled the understanding of the direct impact of starvation zones on traditional biotechnology chassis such as *Escherichia coli* (Löffler et al. 2016). Recently, a single multi-compartment bioreactor (SMCB) device achieved industrial mixing time in lab-scale bioreactors (Gaugler et al. 2023). This new technology, combined with proteomic and transcriptomic analysis, offers the possibility of a deeper understanding of metabolic modulation in large-scale. Therefore, STR-PFR or SMCB will be utilized to assess different limitation scenarios, such as carbon, nitrogen, or oxygen, on an alternative but promising microbial chassis, *Pseudomonas putida*. Multivariate analysis (MVA) will be executed to identify common patterns and construct a genome-reduced strain. As previously reported, gene deletions will target individual genes rather than fragments (Fan et al. 2020; Wynands et al. 2019; Martínez-García et al. 2015; Liang et al. 2020). However, the Design-Build-Test-Learn (DBTL) cycle principles will guide gene deletion and resulting cells will be compared to wild-type and other genome-reduced strains.

Publication bibliography

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The human Atrial Natriuretic Peptide and Osteocrin act as powerful weapons against *Pseudomonas aeruginosa* biofilm

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Abstract

Background:

Pseudomonas aeruginosa is responsible for chronic infections in wounds or lungs where it forms biofilms that are impervious to antibiotics treatments. There is therefore a need for alternative treatments that prevent biofilm formation or disperse pre-established biofilms. In the present study, we evaluate the impact of two human hormones, the Atrial Natriuretic Peptide (ANP) and Osteocrin on *P. aeruginosa* biofilm dispersion.

Methods:

The total 24 h-old biofilms biomasses of *P. aeruginosa* obtained in a dynamic flow-cell system were measured after 2 hours exposure to peptides or a combination of peptide + antibiotics.

Results:

We observed that both ANP and Osteocrin not only prevented the formation of a *P. aeruginosa* biofilm, but also strongly disrupted pre-formed biofilms, in a dose-dependent manner. Interestingly, we demonstrated that the peptides' dispersal activity required the presence of the *P. aeruginosa* AmiC sensor protein, suggesting a specific activity on the bacterial physiology. Since we have noted that these peptides have no antibacterial activity, we validated that ANP acted as an adjuvant agent, enhancing the anti-biofilm action of different antibiotics, allowing almost full biofilm eradication. Finally, we validated *in vivo* the effects of ANP alone or in combination with tobramycin on biofilm dispersion using a chronically infected mouse model. We observed a significant reduction of bacterial load in the lungs of infected mice after three consecutive days of ANP exposition.

Conclusion:

Altogether, these data suggest that ANP and Osteocrin could be new therapeutic tools to control chronic *P. aeruginosa* infections, particularly in cystic fibrosis-suffering patients.

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Mechanisms of resistance to itaconic acid derivatives in *Pseudomonas*

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Abstract

Itaconic acid (ITA) is gaining increasing recognition for its role in mammalian immunity, produced by macrophages to inhibit microbial metabolism. However, some pathogens, such as *Pseudomonas aeruginosa* and *Yersinia pestis*, have evolved mechanisms to degrade ITA (Sasikaran *et al*., 2014). Notably, the ITA degradation operon of the common soil- and plant-associated bacterium *P. aeruginosa* possesses three additional genes (PA0879-0881) that are not directly related to ITA degradation but are recently found to be important for tolerance to the ITA derivatives 2-hydroxyparaconate (2-HP) and itartartarate (ITT) produced by some fungi (de Witt *et al.*, 2023). Inhibition studies with *Ustilago* supernatants and purified 2-HP and ITT on non-pathogenic *P. putida* KT2440, engineered with the ITA operon from *P. aeruginosa* PAO1, identified the putative ring-cleaving dioxygenase Rdo_{PA} (PA0880) as key mediator for tolerance to 2-HP and ITT. Interestingly, the ITA synthesis cluster of several fungal strains such as *Ustilago maydis* and *Aspergillus terreus* contains a gene encoding an enzyme with high sequence similarity to Rdo_{PA}. Through complementation studies, we confirm that the enzymes from these evolutionary distant hosts have the same function. We currently hypothesize a mechanism based on CoA sequestration by a side activity of the itaconyl-CoA transferase Ict (PA0882) on ITT. In this hypothesis, resistance would be mediated by Rdo_{PA} through binding of ITT. Furthermore, it was found that *Ustilago* produces two enantiomeric forms of 2-HP, whereas only (S)-2-HP is converted to (L)-ITT by *Pseudomonas*. Ongoing research aims to elucidate the inhibition and resistance mechanisms related to 2-HP and ITT.

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Host-Induced Signalling and Warfare in a Polymicrobial Community

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Abstract

The Post-Antibiotic Era describes the rise in antimicrobial resistance (AMR) and the lack of novel antibiotic discovery. Thus, alternative methods are required for effective pathogen control. Small molecules have gained considerable traction in selective pathogen targeting and can be viewed as an anti-infective strategy. The chemical languages evolved in dominant pathogenic organisms could offer a novel strategy for suppression of key virulence phenotypes and competitiveness in co-colonising fungal and bacterial pathogens. Alternatively, efflux inhibition and cross-regulation from anti-viral frameworks are emerging as promising alternatives to growth limiting control.

One major challenge to the clinical development of anti-virulence interventions is that microbes exist in diverse polymicrobial communities that undergo significant genotypic and phenotypic diversification. Co-colonising microbes present in the cystic fibrosis (CF) lung, encompassing bacterial (*Pseudomonas aeruginosa*) and fungal (*Candida albicans*, *C. dubliniensis* and *Aspergillus fumigatus*) lab and clinical strains were studied in response to host/microbial signals including *N*-acetyl-glucosamine (NAG), bile, and bile salts. Fungal and bacterial pathogens were found to alter their virulence through biofilm formation, pigmentation, and toxin production in response to specific host signals, adopting distinct morphological and pigmentation profiles when co-cultured in the presence of competing organisms. Emergence of distinct pigment-secretion and pigment-retention profiles indicated a context-dependent shift in pathogen behaviour.

Selective control of these mixed consortia and their distinctive pigmentation patterns was explored using a range of small molecule frameworks. While some retained activity similar to the individual species studies, others were diminished in their anti-*P. aeruginosa* potency when other ESKAPE or fungal pathogens were present.

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Translocation and Assembly Module (TAM) as a Potential Drug Target in *Pseudomonas aeruginosa*

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Abstract

The outer membrane (OM) of Gram-negative bacteria is involved in virulence, cell stability and acts as a permeability barrier. The biogenesis, assembly, and regulation of proteins in the OM are therefore attractive as potential novel drug targets. The Translocation and Assembly Module (TAM) comprises of TamA and TamB and functions to assemble and insert certain β-barrel proteins into the OM in *E. coli* and *Klebsiella pneumoniae.* It has also recently been shown to be involved in lipid homeostasis.

The TAM, however, has not been studied in *P. aeruginosa* and given its robust OM and its reliance on the OM for antimicrobial resistance, the TAM is a potential drug target that may impact on the virulence of *P. aeruginosa*.

Using homologous recombination, *P. aeruginosa tamA, tamB and tamAB* knockouts have been constructed and verified by whole genome sequencing to investigate their effects on OM integrity and by extension virulence of *P. aeruginosa*. Based on a competition growth analysis of the wild type *P. aeruginosa* versus the *tamA*, *tamB* and *tamAB* knockouts, the wild type outcompetes the mutants. Additionally, the mutants show defective growth phenotypes with significant reduction in biofilm formation. However, no differences have been observed in their susceptibility to outer membrane-targeting antibiotics.

The OM proteome of the knockouts will be investigated via mass spectrometry and compared to the wild type to monitor changes in the OM proteome to gain insights into the role of the TAM in *P. aeruginosa* virulence and its potential as a drug target.

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Identifying Intracellular Partners of the Bacterial Toxin ExoU by Proximity Labeling

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Abstract

Pseudomonas aeruginosa (*Pa*) assembles on its surface an arsenal of virulence factors including the Type 3 Secretion System (T3SS), which is a multiprotein needle-like apparatus that injects toxins into the eukaryotic cell. The Exotoxin U (ExoU) is the most aggressive T3SS toxin with a phospholipase activity on the host membrane lipids therefore leading to a rapid necrotic cell death. Host chaperone DNAJC5 is required for ExoU activity. Here, by combining molecular biology, proximity labeling tools and proteomics, we aimed to identify other eukaryotic actors contributing to the activity and trafficking of ExoU. First, ExoU was C-terminally fused to the 19.4 kDa biotin ligase UltraID and we showed that this fusion protein was able to pass through the T3SS needle. A cytotoxicity assay consisting of infecting A549 pulmonary cells by modified *Pa* showed that ExoU-UltraID exhibits a phospholipase-dependent killing, albeit delayed compared to that obtained with ExoU alone. The proximity labeling assay (PLA) was therefore optimized leading to the detection of biotinylated proteins. Streptavidin-affinity capture of proteins from infected cells lysates, followed by mass spectrometry analysis showed an enrichement of different human proteins compared to the negative control. The top two hits were RAB27B and SLC3A2, both of which have roles in a variety of trafficking pathways. Moreover, SLC3A2 was shown to interact with DNAJC5 in the context of the misfolding-associated protein secretion (MAPS) pathway. Several undergoing experiments including single and double knock-down of the hit proteins, co-immunoprecipitation with ExoU and proteins co-localization by fluorescence microscopy will be presented.

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Significance and mode of action of the pseudopaline pathway involved in zinc import in Pseudomonas aeruginosa

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Abstract

Pseudomonas aeruginosa (*Pa*) exhibits a high adaptability to hostile environments such as the ones encountered during infection. It is notably able to survive metal scarce conditions thanks to the production of metallophores, small molecules with high metal affinity ensuring their import when their bioavailability is low. *Pa* therefore adapts to zinc starvation by producing and secreting pseudopaline, a metallophore dedicated to zinc acquisition. We and others have shown that pseudopaline plays a pivotal multifactorial role during *Pa* infections. However, little is known about the mechanisms allowing *Pa* to cope with the high metal affinity of this molecule which needs to be finely controlled to avoiding any unwanted metal chelation during synthesis and secretion. Based on previous evidences of the existence of such a mechanism, we looked for pseudopaline-modifying enzymes. We identified the cytoplasmic methyltransferase CntT, which methylates the pseudopaline and inactivates its intracellular toxicity upon cytoplasmic accumulation. We demonstrated that CntT production relies on two signals: zinc starvation under heat-shock-like stress conditions. Based on these data, we postulated that *Pa* protects itself from potential pseudopaline-mediated damage by inactivating it upon methylation by CntT in particular stress conditions. These results provide new insights into the comprehension of *Pa*'s adaptation to stressful conditions.

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Preliminary characterization of novel genetic determinants associated with *Pseudomonas aeruginosa* pathogenicity in cystic fibrosis patients

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Abstract

Background: *Pseudomonas aeruginosa* (*Pa*) chronic infections in cystic fibrosis (CF) patients are difficult to eradicate. Transcriptional analysis of *Pa* communities from sputum samples of CF patients suggests that *Pa* growth in the airways is associated with a specific transcriptional profile. Yet, most of the genes modulated *in vivo* are poorly characterized, representing new virulence determinants important for *Pa*'s pathogenicity.

Methods: *In silico* prediction tools and genetic approaches were used to reconstruct the regulatory network of the target genes, and to decipher the signals driving their expression. Knockout and overexpression mutants of the *Pa* PA14 strain were tested for their ability to affect virulence-related phenotypes (*i.e*. biofilm formation, antibiotic and oxidative stress resistance, virulence factor production, and pathogenicity in *Galleria mellonella*).

Results: In agreement with their *in vivo* expression, the genes PA14_28530, PA14_58030 and PA14_60480 are controlled by several regulators important for *Pa* virulence (*i.e*. Vfr, AlgU, Fur, and CzcR). Phenotypic analysis on *Pa* PA14 knockout mutants showed that inactivation of PA14_28530 stimulates biofilm formation, virulence factor production, and overall pathogenicity in *G*. *mellonella*, whereas PA14_58030 is connected with oxidative stress resistance. In contrast, PA14_60480 did not show any effect in our test conditions.

Conclusions: We partially characterize the role of two new genes whose expression is stimulated in CF airways, identifying their potential influence on phenotypes important for *Pa* adaptation to the host environment. Overall, our data indicate the importance of mining *in vivo* gene expression data to uncover novel potential determinants for *Pa* success in the host.

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Beyond Expectations: Unraveling the role of proteases produced by Cystic Fibrosis isolates of *Pseudomonas aeruginosa* in Suppressing Inflammation and Remodeling the Extracellular Matrix in 3-Dimensional Lung Epithelial Cells

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Abstract

Background

Pseudomonas aeruginosa produces an array of proteases which contribute to the disease process in people with CF (pwCF). While some studies suggest that these proteases induce inflammation, others indicate degradation of pro-inflammatory cytokines. To unravel the role of *P. aeruginosa* proteases in CF, we studied the influence of CF isolates with/without protease activity on host inflammation, cytotoxicity, and cytokine- and extracellular matrix degradation.

Methods

Fifteen *P. aeruginosa* isolates from pwCF were cultured in synthetic CF sputum medium [1]. Proteolytic activity of the bacterial supernatant was examined with Azocasein and Elastin-Congo Red assays. An organotypic 3-D lung epithelial model [2] was exposed to the bacterial supernatant to evaluate host inflammation (using IL-6/IL-8 ELISA); and integrity/cytotoxicity of the 3-D model (based on microscopy/LDH) and basement membrane (BM) (using Western Blot). In addition, degradation of IL-6, IL-8, IL-10, IL-1β, GM-CSF, MCP-1 and TNFα was evaluated by ELISA.

Results

Proteolytic activity of *P. aeruginosa* isolates varied between and within pwCF. Intriguingly, most isolates with high proteolytic activity induced an anti-inflammatory response, while isolates with low activity triggered a pro-inflammatory response. These findings could be explained by cytokine degradation, as we found that isolates with low activity showed no degradation of cytokines, and vice versa. Similarly, the BM was degraded by isolates with high proteolytic activity, resulting in host cell detachment but no cell death.

Conclusion

Our study unveils a diverse proteolytic activity of *P. aeruginosa* isolates between and within pwCF, and provides new insights into the role of proteases in the infection process.

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Pseudomonas aeruginosa Cystic Fibrosis Isolate Displays Hyperaggressiveness Towards Staphylococcus aureus

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Abstract

The bacteria *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa) frequently co-infect the lungs of patients with cystic fibrosis (pwCF) and co-infection is linked with worse clinical outcomes. Interactions between these bacteria have mainly been studied with strains isolated from different environments and how strains derived from the same environment interact is not fully understood. Our lab previously surveyed the interactions between concurrently isolated (co-isolated) pairs of Pa and Sa from lungs of pwCF and observed that co-isolated Pa and Sa generally co-existed with one another. However, they also identified outlier pairs that did not promote co-existence; Pa32-Sa46 are one such pair. Pa32 significantly inhibited Sa46 compared to the laboratory Pa strain PAO1, suggesting that Pa32 is hyperaggressive towards its co-isolated Sa. These interactions were initially characterized using standard laboratory media, however since the Pa32-Sa46 were isolated from pwCF, we tested their interactions in synthetic cystic fibrosis medium (SCFM2) to observe their interactions in a more clinically relevant environment. We observed that Pa32 aggressiveness towards Sa compared to PAO1 was even more dramatic in SCFM2. We also found that the supernatant of Pa32 cultured in SCFM2 led to a more sustained inhibition of Sa46 growth compared to the supernatant of PAO1. By treating Pa32 supernatant, we identified that heat and amylase liable exoproducts contribute to the observed phenotype. Altogether, these results show that Pa32 is hyperaggressive towards Sa46 compared to PAO1 in both laboratory media and SCFM2 and this same effect was also observed with the supernatants of these Pa strains.

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Roles of the *fro* gene operon in sodium hypochlorite-induced oxidative stress responses of *Pseudomonas aeruginosa*

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Abstract

Reactive chlorine species (RCS), such as hypochlorous acid (HOCl) within household bleach (NaOCl), are powerful antimicrobial oxidants widely utilized for disinfecting industrial, domestic, and clinical contexts. Furthermore, phagocytic immune cells generate abundant RCS to eliminate invading pathogens during infection. Although *P. aeruginosa* can withstand RCS-induced oxidative damage, bacterial RCS stress responses remain largely uncharacterized. Based on previous transcriptional analyses with bleach-treated *P. aeruginosa*, we screened HOCl-overexpressed genes for altered NaOCl susceptibility using *P. aeruginosa* transposon insertion mutant libraries to identify phenotypically strong candidate genes involved in RCS stress resistance. 24-hour growth analyses were conducted by exposing mutant strains to sublethal NaOCl treatment. In addition to mutant strains of known ROS stress response genes (including Δ*katA,* Δ*msrA,* and Δ*ohrR)*, we identified thirty-three mutants with increased NaOCl susceptibility compared to wild type strains, with mutant ΔPA3282 demonstrating a particularly strong phenotype in response to NaOCl treatment. PA3281-84 was recently deemed important for bacterial colonization of fluid flow environments and was thus renamed *froABCD* (flow-regulated operon). Upon further testing, mutants Δ*froA*, Δ*froB*, and Δ*froR* also presented delayed growth in the presence of sublethal NaOCl treatment. Our constructed deletion strain (PAO1 Δ*froABCD*) exhibited increased NaOCl susceptibility, whereas our complemented strain (PAO1 Δ*froABCD* pUCP20::*froABCD*) restored growth to wild type levels, demonstrating that *fro* plays a role in the resistance of *P. aeruginosa* to NaOCl. Ongoing research aims to elucidate *fro*'s specific roles in RCS stress responses of *P. aeruginosa*, offering insights into stress responses, antimicrobial resistance mechanisms, and intervention biotargets for *P. aeruginosa* infections.

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Discovering the metabolic impacts of the aminoglycoside resistance gene '*aph*' of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa, challenging to eradicate, intrinsically encodes sophisticated antibiotic resistance mechanisms, which understanding is crucial to develop new antimicrobial strategies. The conserved *aph* gene, always associated with the same catabolic genes, confers resistance to kanamycin-class antibiotics by encoding a unique aminoglycoside phosphotransferase (Aph). It was previously reported that *aph* expression depends on the transcriptional regulator PA4120 via 4-hydroxyphenylacetic acid, yet, for unexplained reasons.

This study investigates the involvement of Aph in different metabolic pathways, as *aph* seems to be regulated by environmental factors. To achieve this, an RNA-Seq analysis was conducted to explore the global impact of *aph* on the *P. aeruginosa* transcriptome. RNA samples from the wild type PAO1, its in-frame Δ*aph* mutant, and PAO1 overexpressing *aph* were deep-sequenced.

The transcriptional analysis revealed that *aph* significantly affects the mRNA levels of major virulence genes in *P. aeruginosa*, potentially contributing to host damage. Among 83 differentially regulated genes, 69 were found to be upregulated by the overexpression of *aph*. Most of these differentially expressed genes belong to iron-associated processes, pyoverdine biosynthesis, and biofilm formation. These findings were validated by RT-qPCR, lux-based reporter constructs, and phenotypic assays.

This study reveals that *aph* not only encodes an antibiotic resistance enzyme, but also a factor that could contribute to the pathogenicity of *P. aeruginosa*. Elucidating its metabolic functions may enable the development of specific inhibitors of this gene, thereby reducing the production of Aph-associated virulence factors and eventually enabling the use of kanamycin-class antibiotics in the treatment of *P. aeruginosa* infections.

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A Double Edged Sword: Neutrophil Elastase Promotes Aggregation and Antibiotic Tolerance of Pseudomonas aeruginosa in Chronic Lung Infection.

