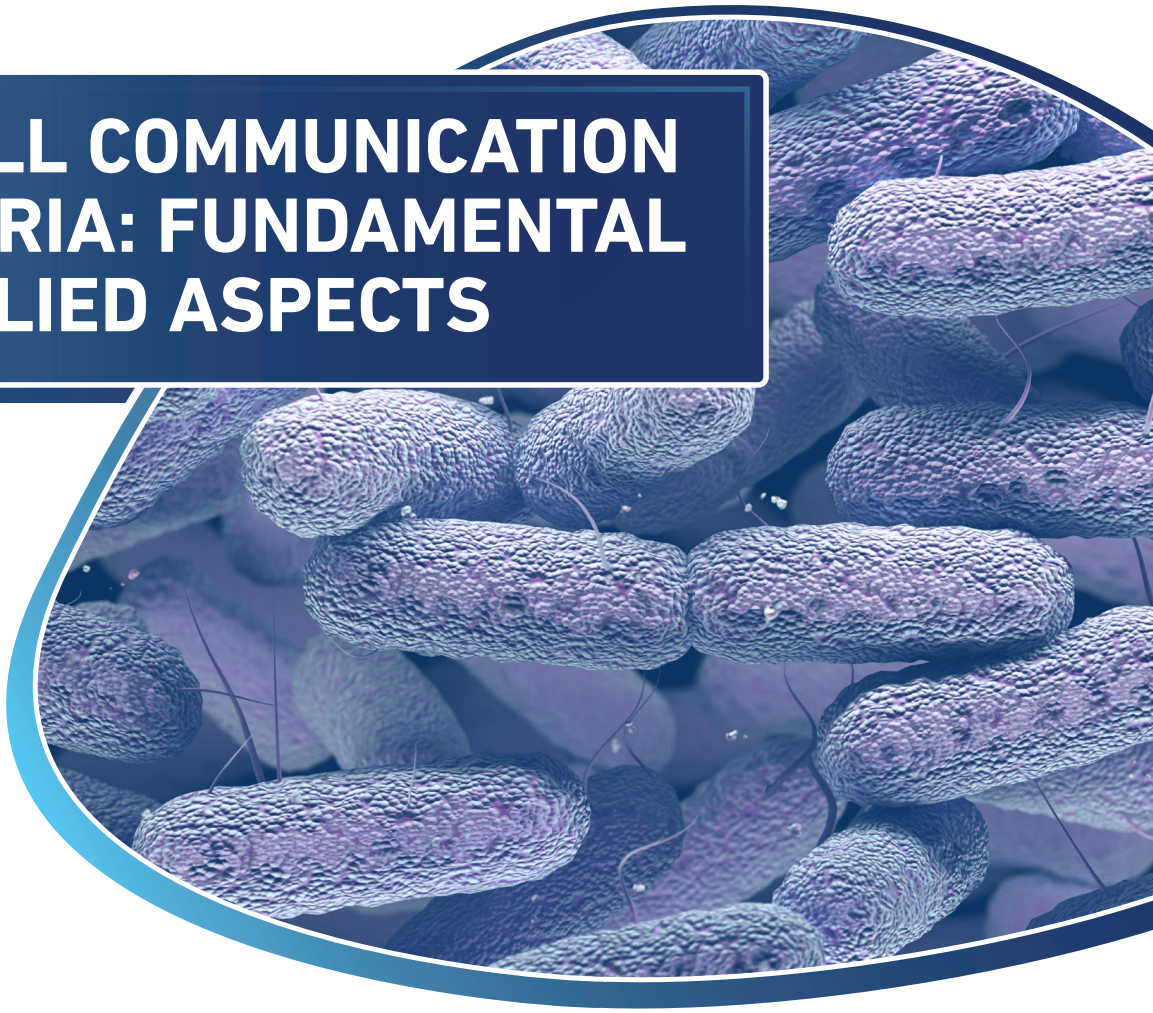
MICROBIOLOGY SOCIETY

CELL–CELL COMMUNICATION IN BACTERIA: FUNDAMENTAL AND APPLIED ASPECTS



POSTER ABSTRACT BOOK

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1

Using social cheats to fight *Pseudomonas aeruginosa* infections

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Abstract

Antimicrobial resistance is an increasing global threat and traditional drug development methods are becoming less effective in the fight against resistant infections. Therefore, there is an increasing incentive to search for novel alternatives to existing strategies. The role of social cooperation in pathogen virulence is underexplored in the search for new therapies. Bacteria cooperate by secreting molecules that serve as public goods. These molecules can be exploited by cheats which do not produce the public good but rely on and benefit from others' production. Empirical evidence has shown that non-cooperating social cheats can successfully invade cooperator populations and reduce virulence in vivo. This suggests the use of invasive ability of cheats as a novel alternative method to drive medically beneficial traits to fight infections where coopertors exist. Quorum sensing molecules in *Pseudomonas aeruginosa* has been shown to act as public goods and quorum sensing deficient cheat invasion has been shown to reduce virulence in mice models. In this study, we used antibiotic sensitive *P. aerguinosa* quorum sensing cheats to drive antibiotic sensitivity to populations of antibiotic resistant cooperators. We tested the effectiveness of the new strategies as proof of principle using in vitro fitness competition assays, and we were able to show improved antibiotic efficiency in cheat invaded populations compared to clonal cooperator populations.

2

Characterization of growth and quorum sensing responses in *Barnesiella* and *Muribaculum* gut microbiota members

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Abstract

The mammalian gut microbiota is a complex community composed of many microbial species. Gut bacteria play essential functions in many aspects of host health. However, the use of antibiotics can compromise the microbiota composition with negative consequences for the host. The quorum sensing signal AI-2 can foster inter-species communication by regulating gene expression in bacterial communities. Previously, we showed that by increasing the levels of AI-2 directly in the mouse gut, it is possible to ameliorate the effect of long-term antibiotics on the microbiota.

Here we focus on the *Barnesiella* and *Muribaculum* genera members, as these bacteria are very abundant in the homeostatic gut but are highly affected by antibiotics. Additionally, we have evidence that these bacteria respond to AI-2 in a murine model. Therefore, we aimed to identify their AI-2 regulated phenotypes specifically focusing on the utilization of fucosylated glycans because of their importance in the gut, but also because we have evidence for AI-2-mediated regulation of fucosidases in a closely related bacterium.

To study AI-2-regulated phenotypes in *Barnesiella* and *Muribaculum* we focused on two representative species – *Barnesiella intestinihominis* and *Muribaculum intestinale* - and assessed bacterial growth in fucosylated compounds with or without synthetic AI-2. Our results show that both species consume fucosylated glycans and provide preliminary evidence that AI-2 can enhance their utilization. This suggests that these species might have an advantage in the gut, where the mucus layer is rich in fucosylated compounds. We are using this knowledge to establish strategies that promote microbiota recovery after antibiotic-induced imbalances.

3

Acylhomoserine lactones increase biofilm formation and upregulate virulence traits in *Enterococcus faecalis*

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Abstract

Acylhomoserine lactones (AHLs) are common quorum sensing signalling molecules among Gram-negative bacteria. Although recent evidence suggests that AHLs may also affect metabolic regulations in some Gram-positive bacteria, knowledge of AHL-responses in Gram-positives remains scarce. Here, we aimed to assess the effect of AHLs on biofilm formation and gene expression in various *Enterococcus faecalis* strains, a Gram-positive nosocomial pathogen.

E. faecalis cultures were incubated with two different AHL mixtures (short- and long-chains) to evaluate their effect on biofilm formation. The amount of biofilm formed was measured spectrophotometrically after crystal-violet staining (OD_{595nm}). Differential expression of selected genes was evaluated using reverse-transcription-qPCR.

Of the five strains investigated, three demonstrated significant increases in biofilm formation in the presence of long-chain AHLs respect to the control without AHLs (Mann-Whitney tests, $p<0.05$), i.e., strains ATCC®29212 and two isolates from infected dental root canals, UmID4 and UmID5. Exposure to long-chain AHLs induced biologically relevant upregulations (>2-fold) of virulence-related (asa1, cylA) and adhesion-related genes (ace, efaA) in ATCC®29212. In strain UmID7, short- and long-chain AHLs upregulated stress response genes (sigmaV, groEL), whereas long-AHLs only upregulated the histidine kinase sensor involved in quorum sensing, fsrC, and several adhesion-related genes (efaA, epaQ). Further characterisation pointed towards the individual AHL C18-HSL as the main inducer of changes in biofilm formation.

Altogether, our results demonstrate that AHLs promote biofilm formation in several *E. faecalis* strains. Furthermore, short- and long-chain AHLs appear to upregulate a transcriptional network involved in virulence, adhesion and survival to stress, all of which was previously unreported.

4

Autoinducing peptides from *Listeria monocytogenes* spontaneously rearrange to form homodetic cyclopeptides

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Abstract

Quorum sensing (QS) is a crucial regulator of virulence in *Staphylococcus aureus*, and it has been linked to the virulence traits of *Listeria monocytogenes*. Structural knowledge of the QS signals, termed autoinducing peptides (AIPs), is important for understanding this signaling. In staphylococci, AIPs usually contain a conserved thiolactone functionality. Previous structure determinations of AIPs from *L. monocytogenes* are ambiguous and we therefore wanted to clarify the AIP identity. Using a chemoselective trapping method for thiolactone-containing AIPs, we could not detect any of the previously reported structures. Employing solid-phase extraction from culture supernatant and thiol-selective chemical labelling, we concluded that AIPs from *L. monocytogenes* are biosynthesised as thiolactones and rapidly undergo spontaneous S->N acyl shift to form homodetic cyclopeptides. We synthesised the thiolactone form to study rearrangement kinetics and its pH dependence, which showed it has a half-life of 2.6 min at neutral pH. We also synthesised the homodetic peptide and *N*-acetylated analogue of the thiolactone to assess their effects on QS in luciferase reporter strains of *L. monocytogenes*. The thiolactone analogue functioned as an inhibitor, while the homodetic peptide functioned as an autoinducer. However, the observed EC_{50} for the homodetic peptide was higher than those observed for staphylococcal AIPs. In this work we demonstrated that AIPs of *L. monocytogenes* readily rearrange to form homodetic cyclopeptides, and that these peptides can induce QS in the bacterium. These findings broaden our general understanding of QS and adds new complexity to AIP signaling and the virulence regulation of *L. monocytogenes*.

