Oral Presentation Abstracts

Control Number: 2022-A-68-ASM-BIO

Topic 1: Innovative Approaches and New Technologies in Biofilm Research

Publishing Title: Morphogenesis and cell ordering in confined bacterial biofilms

Author Block: J. Nijjer[1], Q. Zhang[1], T. Henzel[2], C. Li[3], S. Zhang[3], T. Cohen[2], **J. Yan**[1];
[1]Yale University, New Haven, CT, [2]MIT, Boston, MA, [3]The Pennsylvania State University, University Park, PA.

Abstract Body: **Background:** Biofilms are aggregates of bacterial cells surrounded by an extracellular matrix. Much progress has been made in studying biofilm growth on solid substrates; however, little is known about the biophysical mechanisms underlying biofilm development in confined environments, in which the biofilm-dwelling cells must push against and even damage the surrounding environment to proliferate. **Methods:** Here, combining single-cell live imaging, mutagenesis, and rheological measurement, we reveal the key morphogenesis steps of *Vibrio cholerae* biofilms embedded in hydrogels or at the gel-substrate interface as they grow by four orders of magnitude from their initial size. **Results:** We show that the morphodynamics and cell ordering in embedded biofilms are fundamentally different from those of biofilms on flat surfaces. Treating embedded biofilms as inclusions growing in an elastic medium, we quantitatively show that the stiffness of the environment and cell-to-surface adhesion jointly determine biofilm shape, growth dynamics, cell fate, and internal architecture. When embedded in stiff gels, cells self-organize into a bipolar structure that resembles the molecular ordering in nematic liquid crystal droplets. *In vitro* biomechanical analysis shows that cell ordering arises from stress transmission across the biofilm-environment interface, mediated by specific matrix components. At the gel-substrate interface, a collective cell reorientation cascade leads to a biofilm with radially organized cells at the bottom layer, and an onion-like organization in the bulk.**Conclusions:** Our imaging technique and theoretical approach are potentially generalizable to other biofilm-forming species, and to biofilms embedded in mucus or host tissues as during infection. Our results open an avenue to understand how confined bacterial communities grow by means of a compromise between their inherent developmental program and the mechanical constraints imposed by the environment.

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Control Number: 2022-A-72-ASM-BIO

Topic 1: Host-associated Biofilms

Publishing Title: SORBED HOST PROTEINS MEDIATE NEUTROPHIL ADHESION, MOTILITY, AND DISCOVERY OF *STAPHYLOCOCCUS AUREUS* ON AN ABIOTIC SURFACE

Author Block: B. A. Pettygrove, T. R. Borgogna, J. M. Voyich, **P. S. Stewart**;
Montana State University, Bozeman, MT.

Abstract Body: **Background.** The vulnerability of implanted medical devices to biofilm infection may be due to defects in innate immunity around abiotic surfaces. **Methods.** Video

microscopy was applied to directly image the *in vitro* contest between bacteria (GFP-tagged *S. aureus*) attached to an abiotic surface (surrogate biomaterial) and human neutrophils. This approach allowed neutrophil functions including adherence, motility (random, chemotactic, and swarming), and bacterial discovery and killing to be visualized and quantified. **Results.** In normal human serum, bacteria were readily cleared if neutrophils discovered the bacterial aggregate before it grew too large. When heat inactivated serum was used in place of normal human serum, neutrophil surface adhesion, motility, and discovery were abolished, resulting in bacterial persistence. This strongly suggests that heat labile proteins such as fibrinogen and complement are required for effective neutrophil function on biomaterials. Inhibition of the neutrophil adhesin MAC-1, a receptor that recognizes ligands include fibrinogen and certain complement proteins, reduced motility and efficacy (neutrophil track length 651 ± 113 μm in untreated wells vs 298 ± 33 μm in NIF treated wells), leading to delayed discovery and persistence of bacteria. Attachment and subsequent neutrophil motility were impaired in complement protein C3-depleted serum but not cobra venom factor depleted serum, suggesting that attachment to a serum coated surface is mediated by the complement C3 degradation product iC3b. Consistent with previous reports, adsorbed fibrinogen restored adhesion and motility, but a defect in *S. aureus* discovery persisted. In some instances, rapid, aggressive neutrophil chemotaxis was observed that resulted in fragmentation and phagocytosis of even relatively large bacterial aggregates. **Conclusions.** Sorbed host proteins play a critical role in mediating effective neutrophil function on an implant surface, pointing to possible immunotherapeutic approaches for preventing medical device infection.

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Control Number: 2022-A-102-ASM-BIO

Topic 1: Social and Asocial Interactions in Biofilms

Publishing Title: Predation within biofilms dissected at cellular resolution

Author Block: **C. Nadell**, B. Wucher, A. Goldstein-Plesser, J. Winans;
Dartmouth College, Hanover, NH.

Abstract Body: Through strength in numbers, biofilm-dwelling bacteria exert a powerful influence on their environments in contexts ranging from particulate matter degradation to the establishment of infections that can be difficult or impossible to eradicate. One of the many functions of biofilm formation is protection from exogenous threats, including attack by other microbes attempting to displace or prey upon biofilm-residing cells. An ongoing goal of our lab is to understand the dynamics of predator-prey interaction at the scale of individual bacteria and their viral and bacterial predators within biofilms. I will present some of our recent work in this area, focusing in particular on the interaction of *Vibrio cholerae* and *Escherichia* coli prey with bacteriophages and the bacterial predator *Bdellovibrio bacteriovorus*. Using microfluidic culture methods and high-resolution confocal microscopy, we explore the relationship between prey biofilm architecture and susceptibility to

different predator classes. Notably, different prey species very substantially in their ability to protect themselves from predation in the biofilm context. Further, dual-species prey biofilms exhibit qualitatively different architectures from those seen in respective mono-species biofilm growth conditions, often with significant consequences for predation exposure. In prey biofilms of *V. cholerae*, we discovered a cell packing threshold past which prey are protected from predator exposure; the net result of this protection threshold is a dramatic alteration of biofilm spatial arrangement and community assembly after predation by *B. bacteriovorus*. *E. coli*, on the other hand, is usually not on its own able to protect itself against *B. bacteriovorus* due to its more open cell packing structure. If *V. cholerae* and *E. coli* produce biofilms in co-culture, both prey species' abilities to evade predation are changed substantially due to altered cell arrangements that occur among the prey. Overall, our studies point to the importance of exploring predator-prey dynamics at the scale of individual predator and prey to best understand how macroscopic ecological patterns emerge from cell-cell and cell-phage interaction at the microscale.