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Abstract

Pseudomonas aeruginosa (*Pa*) is a problematic pathogen in people with cystic fibrosis (CF). Inflammation in the CF airway is dominated by neutrophils, which release antimicrobial effectors and extracellular DNA through degranulation and neutrophil extracellular traps (NETs). Failure by neutrophils to clear pathogens such as *Pa* results in a vicious cycle of unproductive inflammation; NETs and their accompanying effectors such as neutrophil elastase (NE), are ineffective at eliminating *Pa*. In addition, antibiotic treatment failure is common for chronic *Pa* infections. We sought to understand the impact of neutrophils on antibiotic treatment outcomes. We co-cultured primary human neutrophils with *Pa* in synthetic CF sputum media, SCFM2, and assessed how the presence of neutrophils impacts antibiotic treatment outcomes. The presence of neutrophils led to increased survival to tobramycin treatment, which was driven by NETs. Antibiotic susceptibility was restored through the use of a NE inhibitor, sivelestat. Treatment with NE alone increased tolerance to tobramycin. We delineated that NE negatively impacts *Pa* motility, which promoted aggregation. Using single cell motility tracking, we found that perturbations to motility directly correlated with survival to tobramycin; increased motility resulted in higher susceptibility and decreased motility reduced susceptibility. Lastly, we identified pairs of clonal clinical isolates with differential motility and found that the isolate within each pair with the lower motility exhibited decreased susceptibility to tobramycin. These results show that motility and aggregation are tightly linked to antibiotic susceptibility in *Pa* and identified NETs and NE as potential therapeutic targets for improving antibiotic treatment outcomes.

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Investigating the role of polysaccharide lyase Smlt1473 against *Pseudomonas aeruginosa* alginate production

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Abstract

Pseudomonas aeruginosa is one of the most frequently occurring opportunistic pathogens and its robust biofilm is a major factor contributing to its widespread hospital occurrence. One of the main components in *P. aeruginosa* biofilm is the exopolysaccharide alginate, which serves as a protective barrier to the bacteria. As a critical virulence factor, alginate is an obvious target to improve treatment of multi-drug resistant *P. aeruginosa*. Our lab previously demonstrated that polysaccharide lyase, Smlt1473, from *Stenotrophomonas maltophilia* can depolymerize alginate. In this work, we characterized the activity of Smlt1473 against alginate produced by *P. aeruginosa* clinical isolates. We explored the ability of Smlt1473 to inhibit alginate secretion by growing *P. aeruginosa* in the presence of enzyme, followed by quantification of alginate production using the carbazole assay. Additionally, we used the thiobarbituric acid assay to determine the ability of Smlt1473 to degrade established *P. aeruginosa* alginate. We concluded that Smlt1473 can inhibit *P. aeruginosa* alginate secretion and degrade preformed alginate, but that efficacy is isolate dependent. Given these data, we sent polysaccharide fractions for carbohydrate analysis and NMR spectroscopy to determine if differences in total alginate levels or M/G ratio could explain observed variability in Smlt1473 efficacy. However, we found that these characteristics are not major contributors to differences in Smlt1473 efficacy. Overall, this work displays that Smlt1473 has activity against *P. aeruginosa* alginate, but that additional factors such as rate of alginate production and percentage of non-alginate secreted components need to be explored to better understand variations in Smlt1473 efficacy.

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Diversifying receptors meet chemical flexibility in *Pseudomonas aeruginosa* virulence, signalling, and host interaction systems

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Abstract

Transcriptional regulators present a tuneable response system for antimicrobial resistance, nutrient availability, metabolic reprogramming, quorum sensing, cell-cell communication, and biotransformation. Acting as receivers and transducers for distinct chemical languages, members of the LysR- and LuxR- families of transcriptional regulators play key roles in the pathophysiology of infection. However, aside from a subset of well characterised members, many of the proteins in these families remain uncharacterised. Less still is understood about the chemical and structural flexibility that underpins their evolutionary trajectory at the strain and species level.

Comparative analysis of *P. aeruginosa* available genomes revealed a wide distribution of LysR-type transcriptional regulators across the species, with core LTTRs present in >90 % of the genomes and accessory LTTRs present in <2 %. Strikingly, AmpR and PqsR/MvfR were found to be amongst the most variable in the dataset. Variant complementation of the PAO1 *pqsR*- mutant suggests a degree of structural promiscuity within the high variance LTTRs. Surprisingly, a large >600 kb region devoid of LTTR encoding genes was identified, with GO analysis revealing a distinct reduction in frequency of transcriptional control systems therein. A similar trend of promiscuous diversification was also seen following comparative genomics analysis of the LuxR-type transcriptional regulator family. Coumarins and Photopyrones, the latter recently identified as a new chemical language operating through the LuxR system, were found to have anti-biofilm and/or negative growth impacts on *P. aeruginosa* and other ESKAPEE pathogens. Together, these findings suggest that chemical flexibility matches the diversification signatures identified in these keystone regulator systems.

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Global analysis of the RNA targets of an interbacterial ADP-ribosyltransferase toxin

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Abstract

One of the ways bacteria interact with surrounding microorganisms is through the release of antimicrobial protein toxins. In recent work, we reported that the opportunistic pathogen *Pseudomonas aeruginosa* exports the antibacterial toxin RhsP2 to gain a fitness advantage over nearby competitors. RhsP2 exerts its toxicity on competitor bacteria by ADP-ribosylating essential RNA molecules. However, the range of RNAs targeted by this toxin and its RNA sequence specificity remain unknown. Here, we used enhanced UV cross-linking immunoprecipitation coupled with high-throughput sequencing (eCLIP-seq) to identify over 800 high-confidence RNA targets of RhsP2. These RNAs include mRNA, tRNA, rRNA, and sRNA species that each possess a conserved "RAAN (R=C/U; N= C/G)" motif likely involved in RhsP2 target recognition. Guided by a structure of RhsP2, we find that the toxin interacts with diverse RNA substrates via a clustered patch of positively charged residues that lie adjacent to its catalytic center. Overall, our findings suggest that substrate recognition by RhsP2 is primarily driven by electrostatic interactions between the toxin and the RNA backbone, providing a plausible molecular mechanism for how this unique antibacterial toxin targets such a diverse pool of RNAs.

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Uncovering the link between bacterial environment and the development of antibiotic resistance

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Abstract

Antibiotic resistance is traditionally associated with modulation of membrane permeability and upregulation of efflux pumps among other mechanisms, however little research focuses on the modulation of bacterial metabolism for developing resistance. Additionally, it is unclear how the metabolic environment contributes to the development and maintenance of antibiotic resistance. To address these questions, we evolved *P. aeruginosa* to three different antibiotics (ciprofloxacin, piperacillin and tobramycin) in four different media (synthetic urine media, artificial chronic wound exudate, synthetic cystic fibrosis media (SCFM) and LB). We also evolved P. aeruginosa in SCFM to the same three antibiotics at varying pH levels and in both aerobic and anaerobic conditions. *P. aeruginosa* developed different levels of resistance to each antimicrobial as a function of the specific host-relevant growth media. Varying the pH of the media caused a significant effect on the starting minimal inhibitory concentration (MIC) but had no effect on the rate at which resistance developed. Interestingly, the absence of oxygen resulted in differing starting MICs as well as a difference in the rate of development of resistance for all three drugs. This data supports the hypothesis that the metabolic environment plays a role in the development of antibiotic resistance. A better understanding of how the environment potentiates specific resistance mechanisms will better inform the course of treatment for chronic infections.

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Cyclic AMP controls production of the R-body, a virulence factor that protects Pseudomonas aeruginosa populations from phagocytic cells

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Abstract

The opportunistic pathogen *Pseudomonas aeruginosa* PA14 produces virulence factors called R-bodies, which are coiled, proteinaceous polymers that rapidly extend in acidic conditions to form 10-µm spear-like structures. R-body production is conferred by a chromosomal locus that includes the structural gene rebP1 and the regulatory gene *rcgA*. The transcription factor RcgA controls rebP1 expression and contains a binding site for cyclic AMP (cAMP)*;* we therefore sought to test whether this major cellular messenger modulates R-body production. We examined rebP1 promoter activity in samples of biofilms formed by mutant strains expressing a fluorescent reporter construct. Compared to the wild type, promoter activity was lower in mutants lacking cAMP adenylate cyclases (Δ*cyaA* or ΔcyaB), while removal of the cAMP-degrading phosphodiesterase CpdA resulted in increased activity. Interestingly, we also found that deletion of either *cyaA* or cyaB in the Δ*cpdA* background produced intermediate levels of expression (i.e. between those of the wild type and ΔcpdA), while deletion of both cyclases in Δ*cpdA* yielded wild-type levels of expression. These results suggest that both CyaA and CyaB play roles in modulating rebP1 expression. In addition to this work, we are testing the role of R-bodies in PA14 protection from the protist *Dictyostelium discoideum*, which also employs cAMP as a regulator of gene expression and as a signal that controls developmental transitions. Our findings indicate that R-bodies limit grazing by Dictyostelium discoideum on PA14-containing bacterial lawns. Together, these observations contribute to understanding of cAMP-mediated R-body production and help determine the role of R-bodies in killing phagocytes.

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Role of *Pseudomonas aeruginosa* virulence factor ExoY during lung cell infection

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Abstract

P. aeruginosa (*P. a.*) represents a major cause of lung infection. The expression of its type III secretion system (T3SS) is linked to high toxicity and poor clinical outcomes in infected patients. ExoY, one of the effectors secreted in cells by the T3SS, produces supra-physiological amounts of cyclic nucleotide monophosphates (cNMPs), with a preference for cGMP. cNMPs are potent eukaryotic second messengers involved in numerous cellular processes. While other T3SS effectors are well characterized, ExoY role requires further elucidation.

This project aims to study the impact of ExoY on cell physiology during *P. a.* pulmonary infection. To identify the full range of molecular pathways modulated by ExoY, we performed an RNAseq analysis on primary bronchial epithelial cells cultivated at the air/liquid interface. This model is more complex than immersed cell lines and mimics a differentiated *in vivo*-like bronchial epithelium.

Primary cells from 4 donors were infected by different *P. a.* mutants secreting or not an active ExoY, and mRNAs were sequenced using NovaSeq X (Illumina). Genes differentially expressed between our conditions revealed distinct transcriptional signatures. This result supports the idea of a significant and specific effect of ExoY activity in cells. We are currently exploring functional enrichment to identify relevant pathways for further investigation in our cell model.

We will better understand how ExoY hijacks eukaryotic cellular pathways and manipulates the host response to promote *P. a.* survival.

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The *Pseudomonas aeruginosa* PrrF sRNAs contribute to biofilm formation at body temperature

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Abstract

Pseudomonas aeruginosa (Pa) is an opportunistic pathogen that causes chronic respiratory infections in individuals with cystic fibrosis (CF). Biofilm formation during CF infection results in increased antimicrobial tolerance, complicating treatment. Iron is a critical nutrient for *Pa* virulence and biofilm formation, and the PrrF sRNAs play a critical role during iron starvation by downregulating the production of nonessential iron cofactored proteins. Previous flow cell biofilm studies performed at 25°C implied the PrrF sRNAs are not required for biofilm formation. As Pa is both a human and an environmental pathogen, we hypothesized that there are differences in gene regulation and iron homeostasis at 25°C and 37°C. To test this, we optimized a flow cell biofilm model for growth at 37°C, and imaged *Pa* biofilms using confocal scanning laser microscopy. Using a PAO1 transcriptional reporter strain for the PrrF sRNAs we determined that at 37°C the PrrF sRNAs are highly expressed in flow cell biofilms. We further found that a PAO1 Δ*prrF* mutant is deficient for biofilm growth at 37°C, while showing no defect at 25°C. Lastly, we show through electron microscopy that the PAO1ΔprrF strain is defective for type IV pili formation at 37°C. These results indicate a temperature dependent requirement for PrrF sRNAs during biofilm formation at body temperature.

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Interactions between Conserved Proteins in *Pseudomonas aeruginosa* Biofilms can be Inhibited to Reduce Antimicrobial Tolerance

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Abstract

Biofilms are significantly more resilient to killing by antimicrobials than planktonic cells, a phenomenon referred to as biofilm antimicrobial tolerance. Recent evidence suggests biofilm antimicrobial tolerance by *Pseudomonas aeruginosa* to require several conserved, uncharacterized proteins that form an interactome, namely SagS, PA0918, PA2146, PA2184, and PA3915. We therefore asked whether the interactions between these proteins contribute to biofilm antimicrobial tolerance by *P. aeruginosa*. We first identified peptides capable of interfering with protein-protein interactions (PPIs) using a modified bacterial adenylate cyclase two-hybrid system. Interfering peptides of interest were then overproduced in *P. aeruginosa* and their effect on biofilm architecture and antibiotic susceptibility assessed using confocal laser scanning microscopy and susceptibility assays. Thus far, we have identified several peptides that can inhibit the interaction between the sensor kinase, SagS, and PA0918, a predicted oxidoreductase that may attenuate oxidative stress. These peptides impeded biofilm formation, discernible by the appearance of thin, unstructured biofilms that were characterized by reduced biofilm biomass and maximum thickness relative to wild-type biofilms. Moreover, overproduction of interfering peptides rendered biofilms highly susceptible to tobramycin. The peptides had no effect on the susceptibility of planktonic *P. aeruginosa* cells. Our findings indicate PPIs to play an important role in biofilm formation and antimicrobial tolerance by *P. aeruginosa*, with the PPI by SagS and PA0918 likely serving as a possible target for anti-biofilm therapeutics. Moreover, the molecular mimicry techniques used here to inhibit PPIs by conserved proteins may be applicable as alternative treatment methods for other biofilm-forming pathogens.

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Development of Biosensors for Screening *Trans*-translation Inhibitors in *Pseudomonas aeruginosa.*

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Abstract

The World Health Organization has identified *Pseudomonas aeruginosa* as one of the bacteria for which there is a critical need to develop new antibiotics (1). In this bacterium, the deletion of the ribosome rescue pathway, *trans*-translation, increased antibiotic susceptibility and decreased tolerance (2). Consequently, *trans*-translation emerges as an attractive target for novel antimicrobial development (3,4). We present the development of a system designed to facilitate simple and reproducible in vivo screening of antimicrobial compounds specifically targeting trans-translation in *P. aeruginosa*. To achieve this, the *hemH* gene has been engineered for conditional degradation of the HemH-ferrochelatase within the cell. HemH catalyzes the conversion of red fluorescent protoporphyrin-IX to heme. The system operates such that, when the *trans*-translation system is active, protoporphyrin-IX accumulates, generating a detectable signal. Conversely, inhibition of *trans*-translation leads to HemH persistence within the cell, resulting in a reduction of the signal. This system offers multiple benefits: measurements are conducted on whole cells, the system does not confer antibiotic resistance, and it is extendable to other emmerging pathogenic bacteria. Armed with this hemH-based detection system, we aim to discover molecules capable of inhibiting *trans*-translation to enhance the susceptibility of *P. aeruginosa* to clinically used antibiotics.

Grants: “Vaincre la mucoviscidose” ; “Association Gregory Lemarchal”

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Optimized stress for pyocyanin production in *Pseudomonas aeruginosa*

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Abstract

Nanomaterials may stimulate secondary metabolism in bacteria by triggering stress response. The main question of our work was whether it is possible to optimize this effect to produce more pyocyanin in *Pseudomonas aeruginosa* and what its physiological state was during this process.

The concentration of zinc oxide nanoparticles (ZnO NPs) and process temperature have been optimized using the Design of Experiment (DoE) approach. The physiological state of cells was controlled by quantitative RT-PCR, confocal microscopy, flow cytometry, fluorimetry, and spectrophotometry.

Optimized conditions were 6.06 µg/mL of ZnO NPs and 32.6°C, while the production yielded 44.16 mg/mL of pyocyanin, with the stimulation ranging from 8.71% to 16.5%. The stimulation was also pronounced in scaled-up conditions (1L). The studies have shown a range of changes in bacterial physiology that included overexpression of genes coding efflux pump components (*czc*A and *czc*D), increased activity of superoxide dismutase, altered membrane stability, as well as increased eDNA content and biofilm formation. Pyoverdin production in the optimized process was transversed from higher levels for the first eight hours to a lower level than the control culture after the 10^{th} hour.

We have shown that the optimized concentration of zinc oxide nanoparticles successfully increases the pyocyanin production in *P. aeruginosa*. The traces of Zn ions in biomass, upregulation of efflux pumps, and increased superoxide dismutase activity suggested the simultaneous activation of stress response. Interestingly, the optimized process was effective only with nanoparticulate ZnO and could be scaled up to 1L.

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CbrA activity promotes PhoB-mediated virulence and fungal antagonism in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is co-isolated with the fungus *Candida albicans* in many people with cystic fibrosis (CF) and co-infection is associated with worse lung function. Fungal killing by *P. aeruginosa* requires PhoB, the transcription factor associated with the low inorganic phosphate (Pi) response, even at physiological Pi concentrations. PhoB induces expression of phospholipases and toxic phenazines which damage both fungal and host cells. We found that clinical isolates with loss-of-function mutations in *lasR* have higher levels of PhoB activity and increased antagonism toward *C. albicans*. To quantify PhoB activity in *P. aeruginosa* we utilized both transcriptomic and enzymatic analyses. To assess antagonism toward *C. albicans* we quantified the production of an anti-fungal phenazine and fungal killing with flow cytometry. Lastly, we used genetic screens to identify factors that modulate PhoB activity in co-cultures. We found that activity of the CbrAB two-component system induces PhoB activity at physiological Pi concentrations and CbrAB is required for PhoB-mediated *C. albicans* antagonism. Isolates with loss-of-function mutations in *lasR* have even higher levels of PhoB activity, likely due to increased CbrAB activity, which leads to increased virulence factor production. We also found evidence for PhoB activity in RNA from clinical sputum samples and laboratory *P. aeruginosa* grown ex vivo in CF sputum. To complement previous studies that have shown PhoB can induce the activity of RhlR, a quorum sensing regulator, this work shows that quorum sensing also modulates PhoB activity at physiological Pi concentrations. Further, these relationships appear to be important in microbe-microbe and microbe-host interactions.

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Establishing a straightforward I-SceI mediated recombination one plasmid system for efficient genome editing in *P. putida* KT2440

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Abstract

Pseudomonas putida has become an increasingly important chassis for the production of valuable bioproducts. This development is not at least due to the ever-improving genetic toolbox, including gene and genome editing techniques. Here, we present a novel, one plasmid design of a key genetic tool, the pEMG/pSW system, guaranteeing one engineering cycle to be finalized in three days. The pEMG/pSW system proved in the last decade to be valuable for targeted genome engineering in *Pseudomonas*, as it enables the deletion of large regions of the genome, the integration of heterologous gene clusters or targeted generation of point mutations. Here, to expedite genetic engineering, two alternative plasmids were constructed: 1) the *sacB* gene from *Bacillus subtilis* was integrated into the I-SceI expressing plasmid pSW-2 as counterselection marker to accelerated plasmid curing; 2) double strand break introducing gene I-SceI and SacB counterselection marker were integrated into the backbone of the original pEMG vector, named pEMG-RIS. The single plasmid of pEMG-RIS allows rapid genome editing despite the low transcriptional activity of a single copy of the I-SceI encoding gene. Here, the usability of the pEMG-RIS is shown in *P. putida* KT2440 by integrating an expression cassette including a *msfGFP* gene in three days. In addition, a large fragment of almost 16 kb was also integrated. In summary, an updated pEMG/pSW genome editing system is presented that allows efficient and rapid genome editing in *P. putida*.

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How to control pyoverdine synthesis in *Pseudomonas* by light

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Abstract

Pyoverdine (PVD) is a fluorescent siderophore found in various *Pseudomonas* species. It plays a pivotal role in microbial social dynamics, contributes as a virulence factor to the pathogenesis of *Pseudomonas aeruginosa*, and is of biotechnological relevance, e.g., due to its plant growth-promoting effects or applicability for soil bioremediation.