5

Mutations in the nitrogen phosphotransferase gene *ptsP* destabilize cooperation in *P. aeruginosa*

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Abstract

The *Pseudomonas aeruginosa* LasR-LasI system coordinates cooperative activities such as exoprotease secretion. Exoproteases are prone to exploitation by freeloading social cheaters, such as individuals with LasR-null mutations. LasR mutants spontaneously emerge in populations grown on casein, where exoproteases are needed for growth. These mutants proliferate in the population because they avoid the metabolic burden of activating the LasR regulon. The proliferation of LasR-null cheaters is predicted to drive the population to collapse, however, collapse rarely occurs in these conditions. Collapse is thought to be avoided in part due to a policing mechanism whereby cooperator-produced toxins selectively target cheaters. One policing toxin in quorum-sensing *P. aeruginosa* populations is hydrogen cyanide. We have discovered mutations that cause collapse in these conditions. One of these is in the gene *ptsP*, which codes for the first enzyme in the nitrogen phosphotransferase system. Our results show that *lasR, ptsP* cheaters cause collapse of either wild-type or a *ptsP* mutant. During competition experiments with either cooperator, the *lasR, ptsP* mutants invade and reach higher frequencies than the *lasR* single mutant. This difference was dependent on the cooperator producing hydrogen cyanide. These results support the idea that *ptsP* mutations cause *lasR* cheaters to evade hydrogen cyanide-dependent policing. We posit that the *ptsP* mutation might increase cheater resistance to hydrogen cyanide. Our results highlight how a single mutation can cause a cheater to destabilize cooperation in *P. aeruginosa* communities. These results provide new insight into quorum-sensing evolution and could have implications for new therapeutic treatments or synthetic biology.

6

Quorum sensing as a target for controlling surface-associated motility and biofilm formation in *Acinetobacter baumannii* ATCC17978

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Abstract

Acinetobacter baumannii is one of the most troublesome multi-antibiotic resistant nosocomial pathogens due to its ability to survive on several surfaces producing biofilms. A complex regulatory network, including the QS system, controls the expression of important virulence-related phenotypes such as surface-associated motility and biofilm formation. *A. baumannii* presents a typical *luxI/luxR* quorum sensing (QS) system (*abaI/abaR*) mainly mediated by *N*-(3-hydroxydodecanoyl)-L-homoserine lactone (OHC12-HSL) and *N*-(3-hydroxydecanoyl)-L-homoserine lactone (OHC10-HSL), and several quorum quenching (QQ) enzymes. The effect of the mutation of the AbaI AHL synthase and the exogenous addition of the QQ enzyme Aii20J on virulence phenotypes such as surface-associated motility and biofilm production was evaluated. We have demonstrated that a functional QS system is necessary for surface-associated motility and robust biofilm production by *A. baumannii* ATCC17978. A significant reduction in biofilm formation by *A. baumannii* was observed with the addition of the QQ Aii20J enzyme, confirming its potential use as an anti-pathogenic strategy against this pathogen. Moreover, extracellular DNA emerges as a key component of the extracellular matrix in *A. baumannii* biofilms since the combined action of the QQ enzyme Aii20J and DNase reduced biofilm formation in *A. baumannii*. Results demonstrate that QQ strategies in combination with other enzymatic treatments such as DNase could represent an alternative approach for the prevention of *A. baumannii* colonization and survival on surfaces and the prevention and treatment of infections caused by this pathogen.

7

Acyl-homoserine lactone-dependent eavesdropping promotes *Chromobacterium subtsugae* competition by activating production of hydrogen cyanide

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Abstract

Many soil saprophytes use LuxR-I-type acyl-homoserine lactone (AHL) quorum-sensing systems to activate production of antimicrobials that might be important to compete with other bacteria in polymicrobial communities. LuxR signal receptors specifically interact with cognate AHLs to cause changes in gene expression. Some LuxR-type AHL receptors have relaxed specificity and are responsive to non-cognate AHLs. These promiscuous receptors might be used to sense and respond to AHLs produced by nearby competitors by eavesdropping. We are interested in understanding the role of eavesdropping during interspecies competition. The soil saprophyte *Chromobacterium subtsugae* has a single AHL circuit, CviR-I, which produces and responds to *N*-hexanoyl-HSL (C6-HSL). The AHL receptor CviR can respond to a variety of AHLs in addition to C6-HSL. In prior studies, we showed that CviR allows *C. subtsugae* to compete with another saprophyte, *Burkholderia thailandensis*, by responding to *B. thailandensis* AHLs via eavesdropping. Here, we show that eavesdropping activates production of hydrogen cyanide (HCN) and we use genetic mutants to demonstrate that HCN is needed for *C. subtsugae* to compete with *B. thailandensis* in response to *B. thailandensis* AHLs. We show that the HCN gene promoter is activated by CviR and AHLs produced by *B. thailandensis*. We also use transcriptomics to identify other genes that are activated by CviR and *B. thailandensis* AHLs. Our results provide new information on the *C. subtsugae* quorum-sensing system and are the basis for future studies aimed at understanding eavesdropping and inter-species competition.

8

A novel cell-to-cell communication system based on a LuxR solo

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Abstract

Many proteobacteria possess so-called LuxR solos; these are quorum sensing LuxR-type regulators that are not paired with a cognate LuxI-type synthase. LuxR solos can exist in both AHL (N-acyl-homoserine lactones) communicating bacteria as well as in non-AHL producers. LuxR solos are likely to play an important role in adapting to novel chemical languages, thanks to their flexibility to recognize different signals. The analysis of 600+ genomes of strains belonging to the fluorescent *Pseudomonas* spp. revealed that they are predominant with over 50% of the genomes harboring at least one *luxR* solo gene. Amino-acid sequence homology and mapping of the adjacent genetic loci has allowed the subdivision of the majority of these LuxR solos into 9 sub-groups, suggesting that very likely many more types and sub-groups exists and are not discovered yet.

A unique *luxR* solo was identified in *P. fluorescent* Ps_77, which did not belong to any sub-group previously described. The *luxR* solo designated as *fluR* is located adjacent to a large pigment biosynthetic gene cluster, consisting of fourteen genes, which together likely constitutes a rare bacterial genomic island found in a few *Pseudomonas* genomes. Results show that FluR responds and positively regulates the transcription of the genetically adjacent operon which encodes for a black-bluish pigment. The pigment molecule therefore acts as a signal, inducing directly or indirectly FluR creating a positive feedback loop. It is believed that this is a novel bacterial communication mechanism since our experiments evidence that this system is capable of undergoing inter-cellular cell-cell signaling.

9

Attenuation of *Pseudomonas aeruginosa* Virulence by Cannabinoids

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Abstract

The emerging threat of multidrug resistance in relevant pathogens requires new approaches to reduce their infectivity. One of these approaches that have been explored in recent years is the disabling of virulence and resistance mechanisms through interference with bacterial quorum sensing (QS). *P. aeruginosa* is one of the model bacteria in the QS field due to our advanced understanding of its main QS systems and strong interest in the medical community due to its resistance to conventional antimicrobial treatment.

As many plant natural products have been identified as potent modulators of bacterial QS in recent years, we surmised that cannabis likely contains such compounds, and we set out to examine this hypothesis.

This work aims to identify molecules in cannabis that affect bacterial QS. We use chemical separation and validation techniques. Next, we evaluate the biological activity and virulence factors by microtiter plate assays using different bacterial strains. Furthermore, we aim to use ABPP to identify the proteins involved in the activity mechanism; therefore, we are currently synthesizing chemical probes.

We have successfully separated and purified a series of main cannabinoids in the plant – including THC and CBD. We found that these cannabinoids inhibit QS in *P. aeruginosa* and affect several virulence factors such as pyocyanin and eDNA production.

Our findings provide evidence that cannabis does affect QS, and we currently study the mechanism of action of several cannabis compounds concerning their ability to modulate QS, and in addition, we examine their use as potent inhibitors of infection by resistant bacteria.

10

New insights into the post-transcriptional regulation of gene expression in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen, causing a variety of nosocomial infections and posing a threat to immunocompromised and cystic fibrosis patients. Its pathogenicity is attributed to the expression of a wide array of virulence genes. Many of these genes are controlled by the three quorum sensing (QS) systems: *las, rhl*, and the *pqs*. Two of the QS effectors, RhlR and PqsE, have been shown to have a very close relationship in the control of virulence traits. We have been looking for regulatory elements that link these two effector proteins and identified a small non-coding RNA that we have called PqsX which seems to play a key role in this regulatory interaction. We have detected PqsX by Northern blot analysis in both model strains PAO1 and PA14, and have predicted its secondary structure through in silico analysis. Using a lux-based bioreporter system, we have identified some regulatory connections between PqsX and the *P. aerugionsa* QS systems. The identification of PqsX further supports the interconnectivity of the QS systems at the post-transcriptional level adding another regulatory layer to the control of virulence in *P. aeruginosa.*

11

Molecular determinants of *Pseudomonas aeruginosa* regulating virulence of *Klebsiella pneumoniae*

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Abstract

In nature, microorganisms are rarely found in isolation rather they live in consortia composed of myriad of microbes. The drug-resistant gram-negative bacteria *Pseudomonas* is not an exception as several microbial species like *Klebsiella, Staphylococcus, Cryptococcus, Acinetobacter, Streptococcus, Burkholderia* also occupy similar niches in the environment and at the sites of infection. How *Pseudomonas* respond to these bacteria or to their metabolites is essential to understand the pathogenesis of *Pseudomonas*. We hypothesize that interspecies interactions are major drivers of virulence properties of pathogens in a consortium. To test this hypothesis, we studied two nosocomial bacteria, *P. aeruginosa* and *K. pneumoniae*, that cooccur in life-threatening infections like pneumonia, sepsis, blood stream infections, urinary tract infections etc. Through a plate-based assay to study the interaction between the two, we observed that *P. aeruginosa* causes clearance of cells on a lawn of *K. pneumoniae*. This is dependent on quorum sensing and biosurfactant rhamnolipids of *P. aeruginosa*. In a screen of around 200 virulence factor mutants, we found that *P. aeruginosa* mutants defective in pyoverdine (*pvdE*) production could not clear *K. pneumoniae* efficiently. The phenotype could also be reproduced in *pvdE* knockout strain. Siderophores including pyoverdine are directly linked to bacterium's virulence in mammalian host, lending credence to our hypothesis. We find additional lines of evidence to show that iron limitation is a major driver of *Pseudomonas* response to *K. pneumoniae*. A comprehensive study of such microbial interactions can provide insight into molecular targets for controlling human infections like pneumonia, cystic fibrosis, sepsis, burn wound.