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Control Number: 2022-A-107-ASM-BIO

Topic 1: Synthesis, Assembly and Function of Extracellular Biofilm Determinants

Publishing Title: PHASE SEPARATION OF EXTRACELLULAR NUCLEIC ACIDS AND THEIR ROLE AS STRUCTURAL BIOPOLYMER IN*PSEUDOMONAS AERUGINOSA*BIOFILMS

Author Block: **S. MUGUNTHAN**[1], L. WONG[1], F. WINNERDY[1], S. SUMMERS[1], M. BIN ISMAIL[1], T. JAGGI[1], O. MELDRUM[1], P. TIEW[1], S. CHOTIRMALL[1], S. RICE[2], A. PHAN[1], S. KJELLEBERG[1], T. SEVIOUR[3];
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Abstract Body: **Background:** Biofilms impart antimicrobial resistance to resident bacteria. This phenotype is attributed to extracellular polymeric substances (EPS) such as extracellular DNA (eDNA), polysaccharides and proteins that protect by forming viscoelastic networks. eDNA in particular is important to the viscoelasticity of *Pseudomonas* biofilm. The viscoelasticity arises because it self-assembles into networks by forming higher order structures such as Holliday junction intermediates and Z-form DNA (Buzzo et al., 2021; Devaraj et al., 2019), and as we recently demonstrated, G-quadruplex extracellular DNA (eDNA) (Seviour et al., 2021). Understanding these higher order structures in biofilm matrix could explain differences between the properties, organization and function of extracellular and intracellular DNA.
Methods: Here, we investigated the structural basis for the ability of extracellular nucleic acids of *P. aeruginosa* to impart viscoelasticity and phase separate, specifically with regards to the nature of the extracellular nucleic acids.To address

this the following was performed:(i) Extracellular nucleic acids extraction and purification. (ii) Biochemical and biophysical analysis on the purified nucleic acid gel isolate and biofilm matrix using Nuclear Magnetic Resonance (NMR), Circular Dichroism (CD) and bulk rheology.**(iii)** Single molecule FISH (smFISH) with confocal laser scanning microscopy (CLSM) to visualize the localization of extracellular RNA in *P. aeruginosa* biofilms.
Results: Purified nucleic acid extract the displayed same networked viscoelastic behaviour as biofilm. Matrix and eDNA network dissolution occurred following alkaline transesterification and resulted from extracellular RNA (eRNA) hydrolysis while eDNA preserved its chain structure. Specific eRNA were found by smFISH to be colocalized with and bound to eDNA fibres in model and clinical *P. aeruginosa* biofilms. The identity of eRNA in fibers was independent of total RNA expression.
Discussion: This work suggests an extracellular function for RNA as key structural component of biofilms. smFISH was used for the first time to visualize the distribution of eRNA in *P. aeruginosa* biofilms. The increased versatility of RNA could enable DNA to form networked structures. Understanding the nature and distribution of extracellular nucleic acids is a big step towards explaining viscoelastic *P. aeruginosa* biofilm matrix assembly and, along with an understanding of the nature of the eDNA and eRNA association, will inform new biofilm controls.

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Control Number: 2022-A-142-ASM-BIO

Topic 1: Metabolism, Physiology and Structure of Biofilms

Publishing Title: STRUCTURAL INVESTIGATION OF THE *CANDIDA ALBICANS/STAPHYLOCOCCUS AUREUS* DUAL BIOFILM BY MESOLENS 3D IMAGING

Author Block: **K. J. Baxter**, G. McConnell, P. A. Hoskisson;
University of Strathclyde, Glasgow, UNITED KINGDOM.

Abstract Body:
Background Co-infection with *C. albicans* and *S. aureus* can cause serious infection in those with immunological dysfunction, resulting in poor patient outcomes and increased patient treatment costs. Previous studies show synergy in their co-infection, with increased virulence and greater resistance to antimicrobial treatments than either single species, however the contribution of biofilm topology to increased antimicrobial resistance (AMR) in this relationship is not well understood. Biofilms allow their inhabitants to grow as well as protecting them from the external environment, and must contain architecture facilitating both aspects of their function. Studying large regions of biofilm enables the macrostructures involved in these processes to be placed in the context of global biofilm structure and their role in biofilm function and AMR investigated.
Methods This study uses the Mesolens 3D imaging system to visualise a large area of biofilm with subcellular resolution. By using an objective lens combining high numerical aperture with low magnification, global biofilm structures are imaged in

great spatial detail. Fluorescently labelled *C. albicans* and *S. aureus* strains are used to study dual species biofilm formation on a variety of growth surfaces including agar, glass and polydimethylsiloxane (PDMS), a silicone elastomer used in indwelling medical devices. Biofilm formation is followed over a given time period with the Mesolens, allowing a structural study of global biofilm morphology when grown on different surfaces.
Results Imaging of the dual species biofilm on agar reveals a distinct biofilm structure, established over a period of 12 hours. These biofilms have a central *C. albicans* core interspersed with *S. aureus* microcolonies, corralled by a *S. aureus* halo. We are altering different growth conditions to investigate the impact of these changes on biofilm formation. We are also using an abiotic/biotic surface interface model to follow biofilm formation on glass and PDMS, and developing methods to study *C. albicans* hyphal penetration into PDMS.
Conclusions Biofilm structure contributes to virulence in infection. By investigating global multi-species biofilm architecture, we can develop our understanding of how these complex and heterogeneous structures inherently promote antimicrobial resistance, and develop new tools against them.

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Control Number: 2022-A-149-ASM-BIO

Topic 1: Biofilm Maturation: Pathways, Consequences and Control

Publishing Title: SIGNALS AND UNDERLYING MECHANISMS THAT PROMOTE BIOFILM FORMATION BY SQUID SYMBIONT *Vibrio fischeri*

Author Block: **C. N. Dial**, K. L. Visick;
Loyola University Chicago, Maywood, IL.