Despite extensive studies on pyoverdine-based interactions of *Pseudomonas* with other microbes, plants and hosts, the spatial and temporal aspects of these processes are largely unexplored. We therefore developed a strategy to non-invasively reprogram PVD production in *Pseudomonas putida* with high spatiotemporal resolution by light. To this end, we first developed and applied a versatile optogenetic toolbox in *P. putida* as a proof of concept. By using different caged inducers and photoreceptors as photosensitive switches, precise, gradual, and dynamic control of reporter gene expression was achieved. Next, the light switches were applied to implement a strict, iron-independent control over PVD biosynthesis. Therefore, the expression of PfrI - an alternative sigma factor responsible for concerted transcription of all PVD biosynthesis genes in *P. putida* – was placed under the control of the light-responsive regulators.

To demonstrate the transferability of the optogenetic toolbox to other *Pseudomonas* species, we further applied these systems in the opportunistic human pathogen *Pseudomonas aeruginosa*, demonstrating the functional similarity of PfrI to the homologous sigma factor PvdS from *P. aeruginosa*. This optogenetic strategy thus enables a targeted and dynamic modification of *cellular behavior* and may provide new insights into PVD-dependent microbial social interactions, plant growth-promotion and virulence in the near future.

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Characterization of PA4880: A novel member of the ferritin super-family

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Abstract

Iron is critical for *Pseudomonas aeruginosa's* survival in the human host but can be toxic due to its participation in Fenton chemistry, resulting in the production of reactive oxygen species. Thus, iron homeostasis must be tightly regulated. The ferritin super-family consists of three distinct types of proteins involved in iron storage; one member is PA4880, a gene highly conserved in the pseudomonads that is annotated as a putative bacterioferritin. This annotation is due to the conserved bacterioferritin (Bfr) ferroxidase center residues and heme-coordinating methionine. However, bioinformatic and structural studies indicate PA4880 encodes a Dps protein with a Bfr-type ferroxidase center. Expression studies demonstrate that PA4880 is induced in high iron conditions via the PrrF small regulatory sRNAs, which are predicted to pair with and destabilize the PA4880 mRNA in iron-limiting conditions. We also observed PrrF-independent iron regulation of PA4880, and we found that hydrogen peroxide induced PA4880 expression in the absence of the PrrF sRNAs. Based on these findings, we hypothesize that PA4880 is a novel bacterioferritin-like Dps protein responsive to iron-induced oxidative stress. Combined, these studies are expected to provide insight on the role of this novel ferritin-like protein in the *Pseudomonas* stress response and pathogenesis.

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Pseudomonas aeruginosa novel operon *mgoRD* increases survival in the presence of methylglyoxal.

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Abstract

Pseudomonas aeruginosa often encounters methylglyoxal (MG), a highly reactive electrophile that is produced by other microorganisms and host cells. High levels of MG have been associated with many diseases including sepsis and cystic fibrosis (CF). We found that passage of *P. aeruginosa* in media with MG selected for strains with gain-of-function mutations in PA14_56360 which encodes an uncharacterized protein that we named MgoR. The *mgoR* gene is in an operon with a gene encoding a putative GSH-independent MG detoxification enzyme PA14_56370 (denoted as MgoD). In these studies, we use *in vitro* genetic and biochemical methods to characterize the function of *mgoR* and *mgoD*, and the impacts of evolved mutations. We use available genome sequences and transcriptomes to highlight differences in *mgoRD* across isolates. We found that *mgoR* and *mgoD* null mutants are much more sensitive to MG compared to their parent strains. Furthermore, *mgoR* and *mgoD* are induced by MG and expression levels of *mgoR* and *mgoD* vary across isolates. We propose that *mgoD* encodes a novel mechanism for MG detoxification or MG damage repair and that evolved *mgoR* alleles lead to increased *mgoD* expression. We found that evolved *mgoR* alleles can rescue MG-sensitivity in strains such as LasR loss-of-function (LasR-) mutants which are often more sensitive to MG than their LasR+ counterparts due to differences in metabolism. Studying *P. aeruginosa* evolution in the context of MG will contribute to our understanding of adaptation in the context of infections.

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Pseudomonas aeruginosa Urinary Tract Infections: An Understudied Site of Infection

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Abstract

Pseudomonas aeruginosa frequently causes severe infections in immunocompromised and hospitalized patients. The infectious biology of *P. aeruginosa* has been well-characterized in infections of multiple anatomic sites, including the respiratory system, skin and wound infections, and ophthalmic infections. The pathogenesis of *P. aeruginosa* in causing urinary tract infections (UTIs) has not received adequate research attention. We reviewed laboratory records at our medical center to determine the frequency of *P. aeruginosa* UTIs. We performed whole genome sequencing on a subset of *P. aeruginosa* UTI isolates. We compared the genomes of *P. aeruginosa* UTI and respiratory isolates. We utilized bladder epithelial cell tissue culture models and mouse models of UTI to interrogate the interactions of *P. aeruginosa* with the urinary tract. Over a five-year period, *P. aeruginosa* UTIs were as common as skin/wound infections and more common than lower respiratory infections at our medical center. Comparative genomics and genome-wide association testing identified the cytochrome c oxidase (*ccoN*) of *P. aeruginosa* as a genomic region with different sequences between urinary and respiratory isolates. Using tissue culture models, we identified that *P. aeruginosa* can invade bladder epithelial cells. In a mouse model of UTI, different mouse backgrounds are differentially susceptible to *P. aeruginosa* infection. The urinary tract is a common site of *P. aeruginosa* infection. *Pseudomonas can* invade urothelial cells. Ongoing research will delineate the role of bacterial oxygen utilization and intracellular epithelial infection to *P. aeruginosa* UTI pathogenesis.

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Does a Leopard change its spots? *Pseudomonas aeruginosa* in cystic fibrosis after CFTR modulation

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Abstract

Background. *Pseudomonas aeruginosa* is a multidrug-resistant pathogen causing severe respiratory infections in people with cystic fibrosis (pwCF). CF transmembrane conductance regulator (CFTR) modulators have been developed that partially correct the defective anion channel driving CF disease. Despite the profound clinical benefits, studies in treated adults have demonstrated that while *P. aeruginosa* sputum load can decrease, chronic infection persists. Here, we investigate how *P. aeruginosa* in pwCF may change in the CFTR modulated lung environment.

Methods. *P. aeruginosa* strains (n=105) were isolated on selective media from the sputum of 11 chronically colonized pwCF at baseline and up to 21 months posttreatment with Elexacaftor-Tezacaftor-Ivacaftor (n=8) or Tezacaftor-Ivacaftor (n=3). Comparative genomics and phenotypic characterization were performed on isolates before and after CFTR treatment.

Results. Clonal lineages of *P. aeruginosa* persisted after treatment with no evidence of displacement by alternative strains. These strains accumulated between 19 to 3400 modifying mutations after modulator commencement and these mutations were more frequently observed in previously described pathoadaptive genes. This included 37 genes that were commonly mutated across lineages from different participants inferring positive selection in the CFTR modulated lung environment. Despite the potential impact of these mutations, chronic *P. aeruginosa* phenotypes such as mucoid morphology and lack of O-antigen expression were sustained, and strains remained just as resistant to clinically relevant antibiotics.

Conclusion. *P. aeruginosa* lineages persist with the same chronic phenotypes that may prove just as difficult to clinically manage in the future especially in individuals with advanced lung disease and irreversible lung damage.

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Functional and biochemical characterization of an extendable, proteinaceous spear in Pseudomonas aeruginosa strain PA14

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Abstract

Diverse microbial pathogens persist in hosts by forming biofilms, which are matrix-encased aggregates of cells. Biofilms of *Pseudomonas aeruginosa* PA14 produce R bodies, hyperstable protein polymers shown to contribute to virulence in a nematode chronic infection model. PA14 harbors genes required for R body production, homologous to structural genes for R bodies produced by unrelated proteobacteria, in a "reb" cluster that includes additional uncharacterized genes that are conserved across pseudomonads. Although previous work has shed light onto the large-scale structure of PA14 R bodies, a high resolution structure and a minimal system for R body production have remained elusive. We have obtained R bodies via native purification from a modified PA14 strain, which has enabled in vitro biochemical assays to study R body dynamics and assembly. We have shown that PA14 R bodies can extend–in a pH-dependent manner–from a coiled state into a needle-like state, consistent with previous work and the model that R bodies extend after bacteria are engulfed by host cells and subjected to the conditions of the phagolysosome. In addition, we have employed fluorescent reporters to study the expression of the R body complex in PA14 and identified a putative, novel assembly factor encoded by the reb cluster gene PA14_27650. Deletion of *PA14_27650* results in PA14 R bodies with altered sedimentation coefficients and decreases purification yield by approximately one half. Together, these findings provide insight into the production and function of a virulence factor that may contribute to the survival of P. aeruginosa populations within hosts.

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Identifying the *Pseudomonas aeruginosa* cell-surface proteins that facilitate catheter colonization

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Abstract

Catheter-associated urinary tract infections (CAUTI) are one of the most common healthcare-associated infections. The foreign surface of the catheter allows bacteria to form biofilms and evade bladder defenses including urination and cellular immunity. The environmental bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen that causes about 10% of CAUTI. We have previously used a murine model of CAUTI which involves transurethral implanting of a catheter tubing into the bladder and found that the strain PA14 requires its type III secretion system to cause severe disease. Surprisingly, mutants lacking type III secretion system were equally capable of colonizing the catheter tubing over 14 post-infection days. These results indicate that colonization is an independent process from the ability to cause severe disease. Because chronic colonization of bacteria on the catheter is nonetheless associated with poor clinical outcomes, understanding the mechanism of attachment can advance the development of strategies to reduce CAUTI. To investigate how *P. aeruginosa* attaches to the catheter surface, PA14 strains lacking specific biofilm-associated factors including Pel polysaccharide, type IV pili, flagella, and the five classes of chaperone/usher pathway pili cupA – cupE were generated. Data will be presented on the ability of these mutants to colonize catheters and form biofilms in vitro.

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Modulator Drugs and Cystic Fibrosis *Pseudomonas aeruginosa* (Pa) Infections: A new era for Pseudomonas Infection Research

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Abstract

Cystic fibrosis (CF) infections are among the most-studied and important Pa infections. A revolutionary drug, elexacaftor/tezacaftor/ivacaftor (ETI) corrects the physiological defect causing CF, and understanding its effects on Pa has major implications for patient's health and Pa research. In addition, its effects will shed light on how human infections respond when infection-initiating host-defects are corrected.

This presentation reports results of 2 major studies. The first examined 236 subjects to define the natural history of Pa infections and found sputum density of Pa decreased ~100-fold after 1 month, with little further change through 3 yrs. Notably, most baseline-infected subjects remained infected, and Pa remained PCR-detectable in the few subjects becoming culture-negative. Sequencing found increased sputum bacterial diversity and shifts in bacterial composition that were caused by decreases in a small group of pathogens.

The second study used bronchoscopy to sample the 2 most and 3 least-damaged lung regions in subjects before and 1.5 years after ETI. In subjects remaining infected, Pa persisted in all high and low-damage areas which contradicts the hypothesis that infection persists predominantly in high-damage segments. In addition, while inflammation generally decreased, the coefficient of determination (R^2) between *Pa* density and inflammation markers markedly increased after ETI, indicating that that Pa better accounts for inflammation post- vs. pre-ETI. Moreover, ETI did not diminish the amount of inflammation induced by Pa on a per-cell basis.

These data show that correcting the basic CF defect rapidly reduces Pa, but most patients remain infected, and infection remains associated with lung-damaging inflammation.

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Strain Specific Outcomes of *Pseudomonas aeruginosa* Catheter-associated Urinary Tract Infections

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Abstract

Hospitalized patients with indwelling catheters face an increased risk of developing catheter-associated urinary tract infections (CAUTI). Catheters provide a surface for bacterial colonization, leading to either symptomatic infections or asymptomatic bacteriuria. In a previous study using an outbred murine CAUTI model with the PA14 strain, we identified two distinct phases: an acute phase, with host morbidity and mortality within the first week, and a chronic phase, with asymptomatic colonization in the second week. Host mortality is primarily driven by ExoU activity of the type III secretion system (T3SS) in PA14. However, in Pseudomonads, the T3SS effectors *exoU* and *exoS* are largely mutually exclusive. To determine infection outcomes from ExoS activity, we used the murine CAUTI model with the PAK strain. Mice infected with PAK had similar survival rates to those infected with PA14. However, while mortality in PA14-infected mice occurred rapidly within the first three days, in PAK-infected mice, it occurred steadily over a 10-day period. Moreover, mutants lacking either T3SS or type II secretion system (T2SS) did not induce acute outcomes in PAK, suggesting that in *exoS*-containing strains, both T2SS and T3SS contribute towards host mortality. To evaluate the applicability of these findings to clinical strains isolated from CAUTI patients, we tested three strains encoding *exoS* and five strains encoding *exoU* in a murine CAUTI model. We observed similar outcomes with their respective laboratory strains; however, some isolate-specific differences were noted. This study highlights the importance of the T3SS in all strains and the T2SS in strains with ExoS.

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CarP, a Novel Ca2+ Sensing Regulator of Metabolism and Virulence in Pseudomonas aeruginosa

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Mackenzie Hull

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Abstract

*Pseudomonas aeruginosa (*Pa) is an opportunistic human pathogen that possesses an incredible capacity to adapt to its environment, including the lungs of patients with the genetic disorder, cystic fibrosis (CF). Our lab has shown that elevated Ca^{2+}, a characteristic of the CF nasal cavities, positively regulates virulence factor production in Pa. We have identified a novel component of this signaling pathway, CarP, which mediates Ca^{2+} regulation of pyocyanin production, protection against oxidative stress, and modulates *Pa* interactions with host immune cells. *In silico* analyses predicted that CarP possess a C-terminal periplasmic β-propeller domain, a single transmembrane domain, and a putative cytoplasmic N-terminal DNA-binding (DB) domain. To understand the molecular function of CarP, a global untargeted metabolomics approach was used to determine specific CarP-dependent metabolic pathways. We observed a global dysregulation of functionally diverse pathways in Δ*carP* in response to Ca^{2+}, suggesting that CarP possesses a regulatory function. This was tested by evaluating CarP-dependent gene regulation via global transcriptomics using the clinically relevant synthetic cystic fibrosis media (SCFM). The expression of approximately 50% of Ca^{2+}-regulated genes was significantly altered in Δ*carP*. We have hypothesized that the putative cytoplasmic DB domain is integral to the function of CarP, and to test this, we have generated a *carP* complementing construct that is devoid of the putative DB domain. This strain, WT, and Δ*carP* are currently being tested to determine the role of the DB domain in CarP-dependent phenotypes. Furthermore, the potential of CarP binding protein partners is being determined by a co-immunoprecipitation approach.

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IGLPR01, a *Pseudomonas* isolate from oil spill achieves an enhanced RL and PYO production by mutation of *rpoS* using CRISPR/Cas9 genetic engineering

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Abstract

Fossil fuels represent an important source of global economic development. Moreover, oil handling has been a climatic concern because of the historic accidental spills reported around the world. To mitigate these events, techniques as containment, skimming, burning and dispersion have been applied. Dispersion consists of surfactants that generate small hydrocarbon droplets, making it more prone for degradation by microorganisms. Among the surfactants, those coming from natural sources are relevant, such as rhamnolipids (RL) synthesized by *Pseudomonas*. In our workgroup, a *Pseudomonas* strain was isolated from an oil spill and sequenced using nanopore and Illumina approaches to get a high-quality genome. Upon genomic analysis, we found that strain IGLPR01 belongs to group II on the *Pseudomonas* clade. The capacity of IGLPR01 to produce RL was evaluated using the acid precipitation method. Additionally, the generation of Pyocyanin (PYO) was checked because of its role as an autoinducer in cell signaling processes to enhance RL production. In that regard, CRISPR/Cas9 system combined with APOBEC was used on IGLPR01 to introduce a stop codon in the *rpoS* gene, due to its function in downregulation of PYO synthesis. The IGLPR01Δ*rpoS* obtained was also evaluated to verify the enhancement of PYO and RL synthesis through its quantification and emulsification in a gasoline mix. The results assign the effectiveness of the CRISPR/CAS9 system used to mutate environmental strains since we got a IGLPR01 mutant in *rpoS* that indeed has an increased RL and PYO synthesis in comparison with the wildtype strain, reinforcing its importance for bioremediation applications.

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Electromagnetic field as a modulator of pyocyanin production

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Abstract

Production of pyocyanin in *Pseudomonas aeruginosa* can be modulated by exposure to stressors such as electromagnetic fields. Here, we present the influence of the electromagnetic field on pyocyanin production and the physiological state of the exposed cells under optimized conditions.

The optimization was conducted with process temperature, time of exposure, and intensity of the magnetic field as input factors and pyocyanin production as the output factor. Such optimization was conducted for the rotating and static electromagnetic field. Then, the optimized and control cultures were incubated with DCFH-DA to verify if the cells were exposed to oxidative stress.

The results revealed a diversified effect of electromagnetic field on pyocyanin production as we observed both stimulation and inhibition of pigment production. The optimal process temperature was 32℃. In the case of rotating and static (positively polarized) electromagnetic fields, the optimal time of exposure was 57 minutes, and the intensity of 41.82 hertz or volts, respectively. However, the static electromagnetic field with negative polarisation was characterized by the optimal intensity of 25.00 volts. Moreover, in some cases, the cultures exposed to electromagnetic fields were characterized by higher ROS reads.

To conclude, we showed the double-edged effect of electromagnetic field exposure on pyocyanin production. Moreover, the type and intensity of electromagnetic fields are important parameters when steering the culture toward pyocyanin production.

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Understanding the Role of Actomyosin Regulators During *P. aeruginosa* Infection in *C. elegans* Intestinal Epithelium

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Abstract

During pathogenic infections, interactions between the host and the pathogen can occur either directly through physical adherence or indirectly through secreted molecules. This dynamic determines the trajectory of the pathogenesis. The host employs various strategies to counteract the infection, and the structural integrity of tissues serves as primary mechanical barrier to pathogen assaults. One notable example is the epithelial cells that line various organ systems; their shape plays a pivotal role in defense against pathogens. Any disruption to the epithelial lining triggers the cell's autonomous immunity and leads to the clearance of the pathogen. The *C. elegans* intestinal epithelium, lacking specialized immune cells, provides an intriguing model system to investigate the role of epithelial cells in pathogenesis. *P. aeruginosa,* a pathogen known for affecting human epithelial linings, behaves differently in *C. elegans*, where it acts as an extracellular pathogen residing in the lumen. Our study focused on understanding the impact of *P. aeruginosa* infection on the architecture of the intestinal epithelium. We found that *P. aeruginosa* infection causes deformation in the *C. elegans* intestine, particularly affecting all apical regions of the tissue. Since the cell cortex is the fundamental component of the epithelial support structure, we conducted a candidate RNAi to identify key contributors to this deformation. We identified the GTPase CDC-42 as an upstream regulator inducing intestinal deformation following infection. Our work sheds light on the crucial physiological role of tissue architecture in the context of pathogenesis and provides a foundation for a deeper understanding of the underlying mechanisms.

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Structure-Function Analysis of FlhF, an SRP GTPase, involved in polar flagellation in *Pseudomonas aeruginosa*

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Abstract

P. aeruginosa is an opportunistic human pathogen with a single polar flagellum. Flagella are present in specific patterns on the bacterial surfaces, and the number and placement are species-specific. Flagella are multifunctional and essential for virulence, pathogenesis, colonization, and biofilm formation. The regulatory mechanisms that enable bacteria to maintain the location of flagella post-cell division reproducibly remain elusive. Signal Recognition Particle (SRP) GTPases serve as molecular switches implicated in localizing factors within the bacteria and eukaryotes. FlhF is an SRP-like GTPase found in polarly flagellated bacteria. It is a 429 amino acid long P-loop NTPase essential for flagella placement, assembly, and function in *Pseudomonas aeruginosa*. Knockouts of *flhf* cause mislocalized or aflagellate phenotypes in different bacterial species, leading to compromised motility. We have structurally and functionally characterized FlhF from *P. aeruginosa*. The unique insights obtained from this analysis will be presented. Findings from this study will be highly relevant to other pathogenic monoflagellated bacteria.