12

Development of a biosensor method for quantification of AI-2 signalling molecules and quorum quenching study using *Campylobacter* as a research model

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Abstract

Cell-to-cell communication of bacteria can be studied by classical analytical and biosensor methods. Colorimetry, liquid and gas chromatography are commonly used for the detection and quantification of bacterial AI-2 signalling molecules. Biosensor methods are usually used for qualitative detection of AI-2 signalling molecules. Our aim was to develop a biosensor method for the quantification of AI-2 signalling molecules. The foodborne pathogen *Campylobacter jejuni*, which can produce AI-2, was used as a research model. Different *Vibrio harveyi* strains were tested to find the best candidate for the detection and quantification of AI-2 signalling molecules. To quantify the AI-2 of *C. jejuni*, we compared the biosensor response to the spent medium of *C. jejuni* with unknown AI-2 concentration with the response to the standard molecule 4,5-dihydroxy-2,3-pentandion (DPD) at known concentration. Through modelling and experimental methods, *V. harveyi* MM30 was selected as the preferred biosensor for quantification of AI-2 signalling molecules. The biosensor method was validated with HPLC-FLD. As we were interested in quorum quenching, we decided to treat the *C. jejuni* culture with lavandin essential oil at sub-inhibitory concentrations and compare the response of *V. harveyi* MM30 with untreated and treated *C. jejuni* spent medium. The response of *V. harveyi* MM30 on treated *C. jejuni* spent medium was reduced by more than 50% compared to the response on untreated *C. jejuni* spent medium. In conclusion, *V. harveyi* MM30 was shown to be sensitive and reliable bosensor for the quantification of AI-2 signalling molecules and for the study of quorum quenching.

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Pseudomonas aeruginosa shows swarming behavior on the lawn of Cryptococcus neoformans in response to foraging signal

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Abstract

The coexistence of *Pseudomonas aeruginosa* (*Pa*) and *Cryptococcus neoformans* (*Cn*) in the lungs of immunocompromised patients has been known to the scientific community for decades. However, the interactions between these two coexisting opportunistic microbes still remain a mystery to scientists and medical professionals. Our previous work showed that ethanol acts as a foraging signal for *Pa* and induces swarming on soft agar. We wanted to investigate if the ethanol produced by *Cn* could mediate any interactions between these two microbes. In this study, we have found that *Pa* forms a solar flare-like pattern on *Cn* lawn grown on 1% (w/v) agar in 48 h of incubation. This solar flare pattern grows rapidly after 28 h of incubation. We believed that the reason behind this pattern could be attributed to factors such as the porosity of the *Cn* lawn, interspace ethanol sensing mechanism, and the mobility of rhamnolipid surfactant through the pores. To explore these possibilities, we have used inter-disciplinary tools combining both engineering and classic microbiology approaches. Upon investigation, we have found that rhamnolipids and the chemosensory system of Pa play an important role in deciding the pattern of the flares by promoting bacterial movement through the porous 3D structure formed by *Cn* cells. We have also modeled the interspecies relation using the predator-prey model to explore the possibility of *Pa* swarming through the lawn of Cn to feed on it.

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Deciphering the role of chemical communication in bacterial colonization among children undergoing adenoidectomy and tonsillectomy

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Abstract

It is known that bacterial biofilms play a role in upper respiratory tract diseases, including recurrent tonsillitis and obstructive sleep apnea (OSA).

Adeno-tonsil hypertrophy and OSA are among the most common childhood conditions leading to repeated antibiotic treatment and surgery. Despite increasing pathophysiological data, the molecular mechanism behind adeno-tonsil biofilm formation and its role in the formation of repeated upper airway infections is not clear. The current study aims to identify a potential role for intra- and interspecies signaling molecules in biofilm formation and to clarify their role in this pathophysiology.

A prospective study was performed, collecting tissue samples of adenoid and tonsils from children with OSA or children with chronic tonsillitis. Samples were analyzed by MALDI-Biotyper, and metabolomics analysis was performed by extraction and homogenization of tissue samples, followed by measurements on a Q-Exactive Focus Orbitrap LC-MS/MS system and network analysis through GNPS.

The results obtained through molecular networking (Cytoscape), are based on a cohort of 29 patients: 17 from the OSA group and 12 from the tonsillitis group. One identified cluster (with m/z values 216.08 and 232.08) indicate the presence kinetin, a known cytokinin. This molecule appears only in tonsils tissue from the tonsillitis group (10 out of 12), while there is no evidence for kinetin in adenoids or in the OSA group. Further analysis is performed currently to examine a potential role for kinetin in guiding interactions between bacteria.

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Novel approaches to influence bacterial quorum sensing using nanobiotechnology

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Abstract

In a series of collaborative studies, we have addressed the quorum sensing (QS) inhibition (or "quorum quenching") and biofilm inhibitory activity in both Gram(-) and Gram(+) bacteria for biopolymer-based nanoparticle and nanocapsules formulations. The average hydrodynamic diameter of these systems ranges from d.~150 – ~270 nm and the zeta potential ~+20 – ~+50 mV. To this end, we have examined both blank and bioactive loaded systems. We have demonstrated experimentally and using computational simulations, that drug-free chitosan-based oil-core nanocapsules bind "stoichiometrically" to a E. coli Top 10 GFP QS reporter strain. This is the consequence of bacterial aggregation while reducing the QS activity. Ionotropic and covalent co-gelled chitosan nanoparticles exhibit similar bioactivity. Different type of phytochemicals such as cinnamaldehyde, baicalein, quercetin and naringenin, have been loaded in chitosan-based nanocapsules. Also, novel natural and synthetic compounds have been screened and identified with potent quorum quenching activity. Our results consistently show that the association of different types of bioactive compounds in nanoparticles results in the reduction and modulation of bacterial network communication via quorum sensing and possibly also by enhancement of the anti-biofilm activity. In ongoing studies, we have started to address QS communication at single-cell resolution using biomicrofluidic approaches in trapped agarose microgel beads using a commercial cell phenotyping platform CellCity® of Evorion Biotechnolgies GmbH (Münster, Germany). These studies are shedding light on QS bacterial communication in conditions relevant to the physiological context.

16

3-oxo-C12:2, a Quorum Sensing molecule from the gut, exerts anti-inflammatory effects through a bitter taste receptor

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Abstract

Background: Acyl-Homoserine Lactones (AHLs) are Quorum Sensing molecules involved in the communication network of bacteria that also have an impact on the host's cells. We identified one that has never been described in the gut ecosystem: 3-oxo-C12:2. It was decreased in Inflammatory Bowel Disease (IBD) patients, and its presence was correlated to normobiosis. We intend to describe 3-oxo-C12:2 effects on gut inflammation and to identify which signalling pathways are involved.

Methods: After interferon-γ and lipopolysaccharide exposure, RAW264.7 macrophages were treated with 3-oxo-C12:2 and inflammatory response was monitored by ELISA. A transcriptome analysis was performed to identify involved inflammatory pathways and then were analyzed by capillary Western blot. Probenecid, a known allosteric inhibitor for T2R138, was used to study T2R138 role in AHL signalling. Bitter Taste Receptor (BTR) screening assay was performed to extend the search for 3-oxo-C12:2 receptors.

Results: After LPS/IFN-γ activation, TNFα secretion decreased when cells were exposed to 3-oxo-C12:2, in a dose-dependent manner, reflecting an anti-inflammatory effect. By transcriptomic analysis, we identified the JAK-STAT pathway as differentially down-regulated, 3-oxo-C12:2 prevented JAK1 and STAT1 protein phosphorylation. In addition, we identified T2R38 as a receptor for 3-oxo-C12:2-HSL and observed that the molecule activates also five other BTR (T2R13, T2R8, T2R14, T2R1, T2R10).

Conclusion: 3-oxo-C12:2 exerted anti-inflammatory effects on immune cells by preventing the activation of the JAK-STAT pathway. Those effects were partially mediated via the activation of BTR.AHLs are new actors in host-microbiota interactions and their study is a new lead in the IBD physiopathology.

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Quorum sensing impacts phage-host interactions

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Abstract

Quorum sensing is employed by bacteria to regulate gene expression in response to population cell density. Density is monitored via quorum molecules that are sensed by surface receptors often belonging to two component signal transduction systems. In bacterial pathogens, quorum sensing often controls virulence gene expression with *Staphylococcus aureus* being a prominent example. In this organism adhesion factors are expressed at low cell density while toxins are produced at high cell density with induction of the *agr* quorum sensing system and production of quorum molecules, the autoinducing peptide (AIP). Intriguingly coagulase-negative staphylococci also produce AIP-like molecules and a substantial fraction of these repress *S. aureus* toxin production demonstrating cross-species impact on gene expression via agr.

Staphylococcal pathogenesis is tightly linked with prophages that encode several key virulence factors of which a greater part is regulated by *agr*. In other bacterial species, quorum sensing is furthermore employed by temperate phages to make life-cycle decisions between replication and integration. Here we have examined the interactions between phages and *S. aureus* under *agr* inducing and repressing conditions. We find that *agr* activity affects infection by lytic phages unable to integrate in the bacterial genome as *S. aureus* was only infected by a phage isolated on *S. xylosus* under conditions of *agr* induction accomplished either through addition of synthetic AIPs or in late stationary phase while inhibitory AIPs prevented infection. Our results indicate that cross-species communication mediated via AIP molecules not only controls gene expression but also susceptibility to phage attack.