Abstract Body:
The marine bacterium *Vibrio fischeri* efficiently colonizes its symbiotic squid host, *Euprymna scolopes*, using biofilm formation dependent on the symbiosis polysaccharide (SYP). Historically, genetic manipulation of *V. fischeri* was needed to study biofilm formation *in vitro*; this manipulation made strain backgrounds convoluted and our ability to deduce signaling pathways difficult. Recently, we discovered that the combination of two small molecules, pABA and calcium, induces *syp* transcription and SYP-dependent biofilms in wild-type strain ES114 without the need for genetic manipulation. Further investigation revealed that these SYP-dependent biofilms were reliant on the positive *syp* regulator RscS, as a loss of this sensor kinase resulted in a complete abrogation of biofilm formation and *syp* transcription. This result was of particular interest because, previously, a Δ*rscS* mutant had no biofilm phenotype outside of the squid or a complicated strain background. Deletion of the sensory domain of RscS also resulted in a loss of biofilm formation, phenocopying a Δ*rscS* mutant and suggesting that RscS might be the main sensory regulator. Overexpression of RscS on a multi-copy plasmid, but not the corresponding vector control, permitted biofilm formation on media supplemented with pABA alone, supporting the hypothesis that RscS may be responsible for recognizing the pABA signal. However, the pathway is complicated with multiple sensor kinases that activate the central sensor kinase SypF via its HPT

domain. We therefore developed an RscS-SypF chimera, which contains the 5' half of RscS fused to the 3' HPT domain of SypF. We found that, in the absence of *sypF*, addition of pABA is sufficient to induce biofilm formation as long as the chimera and a full-length copy of *rscS* are present. Taken together, these data point to the possibility that RscS might be responsible for recognizing pABA to coordinately induce biofilm formation. This work thus provides insight into signals and regulators that promote biofilm formation by *V. fischeri* as well as uncovering a key role for RscS in *in vitro* biofilm formation.

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Control Number: 2022-A-165-ASM-BIO

Topic 1: Biofilm Maturation: Pathways, Consequences and Control

Publishing Title: Bacterial Biofilm-Derived H-NS Protein Acts as a Defense Against NETs

Author Block: **A. L. Hendricks**, J. R. Buzzo, A. Devaraj, F. Robledo-Avila, K. More, L. O. Bakaletz, S. D. Goodman;
Nationwide Children's Hospital, Columbus, OH.

Abstract Body: **Background:** Intracellularly**,** Histone-like Nucleoid Structuring protein (H-NS), a nucleoid-associated protein (NAP) is part of bacterial chromatin, acts as a universal repressor of genes in many genera of eubacteria by binding to curved DNA, often condensing DNA structure. Previous studies investigating the extracellular role of H-NS found it possessed no structural function in the extracellular DNA (eDNA)-dependent biofilm matrix. Recently, we discovered that the conformation of biofilm eDNA shifts from the canonical B-form to the novel Z-form as the biofilm matures, disabling B-DNA binding proteins, including H-NS, from interacting with eDNA. Here we tested the retention of H-NS in a developing biofilm, but also ascertained whether the expulsion of H-NS due to the B-to-Z DNA conversion could affect the eDNA of the host's primary biofilm defense, Neutrophil Extracellular Traps (NETs). NETs utilize discharged neutrophil eDNA to entangle invading bacteria, cordon off biofilm proliferation, and concentrate antimicrobial compounds to combat bacterial infections. With DNA being a major component of NETs, DNA-binding proteins, such as H-NS are of particular interest in the defense of biofilm-resident bacteria against host-immune responses. **Methods:** Immunofluorescence was used to visualize steady state levels of the H-NS protein in uropthaogenic *E. coli* (UPEC), *Streptococcus pneumoniae* (*S. pneumoniae*), and non-typeable *Haemophilus influenzae* (NTHI) biofilms at timepoints ranging from 24 hrs to 1 week. The release of H-NS from biofilms into growth media was quantified via ELISA to confirm the loss of H-NS as the biofilm matured. Confocal microscopy was also used to visualize the impact of H-NS exposure to NET formation. **Results:** Over 1 week, the steady state levels of H-NS decreased in the biofilm which indicated that as the biofilm matured, H-NS was being released into conditioned media. Further, exogenous H-NS prevented the formation of PMA-induced NETs resulting in the retraction and condensation of NET-deployed DNA. **Conclusions:** H-NS, a bacterial DNA-binding protein did not have a

structural role in biofilms *per se* but appeared to have a role in defending biofilms from host-derived NETs. H-NS has the capability to both prevent DNA release from neutrophils and cause condensation of active NETs due to its ability to condense DNA once it has been released from the biofilm eDNA. Thus, a consequence of biofilm Z-DNA formation, was the release of H-NS to inactivate NETs.

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Control Number: 2022-A-167-ASM-BIO

Topic 1: Metabolism, Physiology and Structure of Biofilms

Publishing Title: DRIVERS OF BACTERIAL BIOFILM ARCHITECTURES ARE CONSERVED ACROSS SPECIES

Author Block: **H. Jeckel**[1], F. Díaz-Pascual[2], D. Skinner[3], B. Song[3], E. Jiménez-Siebert[4], K. Strenger[4], E. Jelli[5], S. Vaidya[2], J. Dunkel[3], K. Drescher[4];
[1]Philipps-University Marburg, Marburg, GERMANY, [2]Max-Planck-Institute for Terrestrial Microbiology, Marburg, GERMANY, [3]Massachusetts Institute of Technology, Cambridge, MA, [4]Biozentrum Basel, Basel, SWITZERLAND, [5]Max-Planck-Institute for Neurobiology, Bonn, GERMANY.

Abstract Body:

Background:
Biofilm formation is currently estimated to be one of the most abundant lifestyles of microbes on earth. Depending on the context, bacterial biofilms can be both beneficial and harmful, and they are highly relevant in clinical and industrial settings. Recent advances in microscopic imaging techniques have allowed new insights into the internal structure of biofilms at the single cell level, however it is still unknown, which properties determine biofilm architecture and how differences between different bacterial species arise.

Methods:
We performed single-cell resolution imaging of *Vibrio cholerae*, *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa* biofilms and developed a computational framework to quantify and compare biofilm architecture. Complementary to this experimental approach, we employed a mechanistic mathematical model to simulate biofilm growth, allowing us to explore the impact of different properties on biofilm architecture in a controlled and targeted manner.