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Does PlpD play a role in *Pseudomonas aeruginosa* infections?

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Abstract

PlpD is a membrane-bound phospholipase A_1 in *Pseudomonas aeruginosa* which, due to its structural similarity, was classified as the archetype of a novel subclass of type 5 secretion system and thought to be secreted onto the bacterial surface. Recent literature has challenged surface secretion model and has instead proposed PlpD as a membrane regulator. Despite this recent interest in PlpD, the question of its role in bacterial virulence remains unexplored. Here, we further characterised the enzymatic function of PlpD and investigated its potential role as a virulence factor of *P. aeruginosa* infections.

Using PA14 WT, Δ*plpD*, and complemented strains, as well as purified PlpD, we investigated the impact of PlpD on bacterial membrane composition and virulence phenotypes. Mass spectrometry identified clear differences in the lipid composition of PA14 WT and Δ*plpD* membranes. Although various cell membrane integrity assays did not show a significant difference between PA14 WT and Δ*plpD* strains, we did identify a significant difference in sensitivity to human serum. Additionally, the Δ*plpD* mutant had a small but significant attenuation in virulence in a murine acute respiratory infection model. Finally, significant titres of anti-PlpD antibodies were found in the serum of 24% and 14% of patients with chronic or acute *P. aeruginosa* infections (n=171).

These findings implicate PlpD as a regulator in the composition of the bacterial membrane. We have also found that it plays a significant role in serum resistance and bacterial virulence during infection, and our Immunological data suggests it is expressed and immunogenic during human infections.

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NxtGenWood: Converting wood-based phenols to value added products

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Abstract

Lignin, one of the most abundant natural polymers on the planet, is also one of the most underutilized due to its recalcitrant nature. Our project aims to investigate novel ways to valorise lignin breakdown products into value-added products utilizing bacterial cultures as biocatalysts. In order to achieve this, different bacterial species were selected from the literature due to their reported ability to use lignin derived phenols. The bacteria were tested for growth on the main phenolic acids present in lignin. The results from these experiments showed that *Pseudomonas* species showed the most promise for growth on phenolic acids.

Our next objective was to obtain a candidate that showed good PHA accumulation from the mentioned phenolic acids. We compared the different *Pseudomonas* species for their ability to accumulate the biodegradable polymer polyhydroxyalkanoate (PHA). Out of the selected candidates we decided to continue with *P. putida* KT2440 as it accumulated around 33 % of the cell dry weight as PHA and is a promising model for genetic modification. Using this bacterium, we tested different carbon substrate compositions in batch and continuous fermentation. While cells grow well on the phenolic acids their capacity to produce PHA from these substrates is suboptimal. Supplementation of the phenolic substrate with low concentrations of sodium octanoate increased the PHA amount in continuous culture threefold while maintaining phenol consumption (50 % of the CDW). Current experiments are focussed on adding other low value waste streams to the current substrate feed to increase growth and PHA synthesis.

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Building a Lignin Cell Factory: Insights from *Pseudomonas putida*

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Abstract

Lignin, a complex aromatic polymer, poses a significant challenge to its efficient degradation, hindering the utilization of biomass for many industrial applications. Bacterial degradation of lignin may offer a promising solution to this challenge. This project focuses on elucidating the functionality of secreted oxidative enzymes from *Pseudomonas putida* involved in lignin degradation and implementing *P. putida* as a microbial cell factory for the efficient and sustainable utilization of lignin.

Using CRISPR/Cas9 and CRISPR/Cas3 systems, the putative lignin-degrading versatile peroxidase VP and dye-decolorizing peroxidase DyP genes were individually knocked out from *P. putida* strains KT2440 and S12. Lignin oxidation activity of these knockout strains was analyzed through a colorimetric assay for low molecular weight phenolic products, following incubation of the strains with lignin. In parallel, 2-Phenoxyacetophenone (2-PAP), a model compound for the abundant *β*-O-4 linkage in lignin, was used in a large screening set-up to identify novel enzymes that exhibit specific ether bond-cleaving activity involved in targeted lignin degradation.

To initiate an efficient cell factory approach towards lignin degradation and utilization with *P. putida*, several endogenous and heterologous lignin oxidizing enzymes were expressed in *P. putida* strains KT2440 and S12.

Overall, this project strives to advance our understanding of specific enzymatic lignin bioconversion with *P. putida* offering a relatively low-cost alternative to current lignin utilization strategies and aligning with environmentally friendly practices, marking a significant step towards sustainable lignin valorization in industry.

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Rapid genome modifications and characterized genomic “landing pads” for *P. putida*

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Abstract

Designing a microbial chassis involves integrating and expressing heterologous genes, either genomically integrated or with a plasmid-based expression system. Despite its time-consuming nature and the limited number of well-characterized integration sites in microbes like *P. putida* KT2440, genomic integration is often preferred over plasmid usage.

First, for rapid genome modification, we modified pEMG by integrating an inducible I-SceI gene and a constitutively expressed GFP for positive/negative selection. Fluorescent colonies were inoculated and induced in liquid media before plating. Clones showing no fluorescence were tested for the modification by PCR. The modified pEMG vector enables the creation of genome modifications in three days without replicating or curing a plasmid, broadening the range of microorganisms we can modify beyond *Pseudomonas*.

Second, for identifying landing pads that enable reliable expression from genome, we analyzed RNA-Seq data for similar expressed regions in *P. putida* KT2440. These sites were characterized by integrating expression constructs and cultivation in a BioLector. Identified integration sites showed a low coefficient of variation across different cultivation conditions, but overall strength varied between the sites. These results sustain our idea of using publicly available RNA-Seq data to identify genomic landing pads. Expression control through the choice of promoter and integration site, enables new heterologous expression concepts for chassis design.

These tools presented enhance *Pseudomonas* synthetic biology by providing a rapid modification system and new integration sites distributed over the genome. The aim is to support the *Pseudomonas* community by generating modified strains supporting the establishment of a bioeconomy.

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How inflammatory responses shape loss of quorum sensing in *Pseudomonas aeruginosa* populations

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Abstract

Pseudomonas aeruginosa is well-recognized for its ability to undergo a suite of characteristic adaptations during human chronic airway infections. These include the loss of quorum sensing (QS) and production of proteases regulated by QS. Here we use experimental evolution in airway-mimicking environments to show that the loss of QS-regulated protease production is constrained by environmental factors associated with inflammation. Freely available amino acids and oxidative stress prevented fixation and delayed the emergence of protease-deficient mutants (PDMs), respectively. The PDM phenotype was associated with mutations in *lasR*, the main transcriptional activator of AHL quorum sensing, in all conditions. However, under oxidative stress the loss of QS was always preceded by intergenic mutations upstream of *katA*, encoding the major catalase responsible for reducing hydrogen peroxide and associated with increased tolerance of oxidative stress. We complemented our lab experiments by showing a high proportion of PDMs in >16,000 clinical isolates from bronchiectasis patients. Together our findings highlight the importance of host inflammation in shaping the dynamics and targets of selection in evolving *P. aeruginosa* populations.

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Towards a surveillance strategy for carbapenem-resistant *Pseudomonas aeruginosa*?

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Abstract

Background

Our study investigated carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA) presence in humans and the environment, in order to inform the development of a surveillance strategy for this pathogen of critical priority (WHO).

Methods

A 12-month cross-sectional study was performed in Rotterdam (Netherlands), Rome (Italy), and Jakarta (Indonesia) between March, 2022 and June, 2024 (Rome: sample collection pending). We collected throat, navel, and perineal/rectal swabs from healthy persons and patients upon hospital admission, alongside clinical isolates. Environmental samples inside hospitals were taken twice. Water samples were collected monthly from each hospital's water system, wastewater treatment plants, and receiving river. Highly sensitive culture methods, followed by whole-genome sequencing (WGS) were employed to determine lineages by core-genome multilocus sequence typing and detect carbapenem resistance genes (CRG).

Results

We included 194, 217, and 242 healthy individuals, and 469, 270, and 573 patients in Rotterdam, Rome, and Jakarta, respectively. CR-PA carriage among healthy individuals was low (≤0.8%), but significantly higher in Jakarta patients (4.7%) relatively to other cities ($P<0.001$). Most clinical isolates were detected in Jakarta (n=75). CR-PA presence in hospital environment ranged from 0.0% (Rome) to 13.4% (Jakarta). Higher CR-PA concentrations were detected in hospital wastewater than in municipal wastewaters and rivers. WGS revealed some clusters and shared CRG among water and human isolates.

Conclusion

CR-PA carriage in humans was low, except among patients in Jakarta. CR-PA was detected in water outside hospitals in all cities, with clustering across domains. This underscores the need for a country-specific CR-PA surveillance strategy encompassing the environment.

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Pseudomonas putida as emerging cell factory for PET degradation, recycling and upcycling

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Abstract

Polyethylene terephthalate (PET) globally is one of the most utilized plastics. This fossil-based plastic is widespread across various applications, from food packaging and clothing to being a component in asphalt mixtures and medical applications. We aim to develop a *Pseudomonas putida* cell factory that can depolymerize PET and intermediate oligomers into the constituting building blocks terephthalic acid (TPA) and ethylene glycol (EG). The resulting TPA and EG can subsequently be used for repolymerization into PET, or for upcycling into added value compounds. To this end we have introduced the recently characterized *Streptomyces* BHETase LipA and the *Candidatus bathyarchaeota* feruloyl esterase PET46 in *Pseudomonas putida* strains KT2440 and S12. In addition, we combined these heterologous esterase activities with different ethylene glycol utilization routes in the *P. putida* strains. In this way, we will construct different synthetic routes to either fully degrade PET and its oligomers for bioremediation purposes, or to recover PET building blocks for recycling or upcyling strategies. Preliminary results indicate that the introduction of the different heterologous BHETases into the genome allow *Pseudomonas putida* to degrade PET oligomers in solid as well as liquid medium. Developing cell factories for bacterial plastics degradation, recycling and upcyling will open the doors towards a more sustainable future for these abundant polymers.

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How does genetic diversity shape metabolic adaptation in *Pseudomonas aeruginosa?*

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Abstract

Antimicrobial resistance (AMR) presents a significant global health challenge, influenced by multifaceted factors yet to be fully elucidated. Recent advances underscore the importance of genetic diversity within infection niches, yet research often focuses on individual strains, neglecting the broader spectrum of pathogenic variability. While extensive research has explored the versatile genome of *Pseudomonas aeruginosa*, the implications of genetic diversity among pathogenic strains for metabolic functionality remain poorly understood.

In this study, systems biology strategies were employed to analyse a collection of 30 pathogenic *Pseudomonas aeruginosa* strains isolated from Intensive Care Units across Europe. Phenotypic data were gathered under varying growth conditions, manipulating nutrient availability to target central carbon metabolism. Analysis of growth characteristics revealed nutrient-dependent advantages in specific strain-condition pairings, demonstrating diverse responses to altered nutrient availability. Utilizing high-throughput proteomics, the metabolic responses of the strain library was systematically examined. Initial proteomic analyses confirmed known strain-specific metabolic behaviours and unveiled unique responses among natural pathogenic strains, establishing correlations between phenotypic traits and genotypic variations.

Recording the proteomes of the natural strain library under varied growth conditions will provide comprehensive insights into strain-specific metabolic responses to environmental changes, contributing to our understanding of bacterial adaptability and its role in disease mechanisms.

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Mathematical modeling of TX-TL dynamics in Pseudomonas putida KT2440

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Abstract

Transcription-translation (TX-TL) coupling represents a potential rate-limiting factor in the biosynthesis of proteins. Precise and efficient modeling of this process is crucial for enhancing the overall yield of the target protein product.

The study uses a deterministic, dynamic model founded on differential algebraic equations (DAE) designed to simulate the *in vivo* transcription and translation of individual gene sequences into their respective protein sequences in *Pseudomonas putida*. This model originates from an *in vitro* model developed by Arnold *et al*. [1] and was further refined by Nieß *et al.* [2] to accurately represent *Escherichia coli* protein biosynthesis under *in vivo* conditions. Currently, the model is being translated from Matlab to the programming language Julia for application in *P. putida.*

Julia's open-source advantage and computational efficiency make it the optimal choice over Matlab and Python. The TX-TL model currently under development in Julia closely approximates the results of the Matlab-based model when applying kinetic parameters identified for *E. coli*, with a low error percentage for protein concentration. Following the assessment of metabolite concentrations and distinctive physiological attributes, the model will undergo further refinement to suit the requirements of the *P. putida.*

TX-TL modeling can be used for precise simulation of gene-to-protein translation in *P. putida*, crucial for optimizing protein production in synthetic biology. By employing Julia's computational framework, this approach significantly improves the accuracy and efficiency of synthetic biological systems.

1. https://doi.org/10.1007/b136414.

2. https://doi.org/10.1021/acssynbio.7b00117.

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The forgotten, common antigen: A novel target for cloaking antibodies in chronic infection

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Abstract

Background: People with cystic fibrosis (pwCF) are susceptible to chronic lung infections, particularly with *Pseudomonas aeruginosa.* These infections can cause significant functional decline and are difficult to treat. During chronic *P. aeruginosa* lung infection, a subset of patients develop cloaking antibody (cAb) specific to O-antigen lipopolysaccharide (LPS) that impair complement-mediated bactericidal activity and worsen patient outcomes. Removal of this antibody via plasmapheresis has been used as a successful treatment for multi-drug resistant *P. aeruginosa*. However whether a similar mechanism of antibody-mediated serum resistance exists towards common polysaccharide antigen (CPA) LPS which is upregulated during chronic infection is unknown.

Methods: Forty-two serum samples and 63 matched *P. aeruginosa* isolates were collected from pwCF at The Prince Charles Hospital, Brisbane, Australia. Patient serum responses towards bacterial CPA LPS were examined via bactericidal assays, ELISA, and Western blot techniques.

Results: Serum IgG and IgA responses to CPA were elevated in 92.9% and 83.3% of sera (n = 42), respectively. Further, 69% (n = 42) of pwCF were colonised with CPA-expressing isolates, where 27% of isolates (n = 63) expressed CPA solely. Although significant inhibition of bactericidal activity against CPA-only expressing isolates were found at 35% (n = 17) in patient-matched sera, complete inhibition only occurred against a single isolate expressing hypderdense CPA.

Conclusion: This investigation reveals that for the first time that although CPA-specific cAb can indeed exist, CPA is only expressed at a sufficient density for cAb-mediated serum inhibition to occur in a small percentage of isolates.

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Rhizobacteria's love language: decoding the basis of bacterial colonization patterns on roots

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Abstract

Plant roots can functionally be considered as “inverted guts”, with selective nutrient uptake and microbiome recruitment occurring on the roots’ surface instead of being hidden in the lumen of the gut. The endodermis, a specialized root cell layer, develops diffusion barriers that facilitate the absorption of essential nutrients while restricting the entry of harmful substances. Variability in nutrient quality and quantity along the root developmental axes results in preferential microbial colonization of specific root regions. Most bacteria, including *Pseudomonas protegens* CHA0, tend to accumulate around elongation zones and lateral root emergence sites (LRES), where the endodermal diffusion barrier is not yet developed or transiently broken. Our research indicates that endodermal diffusion barriers not only regulate nutrient uptake, but also restrict bacterial attractant leakage. By using the *Arabidopsis thaliana myb36sgn3* mutant, which exhibits a strong endodermal diffusion barrier defect, we observed enhanced CHA0 root colonization. Metabolomic analysis of wild-type and mutant root exudates revealed elevated amino acids levels in the latter, suggesting their role in enhancing bacterial colonization. We show that bacterial attraction to *myb36sgn3* roots and wild-type LRES depends on chemotaxis and amino acid sensing, as mutants deficient in these processes exhibited reduced colonization. To validate that amino acids are important for bacterial colonization at LRES, we attempt to utilize CHA0 fluorescent reporter strains to visualize bacterial metabolic states during root colonization. Our findings underscore the importance of tight control of nutrient provision to the rhizosphere, and provide insights into the biological basis of bacterial colonization patterns.

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Improved Identification of *Pseudomonas putida* Group Species and Detection of Antibiotic Resistance Genes Using Proteotyping

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Abstract

Pseudomonas putida group species play a pivotal role in pharmaceuticals, agriculture, and healthcare, yet accurate species identification using traditional methods remains challenging. Additionally, these species act as reservoirs for antimicrobial resistance, while also posing threats on nosocomial settings.

In a previous study, a culture-dependent approach analysed clinical environments, identifying a cluster of isolates related to the *P. putida* in an ICU ward using MALDI-TOF MS. Whole-genomes of five isolates from three different clusters within this group were sequenced using the Illumina platform after antibiotic susceptibility testing revealed high resistance rates within the isolates, specially two of the same species, resistant to all carbapenems tested. Characterization and identification of microorganisms using proteotyping relies on species-unique peptides, revealed by tandem mass spectrometry with a curated genome data foundation. Phylogenomic analysis, together with proteotyping results allowed to differentiate three species within the *P. putida* group (two strains of *P. putida* species and two potential novel species, closely related). Genome analysis provided the database for peptide search and matching. This approach allowed for a good differentiation between these species, with the presence of more than 200 unique peptides for each species. Furthermore, eight antibiotic resistance genes were identified across all isolates, including five beta-lactamases. Notably, a novel class C beta-lactamase closely related to $bla_{\text{AER-1}}$ was detected in one of the new species, alongside $bla_{\text{VIM-47}}$.

Accurate identification of *P. putida* group species is crucial for microbiological analyses, while the presence of antibiotic resistance genes underscores the urgent need for surveillance and control measures in healthcare settings.

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A novel regulator of type IV pili assembly, PlzR, induces phage resistance in *Pseudomonas aeruginosa*

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Abstract

A continuous arms race drives the unceasing coevolution between phages and bacteria, in which bacteria develop resistance to protect against phage predation and phages respond by the emergence of counter-strategies. Understanding how bacteria become resistant against phage predation is of interest, for example, to improve phage-based therapies. Phage resistance often develops at the bacterial cell surface, preventing phage adsorption. A surface structure commonly used as a receptor by *Pseudomonas aeruginosa* infecting phages are the type IV pili (T4P), which are flexible filaments involved in multiple pathogenesis-related events, including bacterial motility.

We here present the discovery of a novel regulator of T4P in *P. aeruginosa*, identified during a screen for host factors influencing phage infection. Expression of PA2560 (renamed PlzR) inhibits bacterial motility and induces adsorption resistance to T4P-dependent phages. Visualization of the T4P dynamics by fluorescence microscopy showed the absence of T4P on the cell surface upon PlzR expression. A systematic analysis combining pull-down assays, bacterial two-hybrid and three-hybrid and structural modelling using ColabFold revealed that PlzR does this by directly binding PilZ, which in turn regulates the motor protein PilB. This results in disturbed T4P assembly. As the *plzR* promoter is induced by the second messenger cyclic di-GMP, PlzR may play a key role in coupling T4P functions to environmental stimuli.

Taken together, our results show a novel layer of T4P regulation, which has implications for *P. aeruginosa* cell behavior and pathogenesis under specific conditions, and may be an attractive target for the development of novel virulence attenuating drugs.

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Diversity in type VI secretion system genes in a global population of *Pseudomonas aeruginosa*

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Abstract

Bacterial type VI secretion systems (T6SSs) are puncturing molecular machines that transport effector proteins into other cells and the extracellular space. *Pseudomonas aeruginosa* uses multiple T6SSs and a multitude of effector proteins to kill microbes, manipulate eukaryotic cells, or facilitate nutrient uptake. Whilst few *P. aeruginosa* strains were known to differ in genes encoding for T6SS effector and immunity proteins, the diversity in these genes across the entire species was not understood. Here, we analysed a high number of quality existing genomes (1,960) of phylogenetically diverse *P. aeruginosa* strains isolated from all continents and from clinical and environmental sources. We used this global *P. aeruginosa* population for a pangenome analysis of T6SS apparatus and effector genes. We observed tremendous intraspecific diversity in some genes that encode T6SS effector and immunity proteins and belong to the accessory genome of the species. We found striking associations between some of these accessory effectors and the strain's isolation source. Other T6SS effector genes belong to the species' core genome and are likely critical for the biology of *P. aeruginosa*. These findings advance our understanding of individual molecular traits that differ between *P. aeruginosa*strains with potential implications for microbial pathogenesis.