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DNA-PAINT for localizing the quorum sensing receptors AgrC and the adhesive receptors of FnBPs in Staphylococcus aureus

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Abstract

Staphylococcus aureus is a widespread and highly virulent pathogen that can cause superficial and invasive infections. The pathogenicity of *S. aureus* is caused by a broad range of virulence factors including cell wall anchored proteins used for attachment to the host and the quorum sensing system *agr*. Here we have developed a DNA-based imaging approach of DNA points accumulation for imaging in nanoscale topography DNA-PAINT combined with a rigid and stabilized nucleic acid nanoscaffold of chemically modified Hjs to localize and determine the density of two of *S. aureus* virulence factors of AgrC and FnBPs.DNA-PAINT provides an alternative way to circumvent the classical diffraction limit of light allowing imaging with super-resolution, by 'switching' molecules between on- and off- fluorescence states but using the transient binding of DNA functionalized fluorophores. DNA-PAINT allows subdiffraction images of DNA nanostructures with sub 25 nm spatial resolution. We have achieved a resolution down to 38 to 8 nm for localization of FnBPs and the AgrC receptors on *S. aureus* as the first application of DNA-PAINT on bacterial cells.

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Identification of New Proteins in *P. aeruginosa* that Interact with C10-AHL

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Abstract

Background: The opportunistic pathogen *Pseudomonas aeruginosa* produces, secretes and senses small lipid-based molecules called acylated homoserine lactones (AHLs) to modulate gene expression through the LuxR-type receptors LasR, RhlR and QscR. N-decanoyl-L-homoserine lactone (C10-AHL) is not one of the endogenous AHLs produced by *P. aeruginosa*, yet it was found that it has an effect on the induction and repression of multiple genes in a LasR-RhlR-QscR triple mutant (Chugani *et al*, 2010), implying it mediates interspecies communication between *P. aeruginosa* and other AHL-producing bacteria.

Methods: We aim to identify unknown proteins that interact with C10-AHL in the pathogenic bacterium *P. aeruginosa*, and to unravel their mechanism of action through activity-based protein profiling (ABPP). This chemical proteomics methodology includes synthesis of photoreactive probes, assaying their biological activity, performing in-gel labeling followed by affinity labeling and purification of peptide samples and mass spectrometry to identify proteins. Putative candidate receptors are validated using various techniques, such as virulence assays with transposon mutants.

Results: We have successfully synthesized three C10-AHL-based probes and used them in in-gel fluorescence labeling experiments where we consistently observed two distinct bands. Using one of the probes we successfully identified several possible receptor candidates. These proteins are currently being investigated as new potential AHL sensors.

Conclusion: We provide more evidence that *P. aeruginosa* can sense C10-AHL, possibly with a non LuxR-type receptor. These findings further elucidate *P. aeruginosa*'s molecular mechanisms of crosstalk with other species.

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Autoinducing Peptide Biosynthesis and Quorum Sensing Inhibition in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus virulence is largely controlled by the *agr* quorum sensing system which responds to a pheromone autoinducing peptide (AIP). AIPs are produced by post-translational processing of precursor peptide AgrD by cytoplasmic endopeptidase AgrB. AgrD is directed to the cell membrane via an N-terminal amphipathic leader. AgrB recognises AgrD and is predicted to form an enzyme bound intermediate resulting in cleavage of the AgrD C-terminus and formation of a thiolactone. Removal of the N-terminus releases the mature AIP. Once released externally, AIP binds to histidine kinase AgrC on neighbouring cells activating a signal transduction pathway via AgrA, upregulating AIP biosynthesis and virulence gene expression. The exact mechanism of AgrD processing is poorly understood.

Here an *agrBD* expression construct in *E. coli* was used to increase our understanding of AIP maturation. On detection of AgrB via western blot after co-expression of AgrBD, a shift in the size of AgrB was observed. Detection of the N-terminus of AgrD by western blot confirmed it was present in an AgrBD complex representing evidence for the enzyme bound intermediate of AgrD. This complex was not generated in the presence of AgrB protease inhibitors. This is being investigated further to identify residues necessary for AgrBD complex formation. It will also be probed with AIP biosynthesis inhibitors such as ambuic acid to explore their mechanism of action. Investigation into AgrBD interactions should increase our understanding of AIP biosynthesis and aid development of inhibitors that target AIP biosynthesis for use as alternative therapies to prevent S. aureus infections.

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Interactions between *Campylobacter jejuni* isolates resemble kin-discrimination like behaviour

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Abstract

Kin discrimination is a widespread social behaviour that promotes cooperation with highly related individuals and avoidance or exclusion of less related representatives of the same species. Although kin discrimination has important implications for bacterial competition, cooperation, horizontal gene transfer and community assembly, only a few bacterial species have been investigated for consequences of kin discrimination dependent sociality. For example, almost no knowledge exists on intraspecific interactions of an important foodborne pathogen, *Campylobacter jejuni*, which is responsible for ~ 400 million cases of human gastroenteritis each year, can trigger an autoimmune disease (Guillain-Barre syndrome), is resistant to multiple antibiotics and represents an economic burden of almost 3 billion dollars per year.

We address this knowledge gap by testing interactions between 24 isolates of *C. jejuni* with variable phylogenetic relatedness and host origin in all pairwise combinations (300 combinations altogether) during cooperative swarming, co-cultivation and adhesion to surfaces. Results show that isolates with high (>99.13%) genetic relatedness preferentially merged their swarming colonies, whereas boundary phenotype dominated among swarms of lower genetic relatedness (≤99.13%). During co-cultivation and co-adhesion to a polystyrene surface, we observed the dominance of one of the paired isolates over the other. Finally, we also tested the effect of relatedness on antibiotic resistance transfer among strains during co-cultivation. This study provides the first insight into *C. jejuni* kin-discrimination behaviour and supports that kinship affects outcomes during intraspecific competition and horizontal gene transfer of this important pathogen.

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The role of spatial structure on Pseudomonas aeruginosa quorum sensing dynamics

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Abstract

Quorum sensing (QS) involves production of diffusible signaling molecules, which stimulate the production and release of QS-dependent public goods. These public goods are costly for an individual cell to produce but they provide fitness benefits for other cells in a population and because of this, QS is prone to invasion by non-cooperating cheats. A large body of work in planktonic cultures has unraveled how public goods are shared between QS- and QS+ strains, however these studies do not recapitulate an infection environment. In a structured polymer-rich environment mimicking the spatial conditions found in cystic fibrosis lungs, P. aeruginosa forms two types of aggregates (clumping and stacking aggregates), the nature of which is dependent on the lipopolysaccharide (LPS) structure on the surface of cells. Wild-type P. aeruginosa cells form stacking aggregates in a medium structured with eDNA, while LPS mutants form clumping aggregates. These two aggregate types provide a model for studying QS in spatially-structured populations of cells. In this talk, I will describe how cell surface impacts QS-dependent cooperation and conflict. Specifically I will demonstrate that QS+ and QS- cells with an intact LPS and hydrophilic surface co-aggregate which allows QS- cheats to exploit cooperators. Conversely, when hydrophilic cheats are paired with QS+ LPS mutants with a hydrophobic surface, they are unable to co-aggregate and subsequently they are unable to exploit cooperators. Overall, these findings highlight that the surface properties of P. aeruginosa cells determines the outcome of QS social interactions.

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RRNPP-type quorum sensing regulates fermentation metabolism and sporulation in butanol-producing *Clostridium acetobutylicum*

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Abstract

The genus *Clostridium* comprises a large and physiologically diverse group of strictly anaerobic endospore forming bacteria. Despite their medical and industrial importance, the cell-cell communications systems present in these species remain poorly understood, partly due to the organisms' limited genetic amenability.

Here we report on the industrial species *Clostridium acetobutylicum*, a model organism for the study of clostridial solvent and endospore formation and renowned for its ability to produce the biofuel butanol. Our work revealed the presence of a single Agr and at least 8 RRNPP-type quorums sensing systems, several of which contribute to the regulation of acetone, butanol and ethanol (ABE) formation as well as the initiation of sporulation. One system in particular, termed QssB, appears to act as a repressor of both solvent formation and sporulation, by regulating the shift from organic acid to ABE metabolism that in batch cultures typically occurs when cells enter stationary phase. In solvent producing clostridia, these events are strictly dependent on the global regulator Spo0A. QssB appears to contribute to the fine-tuning of the Spo0A phosphorylation by controlling the expression of several orphan histidine kinases.

Analysis of genomes of solvent-producing clostridia suggests that QssB is present in several clostridia, albeit not other ABE producing species. For instance, the ABE producing *Clostridium beijerinckii* NCIMB 8052 possesses at least six Agr-type systems but no RNNPP-type quorum sensing system. It would seem that despite their close relatedness and very similar fermentation metabolism, solvent producing species have evolved to differ in the communication systems they employ.

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Uncovering molecular tools that guide interactions between species

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Abstract

The gut has the most significant bacterial diversity of all niches in our body, and, most of the time, different species coexist in a balanced manner. However, when certain bacteria invade or outgrow other species, this may generate or exacerbate diseases such as diabetes, IBD, celiac, etc. One particular organism that can colonize many niches is Pseudomonas aeruginosa - a Gram-negative, antibiotic-resistant, opportunistic pathogen. The QS system of P. aeruginosa is relatively well studied, but the communication of P. aeruginosa with other bacteria is still poorly understood.

This study aims to unravel unknown proteins that recognize P. aeruginosa signaling molecules in stool samples of Crohn's and ulcerative colitis patients and healthy volunteers. We achieve this using AHL-based photoreactive probes that are developed in our group. These probes bind to specific proteins, and with the use of click chemistry, we can modify the proteins to purify them by affinity chromatography. We also analyzed the samples using liquid chromatography and mass spectrometry of the samples, followed by analysis of the MS spectra in GNPS (Global Natural Product Social networking), to detect relevant bacterial metabolites.

Our results show specific labeling of proteins by the probe using SDS-PAGE and fluorescent imaging. We also observed changes in metabolite diversity between the different subject groups (healthy, ulcerative colitis, and Crohn’s disease).

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How do *V. cholerae* and *E. coli* sense each other? Chemical proteomics as a tool to decipher interspecies communication

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Abstract

In order to thrive, bacteria often utilize their ability to monitor both their own and neighbors` population densities by secreting and detecting small organic molecules called autoinducers (AIs), in a process named quorum sensing (QS). As such, it is important to understand how AIs from one species affect other species, in terms of growth, motility, swarming, biofilm formation, and toxicity. We previously reported that the *V. cholerae* autoinducer-1 (CAI-1) was found to significantly enhance the virulence of *E. coli* (EPEC), at concentrations as low as 2 µM through an as yet unknown mechanism. In this study, we report the development of tools to identify proteins in EPEC that bind CAI-1. This strategy, termed activity-based protein profiling (ABPP) is based on the use of AI-based probes with a reactive group that will bind covalently to the desired protein, and a reporter tag that will bind either to a fluorophore to visualize the protein or to an affinity tag to detect and purify the protein. This platform was used successfully by us to identify proteins in *Pseudomonas aeruginosa* that bind C4-HSL and PQS/HHQ autoinducers. We synthesized similar probes based on CAI-1 and we studied its interaction with EPEC, and its effect on QS in these bacteria.