Results:
Our analysis reveals, that irrespective of the species and their molecular differences, biofilm architecture is determined by two control parameters, which are cell aspect ratio and cell density. Additional experiments in which these properties were systematically varied in *Vibrio cholerae* mutants, confirmed a causal connection. Exploring the same parameter space through mechanistic simulations, we found that the architecture of different species could successfully be predicted, indicating that mechanical cell-cell interactions dominate early biofilm development.

Conclusions:
Our results indicate, that across different bacterial species, early biofilm architecture is dependent on the cell aspect ratio and cell density and that biofilm development is determined by mechanical cell-cell interactions.

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Control Number: 2022-A-170-ASM-BIO

Topic 1: Susceptibility and Tolerance: Antimicrobials and Biofilms

Publishing Title: Pseudomonas aeruginosa aggregation and Psl expression in sputum is associated with antibiotic eradication failure in children with cystic fibrosis

Author Block: **A. J. Morris**[1], Y. C. W. Yau[1], S. Park[1], S. Eisha[1], N. McDonald[1], M. R. Parsek[2], P. Howell[1], L. R. Hoffman[2], D. Nguyen[3], A. DiGiandomenico[4], A. M. Rooney[5], B. Coburn[6], L. Grana-Miraglia[5], P. Wang[5], D. S. Guttman[5], D. J. Wozniak[7], V. Waters[1];
[1]The Hospital for Sick Children, Toronto, ON, CANADA, [2]University of Washington, Seattle, WA, [3]McGill University, Montreal, QC, CANADA, [4]AstraZeneca, Washington, DC, [5]University of Toronto, Toronto, ON, CANADA, [6]University Health Network, Toronto, ON, CANADA, [7]Ohio State University, Columbus, OH.

Abstract Body: **Background:** Antibiotic eradication therapy of early *P. aeruginosa* infection in children with cystic fibrosis (CF) is key in preventing the detrimental impact of chronic infection. We previously demonstrated that *P. aeruginosa* isolates that persisted despite inhaled tobramycin treatment had increased anti-Psl antibody binding *in vitro* compared to those successfully eradicated. We aimed to validate these findings by directly visualizing *P. aeruginosa* in CF sputum. **Methods:** This was a prospective observational study of children with CF followed at SickKids with new-onset *P. aeruginosa* infection who underwent inhaled tobramycin eradication treatment. Using the microbial identification passive clarity technique (MiPACT), *P. aeruginosa* was visualized in sputum samples obtained before treatment and classified as persistent or eradicated based on outcomes. Pre-treatment isolates were also grown as biofilms *in vitro* and visualized using confocal microscopy. **Results:** A total of 11 patients were enrolled; 4 developed persistent infection and infection was eradicated for 7. *P. aeruginosa* biovolume and the number as well as size of *P. aeruginosa* aggregates were greater in the sputum of those with persistent compared with eradicated infections ($p<0.01$). The amount of Psl antibody binding in sputum was also greater overall ($p<0.05$) in samples with increased *P. aeruginosa* biovolume. *In vitro*, compared to eradicated isolates, persistent isolates showed increased Psl antibody binding but not increased aggregation; tobramycin treatment significantly reduced the metabolic activity (but not biovolume) of persistent isolates whereas for eradicated isolates, tobramycin significantly reduced both metabolic activity as well as biovolume. **Conclusions:** When visualized directly in sputum, *P. aeruginosa* had a greater biovolume, with subsequent more expressed Psl, and formed more numerous, larger aggregates in children with CF who failed inhaled tobramycin eradication therapy compared to children who successfully cleared their infection. These results highlight the benefit of direct visualization of infecting bacteria in clinical samples for predicting antibiotic responses in CF.

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Control Number: 2022-A-190-ASM-BIO

Topic 1: Susceptibility and Tolerance: Antimicrobials and Biofilms

Publishing Title: MODELLING ANTIBIOTIC TOLERANCE IN LIQUID CRYSTALLINE BIOFILMS

Author Block: **M. van Rossem**[1], S. Wilks[1], P. Secor[2], M. Kaczmarek[1], G. D'Alessandro[1]; [1]University of Southampton, Southampton, UNITED KINGDOM, [2]University of Montana, Missoula, MT.

Abstract Body:

BackgroundAntibiotic resistance and tolerance are serious medical issues of strong scientific interest. Recently, liquid crystals (LCs) were discovered in *P. aeruginosa* biofilms [1]. These LCs cause increased antibiotic tolerance and are formed by the filamentous bacteriophage Pf4 in combination with polymers in the biofilm matrix, through an excluded volume effect termed depletion attraction. We aim to explain this phenomenon by developing continuum models of liquid crystalline biofilms, which is a novel area of research.

Methods*In vitro* models of Pf4 virion-induced LCs consist of suspensions of Pf4 virions and depleting polymers, with the phages forming LC droplets called tactoids. These tactoids encapsulate bacteria, forming a protective barrier against cationic antibiotics through adsorption by the anionic phages. Our work investigates if this barrier can be effective through physical mechanisms alone, by adsorption and impeded diffusion [2]. To this end we have developed a numerically solvable model (shown in figure 1) and an analytically solvable homogenized model of antibiotic diffusion through a tactoid.

Results

Our results show that the tactoid barrier significantly affects the diffusion of antibiotics, and that the ability of Pf4 virions to adsorb antibiotics is essential for this efficacy. This agrees with the previous experimental findings that the presence of Pf4 virions increases tolerance to cationic antibiotics only [1][3].

ConclusionThe developed model sets a step towards the modelling of liquid crystalline biofilms in their full complexity. In particular, the homogenised model is straightforwardly extendable to more complex geometries. Ultimately, we hope to contribute useful insights into biofilm structure and the treatment of bacterial infections.