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Metabolic modulations by mucin during infection in diverse clinical *Pseudomonas aeruginosa* isolates

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Abstract

The treatment of *Pseudomonas aeruginosa* infections is complicated by the presence of a large variety of metabolic and virulence mechanisms among clinical strains. We hypothesize that the metabolic differences in *P. aeruginosa* are dependent on a complex combination of host and pathogen-specific factors, which can be delineated using a combination of genomic and transcriptomic analyses coupled with genome-scale metabolic modeling.

We performed a multi-faceted analysis of 25 clinical *P. aeruginosa* isolates by employing whole genome sequencing, phenotypic and genotypic clustering, functional annotation, and analyses on core, accessory, and unique traits in the *P. aeruginosa* pan-genome, and genome-scale metabolic reconstruction with analysis of flux samples.

Genotype-phenotype correlation was assessed using a multi-parametric analysis. A genome-scale metabolic network reconstruction was developed for each isolate through KEGG-based annotation. These network reconstructions show diverse metabolic functionalities and enhance the collective *P. aeruginosa* pangenome metabolic repertoire. Five clinical isolates along with the laboratory strain PA14 were selected for transcriptomic study in SCFM medium ± 0.5% MUC5AC. Specific metabolic functions and biological processes modulated by the presence of mucin were identified using differential gene expression analysis. Mucin-driven metabolic shifts were observed in both central and peripheral metabolism and are both shared and across the clinical isolates studied.

Characterizing this rich set of clinical *P. aeruginosa* isolates allows for a deeper understanding of the genotypic and metabolic diversity of the pathogen in a clinical setting and lays a foundation for further investigation of the metabolic landscape of this pathogen and mucin-induced metabolic modulations during infection.

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Novel *Pseudomonas aeruginosa* Calcium Sensor, EfhP: Mechanisms of Signal Transduction through Protein Interactions

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Abstract

A resilient, opportunistic pathogen, *Pseudomonas aeruginosa* (*Pa*) causes severe infections in humans, particularly in the airways of cystic fibrosis (CF) patients. CF is associated with elevated calcium (Ca^{2+}) in bodily fluids, and our lab has established that increased levels of Ca^{2+} induce the production of several virulence factors in *Pa*. We have identified and characterized a novel EF-hand protein, EfhP, to be a Ca^{2+} sensor through its ability to specifically bind Ca^{2+} using two EF-hand motifs, undergo a Ca^{2+}-dependent conformational change, and transduce the signal to promote transcriptional regulation. This study focuses on the mechanisms by which EfhP facilitates its regulatory function through interactions with its binding partners. For this, a His-tagged protein pull-down assay identified several potential EfhP binding partners. Interactions were validated by a ß-lactamase based protein complementation assay, compatible with the periplasmic localization of EfhP. The results discovered EfhP to robustly bind two partners: SbrR, a negative regulator controlling swarming and biofilm formation *via muiA*, and MucB, playing a key role in controlling sigma factor AlgU required for transcription of a large regulon, including alginate biosynthesis. We showed these interactions are strengthened upon EfhP binding Ca^{2+} and that the predicted hydrophobic patches (FLAVA, PMPAGLF) are required for this Ca^{2+} dependency. Genome-wide RNAseq and luciferase reporter assay confirmed *efhP* to downregulate *muiA,* an inhibitor of swarming, and phenotypic studies validated this observation. The undergoing identification of the *algU* regulated processes that require the EfhP-MucB interaction will expand the scale of EfhP significance in controlling *Pa* virulence and resistance.

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Genes important for *Pseudomonas aeruginosa* lung infection revealed by time-resolved dual multi-omics

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Abstract

The opportunistic human pathogen *Pseudomonas aeruginosa (Pa)* is responsible for life-threatening infections of the respiratory system. Most virulence strategies of *Pa* have been defined using cell culture systems and animal models like mice. However, it is often not clear how predictive animal models are for human lung infections and treatment. Moreover, mice experiments lack the experimental power to investigate infection dynamics in detail. To better understand *Pa* virulence strategies under in vivo-like conditions, we are using human airway microtissue models that closely recapitulates several features of the human lung (pseudostratified epithelium, mucus production, ciliae beating, air/liquid interface). Microtissue models are benchmarked by comparing proteomes from pathogens isolated from infected patients with pathogens from microtissue models. We applied time-resolved RNA sequencing, proteomics and metabolomics to samples of *Pa* and lung tissue from the start of infection all the way to tissue destruction. Using machine learning we were able to generate a list of 85 *Pa* genes that might be required for a successful infection of lung tissue. Validation with transposon mutants identified a subset of 17 genes, which, when mutated, showed a delayed infection phenotype, indicating that they are required for the colonization and infection of lung barrier tissue. The identification of potential new drug targets combined with the validated drug screening platform based on human microtissue models, could lay the foundation for the development of novel therapeutic approaches.

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The Complex Molecular Relationship of *Pseudomonas aeruginosa* and CF Airway Epithelial Cells

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Abstract

Pseudomonas aeruginosa (*Pa*) is an opportunistic human pathogen causing deadly infections in the airways of cystic fibrosis (CF) patients. CF is a genetic disease resulting in increased Ca^{2+} in the nasal and pulmonary fluids possibly impacting *Pa* pathoadaptation and host-pathogen interactions. To identify Ca^{2+}-regulated molecular mechanisms responsible for *Pa* adherence to and invasion into host cells, we have utilized genome-wide Dual-RNA-Sequencing. CuFi-5 epithelial cells were used as host cells. Following adhesion and invasion at high and low Ca^{2+}, RNA was isolated from both CuFi-5 and *Pa* cells. Planktonic *Pa* and CuFi-5 grown alone served as control. We discovered that 48-59% of *Pa* genome was differentially regulated. The most positively affected functional category is host integration, showing increased regulation by Ca^{2+}. Importantly, translation and protein processing as well as nucleotide transport and metabolism were downregulated. In adhered *Pa*, we observed a large upregulation in polyamine and sulfur metabolism, as well as the type 6 secretion system, which was lost once *Pa* invaded. Upon invasion, antibiotic and heavy metal resistance genes were upregulated. The CuFi-5 cells responded by significant increase in autoimmune-like processes along with such important signaling networks as MAP Kinases and TNF signaling. In addition, genes associated with membrane structure and organization were upregulated. In-depth transcriptional and mutational studies are underway to validate the observations and to provide a comprehensive understanding of the *Pa* and CuFi-5 host cells interactions and the regulatory role of the host Ca^{2+} signaling with the goal to identify novel therapeutic targets for combating *Pa* infections.

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Metabolic Engineering of *Pseudomonas putida* to Boost Malonyl-CoA Availability and Polyhydroxyalkanoate Production

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Abstract

Malonyl-CoA is a key precursor for the biosynthesis of various high-value compounds, including polyketides, carboxylic acids, biofuels, and polyhydroxyalkanoates (PHAs). However, the intracellular availability of this metabolite is limited due to competition with essential cellular metabolic pathways. To address this limitation, we undertook a systems metabolic engineering approach on *Pseudomonas putida* strain SEM11. We knocked out specific combinations of genes involved in glycolysis, TCA cycle, and fatty acid biosynthesis to enhance the malonyl-CoA pool. To quantify malonyl-CoA accumulation, we employed an enzyme-coupled biosensor based on the *rppA* gene, encoding type III polyketide synthase RppA, which converts malonyl-CoA into flaviolin, displaying a red color. Strains exhibiting enhanced malonyl-CoA accumulation were identified via a colorimetric screening method, displaying increased red pigmentation. HPLC analysis confirmed that four modified strains exhibited statistically significant increases in flaviolin production compared to the parental strain. To assess PHA production, we utilized Nile Red, a lipophilic fluorescent dye that binds to PHA granules, for microscopy analysis and to compare the PHA production of the strains over time. Our results demonstrated increased PHA production in most engineered strains during the early stages of production.

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Biosynthesis of fragrance 2-phenylethanol from sugars by Pseudomonas putida

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Abstract

Petrochemicals contribute to energy consumption, carbon dioxide emissions and pollution. Microbial platforms offer an eco-friendly alternative by producing valuable chemicals under ambient conditions. *Pseudomonas putida* is a suitable organism for the bioproduction of aromatic compounds such as L-phenylalanine and its derivative 2-phenylethanol (2-PE). The aim of this study is to enhance 2-PE production in *P. putida* CM12-5 derivatives by exploring different strategies and utilizing agricultural waste as a carbon source. The strains CM12-5 and CM12-5Δ*gcd* (*xylABE*) were transformed with plasmid pPE-1, and the biosynthesis of 2-PE measured. The results show that 2-PE production was about 50-60 ppm when glucose was used as the growth substrate. To understand the limiting factors in 2-PE production, intermediates of the Ehrlich and shikimate pathways were added to the cultures. It was found that the addition of phenyl acetaldehyde, phenylpyruvate, and L-phenylalanine increased 2-PE production (100-110 ppm), indicating that the initial amount of L-phenylalanine in the cells is a limiting factor. To enhance L-phenylalanine production, a mutant variant of chorismate mutase/prephenate dehydratase was used in *P. putida*. This mutant strain accumulated higher levels of L-phenylalanine (near 400 ppm vs 250 ppm in CM12-5 strain) and subsequently produced more 2-PE. Random mutagenesis was also used to generate strains that overproduce L-phenylalanine, resulting in increased 2-PE production (up to 120 ppm). The analysis of the latter set of mutants revealed that lateral L-phenylalanine metabolism to dead-end product was blocked, hence the primary substrate was more available for synthesis of 2-PE

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Distribution Of Candida Species and Antifungal Susceptibility Pattern Among Hiv Positive Individuals with Oropharyngeal Infection in Selected Mekelle Health Facilities, Tigray, Northern Ethiopia

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Abstract

Background: Oropharyngeal candidiasis (OPC) is the most common opportunistic infection encountered among human immunodeficiency virus infected patients

Objective: The aim of the study was to determine the distribution and antifungal susceptibility pattern of *Candida* species among HIV positive oropharyngeal patients.

Method: A cross-sectional study was conducted among 381 HIV positive individuals with oropharyngeal infection from September 2021 to May 2022. Socio demographic, data on clinical risk factors and oral swabs were collected from the study participants. The antifungal susceptibility patterns of all Candida species were determined using disk diffusion method and data were analyzed using STATA version 13.0.

Result: Among the 381 study participants, the overall *Candida* species isolation rate was 59.8% (228/381). A total of 240 *Candida* isolates were recovered, among this *C. albican* was the most predominant species 151(62.9%). Among the non albican *Candida* species, the most predominant species were *Candida glabrata* 47(19.6%) followed by *Candida tropicalis* 26 (10.8%) and *Candida krusei* 16 (6.7%). Isolated Candida species was from HAART naïve and those on HAART were 127(52.9%) and 113 (47.1%) respectively. Age group 40-49 years (p = 0.019), previous history of antifungal drug treatment (p=0.039), CD4 counts < 200 cells/mm3 (p=0.003), HAART naïve (p=0.000), body mass indices <15.9 kg/m2 (p=0.002)

Conclusion: *Candida* species isolation in this study was (59.8%). both *Candida albican* (62.9%) and non albicans (37.1%) were isolated from a significant number of the study participants. Therefore, early identification of the etiologic agent and antifungal susceptibility testing should be practiced to improve treatment outcome and prevent the emergence of drug resistant.

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Barley cultivars influence their associated *Pseudomonas* consortia at multiple levels through differential root exudate secretion

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Abstract

Plant-associated microbes play important roles in promoting plant growth and health, with plants secreting root exudates into their rhizospheres to attract beneficial microbes. The composition of root exudates defines the nature of microbial recruitment, with different plant species attracting distinct microbiota to enable optimal adaptation to the soil environment. To more closely examine the relationship between plant genotype and microbial recruitment, we analysed the rhizosphere microbiomes of landrace (Chevallier) and modern (NFC Tipple) barley cultivars. We observed distinct differences between the plant-associated microbiomes of the two cultivars, with the plant-growth promoting rhizobacterial genus *Pseudomonas* substantially more abundant in the Tipple rhizosphere. Striking differences were also observed between the phenotypes of recruited *Pseudomonas* populations, alongside distinct genotypic clustering by cultivar. This selection was driven by root exudate composition, with the greater abundance of hexose sugars secreted from Tipple roots attracting genotypes better adapted to growth on these metabolites, and vice versa. Cultivar-driven selection also operates at the molecular level, with both gene expression and the abundance of ecologically relevant loci differing between Tipple and Chevallier *Pseudomonas* isolates. This cultivar-driven selection appears to be important for plant health, with both cultivars showing a preference for microbes selected by their genetic siblings in rhizosphere transplantation assays. Work is now underway to identify the genetic loci in barley that drive this selection, with the ultimate aim to breed novel varieties that preferentially recruit beneficial soil microbiomes.

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Pseudomonas Transmission Dynamics Revealed by Prophage Barcoding

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Abstract

Chronic *Pseudomonas aeruginosa* (*Pa*) lung infections in people with cystic fibrosis (pwCF) characteristically involve many diverse strains; it can be challenging to disentangle the origins of this bacterial diversity within individuals. To better elucidate these relationships, we have developed a novel approach for tracking bacterial strain dynamics using prophages – lysogenic bacteriophages integrated into the bacterial genome. In studies of 680 *Pa* isolates collected from three longitudinal cohorts of pwCF in Denmark, Italy, and California, we find that Pf and other prophages are typically transmitted vertically within *Pa* lineages in patients. This lineage-specificity enables the use of unique combinations of prophages to 'barcode' *Pa* lineages. Prophage barcoding makes it possible to distinguish radiative evolution from superinfection within individuals. We further demonstrate the utility of this approach in identifying inter-patient transmission, hospital outbreaks, and for studying infections with other pathogens. Prophage barcoding is a powerful tool to study bacterial strain dynamics.

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Leveraging Pentamidine to Restore Lost Hope Against Multidrug Resistant *Pseudomonas aeruginosa*

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Abstract

Antimicrobial resistance (AMR) is a rising global crisis as it renders clinically available antibiotics ineffective. This study sought to repurpose pentamidine as an adjuvant in combination with antibiotics, against *Pseudomonas aeruginosa* isolated from the intensive care units of some selected Ghanaian hospitals.

Four *P. aeruginosa* (clinical and environmental) strains were subjected to Kirby Bauer disk/agar well-diffusion, broth-microdilution to determine AMR profiles. Chequerboard, for pentamidine-antibiotic synergistic activity and their efficacy on biofilms was tested with crystal-violet dye. Nucleic acid materials were purified with Qiagen prep-kit, associated AMR markers were profiled with PCR and RT-qPCR. Effect of the cocktails, using cytotoxicity and infection assays were tested on THP-1 immune cells with Alamar blue at MOI of 10:1(bacteria:THP-1 cells).

The strains showed 100% levels of AMR to aminoglycoside, macrolide, cephalosporin, carbapenems, polymyxins, fluoroquinolones and penicillin; however exhibited effectiveness (10 mm zone-of-inhibition) when combined with pentamidine. The MICs of antibiotics against pentamidine were 160-800 µg/ml and 200-400 µg/ml however. pentamidine-antibiotic cocktail reduced AMR levels to 50-100 µg/ml. Pentamidine synergized (FIC) with penicillin, chloramphenicol, cefuroxime, and doxycycline. The combinations inhibited/eradicated strong biofilms to weak (0.701-0.219, 590 nm) phenotype and reduced the expression of *blaTEM/CTX, catB,, tetB, pmrA/B*. Cocktail was non-toxic (about 100% viability) to THP-1 and decrease in bacterial population ($3.3X10^2$ to $0.3X10^3$ colonies).

Pentamidine potentiated activity of penicillin, chloramphenicol, cefuroxime, doxycycline, tetracycline, trimethoprim, amoxyclav, erythromycin, meropenem, and amoxicillin against MDR *P. aeruginosa* strains. It decreases the expression of resistance markers and altered biofilm phenotypes.

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Swarming in clinical strains of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an ubiquitous bacterium. Two strains of *P. aeruginosa* isolated from wounds, PA01 and PA14, readily display swarming motility on semisolid agar. The snowflake-like branched swarming colony is easily observed on low nutrition media. Swarming is a population behaviour which relies on the ability of flagellated bacteria to move on the surface collectively when aided by a surfactant, rhamnolipids produced under the control of quorum sensing system RhlR/RhlI of *P. aeruginosa*. Swarming also provides resistance to antimicrobials and competitive fitness advantage over nonswarmer strains. It is, therefore, logical to think that clinical strains will display swarming and perhaps hyperswarming. We present data for clinical strains from group I (PA01 like) and group II (PA14 like) clades of *P. aeruginosa* pangenome on two different swarming media. We will present evidence to show the relevance of swarming for 32 sequenced, clinical strains of *P. aeruginosa* and discuss relevance of swarming for clinical features of the bacterium.

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The identification of *Pseudomonas aeruginosa* persisters using flow cytometry

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Abstract

Persister cell development is one of the main factors that contributes to recurrent *Pseudomonas aeruginosa* infections, yet persister cell mechanisms are largely unknown for *P. aeruginosa*. Persister cells are a rare and transient subpopulation of cells that survive bactericidal concentrations of antibiotics. Here, we sought to develop a protocol to identify and isolate *P. aeruginosa* persister cells using flow cytometry. *P. aeruginosa* PA14 and CF18 cultures were treated with ciprofloxacin or tobramycin for 24 hours at concentrations ranging from 1-5 times the minimum inhibitory concentration (MIC), surviving cells were considered persisters. Cultures were stained with a fluorescent staining combination that consisted of a dead-cell stain and an all-cell stain and analyzed via flow cytometry. In a separate experiment, live single cells were sorted onto agar using fluorescence activated cell sorting (FACS) to recover persister cells. Using flow cytometry, we were able to successfully identify *P. aeruginosa* persister cells at 1, 5, and 10 times the MIC with two different staining combinations. Of note, the percentage of live *P. aeruginosa* cells significantly increased as the concentration of antibiotic increased ($P<0.05$). Our FACS protocol was able to isolate single *P. aeruginosa* cells however, only our untreated cultures showed consistent recovery. Interestingly, altering the ingredients in the recovery media enhanced the recovery of *P. aeruginosa*. Taken together, this study provides an important steppingstone for isolating and identifying persister cells in heterogenous *P. aeruginosa* populations. Furthermore, we show that increasing the concentration of antibiotics can increase the percentage of live *P. aeruginosa* persister cells.

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Next Generation 3D Bioprinting of Human Lungs for Studies of Pseudomonas aeruginosa Invasion, Pathogenesis, Immune Response and Antimicrobial Resistance

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Abstract

Major limitation in *P. aeruginosa* studies as an opportunistic pathogen and in Cystic Fibrosis is the lack of adequate models for cell-cell interactions during initial invasion and chronic pathogenesis. Using animal models has limitations due to genotypic, phenotypic, and metabolic differences. Classical two-dimensional cell culture may lack the complexity and tissue-like structures relevant to detailed biological analysis. Furthermore, it is not necessarily predictive in treatment and providing poor clinical outcomes.

Recent progress in three-dimensional (3D) bioprinting have been made for tissue engineering and regenerative medicine. We propose a novel approach of 3D bioprinting of 3 human cell lines as an improved *ex vivo* lung model coupled to high throughput transcriptomics analysis. Our strategy is based on the combination of endothelial cells and fibroblasts within bioink's gelatinous support resulting in the production of the 3D extracellular matrix combined with establishment of air-liquid differentiated epithelium. Cell integrity and tissue viability assays were combined with AmpliSeq RNA-Seq profiling. Data mining of gene expression levels was compared to human lung tissues in the Lung Expression Atlas (LGEA Web Portal). Transcriptomic profiling in single and mixed 3D lung constructs identified the top 100 significant differently expressed genes in cell surface interactions, collagen and extracellular matrix organization with changes in cellular secretion, signal transduction, cell-cell communication, adhesion, immune response, and hemostasis.