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Agr quorum sensing regulates sporulation and solvent formation in *Clostridium beijerinckii*

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Abstract

The bacterium *Clostridium beijerinckii* is a gram-positive, anaerobic, endospore forming bacterium of industrial importance due to its ability to produce solvents such as bioethanol and biobutanol through acetone-butanol-ethanol (ABE) fermentation. In industrial settings, fermentations are carried out at high cell densities, making it likely that bacterial cell-cell communication (quorum sensing) is occurring and controlling cellular behaviour.

The genome of *C. beijerinckii* NCIMB 8052 contains several putative *agr* gene clusters, hypothesised to play a role in solventogenesis and sporulation. This study aimed to identify homologues of the *C. beijerinckii* NCIMB 8052 AgrB and AgrD proteins in other *C. beijerinckii* strains through bioinformatic analysis to ascertain their distribution and diversity within the species. Using CRISPR technology, the three most highly conserved systems were targeted for *agrB* gene disruption in the NCIMB 8052 strain genome. The mutants and wild-type (WT) were phenotypically characterised. All *agrB* mutants analysed had a highly similar growth rate to that of the WT, however for two of the *agr* mutants a statistically significant reduction in sporulation, measured as heat resistant CFU/mL, was observed after 24 h and 120 h growth. All *agr* mutants also demonstrated a reduction in butanol and acetone formation compared to the WT. These results suggest that NCIMB 8052 *agr* gene clusters play important roles in sporulation and/or solvent production pathways. Understanding their roles and how to manipulate the underlying regulatory mechanisms could increase the efficiency in the industrial fermentation process.

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Behind the scenes of the complex mangotoxin regulation in *Pseudomonas syringae* pv. syringae

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Abstract

Bacteria have evolved sophisticated molecular mechanisms of communication to organize and coordinate different kind of behaviours, ranging from symbiosis and virulence to biofilm and secondary metabolite production. These mechanisms predominantly include quorum sensing (QS) systems, which count on the diffusion of signal molecules and their concentration to switch on and off the corresponding target gene(s).

A recent study from our group identified a novel signal molecule, leudiazen, to be involved in the intricate regulation of the major virulence factor, mangotoxin, in the *Pseudomonas syringae* pv. syringae UMAF0158 (Pss UMAF0158). Our current study aims to elucidate the upstream regulatory mechanism as well as the structural characterization of mangotoxin. Interestingly, bioinformatic analysis revealed that this strain lacks the usual QS-systems present in the *Pseudomonas* strains. Transcriptomic data revealed that leudiazen is a specific regulator of mangotoxin. In addition, we identified the roles of two other master regulators in this pathogen, a GntR-transcriptional regulator and a hybrid histidine kinase/response regulator.

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Mushroom-shaped structures formed in *Acinetobacter baumannii* biofilms grown in a roller bioreactor are associated with quorum sensing dependent Csu-pilus assembly

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Abstract

There is currently a need to develop simple biofilm models that facilitate investigation of the architecture/biology of mature bacterial biofilms in a consistent/standardised manner given their environmental and clinical importance and the need for new anti-biofilm interventions. This study introduces a novel biofilm culture system termed the rolling biofilm bioreactor (RBB). This easily operated system allows adherent microbial cells to be repeatedly exposed to air/solid/liquid interfaces optimising biofilm growth. The RBB was exploited to investigate biofilm formation in *Acinetobacter baumannii*. High-levels of *A. baumannii* biofilm biomass reproducibly accumulate in the RBB and, importantly, undergo a maturation step to form large mushroom-shaped structures that had not been observed in other models. Based on image analysis of biofilm development and genetic manipulation, we show how N-acylhomoserine lactone-dependent quorum-sensing (QS) impacts on biofilm differentiation, composition, and antibiotic tolerance. Our results indicate that extracellular DNA (eDNA) is a key matrix component in mature *Acinetobacter* biofilms as the mushroom-like structures consist of dense cellular masses encased in an eDNA mesh. Moreover, this study reveals the contribution of QS to A. baumannii biofilm differentiation through Csu pilus assembly regulation. Understanding the mechanisms of the structural development of mature biofilms helps to identify new biofilm eradication and removal strategies.

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Multi-omics analysis of MDR Acinetobacter baumannii upon exposure to colistin

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Abstract

Acinetobacter baumannii release a large set of biomolecules, including proteins, outside the cells. These proteins, alone or incorporated in outer membrane vesicles, possess several significant roles in bacterial pathogenesis: virulence, cell-to-cell communication, defense and antibiotic resistance. Multidrug-resistant (MDR) A. baumannii is a rapidly emerging healthcare-associated bacterial pathogen causing severe infections. The resistance of MDR A. baumannii to the last-resort drug colistin has been increasingly reported. Furthermore, colistin-susceptible A. baumannii isolates can even develop colistin dependence after exposure to colistin. We employed a comprehensive strain-specific multi-omics approach[1] to characterize excretome profile and its correlation to colistin dependence mechanisms of MDR A. baumannii. We combined the proteogenomic data with high-resolution imaging to enable a comprehensive molecular and cellular overview of genome, cellular proteome and excretome rearrangements upon development of dependence. We demonstrated that in the colistin-dependent subpopulations of A. baumannii the lipid A biosynthesis was terminated by the disruption of the lpx genes by insertion sequences resulting in lipooligosaccharide-deficient phenotype. The major cellular proteome differences between colistin susceptible and colistin dependent subpopulations were detected in secretion system components, the resistance-nodulation-division (RND)-type efflux pumps, and in levels of proteins involved in maintenance of outer membrane asymmetry. Analysis of excretomes allowed us to identify over 900 extracellular proteins and revealed the strain specific proteins. Outer membrane proteins, enzymes and transporter proteins were among the most abundant extruded proteins. Our findings suggest that colistin dependence in A. baumannii is associated with an array of cellular responses and events.

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Single-cell gene expression analyses reveal nonsynchronous heterogeneous activation of *Pseudomonas aeruginosa las* quorum sensing system

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Abstract

Nongenetic variation in gene expression can occur between single cells of a clonal bacterial population facing the same environmental conditions. Such phenotypic heterogeneity is emerging as a common feature in many bacterial processes, including quorum sensing (QS). QS communication systems are usually considered as regulatory circuits allowing coordinated reprogramming of gene expression among single cells of a bacterial population once the quorum cell density is reached. However, growing evidence is emerging showing that in different bacterial species QS activation is not synchronous among single cells, and that quorate and non-quorate sub-populations can co-exist even at high cell density. Despite being one of the most studied QS circuits, little is known about possible heterogeneous activation of the *las* QS system in the opportunistic pathogen *P. aeruginosa*. To delve into this issue, we employed single-cell analyses based on confocal microscopy imaging of *ad hoc* engineered biosensor strains, in which *las* QS activation results in fluorescence emission. Our data indicate that activation of the *las* QS system does not occur synchronously in all the cells of the population, with different fractions of quorate and non-quorate sub-populations co-existing along the growth curve. Experiments performed in PAO1 mutant strains revealed that the incoherent feedforward loop generated by the transcriptional activator LasR and the transcriptional repressor RsaL plays a key role in the heterogeneous activation of the *las* QS system. Further experiments are in course to investigate the heterogeneous expression of *las*-controlled virulence genes and of the *rhl* and *pqs* QS systems.

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Decoding bacterial communication using a single-cell resolution phenotyping microfluidic platform

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Abstract

Traditionally, bacterial cell-to-cell communication, *quorum sensing (QS)*, studies were focused on understanding the influence of cell density at the population level. Several studies that investigated entrapments of small groups of bacteria have shown that other factors, such as the mass transport and the microenvironment conditions, might also be at play.

In this study, CellCity®, a gel-bead based microfluidic phenotype profiling platform, was used to examine the bacterial communication at a single-cell level. An *E. coli* strain able to express GFP in response to inducers, such as N-(3-oxohexanoyl)-L-acylhomoserine lactone (OHHL) at sub-nanomolar concentrations (0.5 nM) was studied in this platform. Using CellCity®, individual bacterial cells were embedded in agarose gel beads; then trapped in a multi-channel microfluidic chip and perfused with medium, antibiotics and OHHL for 30 min. The GFP expression was monitored by automated time-lapse fluorescence microscopy (EVOS cell imaging system) to study *QS* inductions at low cell densities. The High-resolution fluorescence microscopy showed growth of GFP-expressing colonies within agarose beads. These preliminary results indicate that agarose-entrapped single *E. coli* cells can be induced by OHHL, which constitutes a new approach to investigate bacterial *QS*. Thus, these results call into question the traditional cell density dependent *QS* hypotheses and provide further insights into how bacteria establish communication networks.

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Altruism and Locality of Quorum Sensing Bacteria

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Abstract

Canonical N-Acyl-Homoserine Lactone (AHL) quorum sensing (QS) systems, which usually regulate costly adaptive traits, contain an AHL synthase, an AHL dependent transcriptional activator and sometimes a repressor element. Both latter elements influence AHL synthase gene transcription. This positive feedback loop can overcome the repressor element and ultimately promote QS activation. In many negatively stringently regulated AHL QS systems however, especially in standard laboratory growth conditions, QS activation is below detection capabilities. This apparent redundancy of QS is unlikely in natural environments, that are selective for the QS regulated adaptive traits.

We present a hypothesis that emphasizes the virtue of temperance, but argue that “altruistic” bacterial cells can nonetheless overcome repression and activate their QS. “Altruists” will influence neighbouring bacteria, causing the emergence of local QS active hotspots (absent in well-mixed, liquid cultures). The stringency of QS repression will inversely correlate with the frequency of altruist emergence. The frequency of “altruist” emergence could mirror the periodicity of environmental selective pressures.