References:[1] P. R. Secor et al., *Cell Host Microbe*, **18**, 5 (2015).[2] M. van Rossem et al., *PLOS ONE*, **17**, (2022).[3] A. K. Tarafder et al., *Proc Natl Acad Sci*

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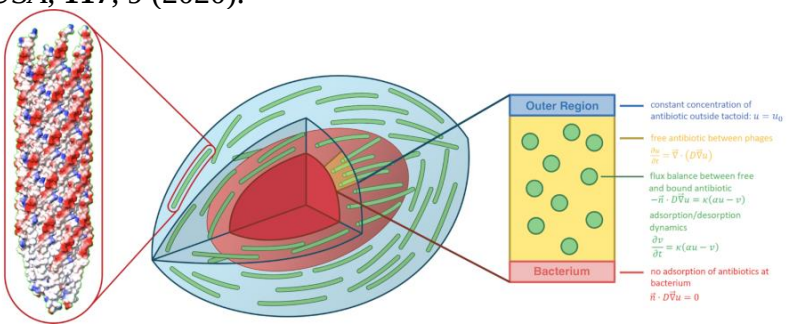

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Control Number: 2022-A-195-ASM-BIO

Topic 1: Initiating Contact: Signals and Mechanisms Promoting Attachment

Publishing Title: THE TYPE OF SURFACE DICTATES SURFACE RESPONSES IN *PSEUDOMONAS AERUGINOSA*

Author Block: **X. Zheng**, M. R. Parsek;
University of Washington, Seattle, WA.

Abstract Body: Surface sensing is the first step for bacteria to form a biofilm. In the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa*, two main types of surface sensing mechanisms have been proposed to trigger the production of c-di-GMP, the central molecule that controls biofilm formation. The Pil-Chp system is a mechanosensor that responds to the forces generated through type IV pili extraction and retraction. Activation of the Pil-Chp system increases the production of cyclic adenosine monophosphate (cAMP) through the function of CyaB and cyclic diguanylate monophosphate (c-di-GMP) through SadC. Meanwhile, the Wsp system is activated by cell envelope stress and the diguanylate cyclase within the system, WspR, synthesizes c-di-GMP. While both cAMP and c-di-GMP are critical second messenger molecules that control various cellular activities, how the bacterium coordinates these two signaling pathways is largely unclear. Here, we developed a tri-color reporter to simultaneously measure the levels of both c-di-GMP and cAMP in a single cell. By exposing *P. aeruginosa* to different types of surfaces, we found that the activation of c-di-GMP and cAMP depends on surface types. In a continuous flow cell system, we mainly observed an increase in c-di-GMP reporter activity but not cAMP during the first 6 hours of inoculation. On the contrary, on a 1.5% agarose pad, the levels of cAMP reporter activity increased but not c-di-GMP. These findings suggest that *P. aeruginosa* senses different surfaces and responds with distinct surface behaviors. Our ongoing work will investigate the regulation of c-di-GMP/cAMP synthesis and heterogeneity on different surfaces.

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Control Number: 2022-A-208-ASM-BIO

Topic 1: Susceptibility and Tolerance: Antimicrobials and Biofilms

Publishing Title: Evolution during combinatorial drug treatment overcomes constraints across a Pareto front

Author Block: F. A. Arroyo[1], **V. S. Cooper**[2];
[1]Fresno State University, Fresno, CA, [2]University of Pittsburgh, Pittsburgh, PA.

Abstract Body: **Background** Combination drug therapies aim to combat antibiotic resistance by either slowing the evolution of resistance or exploiting evolutionary tradeoffs. These therapies challenge bacteria to acquire multiple discrete mutations to combat each antibiotic separately. Alternatively, by combining drug pairs with collateral sensitivities, an evolutionary tradeoff is imposed wherein exposure and evolved resistance to one drug causes a different drug to lose effectiveness. Unfortunately, few antibiotic pairs with collateral sensitives emerge in clinics and outcomes from combination therapy are highly variable. Furthermore, little is known about the influence of biofilm growth on the evolution of collateral effects during combination therapy.**Methods** We challenged *Acinetobacter baumannii* ATCC17978-UN with two drugs simultaneously in a 15-day evolution experiment. Drugs with demonstrated reciprocal collateral sensitivity when tested in monotherapy were selected: fluoroquinolone ciprofloxacin (CIP) and beta-lactam ceftazidime (CAZ). We compared effects of planktonic or biofilm growth in the bead model on resistance trajectories and selected mutations identified by longitudinal deep sequencing. We applied a Pareto optimality front model to summarize the collateral effects of both combination and monotherapy experiments.**Results** We found that monotherapy selection produced tradeoffs, yet combination therapy overcame these constraints. The shape of the Pareto front was dynamic and governed by mutation order. Driver mutations in the *adeIJK* efflux pump locus were selected in all conditions, but the identity of first step driver mutations (*adeN* and *pgpB*) differed between growth lifestyles. These drivers caused cross resistance at sub-inhibitory concentrations, but further adaptation to higher concentrations of either drug required multiple mutations to arise independently, regardless of lifestyle.**Conclusions** Pareto optimality models can characterize constraints between multiple environmental variables, including across time. Collateral sensitivity in monotherapy did not predict whether simultaneous dual treatment would slow adaptation. We hypothesize that treatment failures in this model were caused by *adeIJK* mutations that enhanced efflux of one drug without losing capacity for multidrug resistance. How and why different *adeN* and *pgpB* mutations were selected in biofilm or planktonic lines requires further study.

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Control Number: 2022-A-215-ASM-BIO

Topic 1: Host-associated Biofilms

Publishing Title: HOW NON-FIMBRIAL ADHESINS OF DIARRHOEAGENIC AND UROPATHOGENIC *E. COLI* 'FINE-TUNE' BIOFILM FORMATION

Author Block: **J. J. Paxman**[1], J. Vo[1], G. C. M. Ortiz[1], A. W. Lo[2], L. Hor[1], M. Totsika[3], M. Schembri[2], B. Heras[1];
[1]La Trobe Institute for Molecular Science, La Trobe University, Melbourne,

AUSTRALIA, [2]Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, AUSTRALIA, [3]Centre for Immunology and Infection Control, Queensland University of Technology, Brisbane, AUSTRALIA.