Primitive human 3D lung models will lead to functional near-native microanatomy providing tools for investigation of the *P. aeruginosa* pathogenesis in lung infections, cytotoxicity of novel therapeutics and high-throughput screening assays.

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Evolution of AMR in *in vivo* experimental evolution biofilm model

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Abstract

Background: Treatment of biofilm infections is challenging due to their tolerance and resistance to the immune system and antibiotics. We aimed to examine the evolution of antimicrobial resistance (AMR) in a chronic *P. aeruginosa* lung infection model.

Materials/methods: Experimental evolution using a monitor strain for mutations in *nfxB*, the regulator of the MexCD OprJ efflux pump, was conducted in mice during 4 passages with sub-inhibitory concentration of ciprofloxacin (CIP), 0.25 mg CIP twice/day for one or two days. Bacteria embedded in alginate beads were inoculated into the lung of BALB/c mice and treatment was started 24h after inoculation. The biofilm populations from the lungs were investigated by population analysis profiles and CIP-resistant colonies from lung homogenates were passed to the next passage, cytokine levels in the lungs were measured, and selected isolates were sequenced.

Results: Comparison between the development of AMR using one or two-day treatment revealed that several CIP treatments increased bacterial resistance. After two days of CIP treatment, 22.8% of the bacterial population survived on 8 mg/L CIP plates, compared to 16.5% surviving on 2 mg/L CIP after one-day treatment. Mutations in *nfxB*, efflux pumps (*mexZ*), and two-component systems (*parS*) contributed to CIP resistance. In the initial two passages, the CIP-treated group exhibited an elevated inflammatory response compared to the control group, which might contribute to the release of mutagenic reactive oxygen species and the development of AMR.

Conclusions:

This study illustrates the complex relationship between infection, antibiotic treatment, and immune response.

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Targeting PqsE as an antimicrobial strategy against Pseudomonas aeruginosa

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Abstract

The human pathogen *Pseudomonas aeruginosa* is a leading cause of hospital-acquired infections for which effective treatments are currently lacking. *P. aeruginosa* relies on the cell-cell communication process called quorum sensing to orchestrate pathogenic behaviors. Quorum sensing involves the production, release, and group-wide detection of extracellular signal molecules called autoinducers. Quorum sensing enables bacteria to track changes in cell population density and trigger transitions between individual and collective behaviors. A key *P. aeruginosa* quorum-sensing component is PqsE, an enzyme that is essential for *P. aeruginosa* to produce virulence factors and establish infection. PqsE is known to participate in quorum sensing via the RhlR regulator. Through high-throughput small molecule screening, chemical synthesis, biochemical analyses, and x-ray crystallography we identified and characterized potent inhibitors of PqsE catalytic activity. Based on these results, we used structure-guided mutagenesis, *in vitro*, *in vivo,* and animal infection assays to show that PqsE and RhlR directly interact, and it is this interaction, not PqsE catalytic activity, that drives expression of genes involved in virulence. These findings provide the mechanism for PqsE-dependent regulation of RhlR, identify a unique regulatory feature of *P. aeruginosa* quorum sensing and its connection to virulence, provide a new and unexpected route to drug discovery, and form the basis of projects being launched in the Taylor Lab at William & Mary.

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FimX marks the spot: Mapping twitching motility suppressors to reveal the function of a motility dual regulator

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Abstract

Many bacteria navigate their environment using surface-exposed protein appendages. *Pseudomonas aeruginosa* uses polarly-localized type 4 pili (T4P) to mediate surface-associated twitching and a single flagellum for swimming motility, respectively. These distinct forms of motility enable bacteria to transition between planktonic and surface-attached growth phases, though the molecular details of how cells coordinate a switch from one mode to another are poorly understood. Twitching motility involves dynamic cycles of extension and retraction of a protein filament to pull cells along surfaces. This process is regulated at the level of extension by protein effectors which bind to and modulate the activity of an assembly ATPase, PilB. Mutants of the cyclic-di-GMP binding effector FimX are significantly attenuated in twitching motility, though pilus function is not abolished. We leveraged this deficit by conducting a twitching gain-of-function screen to positively select for mutations which alleviated the loss of FimX. One of the mutations that restored twitching introduced a frameshift at the C-terminus of PilB, the FimX target, extending the reading frame by seven amino acids. FimX mutants encoding the frameshifted PilB exhibited enhanced pilus assembly but also significantly more swimming motility. In follow up studies, deletion of FimX or mutation of the cyclic-di-GMP binding motif increased swimming motility in an otherwise wildtype background, supporting the hypothesis that FimX suppresses flagellar-derived motility while simultaneously upregulating pilus-dependent twitching in response to second messenger binding. Together, these data position FimX as a dual regulator of *P. aeruginosa* motility connecting the function of T4P to that of the flagellum.

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Engineering *Pseudomonas putida* KT2440 for efficient 3-HP production and its evaluation using different carbon sources

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Abstract

The industrial production of 3-hydroxypropionic acid (3-HP) from glycerol is limited by the high cost of cofactor co-enzyme B_{12}. This study developed *Pseudomonas putida* KT2440, a native coenzyme B_{12}-producing strain, as a potential host for 3-HP production. The glycerol synthesis module, consisting of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase, along with the 3-HP synthesis module, including glycerol dehydratase, glycerol dehydratase reactivase, and aldehyde dehydrogenase, were introduced in strain KT2440. The recombinant strain produced 35 mM 3-HP in 24 hr using glucose as a carbon source. 3-HP production was further improved by sequentially deleting *3hpdH*, *3hibdHIV*, *3hibdHII*, and *3hibdHI* genes responsible for 3-HP degradation and glycerol kinase gene to improve glycerol flux towards 3-HP production. The resulting mutant strain produced 403 mM 3-HP in 42 hr, with a yield of 0.91 mol/mol glycerol. Strain KT2440 showed a significantly low requirement for co-enzyme B_{12}, making production more economical. Substituting glucose with gluconate as a carbon source significantly improved the 3-HP titer to 630 mM in 42 hr with a yield of 0.97 mol/mol glycerol. This increase in 3-HP production could be attributed to alleviating the generation of glyceraldehyde-3-phosphate, which directly inhibits the activity of glycerol dehydratase. This study demonstrates the potential of genetically engineered *P. putida* KT2440 as an efficient host for 3-HP production from various carbon sources, highlighting its industrial application prospects.

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Four Branches of Calcium Signaling Network to Control Virulence and Resistance in a Human Pathogen, Pseudomonas aeruginosa

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Abstract

Calcium (Ca^{2+}) regulates host responses and accumulates in pulmonary and nasal liquids during cystic fibrosis (CF). Such Ca^{2+} misbalance may trigger the adaptive and virulent traits in *P. aeruginosa* (*Pa*) during its pathoadaptation in nasal cavities. Previously, we established that *Pa* responds to elevated Ca^{2+} by enhancing production of secreted virulence factors and infectivity in plant and animal models. We determined that this response is achieved by at least four regulatory pathways guided by (1) two-component regulatory system CarSR, (2) Ca^{2+} sensor EfhP, (3) Ca^{2+} sensor/regulator CarP, and (4) Ca^{2+} channel CalC. The transcription of *carSR* is induced by Ca^{2+}, and CarSR exerts Ca^{2+}-dependent regulation of a regulon, including CarP, predicted novel Ca^{2+} sensor/regulator enabling *Pa* resistance against high Ca^{2+}, osmotic and oxidative stress, and regulating several virulence traits and interaction with the host. EfhP belongs to EF-hand family, specifically binds Ca^{2+} and two partners: SbrR, anti-sigma factor that together with sigma factor SbrI controls swarming and biofilm formation, and MucB, controlling sigma factor AlgT controlling alginate biosynthesis and protection against osmotic and oxidative stress. EfhP is also responsible for Ca^{2+}-dependent regulation of iron sequestration. CalC functions as a Ca^{2+} channel responsible for generating Ca^{2+}_{in} transients required for Ca^{2+} regulation of multiple pathways, including production of virulence factors, biofilm formation, and cellular communication. Overall, we identified four branches of Ca^{2+} signaling that orchestrate multiple *Pa* pathways, enhancing its adaptation to the host, tuning biofilm transitions and production of virulence factors. We mapped several essential components of the Ca^{2+} signaling network that can serve as drug targets for future developments of the means to control *Pa* infections.

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Engineering *P. putida* as a whole-cell biosensor for production of chemicals and characterization of PET degradation

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Abstract

Biosensors typically rely on the production of a fluorescent protein mediated by a transcriptional regulator that recognizes an analyte of interest. While simple to implement and useful for the detection of many compounds, such designs also suffer from technical limitations. For example, the dynamic and operational ranges are often narrow and these sensors are specific for a single metabolite. Additionally, the output of biosensors typically reflects the concentration inside the cells, while extracellular concentrations (titers) are the key performance indicator for microbial fermentations.

An alternative strategy is coupling microbial growth to the presence of the compound of interest. This strategy can be challenging to implement as it depends on rendering the analyte essential for the cells, but the versatile metabolism of *P. putida* can be exploited to this end. This soil bacterium grows on structurally unrelated carbon and nitrogen sources. Therefore, the metabolic network of *P. putida* can be engineered to make the cell growth dependent on selected compounds.

Here, *P. putida* SENS was constructed by blocking sugar assimilation. Upon equipping the SENS strain with a constitutively expressed fluorescent marker, reliable fluorescence signals were detected in cultures supplemented with different chemicals. This strategy showed a broad operational range and high sensitivity, with flexibility to detect both analytes of industrial (lactate), and environmental interest (PET-degradation products). Furthermore, we adapted this platform to different screening formats, including co-culture systems, analysis of culture supernatants, and detection of sub-products of enzymatic in-vitro degradation.

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Accessorize for Success: Investigating the Role of TfpY in Type IV Pilus Assembly

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Abstract

Type IV pili are polar filaments that enable bacteria to rapidly colonize their hosts. These filaments are composed of minor pilins at the tip of the pilus, which initiate pilus assembly, and major pilins that undergo cycles of pilus extension and retraction. This dynamic process facilitates motility, is critical for the formation of drug-tolerant biofilms, and upregulates the expression of virulence factors, making pili important targets for novel antimicrobials.

Bacteriophages exploit pili as receptors to infect their hosts. To evade phage predation, bacteria can glycosylate their major pilins using accessory proteins to mask pili from phage recognition. Some major pilins expressed by strains of *Pseudomonas, Acinetobacter,* and *Neisseria* are associated with TfpY, an accessory protein of unknown function.

Here, we use *Pseudomonas aeruginosa* as a model to show that TfpY expression is required for pilus assembly and motility. However, unlike previously characterized accessory proteins that glycosylate pilins, TfpY does not play a comparable role in phage defence. Through protein interaction studies, we show that TfpY interacts with the major pilin and some minor pilins but is not incorporated into the pilus itself. We propose that TfpY, along with the minor pilins at the tip of the pilus, facilitates recruitment of the first major pilin, allowing for subsequent addition of major pilins during pilus extension.

Due to the key role of pili in bacterial pathogenesis and their pervasive nature, understanding TfpY-mediated pilus assembly can inform the design of pilus-targeting vaccines, phage cocktails, and small-molecule inhibitors that target multidrug-resistant bacteria.

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In vivo divergent evolution of cross-resistance to new β-lactam/β-lactamase inhibitor combinations in *Pseudomonas aeruginosa* following ceftazidime/avibactam treatment

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Abstract

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has become a major concern in healthcare settings due to its high mortality rate and limited treatment options. New β-lactam/β-lactamase inhibitor combinations (BLBLIs) were introduced to address the growing problem of antibiotic resistance. To date, CRPA strains have demonstrated resistance to new BLBLIs through various mechanisms. However, clinical reports on the cross-resistance to these BLBLIs are relatively scarce. In this study, we described and characterized the CRPA lineage isolated from a patient who received two courses of ceftazidime/avibactam treatment. The CRPA strains developed high-level resistance to ceftazidime/avibactam and cross-resistance to ceftolozane/tazobactam or imipenem/relebactam through two distinct evolutionary pathways, clustering into clade A and clade B. In both clades, the overexpression of AmpC was crucial to ceftazidime/avibactam resistance, which was driven by AmpD deficiency in clade A and DacB mutation in clade B, respectively. In clade A, *mraY* mutation and a new allele of AmpC ($bla_{PDC-575}$) elevated resistance to ceftazidime/avibactam, with $bla_{PDC-575}$ also conferring resistance to ceftolozane/tazobactam. In clade B, *mexB* mutation was associated with the resistance to both ceftazidime/avibactam and imipenem/relebactam. In addition, the overexpression of both MexAB-OprM and AmpC contributed to the resistance to imipenem/relebactam. Moreover, the fitness costs of *P. aeruginosa* strains typically increased with the higher MICs of ceftazidime/avibactam. Divergent resistance evolution resulted in a complex phenotype in the CRPA lineage, posing significant challenge to clinical treatment. The resistance surveillance needs to be prioritized, and new therapeutic strategies are urgently required.

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Reciprocal action of iron scavengers in *Pseudomonas donghuensis* P482 mediates antibacterial activity against *Dickeya solani*

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Abstract

Pseudomonas donghuensis P482 demonstrates significant antimicrobial activity against a variety of phytopathogens, including the soft rot bacteria *Dickeya solani*.

Our study reveals that under conditions of limited nutrient availability, the antibacterial efficacy of *P. donghuensis* P482 against *D. solani* is contingent upon the combined action of two iron scavengers: 7-hydroxytropolone (7-HT) and a newly identified pyoverdine (PVD_{P482}). This antibacterial activity is diminished in environments with increased iron availability. We discovered that the biosynthesis of 7-HT and PVD_{P482} is metabolically coordinated. Specifically, the functional *BV82_4709* gene, essential for the synthesis of 7-HT, is also required for the expression of the *BV82_3755* gene, which is necessary for pyoverdine production. Conversely, the *BV82_3755* gene is essential for the expression of the *BV82_4709* gene, indicating a bidirectional regulatory relationship between these two genes.

Furthermore, the production of both scavengers is regulated by the Gac/Rsm system, with PVD synthesis also under the control of the Fur regulator.

In conclusion, the antibacterial activity of *P. donghuensis* P482, influenced by nutrient and iron availability, underscores the significance of these factors in its competitive interaction with *D. solani.*

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Mechanism of biofilm inhibition by peptidomimetic polyurethanes

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Abstract

Biofilms are bacterial communities that are encased in an extracellular polymeric substance (EPS) and are inherently tolerant to antimicrobials and antibiotics. Peptidomimetic polyurethanes (PPs) have been shown to be able to prevent the formation of these biofilms. However, the mechanism by which these compounds inhibit biofilm formation is unknown. We hypothesized that PPs inhibit biofilm formation by modulating the levels of the secondary messenger molecule cyclic di-GMP (c-di-GMP). High levels of c-di-GMP are associated with biofilms and EPS production while low levels are associated with planktonic cells. Utilizing enzyme and c-di-GMP reporter assays, we demonstrated that exposure of *Pseudomonas aeruginosa* to PPs coincided with increased c-di-GMP levels and decreased, c-di-GMP-degrading, phosphodiesterase activity. These findings suggest that PPs dysregulate c-di-GMP levels which likely results in EPS overabundance causing biofilm cells to suffocate within its encasement. Ongoing research explores the effect of PPs on the EPS' location, abundance, and composition as well as biofilm cell viability using confocal microscopy. Extracellular DNA will also be assessed biochemically and by confocal microscopy post staining to better understand the stability and resistance of PPs exposed P. aeruginosa biofilms. By defining the mechanism by which these polymers inhibit biofilm formation, PPs can be applied to implanted medical devices to reduce the likelihood of biofilm infections in patients. It will also allow researchers to create more effective antibiofilm therapies in the future.

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Unveiling the microenvironmental drivers of β-lactam resistance evolution in *Pseudomonas aeruginosa* within cystic fibrosis airways.

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Abstract

We have investigated the influence of the microenvironment and the bacterium's inherent mutation rate on the evolution of antibiotic resistance in *Pseudomonas aeruginosa* (PA). We employed air-liquid interface (ALI) models mimicking the human airway epithelium, alongside traditional *in vitro* cultures, to explore the impact of environmental complexity. Our findings highlight the interplay between these factors in shaping the evolutionary trajectories of PA resistance to ceftazidime (CAZ).

Firstly, the results emphasize the combined influence of hypermutability and environmental complexity on fostering diversity and, consequently, higher levels of resistance. Strains with a higher mutation rate displayed greater phenotypic diversity than wild-type strains across all conditions. Interestingly, the *ex vivo* environment significantly increased diversity compared to *in vitro* cultures.

Secondly, the study revealed a trend towards complex phenotypes in the *ex vivo* environment. While both environments led to increased CAZ resistance (MIC), *ex vivo* evolution appeared to favour strategies that also minimize damage to the host tissue, evidenced by lower cytotoxicity and reduced immune response activation.

Finally, our data demonstrate that distinct environments select for different resistance strategies. In the simpler *in vitro* cultures, biofilm formation emerged as a significant contributor to resistance alongside increased MIC. This strategy offers protection from antibiotics by creating a physical barrier. In contrast, the *ex vivo* environment primarily selected for enhanced MIC.

Our findings highlight the emergence of diverse resistance strategies driven by the microenvironment, with *ex vivo* evolution potentially favoring complex phenotypes and distinct resistance mechanisms compared to simpler *in vitro* settings.

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R-loops from replication forks intrinsically link stringent response to viscoelastic biofilm matrix formation

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Abstract

Biofilms impart antimicrobial resistance to resident bacteria by secreting extracellular polymeric substances (EPS) that protect cells against stresses by formation of viscoelastic networks. We have demonstrated that extracellular DNA (eDNA) form viscoelastic networks by the self-assembly into G-quadruplex higher order structures and their association with extracellular RNA (eRNA), thereby stabilizing P. aeruginosa biofilms. One potential source of RNA: DNA hybrids and G-quadruplex DNA in bacteria is that of R-loop structures. These form in response to stress and can regulate central dogma processes. Therefore, confirming and accounting for the presence of-R-loops could inform on *P. aeruginosa* matrix formation as well as biofilm control.

In this study, we describe the distribution and importance of R-loops to viscoelastic biofilm matrix formation and identify factors accounting for accumulation of R-loops in *P. aeruginosa* biofilms.

Our findings suggests that R-loops colocalize with the eDNA of P. aeruginosa, *S. epidermidis* biofilms and chronic lung sputum, and their presence correlates with the biofilm viscoelastic response. A uniform coverage of mRNA in the R-loops suggests that R-loop formation in cells is a pre-transcriptional event. Moreover, extracellular R-loops and biofilm formation increase when the stringent response is amplified and decrease when stringent response induced stresses are removed. Thus, for the first time, we have identified a positive correlation between the highly conserved stringent stress response (SSR), R-loops, and viscoelastic biofilm matrix formation in P. aeruginosa.

In conclusion, we suggest that the starvation induced stringent stress response mediates viscoelastic eDNA networks in biofilm matrices and could potentially be targeted for biofilm mitigation.

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An innovative genomics monitoring of carbapenem resistance in 752 Pseudomonas aeruginosa isolates from Europe and East Asia

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Abstract

Carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA) is a top priority issue according to the WHO. Continuous genomic surveillance of CR-PA allows prospective identification of antibiotic resistance genes (ARG). However, sequencing errors can bias ARG amino acid translations and resulting phenotypic resistance prediction. Among ARGs, several are not *sensu stricto* beta-lactamases such as penicillin-binding proteins (PBPs) where point mutations can confer beta-lactam resistance. Here, we present a pipeline (PointAMR) to both enhance assembly quality and ARG variant prediction by read quality and coverage mapping (RQCM).