In a contrasting interpretation, QS functions in a switch on/off like manner, thus rendering it vulnerable to the paradoxical “tragedy of the commons”., i.e. selfish non-cooperative individuals exploit “altruists”, overgrow them, and lead to a populational collapse. In our interpretation, QS is repressing adaptive traits by default, thus minimizing resource squandering. The stochastic emergence of localized QS activated clusters should increase the population’s phenotypic plasticity by enabling “bet-hedging”. By understanding QS mechanisms, we can also assume more about the selective pressures, which shaped them in the first place.

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Antibacterial Activity of Phenazine-based Natural Products and Synthetic Analogs Against Staphylococcus aureus

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Abstract

Phenazines are redox-active secondary metabolites produced by many Gram-positive and negative bacteria. These compounds exhibit a wide range of activities, and they have been explored as potential therapeutic agents. Since P. aeruginosa is one of the most common phenazine producers, we investigated the activities of an array of phenazine-based natural products and their analogs on Gram-positive bacteria that share the same natural niches with Pseudomonas, such as the human pathogen S. aureus.

Natural phenazines were screened against S. aureus and evaluated as growth inhibitors. Their potential mode of action was examined by spectroscopic measurements, and by investigating the ability of such phenazines to act as iron chelators. Further growth inhibition assays were performed to assess potential iron starvation mechanisms.

Hydroxyl-containing phenazines with a 5,10-dioxide scaffold were found to exhibit potent inhibitory effects on the growth of S. aureus. The natural product iodinin was chosen as a model for 5,10-dioxide scaffold and investigated in further experiments. Iodinin was found to strongly chelate iron, as determined by UV-Vis analysis after being treated with supplemented iron. Furthermore, the chelation ratio and competition with EDTA were explored. Additional potentiation assays showed that iodinin lost its inhibitory activity when 100 μM of supplemented iron was added to the bacteria. Finally, synthetic analogs with enhanced activities were prepared and screened against S. aureus, as well.

These results strengthen the hypothesis that iron starvation may play a role as a mechanism in which phenazines can be utilized by their producers to compete over resources with competing microorganisms.

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'New insights into the control of the quorum sensing *pqsA* promoter by PqsE in *Pseudomonas aeruginosa*'

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Abstract

The *Pseudomonas aeruginosa pqs* quorum sensing (QS) system controls the expression of many virulence traits via the *Pseudomonas* quinolone signal (PQS) synthesized by the *pqsABCDE* operon, which expression is mainly activated by PqsR upon binding to PQS. PqsE not only has a role in the biosynthesis of PQS, but also acts as the main effector of the *pqs* system. Among others, PqsE positively regulates genes involved in the biosynthesis of pyocyanin, elastase, rhamnolipids as well as genes involved in biofilm development. Furthermore, it modulates the production of PQS by repressing the *pqsA* promoter. The lack of a DNA-binding domain in PqsE suggests that this repression may be indirect. Using a DNA promoter pull-down analysis to identify potential mediators of the action of PqsE, we found a protein of unknown function, which we have named PA27NR, and the denitrification regulatory protein NirQ binding to the *pqsA* promoter upon the overexpression of *pqsE*. Moreover, using gene deletion, site-directed mutagenesis, as well as transcriptional and translational reporter assays, we have found that: (i) albeit not essential for the action of PqsE, both PA27NR and NirQ play a role in the regulation of the *pqsABCDE* operon; (ii) the PqsE-mediated repression towards the *pqsA* promoter is at the post-transcriptional level and, (iii) PqsR is indispensable for this repression. These results provide new insights into the regulatory relationships between PqsE and the *pqs* operon and further demonstrate the complex dynamics of the regulation of the *P. aeruginosa pqs* system.

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Simulations of atomic-scale interactions that govern the motion of quorum sensing autoinducer molecules through the mucoid *P. aeruginosa* exopolysaccharide matrix

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Abstract

Upon colonisation of the cystic fibrosis (CF) lung, mucoid *Pseudomonas aeruginosa* establishes stable, cation cross-linked, exopolysaccharide (EPS) biofilm matrices. These EPS scaffolds offer resistance to therapeutic intervention and are critically implicated in chronic infection. Mucoid *P. aeruginosa* utilises quorum sensing autoinducers (QSAI), which are cell to cell signalling molecules, to regulate biofilm proliferation and maintenance. Despite our knowing that QSAI molecules must pass through EPS material to reach neighbouring bacteria, the molecular interactions between QSAIs and the EPS, that directly influence molecular motion, are poorly understood. For the first time, using molecular dynamics (MD) and Density-Functional Theory (DFT), interactions between the EPS and two QSAI molecules (C_4-HSL and PQS) utilised by mucoid P. aeruginosa in the CF lung, have been studied at the atomic scale. DFT modelling elucidated the structural chemistry fundamental for large-scale multi-chain EPS aggregation, and explicit solvent finite temperature MD demonstrated the influence of physiological conditions on EPS scaffold architecture. Critically these simulations rationalised the origin of dendritic discontinuous EPS matrix structures observed *in vivo*. The resulting physiological EPS structure was exposed to the QSAIs and deployed in further MD simulations to study which interactions facilitate molecular motion through the EPS. C_4-HSL has a low EPS binding propensity, partitioning readily into water channels, whereas the binding propensity for PQS is large, forming thermodynamically stable ionic and hydrogen bonded complexes with EPS. These simulations highlight key molecular functionality responsible for EPS binding and, based on its significantly reduced mobility, suggest PQS as a viable target for quorum quenching.

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Metabolomic profiling of heme's catabolic pathway in IBD fecal samples

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Abstract

Crohn's disease (CD) and ulcerative colitis (UC) are two common conditions of Inflammatory Bowel Diseases (IBDs). IBDs are chronic inflammatory conditions that manifest in the gastrointestinal tract (GI). Communication disorders between the immune system and the gut microbiome population (and perhaps also within the microbiota) are suspected to be among the causes of an inappropriate inflammatory response. A healthy microbiome has a crucial role in heme's catabolic pathway to stercobilin through bilirubin. Thus, a comparison made between animal models (healthy and with UC) has previously shown a difference in this metabolic profile. In healthy mice, mainly stercobilin was observed. In contrast, various bilirubin metabolites, including oxidized analogs of stercobilin, were observed in sick mice. Since bilirubin reduction to stercobilin is performed by gut bacteria, we set to identify a potential link between bilirubin metabolism and IBDs, mediated by microbes. In this study, our goal is to identify molecular patterns in fecal samples that distinguish between healthy people and IBD patients. Fresh stool samples were collected from CD and UC patients, and healthy volunteers. After aqueous extraction of samples, LCMS analysis was carried out to identify bilirubin metabolite via an online platform data analysis tool, GNPS. Further analysis was performed to quantify and compare the different groups. Preliminary results show that there are oxidized analogs of stercobilin present in the samples of IBDs patients. We detected significant differences between the relative amounts of several analogs between the groups. Our findings indicate that disorders in heme catabolism may correlate with IBD.

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Influence of ginger metabolites on bacterial communication

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Abstract

Natural products from plant sources have been at the basis of therapeutic development since ancient times. An example of a natural substance with interesting properties and abilities to promote human health is ginger, which is known as both an antioxidant and anti-inflammatory agent, and more.

Currently, the use of antibiotics is increasing due population pressures and to the increased presence of bacteria that thrive in different, diverse and even extreme living environments. Pathogenic bacteria have become increasingly resistant to common antibiotics and therefore their effectiveness is reduced.

An example of a bacterium that is highly resistant to a variety of antibiotics is Pseudomonas aeruginosa, which is a common pathogen in patients with a weakened immune system, such as patients with cystic fibrosis or pneumonia.

This pathogen, like most other bacteria, can communicate on a population-wide scale, and can regulate their behavior such as biofilm production and secrete toxins and enzymes that cause disease in the host. Bacterial behavior is coordinated using a quorum-sensing (QS) system that regulates behavior using small molecules called autoinducers (AI).

In this study, we prepared a series of ginger molecules and their synthetic analogues, and we evaluated them for their ability to modulate three of the known P. aeruginosa QS systems: Las, Rhl and PQS.

Our preliminary results show that several of the tested analogs and natural compounds can effectively inhibit QS in P. aeruginosa by inhibition of one or more of its QS systems, and we studied their mechanism of action.

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Antiactivator-dependent self-sensing in *Pseudomonas aeruginosa* quorum sensing

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Abstract

Quorum sensing is described as a widespread cell density-dependent signaling mechanism in bacteria. Groups of cells coordinate gene expression by secreting and responding to diffusible signal molecules. Theory however predicts that individual cells may short-circuit this mechanism by directly responding to the signals they produce irrespective of cell density. In this study, we characterize this self-sensing effect in the acyl-homoserine lactone quorum sensing system of *Pseudomonas aeruginosa*, and we show that antiactivators, a set of proteins known to affect signal sensitivity, are involved in it. Measuring quorum-sensing gene expression in individual cells at very low densities, we find that successive deletion of antiactivator genes *qteE* and *qslA* produces a bimodal response pattern, in which increasing proportions of constitutively induced cells co-exist with uninduced cells. Comparing responses of signal-proficient and deficient cells in co-cultures, we find that signal-proficient cells show a much higher response in the antiactivator mutant background but not in the wild-type background. Our results experimentally demonstrate the antiactivator-dependent transition from group to self-sensing in the quorum-sensing circuitry of *P. aeruginosa*. They broaden our understanding of the functional capacity of quorum sensing, provide a mechanistic basis for the quorum-sensing response heterogeneity observed in other bacterial species, and have implications for the design of cell-cell signaling circuits in synthetic biology.

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Measuring conjugation dynamics in bacterial communities

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Abstract

DNA conjugation is the process through which genetic information is directly transferred from one bacterial cell to another and is one of the primary mechanisms facilitating horizontal spread of genes required for environmental adaptation and antibiotic resistance. In this study we investigated the dynamics of conjugative plasmids in different bacterial species, including the rate of plasmid propagation within the population, how quickly newly transferred genes are expressed, and the factors affecting this process. We focused on two conjugative plasmids in *Escherichia coli* and *Vibrio cholerae*: RP4, a broad host range plasmid and J13, a *Vibrio*-specific plasmid. Conjugation efficiency assays revealed that, although RP4 could move between both *E. coli* and *V. cholerae* species, its efficiency dropped when *V. cholerae* was the donor cell. Between *E. coli* cells, RP4 reached full efficiency within an hour of mating. We also verified that J13 is a *Vibrio*-specific plasmid. By creating fluorescence tools, we visualized conjugation in real-time with time-lapse microscopy imaging. Donor cells in *E. coli* communities transfer RP4 to recipient cells within minutes. However, new donors take longer to transfer the plasmid, requiring as much as 2 hours before successfully conjugating to a new recipient. J13 preliminary data suggest different dynamics to RP4 with mating taking longer to start, but then quickly spreading within the community. We are currently performing screens to identify plasmid-based genetic factors altering these transfer dynamics. Ultimately, our enhanced understanding of how conjugative plasmids spread in microbial populations can be exploited to directly manipulate the genes of bacterial communities.