Abstract Body:

Background: The involvement of bacterial surface fimbrial adhesins in the formation of biofilms has been well established. However, apart from these complex multi-component structures bacteria are also known to employ much simpler non-fimbrial adhesins to promote biofilm formation[1]. The largest group of these non-fimbrial adhesins are the autotransporter proteins from Gram-negative bacteria[2,3]. Here we reveal the underlying molecular mechanisms as to how autotransporter adhesins both promote and modulate biofilm formation by uropathogenic, enterohemorrhagic and entertoxigenic *E. coli*, that are responsible for urinary tract and diarrheal infections.**Aims:** We sought to progress from the multitude of genotype-phenotype studies to gain a molecular understanding of how non-fimbrial adhesins contribute to biofilms.**Methods:** In order to dissect the mechanisms of biofilm formation we worked with expert collaborators using a multidisciplinary approach that involved X-ray crystallography with biophysical, biochemical, cellular, and microbiological analysis.**Results:** Using insights from our further 5 new crystal structures of self-associating autotransporter adhesins, we have shown how they promote bacterial aggregation and biofilm formation in the absence of fimbrial adhesins[4]. Although they share similarities in both structure and mechanism, differences in their modes of self-association influence the kinetics of aggregation and biofilm formation.**Conclusions:** Variations in both the structure, self-association and post-translational modification of autotransporter adhesins have given bacteria such as *E. coli* another means to 'fine-tune' their levels of aggregation and biofilm formation[4]. Given that biofilms are a tradeoff, between the protection they afford at the cost of reduced dissemination, the ability of bacteria to modulate biofilm formation to suit their different environments is highly beneficial. These findings are contributing to our further overall understanding of bacterial biofilms including those associated with human infections. Importantly, our research has identified new targets to control biofilms which we are currently utilising in our recently patented biofilm inhibitor under preclinical trials[5]. [1]Heras,..Paxman et al. (2014). PNAS. [2]Clarke...Paxman* and Heras*.(2022). Front. Immunol. [3] Paxman et al., (2019). Nat. Commun. [4]Vo,.....Paxman* and Heras*(2022). Nature NPJ Biofilms Microbiomes. [5] Heras, Paxman et al., PCT/AU2019/050893.

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Control Number: 2022-A-224-ASM-BIO

Topic 1: Social and Asocial Interactions in Biofilms

Publishing Title: The impact of cell surface hydrophobicity on quorum sensing in *Pseudomonas aeruginosa*

Author Block: **K. O'Connor**, S. P. Diggle;
Georgia Institute of Technology, Atlanta, GA.

Abstract Body:

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. Here we show how the cell surface impacts QS-dependent cooperation and conflict. Specifically, we demonstrate that depending on the environmental requirements for QS-regulated public behaviors, QS- cells differentially arrange based on their social needs to remain close to QS+ cells. When QS-regulated public behaviors are not required for survival, QS+ and QS- cells spatially assort depending on their LPS structure, preferring to structure "like with like". However, when QS-regulated public behaviors are required for survival, QS- cells overcome physical differences in hydrophobicity to aggregate together with QS+ cells. Overall, these findings highlight that the surface properties of *P. aeruginosa* cells, and environmental requirements for sociality determine the outcome of QS social interactions.

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Control Number: 2022-A-225-ASM-BIO

Topic 1: Natural Biofilms, Consortia and Simulations

Publishing Title: The Formation of Biofilms on Microplastics

Author Block: **K. Crandall**, S. Pasqualetti, K. Milligan-McClellan;
University of Connecticut, Storrs, CT.

Abstract Body: The Formation of Biofilms on Microplastics **Background**: Microplastics (MPs) have been found in every environment, including humans[1-4]. MPs have a diameter less than 5mm and biomagnify through ecosystems causing detriments to the health and development of all organisms[5-7]. Bioremediation uses environmental microbes to break down and consume contaminants. MP bioremediation begins with biofilm formation on the hydrophobic plastic for microbes to adhere to the surface. Then, microbes secrete extracellular enzymes to break the bonds of MPs. The depolymerized plastic can be used for energy or creating biomass[8-11]. Identifying the microbes that adhere and produce MP-degradation enzymes has not been shown in microbes from the gut microbiome of species living in MP-polluted environments. Additionally, the comparison of biofilm formation on different types of MPs has not been shown. The ability to form a biofilm is critical for MP bioremediation and identification of microbes capable of producing a MP biofilm could lead to identification of MP-degrading microbes. **Methods**: I screened over 350 microbes from the gut of Alaskan stickleback, a host known to live in MP-contaminated

waters, for the ability to form MP biofilms and for the genes required for the depolymerizing enzymes, lipase and esterase. I grew pure cultures with a MP-containing minimal media using one of four types of MPs for 6 weeks. I fixed and stained the MP films using crystal violet for the presence of biofilms. Then, I identified if these strains contained lipase or esterase using primers designed against the genes. **Results**: 43 environmental strains were capable of biofilm formation in minimal media on MP films. I found there was a microbe-dependent MP preference. 14 of those strains contained at least one of the depolymerization genes, lipase or esterase. **Discussion/Conclusions**: The environmental genera that formed a biofilm on MPs was predominately *Pseudomonas* and *Bacillus*, genera in the core gut microbiota in stickleback[12]. These microbes showed a preference for some MPs over others, likely from the difference in how these microbes form a biofilm. Some strains were also found to contain the depolymerization genes required. Environmental strains from the stickleback gut can and do form biofilms on the hydrophobic MP surface and have the potential to depolymerize the MPs as a source of energy. Using microbes and their enzymes to degrade MPs could aid in the bioremediation of natural environments or applied to wastewater treatment to remove and prevent the spread of MPs in aquatic systems.

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Control Number: 2022-A-231-ASM-BIO

Topic 1: Social and Asocial Interactions in Biofilms

Publishing Title: Cooperation between mucoid and nonmucoid *Pseudomonas aeruginosa* during biofilm formation

Author Block: P. Hill[1], D. Wozniak[1], **E. Gloag**[2];
[1]The Ohio State University, Columbus, OH, [2]Virginia Tech, Blacksburg, VA.