The PointAMR workflow was developed for the SAMPAN collection of 752 CR-PA clinical and environmental isolates from The Netherlands, Indonesia, and Italy. Chromosome-level assemblies were done using Nanopore and Illumina reads (NanoLite v1.1), followed by ARG annotation (RGI v6.0.3) and RQCM over predicted genes (bwa, minimap2, R). The genomic context of ARGs was investigated with MOB-Suite v3.1.8. A web interface was developed with R Shiny for visualizing ARG variant profiles.

The 752 CR-PA genomes were complete according to CheckM analysis (98-99% completeness; 0.1-0.5% contamination). Total of 73 beta-lactamase genes were identified, of which 51% were either exclusively plasmid-encoded or chromosomal from which IMP, OXA, and *mdsB* genes variants were predicted by RQCM.

PointAMR is an efficient standalone approach to assemble high quality genomes, detect ARGs and evaluate variants with quality control metrics. These variants could not have been detected using RGI alone as it does not evaluate variants outside of its own database. More analysis are planned to validate *in silico* the effect of these variants.

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A bifunctional anti-biofilm agent for treatment of *Pseudomonas aeruginosa* infections

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Abstract

Biofilm matrices encase microbes, impeding antibiotic agents. We describe development of a bifunctional enzyme biologic that degrades biofilms and prevents formation, empowering antibiotic treatment and host immunity. *P. aeruginosa* produces *N*-acyl-L-homoserine lactones (AHLs) to coordinate biofilm formation. Quorum-quenching lactonases degrade AHLs, blocking biofilm formation. However, quorum quenching is less effective against mature biofilms. To overcome this, AiiA, an AHL lactonase from *B. thuringiensis*, was coupled with PslG, an amylase from *P. aeruginosa* that cleaves Psl, the main polysaccharide component of PAO1 biofilms. Fused PslG-AiiA is well expressed in E. coli with a yield of 4mg per liter of culture. AiiA is a Zinc-metalloprotein with a Kcat/Km of $1.6x10^4$ $M^{-1}s^{-1}$ for C6-HSL, a representative AHL. Substitution of Cobalt for Zinc increases catalytic efficiency 100-fold in the monomeric lactonase (Kcat/km = $1.4x10^6$ $M^{-1}s^{-1}$) and fusion protein (Kcat/km = 1.1 $x10^6$ $M^{-1}s^{-1}$). Using a Crystal violet assay to measure biofilm biomass, 300nM fusion reduces biomass by 85% following 1-h treatment of PAO1 biofilms compared with a 45% reduction from treatment with monomeric amylase. A checkerboard assay shows a synergistic effect between the fusion protein and tobramycin for biomass disruption. Further testing will define a dose-response relationship between biofilm disruption and fusion protein concentration alone and in combination with antibiotics as measured by CV and XTT. We report the development of a bifunctional biologic that acts as a quorum quencher and disrupter of biofilm matrices in *P. aeruginosa.* When co-administered with antibiotics, it will improve treatment of biofilm-embedded *P. aeruginosa* infections.

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Pseudomonas putida biofilm depends on the vWFa-domain of LapA and extracellular peptides

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Abstract

Formation of Pseudomonas putida biofilm is a complex process involving various intercellular and extracellular factors. One of the significant key players is the surface adhesin LapA, specifically its von Willebrand factor A-like (vWFa) domain. We noticed that in the presence of peptides in the medium, the biofilm of *P. putida* is enhanced. Although *P. putida* control strain PSm has peptide-dependent biofilm enhancement, it was more pronounced in *P. putida* strain F15, where it is possible to overexpress *fis* and thereby *lapA*. We created *P. putida* strains with inducible *lapA* expression and LapA variants lacking the vWFa domain to ascertain the mechanisms involved in biofilm enhancement. Our findings indicate that the vWFa domain is crucial for the peptide-driven enhancement of biofilms. Specifically, in the Fis-overexpressing strain, the absence of the vWFa domain reduces the biofilm formation, indicating the connection of the LapA vWFa domain and extracellular peptides.

The interaction between LapA and extracellular peptides suggests a complex regulatory network in which the vWFa domain plays an important role in biofilm formation. This domain, known for its similarity to von Willebrand factor, appears to facilitate the integration of environmental signals (e.g., the presence of peptides) into biofilm formation.

Our study adds to and refines the knowledge of *Pseudomonas putida* biofilm formation, highlighting the importance of extracellular factors in biofilm formation. In the future, the question remains whether a specific peptide is recognised by the vWFa domain and whether there are still regulatory nodes of *lapA* expression that need to be unravelled.

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Exploring the use of ancient phasins as protective agents during environmental stress conditions in rhizobacteria

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Abstract

The ongoing climate change is threatening to decimate future harvest yields around the globe through heat waves and droughts. Although plants have survived warmer periods throughout Earth's eras, the current rate of climate change is unprecedented, necessitating intervention to help crop plants adapt. Our approach here involves engineering plant growth-promoting rhizobacteria that can potentially be employed in soils compromised by heat, drought, or pollutants. To this end, we revive ancient genes and biosynthestic gene clusters from previous warm periods, to equip modern rhizobacteria with the tools to combat the effects of today's climate change for plant protection.

Here we focus on reviving phasins, proteins with chaperone-like functions regulating the dynamics of carbonosomes. As granules made of polyhydroxyalkanoates (PHAs), carbonosomes serve as carbon and energy reservoirs in multiple rhizobacteria (including *Pseudomonas putida*) during the response to diverse environmental stresses. Taming the bacterial mobilization of PHAs via phasin-utilization may pave the way for the development of strains useful for bioremediation, and for the synthesis of chemicals with biotechnological applications.

Using strains deficient either in the PHA biosynthetic cluster, in the phasins or in both, we attempt to clarify the mechanisms through which PHA-related proteins perform their protective functions during growth in the presence of drought-mimicking agents and chemical solvents. To extend the range of potential functionalities, we retrieved phasins from paleo-environmental samples, and selected protein sequences without significant amino acid similarity across different ages. Their recombinant expression was therefore tested *in vivo,* and evaluated in terms of host fitness under stress conditions.

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Municipal wastewater containing clinical effluents with antimicrobial pharmaceuticals disseminates carbapenemase-producing Pseudomonas aeruginosa

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Abstract

Wastewater from clinical sources containing antimicrobials administered in healthcare is a key input route for pharmaceutical residues in various water systems where they contribute to the evolution and dissemination of antibiotic resistance. This study determined the occurrence of antibiotic residues in hospital effluents using HPLC and characterized the prevalence of β-lactamases in *Pseudomonas. aeruginosa* from hospital effluents, urban municipal wastewater and a proximate wastewater treatment plant through antibiograms, genotyping and capillary sequencing. Eight antibiotics belonging to macrolides, fluoroquinolones, sulfonamides, and trimethoprim were recovered from the wastewater samples. The highest concentrations were, trimethoprim (24907 ngL^{-1}), ofloxacin (21916 ngL^{-1}), and ciprofloxacin (11710 ngL^{-1}). Resistance profiles of 151 *P. aeruginosa* isolates to three chemical classes demonstrated a slightly higher multiple antibiotic resistance (MAR) index in hospital effluents (0.8) compared to wastewater treatment plant and urban wastewater (0.7). The species was resistant to gentamicin (92.7%), ceftazidime (85.4%), imipenem (83.4%), ciprofloxacin (74.8%), and meropenem (57.6%). Metallo-β-lactamase VIM-4 was the predominant β-lactam degrading enzyme detected among the isolates that exhibited a resistance phenotype towards carbapenems. Continuous environmental exposure to the antimicrobial residues may lead to the emergence of resistant strains, and may result in the build-up of environmental reservoirs of carbapenamase producing bacteria emanating from the pool of resistance genes circulating in the broad microbial population entering the ecosystem. Pre-treatment of hospital effluents prior to discharge into the wastewater network is a feasible management strategy.

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Integrative structural and functional study of a major histidine kinase controlling Pseudomonas aeruginosa infection modes

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Abstract

Two component systems (TCS) detect an environmental signal and trigger an appropriate cellular response. In *Pseudomonas aeruginosa,* the GacS/GacA system is a central and critical TCS that controls the switch between its acute (where bacteria are disseminated in the bloodstream) and chronic mode of infection (characterized by the formation of an antibiotic resistant biofilm). Our research focuses on the structural aspects of this master regulatory system, aiming to elucidate the molecular mechanisms underlying GacS autophosphorylation.

We used crystallography to determine the structure of the histidine kinase catalytic core and autophosphorylation assays with g^{32}P-ATP to assess the enzyme's activity. Additionally, we used various biophysical techniques to gain deeper understanding of GacS's functioning such as Site-Directed Spin Labelling coupled to Electron Paramagnetic Resonance (SDSL-EPR) or Size Exclusion Chromatography coupled to Small-angle X-ray Scattering (SEC-SAXS).

The crystallographic structure of GacS catalytic core in its inactive form, in addition to providing us with structural insights into this histidine kinase, revealed an uncharacterized domain (ND domain). Located between the ATP catalytic domain (CA domain) and the receiver domain (D1), this domain was found to be essential for the autophosphorylation of GacS without being directly involved in the ATP binding. This result enlightens an atypical autophosphorylation mechanism and raises new questions about the functioning of this system.

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Collective immunity - how groups of bacteria sense and respond to danger

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Abstract

Quorum sensing (QS), is the bacterial cell-to-cell communication process that promotes the collective undertaking of group behaviors. In addition to regulating bacterial virulence programs, it shapes the outcomes of encounters between bacteria and the bacteriophage (phage) viruses that prey on them.

Since phages require a host to proliferate, at low cell density, bacteria rarely encounter a phage. Conversely, at high cell density, phage infection can spread rapidly in a dense microbial environment. We have shown that Pseudomonas aeruginosa uses elevated QS levels at high cell density to launch its CRISPR-Cas anti-phage defense system when the risk of phage infection is highest. If phages successfully infect *P. aeruginosa*, despite defenses, it emits the PQS QS signal, warning and redirecting healthy neighboring bacterial swarms away from the infected cells. Not only does phage infection activate QS in wild type cells, it also restores PQS quorum sensing in a *lasI* quorum sensing mutant, in which the PQS system is otherwise mute. Hereby, we demonstrate that a *P. aeruginosa lasI* mutant can bypass the otherwise hierarchal QS organization in response to phage infection, further underscoring the importance of QS in regulating phage defenses.

Clinical isolates of *P. aeruginosa* frequently harbor mutations in particular QS genes. Thus, phage infection of such *P. aeruginosa* strains may inadvertently increase bacterial virulence. This highlights the importance of studying phage-host interactions in the context of bacterial mutants that are relevant in clinical settings for developing phages to eliminate *P. aeruginosa* infections.

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Transthyretin mediates the agglutination and death of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a leading cause of nosocomial bloodstream infections. The outcome of these infections depends on the virulence of the microorganism as well as host-related conditions and factors.

To investigate the factors that interact with *P. aeruginosa* when enters the bloodstream, we conducted a global proteomic analysis of the proteins bound to the microorganism incubated in human serum. About 45 proteins identified by mass spectrometry were found to interact with *P. aeruginosa*. Among these proteins, we identified transthyretin (TTR), a homotetrameric protein primarily responsible for transporting thyroxine and retinol-binding protein (RBP).

We performed TTR binding assays using either whole cells of *P. aeruginosa* or lipopolysaccharides (LPS) isolated from various LPS isogenic mutants with progressively truncated LPS structures, to show that TTR binds to *P. aeruginosa* via lipid A in a manner dependent on the LPS O antigen.

To localize the binding region of lipid A in TTR, we used a library of synthetic N-terminal biotinylated peptides spanning the entire human TTR molecule. Quantitative ELISA analysis revealed that lipid A exhibited the highest reactivity with a 42-mer peptide located in the N-terminal region of TTR.

Bacterial viability assays and confocal microscopy with various *P. aeruginosa* bloodstream infection isolates demonstrated that purified human recombinant TTR induces bacterial agglutination and cell death. Additionally, amyloid diagnostic dyes detected the formation of protein amyloid-like aggregates on the bacterial surface.

We concluded that human TTR forms extracellular amyloid deposits on *P. aeruginosa* that induce the killing of the microorganism.

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Novel Cell-Machine Interface Reveals Pulse Frequency-Dependent Heterogeneity in *P. putida* KT2440 During Benzoate Assimilation in Continuous Culture

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Abstract

Modern strategies to address bioremediation involve plastic pretreatment, synthetic division of labour, and techniques to mitigate toxic compound flux. However, these strategies are rarely adapted to continuous cultivation systems, which offer higher productivity but face stability challenges, mainly due to compounds with a toxic effect on cell physiology.

We employed a novel cell-machine interface, which integrates an online flow cytometer with various regulation options. This system monitors cellular physiology and specific phenotypes at single-cell resolution, allowing the calculation of system entropy, which reflects population heterogeneity. Recent studies show that microbial populations increase entropy to compensate for their fitness loss. Different diversification regimes emerge depending on the cost of phenotype activation, providing insights into cellular physiology and process control.

In this study, *Pseudomonas putida* KT2440 was subjected to benzoate continuously or in increasing pulse regimes during continuous cultivation. A biosensor expressing sfGFP under *ben*-operon regulation indicated degradation activity. In a chemostat, benzoate-degrading enzyme expression showed low entropy, while the population exhibited short-term increases and long-term decreases in cellular size, possibly due to reduced surface area and solvent uptake rates. Under varying pulse frequencies, *ben* system expression exhibited higher entropy at low frequencies and lower entropy at higher frequencies until the environmental concentration caused collapse. This entropy change may relate to other solvent stress phenotypes.

This work aims to enhance understanding of bioremediation and population dynamics under toxic compound exposure. Furthermore, we aim to explore ethylene glycol and benzoate assimilation using a synthetic consortium with a division of labour in continuous cultivation.

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Detection of potential pan-resistant urinary tract *Pseudomonas aeruginosa* isolates in a comparative study between two distinct geographical locations

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Abstract

Urinary tract infections (UTIs) have a significant impact on morbidity and mortality worldwide. *Pseudomonas aeruginosa (P. aeruginosa)* utilise a wide range of virulence factors, such as biofilm formation and motility, to induce infections. *P. aeruginosa* exhibit high levels of antibiotic resistance, and the World Health Organisation has classified it as a pathogen in need of the development of new antimicrobials. Despite the emergence of highly pathogenic antimicrobial-resistant (AMR) strains around the world, UTIs caused by *P. aeruginosa* are underreported in the literature.

We obtained a total of 23 *P. aeruginosa* UTI isolates from the United Kingdom (UK) and Kuwait. In order to determine the characteristics of UK isolates, we conducted growth rate analysis, biofilm formation, and antibiotic disc diffusion assays. Additionally, both sets of isolates underwent whole genome sequencing, antimicrobial susceptibility testing, and *in silico* analysis of AMR-associated genes.

The UTI isolates exhibited variations in both their clinical features and genetic composition. Isolates from Kuwait have been found to possess several resistance genes that confer resistance to different kinds of antibiotics, including aminoglycosides, fluoroquinolones, and β-lactams. Particularly, OXA-10 Class D β-lactamase was detected for the first time in *P. aeruginosa* UTI isolates in Kuwait.

This study emphasises that the isolates obtained from UTIs exhibit a wide range of genetic variations and have the potential to exhibit very high levels of resistance to antibiotics. Surveillance in countries such as Kuwait is lacking, prompting the need for immediate intervention.

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Engineering hypervesiculation of outer membrane vesicles in *Pseudomonas putida KT2440* for improved production of 3-hydroxyacids

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Abstract

The soil bacterium *Pseudomonas putida* KT2440 is valued in biotechnological applications for its robust growth in cytotoxic conditions, diverse metabolic capabilities, and genetic tractability, making it well-suited for the valorization of lignocellulose-derived feedstocks into biochemicals. We recently demonstrated that *P. putida* KT2440 secretes outer membrane vesicles (OMVs) packaged with enzymes involved in the catabolism of lignin-derived aromatic compounds. OMVs are ubiquitous among studied Gram-negative bacteria and are increasingly recognized as valuable biotechnological tools, customizable for bespoke functions. Here, we (1) identified genetic targets for engineering hypervesiculation in *P. putida,* (2) characterized the effects of hypervesiculation on cellular physiology and OMV protein cargo, and (3) leveraged hypervesiculation to increase 3-hydroxyacids production. We found that deletion of *oprI* and *oprF* resulted in increased hypervesiculation in *P. putida*, analyzed using nanoparticle tracking analysis to enumerate and measure OMV size. This affected cell permeability and utilization of lignin-derived aromatics, and differences in OMV cargo were observed between hypervesiculation strategies through proteomic analysis. Finally, leveraging our hypervesiculation phenotype along with the overexpression of acetyl-CoA carboxylase and enzymes involved in cell wall biosynthesis aimed to increase flux through acetyl-CoA and augment membrane components supporting OMV formation, enhancing production of 3-hydroxyacids, a useful building block for biochemicals, from lignin-derived aromatics. Together, this resulted in a two- to three-fold increase in 3-hydroxyacid titers. Overall, our work underscores the potential of leveraging OMVs as a bioengineering tool that complements current engineering efforts and highlights the versatility of *P. putida* as a promising host.

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Enhanced Treatment Strategies for Lung Infections: The Role of PEGylated Tobramycin in Disrupting Pseudomonas Aeruginosa Biofilms

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Abstract

Purpose:

Chronic bacterial lung infections develop highly dense 3-dimensional biofilms that have been shown to reduce the diffusivity of antibiotics. In previous work, we synthesized a modified version of tobramycin via a site specific PEGylation in an attempt to improve antibiotic diffusion through biofilms. The objective of this study was to evaluate the *in vitro* and *in vivo* performance of PEGylated tobramycin in models of *Pseudomonas aeruginosa* (PA) biofilms.

Methods:

Tobramycin was PEGylated (PEG-TOB) with 5kDa mPEG through carbodiimide-mediated coupling. In-vitro antibacterial activity was evaluated on PA (ATCC:15692) biofilms using XTT and crystal violet assays. Biofilm penetration of PEG-TOB was imaged using confocal microscopy. In-vivo antimicrobial activity was evaluated with a chronic PA pulmonary infection rat model. Infection characteristics were measured following three therapeutic doses (100uM/kg).

Results:

In *in vitro* PA biofilms (24hr growth), PEG-TOB exhibited a 7-fold reduction in MIC_{80} (14uM) compared to free tobramycin (100uM). In mature biofilms (48 and 72hr growth) anti-biofilm efficacy of PEG-TOB was further enhanced. Microscopic analysis showed that PEG-TOB had greater bacterial cell death with increasing biofilm depth compared to tobramycin controls. In the *in vivo* rat studies lower PA CFU lung counts were observed for PEG-TOB (55 CFU/g lung weight) compared to tobramycin (200 CFU/g). Additionally, PEG-TOB continued to inhibit PA proliferation 7 days post treatment, reaching ~15 CFU/g, and statistical reduction in IL-6, TNF-α, C-reactive protein, and procalcitonin.

Conclusions:

PEG-TOB significantly improved antimicrobial efficacy compared to tobramycin in both *in vitro* and *in vivo* models of PA biofilms.

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Exploring Pseudomonas Species Diversity and Pathogenic Potential in Hospital Settings

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Abstract

Pseudomonas species are known for their ubiquitous presence across diverse environments and their great adaptability to a multitude of hosts. In clinical settings, *Pseudomonas* species, such as *P. aeruginosa* can be dangerous pathogens, but they also play a major role in defining the hospital microbiota.

In this study, a total of 758 *Pseudomonas* isolates were retrieved from hospital sink drains and bed rails, along with 13 clinical isolates. All isolates were identified using MALDI-TOF MS, and further identification was confirmed by sequencing the 16S rRNA gene, in combination with the *rpoD* gene, specific for the genus. The preliminary analysis of all isolates revealed at least 17 different species, 6 of which may represent new species within this genus, five from the environment and one clinical. *P. aeruginosa* was the most common species in both the environment and clinical isolates, highlighting the importance of this species in these environments. Although *P. putida* group species are often overlooked regarding their pathogenic potential, this study highlights their dangers and potential threats to public health. The high diversity of this genus in these environments also poses risks, as it may indicate that newer species can develop more antibiotic resistance and pathogenic potential.