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Targeting bacterial communication via LsrK Kinase: an anti-virulence approach

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Abstract

Anti-virulence strategies are promising in the era of rising microbial resistance to existing antibiotics. One such strategy is to target bacterial communication processes, such as quorum sensing (QS). QS controls several functions such as virulence factor secretion, biofilm formation, adhesion, and host colonization. QS in enteric species such as Salmonella enterica is mediated by an autoinducer signaling molecule, AI-2[1]. AI-2 molecules are derived from the precursor molecule (4S)-4,5-dihydroxy-2,3-pentanedione (DPD) and undergoes structural rearrangements leading to species-specific bacterial communication. The LsrK kinase is involved in the phosphorylation of DPD and plays an important role in AI-2 signaling, since only phosphorylated DPD can further initiate QS signaling in S. enterica. Thus, LsrK inhibition is an attractive strategy for abrogating pathogenicity in this species. To achieve this goal, in silico (computational) virtual screening using commercially-available compound libraries was conducted to identify potential inhibitors of an in-house-generated LsrK protein structure. Experimental testing of the “hits” obtained this way led to the identification of two inhibitory compounds which were further improved by subsequent analogue screening. This yielded compounds with inhibitory activity in the low micromolar range[2]. These first-in-class LsrK inhibitors have the potential to be further optimized and are a useful starting point in the design of novel compounds targeting S. enterica virulence.

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Polymer Based Inhibition of QuorumSensing in Gram Negative Bacteria

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Abstract

An anti-quorum sensing polymer, HB-PNIPAM-HL, has recently been developed by our group. HB-PNIPAM-HL is a hyperbranched Poly(NIPAM) polymer with chain ends uniquely functionalized with homoserine lactone, that interferes with the AHLs QS system found in Gram-negative bacteria. Targeting QS circuits could present a possible control over several virulence factors at once and thus limit infection. We have investigated the extent of the circuit disruption via HB-PNIPAM-HL in a biosensor strain of Gram-negative bacteria, Chromobacterium violaceum CV026, and the Gram-negative pathogen Pseudomonas aeruginosa.The anti-QS ability of the polymer was first measured in the cviI mutant strain of Chromobacterium violaceum CV026 that cannot produce AHLs, wild types of which produce a characteristic purple pigment, violacein. The mutant strain of C.violaceum, CV026, has colonies that appear white; violacein production can be restored by adding the AHL signalling molecule, making it an ideal bioassay for AHLs signalling and blocking. Gene expression of receptors involved in AHL-type signalling, in the presence or absence of HB-PNIPAM-HL was investigated using RT-qPCR. The effect of the polymer on Pseudomonas aeruginosa virulence factors regulated by QS was then evaluated via several virulence factors assays. Initial tests using the CV026 biosensor assay indicated HB-PNIPAM-HL was able to block AHL signalling in CV026 bacteria. Interference with QS signalling by the polymer, also downregulated the expression of some P.aeruginosa virulence related phenotypes that are controlled by QS. The initial data suggest that HB-PNIPAM-HL can interfere with QS signalling. This novel anti-quorum sensing polymer could limit some Gram-negative infections

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Type VI Secretion System effector toxicity towards Pseudomonas aeruginosa

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Abstract

The type VI secretion system (T6SS) is a nano-machine used by many Gram-negative bacteria to deliver toxic effector proteins into neighbouring cells. Although Vibrio cholerae T6SS can successfully eliminate several different enteric bacteria species, Pseudomonas aeruginosa is largely unaffected by V. cholerae attacks. Since the T6SS of several other bacterial species can eliminate P. aeruginosa, P aeruginosa is not universally immune to T6SS attack. To determine if the species-specific resistance to the T6SS of V. cholerae is due to intrinsic resistance to the unique complement of toxic effectors carried by V. cholerae, we have endogenously expressed three V. cholerae effectors (VgrG3, VasX and TseL) individually in P. aeruginosa and localised them to both the cytosol and periplasmic compartments. When each effector was expressed in the P. aeruginosa cytosol, no toxicity was observed. However, when localised to the periplasm, each of the effectors was toxic. These results suggest that P. aeruginosa resistance to V. cholerae may be due to its T6SS being unable to properly deliver toxic effectors into the correct subcellular compartment of P. aeruginosa. Understanding this type of species-specific interactions contributes to our understanding of cellular dynamics in multi-species bacterial communities.

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The study of the pheromone exporter PptAB reveals crosstalk between different quorum sensing systems in *Streptococcus thermophilus*

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Abstract

In Gram-positive bacteria, autoinducing peptides (AIPs) are the signaling molecules of quorum sensing (QS) mechanisms. Their life cycle can be divided in five steps: intracellular production, maturation, secretion, detection and regulation of the expression of target genes. *Streptococcus thermophilus* strain LMD-9 possesses 7 QS systems. Five of them involve AIPs named short hydrophobic peptides (SHPs) that function with transcriptional regulators of the Rgg family.

In this bacterium, we have confirmed the role of PptAB as the SHP exporter using transcriptional fusions in different genetic backgrounds. We compared the transcriptomes of a Δ*pptAB* mutant with that of a wild-type strain. We showed that the transcription of the genes located downstream of each of the 5 *rgg* genes occured in our condition and required the presence of intact *pptAB* genes. These downstream genes were therefore identified as target genes of a SHP/Rgg QS mechanism. However, only three *shp* out of five were expressed and their corresponding peptides detected by mass-spectrometry in our experimental condition. Thus, these results highlight the complex interconnected network that exists between SHP/Rgg systems. We confirmed this interconnection with the study of one locus and showed that the genes located downstream of the *rgg* gene of this locus are controlled by different distal SHP and Rgg.

Redundant *shp*/rgg loci in genomes of S. thermophilus but also in other streptococci genomes and the evidence of cross-talks between these systems point out the complexity of QS regulation. This complexity must be taken into account to control these systems.

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Quorum Sensing and Biofilm-formation in multi-drug resistant *Klebsiella pneumoniae* strains.

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Abstract

Background

Biofilm-formation, a key feature for the pathogenesis of *Klebsiella pneumoniae*, has been correlated with the Quorum Sensing (QS) signal AI-2. However, the role of acyl-homoserine lactones (AHLs) is unclear since the production of AHLs has been reported in some strains, but no putative synthase has been identified. Moreover, SdiA, a conserved AHL orphan receptor, has been recently reported to act as an inhibitor of biofilm-formation. Therefore, a deeper study of the role of QS in biofilm-formation is needed to develop novel antimicrobial applications based on anti-biofilm strategies.

Methods

Biofilms from 24 *K. pneumoniae* multi-drug resistant strains of different origin were studied using the Active Attachment method under aerobic/microaerobic conditions in LB and LB+0.4% glucose, plus the effect of the exogenous addition of long/short AHLs. The presence of AHLs was analysed in the supernatants by HPLC-MS. AI-2 production and Quorum Quenching (QQ) activity were studied using the strains *Vibrio harveyi* BB170 and JMH597, and *Chromobacterium violaceum* VIR07 and CV026, respectively.

Results

Culture conditions strongly affected biofilm-formation, with significant variability and biofilm-formation among strains of human origin. AI-2 production was detected in all strains and was higher with glucose supplementation. AHLs could not be detected in the conditions tested, while QQ activity was detected in 3 food-origin strains from ST-307. However, the exogenous addition of C14-HSL produced a significant increase of biofilm-formation in a hypervirulent strain under biofilm-inducing conditions.

Conclusion

Additional studies are required on the role of SdiA in AHL detection and regulation of biofilm-formation in *K. pneumoniae*.

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P. aeruginosa and *S. aureus* Communicate using Small Signaling Molecules in Dual-Species Biofilms

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Abstract

The communication between bacteria in multi-species environments is a tool that may enable both species to survive and perhaps even thrive. *P. aeruginosa* and *S. aureus* are two bacterial pathogens found in natural biofilms, especially in the lungs of cystic fibrosis (CF) patients, as this is a niche that appears to favor their coexistence. Recent studies on CF isolates that contained both pathogens showed that there is often cooperation between the two species, leading to increased disease severity and antibiotic resistance. However, the mechanisms behind this cooperation are not fully understood.

We explored conditions that enable co-cultured biofilms, and secreted molecules were extracted and investigated using Q-Exactive Orbitrap tandem mass spectrometry. The metabolic profile of mutual biofilms was analyzed using online platforms and databases such as GNPS, MetaboAnalyst, and Xcms. The analysis revealed one metabolite that was found in the mixed biofilms, while it was barely detected in *P. aeruginosa* and *S. aureus* monoculture biofilms. This metabolite is an analog of a known *P. aeruginosa* siderophore that is regulated by the quorum sensing (QS) system of the bacterium. Its concentration increase when the *S. aureus* abundance in the co-cultured biofilm increases, suggesting *S. aureus* is converting the known siderophore into the observed derivative. These preliminary results indicate that QS molecules of *P. aeruginosa* can be used for inter-species communication with *S. aureus* to coordinate group behavior and increased pathogenesis.