Abstract Body: **Background:**In the CF lung biofilms are observed as aggregates, that range in size from 10 - 300 μm, suspended in the mucus lining. Biofilm-aggregates of *Pseudomonas aeruginosa* are segregated spatially within the CF lung. These isolated *P. aeruginosa* populations evolve independently from neighboring aggregates, resulting in diverse biofilm subpopulations. This suggests that evolved variants coexist in spatially isolated biofilm-aggregates with the evolved ancestor, contributing unique functions to the biofilm. One such variant that commonly evolves during CF is the mucoid variant, which overproduces the exopolysaccharide alginate. These variants have increased tolerance to antimicrobials and can extend this protection to the nonmucoid subpopulation when grown in planktonic coculture. However, it is unclear if this cooperation also occurs during biofilm development. **Methods:**To assess cooperation between mucoid and nonmucoid *P. aeruginosa* during biofilm formation, mixed biofilms were grown with a starting inoculum of 1, 10, 50 and 90% mucoid cells, and compared to single biofilm counterparts. Biofilms were grown in flow cells for 2 days and imaged by confocal laser scanning microscopy (CLSM) to determine if mucoid cells localize to specific regions of the biofilm. Lawn biofilms were grown for 24h, and creep-recovery analyses performed to determine if mixed biofilms display altered mechanical

properties.
Results:CLSM of mixed biofilms revealed that mucoid cells displayed niche specialization, localizing specifically to the substratum of the biofilm, with the nonmucoid cells forming microcolonies on top. Mixed biofilms grown with the 50% mucoid inoculum also had increased biomass compared to single mucoid and nonmucoid biofilms. Creep-recovery analyses revealed that mixed biofilms had increased viscoelasticity, compared to single mucoid and nonmucoid biofilms.
Conclusions: Our results indicate that mucoid and nonmucoid *P. aeruginosa* display cooperation during biofilm development, forming biofilms that have altered biomass, architecture, and mechanical properties. This points towards an adaptive advantage of mixed *P. aeruginosa* mucoid and nonmucoid biofilms. We hypothesize that this cooperation could lead to increased persistence of *P. aeruginosa* biofilms during infection in the CF lung.

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Control Number: 2022-A-238-ASM-BIO

Topic 1: Synthesis, Assembly and Function of Extracellular Biofilm Determinants

Publishing Title: THE TPR DOMAIN OF PgaA BINDS PNAG AND FACILITATES PERIPLASMIC POLYMER MODIFICATION AND EXPORT

Author Block: **A. Shankara Subramanian**[1], R. Phoh[1], A. Forman[2], B. R. DiFrancesco[2], N. Balouchestani-Asli[1], E. N. Kitova[3], J. S. Klassen[3], M. Nitz[2], P. L. Howell[1]; [1]Hospital for Sick Children, Toronto, ON, CANADA, [2]University of Toronto, Toronto, ON, CANADA, [3]University of Alberta, Edmonton, AB, CANADA.

Abstract Body: Exopolysaccharides (EPSs) are diverse macromolecules that provide structural integrity to biofilms produced by a wide variety of bacteria. EPSs protect the embedded bacteria against environmental stress and confer antibiotic tolerance. In Gram-negative bacteria, synthase-dependent EPS biosynthetic systems chemically modify the polymer in the periplasm prior to its export into the extracellular milieu. These operons also contain a gene that encodes an outer membrane-associated tetratricopeptide repeat (TPR) domain-containing protein. While the TPR domain has been shown to bind periplasmic polymer modification enzymes, the functional consequences of these interactions for the polymer remain poorly understood. Using the poly-β-1,6-*N*-acetyl-D-glucosamine (PNAG) biosynthetic system as a model, we demonstrate that the central and C-terminal TPR region of PgaA interacts with the N-terminal de-*N*-acetylase domain of PgaB. This interaction leads to increases in the deacetylase and hydrolase activities of PgaB. We determined the structure of PgaA-220-367 and observed that the crystallized TPR module displays a superhelical curvature with two conserved grooves. Tryptophan quenching and mass spectrometry analyses suggest that the TPR module binds PNAG via an electronegative groove on its concave surface. Taken together, our results suggest that the TPR domain of PgaA acts as a multifunctional scaffold and can potentially guide the polymer towards the porin for export by interacting with PgaB.

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Control Number: 2022-A-240-ASM-BIO

Topic 1: Metabolism, Physiology and Structure of Biofilms

Publishing Title: BIOFILM SUBPOPULATION PROFILES AND THE ROLE OF ANAEROBIC RESPIRATION

Author Block: **K. Jessel**, J. Price, M. Chapman;
University of Michigan, Ann Arbor, MI.

Abstract Body: **Background:** Bacteria primarily exist in biofilms—communities of cells living together that are protected from environmental stressors like antibiotics, predation, extreme temperatures, among others. The extracellular matrix (ECM) confers this protection upon the bacterial community; for *Escherichia coli* (*E. coli*) biofilms, the ECM consists primarily of protein and carbohydrate polymers known as curli and cellulose, respectively. The production of curli and cellulose are regulated by curli specific gene D (csgD). A hallmark of *E. coli* biofilms is the stratification of cells into two distinct subpopulations: ECM component-producing cells and cells not producing ECM components. **Methods:** To understand the difference in ECM component production between the two subpopulations in a biofilm, we performed RNAseq on each individual subpopulation. **Results:** The two subpopulations showed distinct transcriptional profiles. We identified over 200 transcripts as having at least a 2-fold difference between the two subpopulations. The differentially expressed transcripts are involved in motility, curli production, environment sensing, anaerobic respiration, stress response, among other genes. To connect the role of anaerobic respiration in biofilm development, we grew biofilms under aerobic and anaerobic conditions. Biofilms grown under anaerobic conditions had little to no ECM production compared to biofilms grown under aerobic conditions, demonstrating the effects of aerobic changes on biofilm development. **Conclusions:** The two biofilm subpopulations have distinct transcriptional profiles that provide insight on potential mechanisms important for biofilm formation and maturation. To understand the protein drivers of anaerobic respiration differential expression between the two subpopulations, we plan to follow up on the anaerobic respiration RNAseq candidate transcripts *nrfA*, *narG*, and *fnr* to explore the role of anaerobic respiration in biofilm development and subpopulation establishment.

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Control Number: 2022-A-247-ASM-BIO

Topic 1: Signals and Mechanisms Promoting Biofilm Disassembly and Dispersal

Publishing Title: The role of lactate leading to *Salmonella enterica* serovar Typhimurium biofilm dispersal through curli repression in the intestinal inflammatory environment

Author Block: **F. Albicoro**, S. Bessho, S. Olujabo, C. Tukel;
Temple University, Philadelphia, PA.