The presence and similarity of species from both environmental and clinical origins underscores the potential of nosocomial infections from environmental sources (with particular emphasis on sink drains and bed rails) while also highlighting necessity for rigorous hospital monitoring and infection control measures.

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Interactions between hibernation promoting factors and ribosomes in Pseudomonas aeruginosa

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Abstract

When *Pseudomonas aeruginosa* is dormant, the small accessory proteins, ribosome modulation factor (RMF) and hibernation promoting factor (HPF), lock ribosomes in an inactive state. During dormancy, HPF protects a critical number of ribosomes from degradation. Here, we developed single-copy bi-molecular fluorescence complementation (BiFC) vectors to study the *in vivo* kinetics as HPF and RMF interact with ribosomes. We first tested the efficacy of the BiFC system using epifluorescence microscopy, showing interactions of the chemotaxis proteins, CheY and CheZ, on the cell pole. We next characterized HPF/ribosome and RMF/ribosome interactions as these ribosomal accessory factors load onto the mRNA channel adjacent to the ribosomal proteins, S9 and S13 (for HPF), and S1 and S21 (for RMF). Immunoblots and cell imaging verified that the BiFC fluorescent signal occurs as the hibernation factors interact with ribosomes. Interestingly, the HPF/ribosome BiFC signal occurs early in the cell growth, while RMF loading occurs primarily in stationary phase. The RMF/ribosome interaction is reduced in a *P. aeruginosa* Δ*hpf* mutant and enhanced when *hpf* is overexpressed. In contrast, the *P. aeruginosa* Δ*rmf* mutation does not influence the rate of HPF/ribosome interactions. In addition, the stringent response influences RMF/ribosome interactions, but does not affect HPF/ribosome interactions. Overall, the results indicate that in *P. aeruginosa*, HPF loads onto ribosomes throughout cell growth, and that HPF plays a role in recruiting RMF to the ribosome. RMF is not required for HPF/ribosome interactions. The stringent response helps recruit RMF to ribosomes but does not affect HPF loading.

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The role of polyamines in the virulence of *Pseudomonas aeruginosa* during chronic pulmonary infection in cystic fibrosis patients

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Abstract

Polyamines (polyA) are polycationic metabolites involved in several cellular processes. Our group has previously shown, by non-targeted comparative metabolomics analysis on clinical strains of *Pseudomonas aeruginosa* (*Pa*) from the airways of cystic fibrosis (CF) patients, a correlation between increased polyA production by *Pa* and instability of lung function in these patients.

This multi-omics study aims to understand how *Pa* modulates its polyA production in CF patients, how this affects the expression of virulence factors and whether *Pa* controls its virulence phases by modulating polyA production during chronic infections in these patients.

Genomic and transcriptomic analyses were used to elucidate the mechanisms by which polyA is modulated. Isogenic mutants of *Pa* that partially or completely block polyA production pathways were generated. Experiments were also carried out to measure the virulence of mutant strains producing different levels of polyA, as assessed by LC/HRMS.

The results identified thirty-eight genes that were over-expressed in isolates with high polyA production, including type 3 secretion system (T3SS) and motility genes. Among these genes, speE2 and speD2 appeared to be responsible for the increase in spermidine levels, highlighting the role of alternative pathways in polyA production. Polyamines synthesised via the alternative pathway contribute more significantly to cytotoxicity than those produced via the classical pathway.

In conclusion, this study demonstrates the crucial role of alternative polyA biosynthetic pathways in *Pa* virulence and suggests new therapeutic targets for the management of chronic infections in CF patients.

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Pseudomonas strains isolated from seawater in Kuwait: a possible source of novel antibiotics.

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Abstract

Background: With rise in the spread of antibiotic resistant bacteria and the decline in antibiotic discovery, novel antibiotics are urgently needed. The aim of this research was to use culture-based approaches and bioinformatics algorithms to estimate the possible antibacterial production capabilities of 3 Pseudomonas sp. strains initially isolated from seawater, as well as to determine their antibacterial spectrum.

Methods: The possible antibacterials produced by Pseudomonas sp. strains KD1, KD2 and KD3 were predicted using bioinformatics platforms including antiSMASH and BAGEL. These strains were evaluated for antibiotic production by growing them in different culture conditions, including different growth medium, incubation temperature and incubation time. The supernatants were collected and tested against several indicator bacteria including the ESKAPE pathogens and pandrug-resistant Escherichia coli, using agar well diffusion assays.

Results: All Pseudomonas sp. strains were inhibitory to several indicator bacteria, including multidrug-resistant Acinetobacter baumannii, Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA). Pseudomonas sp. strain KD1 produced broadest antibacterial activity, inhibiting pandrug-resistant Escherichia coli, A. baumannii, MRSA, P. aeruginosa and extended-spectrum-beta-lactamase-producing Klebsiella pneumoniae (ESBL-KP). Bioinformatics analysis predicted the capability of Pseudomonas sp. strain KD1 to produce novel secondary metabolites with antibacterial properties. This strain was also predicted to produce compounds related to viscosin and syringomycin.

Conclusion: Pseudomonas sp. strain KD1 outperformed other strains in its capability to produce novel, broad spectrum antibiotics. This strain therefore offers a potential source of novel antibacterials in the future

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Establishment of a airway infection model utilizing a pseudostratified airway epithelial cell and human monocyte derived macrophages in a Air liquid interface model

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Abstract

Airways of CF patients often get infected by *Pseudomonas aeruginosa (Pa)*, which initially is antibiotic-susceptible, but over time develops resistance towards several antibiotics. Moreover, CF patients have difficulties regulating the inflammatory response. Much is known about the neutrophil response during Pa infection since polymorpho-nuclear leucocytes can be found in large numbers in the sputum. However, the dysregulation of the innate immune response is not elucidated to the same degree. In recent years researchers have started to investigate the role of other innate immune cells, such as macrophages. Macrophages play key roles as a first line of defense against pathogens, but they also participate in immune regulation. There is some disagreement whether macrophages have a hyper-response to Pa, or if there is a lack of a response. To elucidate the role that macrophages play during Pa airway infection, we have developed an air-liquid-interphase (ALI) infection model, which allows us to investigate how Pa, airway epithelial cells, and macrophages interact. This model closely mimics the infection in CF airways. We have used flow cytometry and confocal microscopy to monitor the differentiation of the macrophages and to visualize the adhesion to the basolateral side of the ALI cultures.

In conclusion, we have successfully established an ALI airway culture model and we have managed to differentiate monocytes to macrophages and get them to adhere to the basolateral side of the ALI culture. The next step will be in depth-characterization of the bacterial immune interaction in the ALI model.

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High-resolution mapping of Sigma Factor DNA Binding Sequences using Artificial Promoters, RNA aptamers and Deep Sequencing in *Pseudomonas putida*

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Abstract

The variable sigma (σ) subunit of the bacterial RNA polymerase holoenzyme determines promoter specificity and facilitates open complex formation during transcription initiation. Understanding σ-factor binding sequences is therefore crucial for deciphering bacterial gene regulation. We have devised a high-throughput approach that utilizes an extensive library of 4.5 million DNA templates to provide artificial promoters and 5'prime UTR sequences for σ-factor DNA binding motif discovery. This method combines the generation of extensive DNA libraries, in vitro transcription, RNA aptamer selection, and deep DNA and RNA sequencing. It allows direct assessment of promoter activity, identification of transcription start sites, and quantification of promoter strength based on mRNA production levels.

Using this method, we determined the DNA binding sequences of 17 sigma factors from three bacterial species: *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas putida*. From the library of 4.5 million DNA templates, we identified more than 2.5 million functional RNA sequences across 17 different σ-factors.

In this presentation, I will introduce our approach for high-resolution mapping of σ-factor-DNA binding sequences, with a particular focus on the findings for *P. putida*. Additionally, I will also share our efforts in updating annotation for the regulatory sequences in the *P. putida* genome. I will conclude my presentation with our ongoing efforts in developing deep learning-based promoter prediction tools and future plans.

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Loss of repressor MexZ function commonly found in the clinic accelerates induction of aminoglycoside response in *P. aeruginosa*.

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Abstract

P. aeruginosa is a leading cause of mortality for cystic fibrosis (CF) patients. Opportunistic infections in the CF lung often progress into chronic infections with phenotypically heterogeneous populations and increased antibiotic resistance. Aminoglycoside antibiotics are often used in the treatment of chronic infections, but success is limited mostly due to high expression of a multidrug efflux mechanism MexXY-OprM. Although about half of *P. aeruginosa* lineages evolved in the CF lung eventually acquire mutations disrupting MexXY-repressor MexZ, such mutations do not increase the minimal inhibitory concentration (MIC) of aminoglycosides. Here, we test the hypothesis that *mexZ* mutations increase antibiotic resistance not by upregulating fully induced MexXY levels, but by delivering a faster response upon drug exposure. We use a microfluidic device to follow *mexXY* expression in single cells of both WT and *mexZ* mutants during drug responses, to determine how the dynamics of MexXY induction affects survival to antibiotic treatments. We use an automated continuous-culture device to study the progression of heterogeneous populations subjected to drug treatments, showing that *mexZ* mutants better survive treatment and have a temporary fitness advantage over WT cells upon exposure to aminoglycosides. Finally, we use a simple assay where liquid cultures are exposed to the drug during mid-log phase to estimate cell survival upon sudden drug exposures, capturing gains in resistance that are not detected by typical MIC assays. A better understanding of how heterogeneous microbial populations respond to dynamic drug regimens is essential to design antibiotic treatments that address specific resistance profiles.

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Identifying novel drug targets of the ESKAPE pathogen Pseudomonas aeruginosa

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Abstract

The ESKAPE pathogen *Pseudomonas aeruginosa* is a priority pathogen that requires the urgent development of new antimicrobials. With limited antibiotics in clinical development, and stagnation of the discovery pipeline, new drug targets and approaches are needed. For example, targeting bacterial virulence factors should reduce the pathogenesis of an organism while slowing its infection progression. However, one challenge in targeting virulence mechanisms is that they vary among bacteria, often being genus or even species specific, and this makes identification of a broad-spectrum virulence target challenging.

The Macrophage Infectivity Potentiator (Mip) proteins provide such a candidate [1]. They are ubiquitous and modulate virulence in numerous gram-negative pathogens including *Legionella pneumophila* and *Burkholderia pseudomallei* where they are essential for invasion of macrophages, pathogenesis in murine-infection models, and for other virulence determinants [2-4].

Here we show that *Pseudomonas aeruginosa* (PA01) also possesses Mip virulence factors. Through phylogenetic analysis we identified three genes encoding Mip-candidates that we have shown are required for the full virulence of *P. aeruginosa* in *Galleria mellonella* infection and macrophage infection models, and in a range of *ex vivo* models including biofilm formation, haemolysis, and motility assays. Furthermore, Mip control of virulence can be addressed pharmacologically using bacterial natural products, reducing invasion of macrophages by up to 52%. Additionally, we have begun to unpick molecular basis through which Mips exert their virulence role.

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Pseudomonas aeruginosa and its interaction with lung epithelial cell cultures at the Air-Liquid Interface

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Antonella Colque

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Abstract

Pseudomonas aeruginosa remains one of the biggest challenges causing chronic airway infections in people with cystic fibrosis (pwCF). Profiling the early colonization of *P. aeruginosa* is of great importance since it is still poorly understood how bacteria use their virulence machinery to become persistent. To model mechanisms of host colonization, we performed infection assays, using primary epithelial cells from healthy and CF donors and the BCi-NS1.1 cell line cultured under Air-Liquid Interface (ALI) conditions, and early and late clinical strains isolated from pwCF. As controls, we used the laboratory strain PAO1 and a less virulent Type III Δ*pscC* strain. Principal component analysis of epithelium integrity, cytotoxicity levels, cytokine secretion and bacteria count over 14h or 38h of infections, showed grouping of *P. aeruginosa* strains depending on their intrinsic infection capabilities. Dual-species transcriptomics of PAO1 and Δ*pscC* mutant infected ALI cultures showed that global gene expression profile from primary cells was highly different from that of the BCi cell line. Upon colonization with PAO1, all host cells showed upregulation of inflammatory pathways with CF cells showing a less active pathogen-associate response. Strikingly, bacterial gene expression profiles were dependent on the host environment. While transcriptional profile was similar in healthy and BCi cell line environment, upon colonization of the CF epithelium, Δ*pscC* showed downregulation of dozens of genes related to quorum sensing signaling pathways. Altogether, our infection data captures a snapshot of the interplay between *P. aeruginosa* and lung cells and recapitulates some of the features from *in vivo* CF lung infections.

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Catabolism of Acetosyringone and Co-metabolic Degradation of Phenaclor by a Novel FAD-dependent monooxygenase from *Pseudomonas rhizophila* ASC12

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Tomáš Engl

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Abstract

Chlorophenols (CPs) pose a significant environmental threat due to their toxicity, persistence and bioaccumulation. Co-metabolic biodegradation shows great promise in the removal of these pollutants. Given the structural similarity between CPs and various plant metabolites (PM) such as acetosyringone (AS) or other structural motives in lignin, enzymes involved in the catabolism of PM are hypothesized to play a crucial role in the degradation of CPs. In this study, we aimed to identify novel enzymes involved in the catabolism of AS and evaluate their co-metabolic activity toward CPs. Using the enrichment culture on AS as the sole carbon source, a bacterial consortium was obtained that exhibited degradation of phenaclor (2,4,6-triCP) and 2,6-diCP. Metagenomic analyses revealed that the consortium was dominated by *Pseudomonas rhizophila* and *Methylotenera versatilis*. Metagenome-assembled genomes (MAGs) of the bacterial consortium were searched against a database of genes implicated in the degradation of S-lignin and CPs. Through heterologous expression and functional assay, a phylogenetically unique FAD-dependent oxidoreductase AsdA was identified in *Pseudomonas rhizophila* MAG. AsdA showed biotransformation of both AS and phenaclor, moreover, non-targeted LC-MS analysis of biotransformation products further elucidated the reaction mechanisms catalyzed by AsdA. In summary, this work shows that metagenomics linked with bioinformatic predictions and functional assays represents a powerful approach for discovering novel enzymes for green biotechnology applications ranging from lignin valorization to pollution control.

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Metabolic Determinants Involved in the Increased Fitness of *Pseudomonas aeruginosa* LasR- Strains

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Igor Todorovic

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Abstract

Pseudomonas aeruginosa is a common pathogen that causes many types of infections. Analyses of evolved lineages that arise in infections have identified mutations that commonly occur during infection. One such class of *P. aeruginosa* mutations are those impacting the quorum sensing (QS) master regulator, LasR. We previously published that in both clinical and laboratory strains, the evolution of LasR- mutants is dependent on the carbon catabolite repression (CCR) system controlled by the CbrA sensor kinase and CbrB response regulator. The CbrAB pathway grants *P. aeruginosa* improved growth in post exponential phase. We also showed that LasR- strains have increased PhoB activity at physiological phosphate concentrations. We show that complementation of LasR- clinical isolates with a functional *lasR* restores protease activity, reduces growth in post-exponential phase, and decreases PhoB activation. To better understand the fitness benefit of LasR- mutants, we performed a transposon mutant screen using the *P. aeruginosa* LasR- clinical isolate J215 for mutants that lacked some of the metabolic changes that occur in the absence of LasR function. Out of the twenty-five mutants identified in this screen, several were involved in cysteine biosynthesis, ppGpp signaling, and central carbon metabolism. Mutants are phenotypically categorized and the pathways that were targeted were analyzed in existing transcriptomics and metabolomics datasets. Together, these data bring us closer to understanding the metabolic basis for the rise of LasR- strains and their virulence-relevant phenotypes.

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Regulatory mechanism of autonomous disaggregating chaperone ClpG in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa, a human opportunistic pathogen, thrives a various environmental and host niches. Molecular chaperones maintain protein homeostasis and contribute to its survival in these circumstances. *P. aeruginosa* possesses two Hsp100 disaggregating chaperones, ClpB and ClpG, which unfolds aggregated proteins in ATP-dependent manner. Since indiscriminate protein disaggregation can lead to excessive ATP consumption and the unfolding of native proteins, finely tuned regulation of disaggregating activity is required. In case of ClpB, the interaction between M-domain of ClpB and its co-chaperone DnaK-DnaJ regulates its activity. However, unlike ClpB, ClpG performs disaggregation activity without a co-chaperone, and its regulatory mechanism has not yet been characterized. Notably, M-domain of ClpG is shorten than M-domain of ClpB, and ClpG has unique N1 domain and C-terminal extension domain (CTE), which is not found in ClpB. In this study, we focused how each domain of ClpG contributes to regulate its autonomous disaggregating activity. We constructed variants of ClpG containing mutation in N, M, CTE, and ATPase domains, respectively. Then, we examined disaggregating activity by testing heat shock resistance which is conferred by ClpG. Meanwhile, testing ClpG protein solubility and monitoring the in vivo fluorescent signal of YFP-fused ClpG variants using fluorescent microscopy identified the roles of the respective domains in substrate binding and ATP-dependent substrate unfolding activities.

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Employing a 3D human lung infection model to unravel host-microbe interactions, by dual-species proteomic and phosphoproteomic analyses.

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Abstract

Cystic fibrosis (CF) classically manifests in the airways, leading to impaired respiratory capacity. Persistent infection is a hallmark of CF, where Pseudomonas aeruginosa (Pa) is the predominant pathogen. Despite treatment, Pa infections persist throughout the lifetime of the patient. During this time, Pa undergoes genetic, phenotypic, and metabolic adaptations, which are often shaped by the host environment. Yet, our understanding of the mutual impact between Pa and host remains limited. The aim of this study is to unravel key factors and molecular events of host-microbe interactions, during Pa infection. To this end, we perform dual-species proteomics on human lung air-liquid-interphase (ALI) models infected with different Pa strains. Our preliminary data indicate an upregulation of immune system factors and a downregulation of extracellular matrix proteins during the early stages of infection. Future dual-species phosphoproteomic studies will complement the current data. Thus, we expect the outcome of this project will identify new potential drug targets, and highlight signaling pathways important in the early stages of Pa infection, with the overall goal of preventing Pa infection.

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Characterizing microbial community interactions among pathogens in an in vitro chronic wound model

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Abstract

Chronic wounds often harbor polymicrobial biofilms that frequently display high antimicrobial tolerance and persistence, therefore leading to worse patient outcomes relative to mono-species infections. This project set out to determine the best *in vitro* model to investigate the dynamics between four opportunistic human pathogens, namely Acinetobacter baumannii, *Enterococcus faecalis*, Pseudomonas aeruginosa and *Staphylococcus aureus*, that are frequently co-isolated from chronic wounds and are on the ESKAPE pathogen priority list. Traditional rich-media in vitro experiments often favor a singular pathogen out of a multi-species mixture, with one species dominating within 24 hours. This makes it complicated for multi-omic analyses due to the scarcity of RNA and metabolites produced by low-abundant members in these communities. We found that in an *in vitro* wound-like model (WLM), these four pathogens proliferate within 24 hours and are able to be passaged together over the course of 5 days, making it a suitable model to study community dynamics and co-evolution. Through whole genome sequencing, we identified specific mutations that occur when these bacteria are passaged together. Carrying out combinatorial co-culture experiments, we found that this chronic wound model allows otherwise out-competed gram-positive bacteria to survive likely due to coagulases produced by S. aureus, creating anoxic microniches. This is evidenced by the formation of microaggregates that penetrate deeper into the fibrous clots in the WLM. To further elucidate the complex interactions between these bacteria, we have applied a combination of fluorescence in-situ hybridisation, metatranscriptomics, and metabolomics.

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