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Targeting quorum sensing with monoclonal antibodies as a novel approach to tackle biofilm-mediated *Pseudomonas aeruginosa* infections

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[Abstract redacted at author's request]

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Deciphering inter-species communication for the development of anti-virulence therapies against *Staphylococcus aureus* – a focus on lactic acid bacteria

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Abstract

Staphylococcus aureus is an opportunistic pathogen that causes a wide range of hospital and community-acquired infections, and treatment remains challenging due to the emergence of multidrug-resistant strains. Quorum sensing inhibition is a promising alternative once it diminishes *S. aureus* virulence, reducing disease severity and facilitating clearance by the host immune system. The accessory gene regulator (*agr*) quorum sensing system regulates the expression of most *S. aureus* virulence factors. Communication relies on a two-component signalling system, agrCA, responding to auto-inducing peptides (AIPs) encoded and secreted by AgrBD. Previous research has shown that other staphylococci produce AIPs able to inhibit the *S. aureus* quorum sensing system, indicating that *agr* is an interspecies communication system. This project aims to study the effects of lactic acid bacteria on the staphylococcal quorum sensing system and understand the benefits of using probiotics to treat *S. aureus* infections. An extensive collection of lactic acid bacteria was screened against *S. aureus* using quorum sensing transcriptional reporters from all four agr-types, and strains capable of suppressing staphylococcal quorum sensing were identified. Cell-free supernatants from some lactic acid bacterial strains demonstrated quorum-sensing inhibition in *S. aureus*, particularly *Lactiplantibacillus plantarum*. Auto-inducing peptides from *L. plantarum* have also been synthesized and found to inhibit quorum sensing without affecting staphylococcal growth. Quorum sensing inhibition activity depended on the tested AIPs sequence and the *S. aureus agr* group. Lactic acid bacteria, including well-known probiotics, are promising candidates for developing anti-virulence therapies against *S. aureus* infections.

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Quorum sensing-mediated repression of solvent formation in *Clostridium acetobutylicum* ATCC 824

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Abstract

The Gram-positive obligate anaerobe *Clostridium acetobutylicum* is recognised for its ability to metabolise sugars into organic acids and solvents, particularly the biofuel butanol. *C. acetobutylicum* has been historically used for industrial-scale production of organic solvents like acetone and butanol however improvements are required for a viable economic process. Signalling peptide-based regulation of solvent formation and sporulation in *C. acetobutylicum* ATCC 824 has been recognised and a regulatory role for the RRNPP-type Quorum sensing system B (QssB) comprising Quorum sensing regulator B (QsrB) and its cognate signalling peptide Quorum sensing peptide B (QspB) has been established. The aim of this study was to determine how *qsrB* alters the switch from the acidogenic to the solventogenic phase in *C. acetobutylicum* ATCC 824. Overexpression of *qsrB* led to a significant reduction in solvent and endospore formation in comparison to wild type *C. acetobutylicum*, establishing a role for *qsrB* in the regulation of solvent formation and sporulation. RNA samples of wild-type and *qsrB* overexpressing strains just entering the solventogenic phase of growth were acquired and submitted for differential expression (RNAseq) analysis. Considerable changes for several well-defined gene clusters were observed with 76 of the differentially expressed genes being more than 10-fold up/down regulated and 16 genes being more than 70-fold up/down regulated. The data suggests that QsrB may interfere with phosphorylation-dependent activation of the sporulation-controlling global regulator Spo0A.

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A foraging signal is necessary for quorum-dependent swarming motility in *Pseudomonas aeruginosa*.

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Abstract

The opportunistic human pathogen *Pseudomonas aeruginosa* exhibits diverse forms of collective behaviors, like swarming motility and biofilm formation. The collective behavior is also intimately linked to both chronic and acute lung inflammation. Does swarming allow this bacterium to spread in mucus-covered organs such as the lungs? Swarming in *P. aeruginosa* is a coordinated movement of the bacterial population over a semisolid surface, but specific signals or molecular inducers for swarming are not clear. We hypothesize that specific environmental signals induce swarming. We show that under nutrient-limiting conditions, a low concentration of ethanol provides a strong motivation for swarming in *P. aeruginosa* strain PA14. Ethanol serves as a signal and not a source of carbon under these conditions. Moreover, ethanol-driven swarming relies on the ability of the bacteria to metabolize ethanol to acetaldehyde using a periplasmic quinoprotein alcohol dehydrogenase, ExaA. An orphan response regulator, ErdR linked to ethanol oxidation, is necessary for the transcriptional regulation of a cluster of 17 genes, including *exaA*, during swarm lag. We show that *P. aeruginosa* displays characteristic foraging motility on a lawn of yeast *Cryptococcus neoformans* in a manner dependent on ethanol dehydrogenase and on rhamnolipids. Finally, we show that ethanol provided in trans, as a volatile, could induce swarming in *P. aeruginosa* at a distance. Our study suggests ethanol as a possible motivator for the spread of *P. aeruginosa* in human lungs where many ethanol-producing microbes such as *Staphylococcus aureus* and *C. neoformans* are readily found.

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Anti Quorum-Sensing, Anti-Virulence and Anti-Inflammatory Activities of PL-18, a Derivative of Piperlongumine from Piper longum

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Abstract

Bacterial communication, termed Quorum Sensing (QS), is a promising target for virulence attenuation, thus providing an alternative treatment to decrease antibiotic-resistance infections. In this work, PL-18, an amide alkaloid derivative of Piperlongumine from *Piper nigrum*, inhibited bacterial QS. It has Quorum Sensing Inhibitory (QSI) activity on *Agrobacterium tumefaciens* (KYC55) and *Chromobacterium violaceum* (CV026) bacteria, also against the clinically relevant *Pseudomonas aeruginosa* (PA01), both against lasR-lasI and rhlR-rhlI QS systems. Pyocyanin, rhamnolipids and the buildup of *P. aeruginosa* biofilm are virulence important elements produced by *P. aeruginosa.* Their expression is controlled by QS. These three elements were significantly inhibited by PL-18. Interestingly, PL-18 is not toxic to P. aeruginosa, even at high doses as 0.5mM. PL-18 also efficiently inhibits the activation of Nuclear Factor kappa B (NF-kB), a pro-inflammatory transcription factor that is upregulated in many inflammatory diseases, including those induced by bacterial infections. Furthermore, PL-18 was found to has some cytotoxic to transformed cell lines. QS inhibition is a promising approach to prevent bacterial virulence, focused on the disturbance of bacterial communication. Coupled with the ability to inhibit NF-κB induced inflammation, we believe that PL-18 is a suitable candidate as an effective compound against bacterial infection, antibiotic resistance and biofilm formation.

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The role of AHL cell-to-cell signaling in the rice endosphere

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Abstract

The plant microbiome represents many diverse microorganisms that interact and colonize different plant-associated compartments; one of these is the endosphere, which consists of the inner plant tissues. In this complex multispecies community, many different mechanisms of cell-cell interactions are likely to take place among bacteria. Quorum Sensing (QS) is one type of interaction among bacteria, which regulates gene expression in response to cell density via the release and detection of chemical signals, such as acyl-homoserine lactone (AHLs). However, very little information is currently available on the role of QS in the plant endosphere in shaping and establishing microbial communities. Studies have shown that most commonly the endophytic microbiome has a prevalence of Proteobacteria, representing over 50% of the bacterial community. We performed a bioinformatics study searching for the presence of complete AHL-QS systems among publicly available endophytic bacterial genomes belonging to the proteobacteria phylum. Among the hits obtained the *Pseudomonas fluorescens* L111 was selected as a model to study the role of AHL-QS, wild type and the QS systems knock-out mutants have been molecularly and genetically studied to understand the organizational hierarchy of the two signaling mechanisms and possible target genes in the rice endosphere, since it harbors two AHL-QS systems. Via in planta colonization assays and microbiome 16S rRNA amplicon sequencing analysis the possible role of the AHL-QS systems in the plant endosphere has been studied. This study highlights the function of the QS communication systems and AHLs signals in the establishment of a multispecies community in rice plants.

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An engineered *E. coli* consortium for studying quorum sensing

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Abstract

Quorum sensing (QS) is a cell-to-cell communication process that coordinates bacterial activities (eg. virulence) according to bacterial population density. QS inhibitors have shown potential as a strategy against bacterial pathogens *in vitro* and *in vivo*. A fluorescence-based *E. coli* consortium has been designed to monitor QS activity. It comprises three recombinant strains that either sense, produce or regulate (degrade) the QS signalling molecule N-3-oxohexanoyl-L-homoserine lactone (OHHL). Each construct is under the control of different inducible promoters and can be induced at any point during the observation. Upon induction, the producer strain can express the gene encoding the OHHL synthase (LuxI), the regulator strain can express the gene encoding the OHHL lactonase (AiiA), and the sensor strain can express LuxR, which forms a complex with OHHL, thereby activating another downstream promoter to express a gene encoding EGFP. This system thereby enables the direct quantification of OHHL concentration via a fluorescent signal, with a sensitivity within the nanomolar range. Two other fluorescent proteins, mCherry and EBFP2, are located downstream of the genes encoding the OHHL synthase and lactonase, respectively. These fluorescent proteins are designed to report the localisation of strains, the relative OHHL concentrations, and the relative expression of the genes in the community. Overall, this system allows the flux between OHHL degradation and production to be determined in the environment to help provide insight into this QS molecule. The functional parts of the strains could also be replaced via subcloning to study other QS signalling molecules and inhibitors.

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Identification of *Desulfuromonas carbonis* sp. nov. Metabolites that are Secreted in Response to Electron Acceptors

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Abstract

Chemical profiles in anaerobic porewater of sediments indicate that various microbes use several electron acceptors in anaerobic respiration. One of those bacteria is *Desulfuromonas carbonis* sp. nov.. Here we investigated the changes in secretion of these metabolites due to the presence of various common electron acceptors (EAs), focusing on microbial secondary metabolites that are used in bacterial signaling processes.

Pure cultures of *D. carbonis* were incubated with several different EAs, extracted by solid-phase extraction methods, and examined by liquid chromatography-tandem mass spectrometry (LC-MS\MS). The LC-MS\MS data was analyzed using the global natural products social molecular networking (GNPS) online platform, to identify potential bacterial signals that may play a role in population-wide processes related to the use of EAs to enhance fitness. We examined the presence of such compounds in complex cultures containing D. carbonis, natural anoxic lake sediment, and iron oxide, in order to mimic their ecological niche.

Our results indicate that bacteria produce unique metabolites depending on the presence of different EAs in the cultures. Indole-3-carboxaldehyde (I3C) was found in significant amounts in iron-oxide containing cultures. This compound has been found to play a role in tryptophan metabolism and is known to affect chemical communication among various bacterial species. Incubation of *D. carbonis* with increasing amounts of iron resulted in increased production of I3C.

These results establish the potential of I3C to be involved in natural processes specific to dissimilatory iron reduction. We will continue to investigate these processes and the connection between I3C signaling and iron.

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