Abstract Body: **BACKGROUND:** *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) survives in the gut environment through the formation of biofilms. Curli, a major

component of *S.* Typhimurium biofilms, is expressed and synthesized in the intestinal tract and activates pattern recognition receptors leading to joint inflammation after infection with this pathogen. Recently, we discovered that host-generated nitrate during inflammation represses curli production and activates flagella via the modulation of intracellular cyclic-di-GMP levels. To obtain a bigger picture of how gut inflammatory environment regulates the sessile-to-motile switch in *S.* Typhimurium we tested the effect of lactate -a dominant metabolite produced during intestinal inflammation- in biofilm development.
METHODS: Colony biofilm morphologies were analyzed on YESCA agar containing Congo Red and Coomassie Blue in increasing concentrations of nitrate and lactate. Plates were incubated 72h 26°C. To analyze gene expression, promoter-GFP plasmids were used. Each plasmid contained the promoter region of *csgD* or *csgBAC* open reading frames. Cells were grown in biofilm inducing conditions with or without nitrate or lactate. GFP fluorescence were normalized with cell growth.
RESULTS: We observed that the typical colony morphotype that evidences curli and cellulose production in *S.* Typhimurium were reduced in the presence of lactate in a dose-dependent manner, suggesting that lactate play a role in initiating dispersal of bacteria from the biofilm. By comparing the results of lactate to the same concentrations of nitrate, we determined that lactate had a stronger effect on the biofilm. Also, using GFP reporter plasmids, we detected that lactate reduced the expression of *csgD* biofilm master regulator, and *csgBAC* curli operon expression when cells were incubated in biofilm inducing conditions.
CONCLUSIONS: Lactate plays an important role in *S.* Typhimurium fitness by regulating the sessile-to-motile lifestyle switch even at low concentrations. These results suggest that host-originated lactate in the inflamed intestine upon *S.* Typhimurium invasion may act as a cue that triggers biofilm dispersal, allowing bacteria to colonize other niches. To reveal the mechanism by which lactate represses biofilm development, we currently are monitoring the levels of intracellular c-di-GMP upon lactate incubation.

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Control Number:	2022-A-263-ASM-BIO
Topic 1:	Control, Prevention and Elimination of Biofilms
Publishing Title:	MULTIPLEXING PEPTIDE NUCLEIC ACID THERAPIES TO PREVENT FORMATION OF BACTERIAL BIOFILMS
Author Block:	**d. F. stamo**, A. Chatterjee; University of Colorado Boulder, Boulder, CO.
Abstract Body:	**Background** Antimicrobial resistance (AMR) is emerging as a “silent pandemic,” threatening modern treatments for bacterial infections around the world. This issue is further exacerbated by the formation of biofilms—established bacterial colonies strengthened by diversified gene expression, tough extracellular matrices, and increased resistance to both small-molecule antibiotics and host immune defenses. Peptide nucleic acid (PNA) antisense therapy is an intriguing alternative

antimicrobial due to the molecule's highly specific targeting, tolerance to biological degradation, and sequence tunability. PNA targeting specific essential genes are notably efficacious for bacterial clearance, but this specific targeting can deliver a similar evolutionary pressure to that of traditional small-molecule antibiotics, thereby perpetuating conditions for the development of AMR. Biofilm formation involves the precise coordination of hundreds of genes, some of which are necessarily non-essential. Despite their non-essentiality, PNA perturbation of these interconnected gene networks may nevertheless culminate in profound and wide-reaching transcriptomic effects. This work explores the rational design of a PNA multiplex system targeting several fitness-neutral biofilm genes to prevent the formation of early-stage biofilms.
Methods We seeded *in vitro* biofilms in microtiter plates from *Escherichia coli* cultures grown with PNA targeting 3 non-essential, biofilm formation genes (yfaL, fimA, and csgD). Post-treatment cultures were also diluted to seed biofilms on cellulose discs to model a burn wound.
Results All three PNA significantly decreased the size of biofilms compared to the no treatment control, with more profound decreases associated with PNA combinations. All 3 treatment conditions established biofilms on cellulose discs; however, pre-treatments targeting yfaL showed significant bacterial migration away from the cellulose disc, suggesting interference with biofilm formation contributes to increased expression of motility genes.
Conclusions Our results suggest the potential for synergy with existing small-molecule antibiotics as bacteria in their planktonic state are resensitized to these drugs. Additionally, efficacious PNA may be pre-loaded onto implantable biomaterials to decrease the risk of biofilm formation following surgical procedures. Both implications contribute to the clinical mitigation of the AMR crisis.

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Control Number: 2022-A-286-ASM-BIO

Topic 1: Initiating Contact: Signals and Mechanisms Promoting Attachment

Publishing Title: REGULATION OF DEVELOPMENT BY THE BACTERIAL FLAGELLUM

Author Block: **D. M. Hershey**;
University of Wisconsin - Madison, Madison, WI.

Abstract Body:
Regulation of development by the bacterial flagellum
Background: Bacteria often activate surface colonization programs when they encounter solid substrates. A molecular machine called the flagellum plays a central role in these surface sensing events, but how the flagellum transmits physical cues has been difficult to define. The aquatic bacterium *Caulobacter crescentus* responds to surface contact by producing an adhesin called the holdfast. We showed that mutating genes involved in the assembly of the flagellum stimulates holdfast production by mimicking surface contact, and my laboratory uses this gain of function phenotype to characterize the surface sensing pathway in *C. crescentus*.
Methods: We developed a genome-wide approach for studying the genetic basis of

adhesion in *C. crescentus* and leveraged this method to identify dozens of *flagellar signaling suppressor* (*fss*) genes that regulate holdfast production downstream of the flagellum. Measuring surface adhesion, motility behavior, cellular morphology and cell cycle progression in strains with individual *fss* genes deleted has provided surprising insights into the breadth of cellular responses to surface contact.
Results: *C. crescentus* has an unusual cell cycle. Cell division is asymmetric and yields two cell types: a motile swarmer cell and a sessile stalked cell. Deleting many of the *fss* genes affects specific events in this developmental program. A subset of the genes we identified codes for novel flagellar proteins that support motility in swarmer cells. Another class of genes influences the assembly of a cellular appendage called the stalk, and we are dissecting how these genes link disassembly of the flagellum to stalk development. Finally, we identified a number of genes that influence the timing of cell cycle progression. Deleting one gene in particular elongates the G1 phase of the cell cycle, and we are characterizing this protein's role in a novel pathway for regulating DNA replication.
Conclusions: Our results show that surface sensing by the flagellum affects morphogenesis, cell cycle progression, cell fate determination and other developmental processes. These responses are mediated by two-component signaling, second messenger dynamics and even alterations to central metabolism. We propose a model in which the flagellum sits atop a complex signaling network that orchestrates surface adaptation by reprograming how cells proliferate.

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Control Number:	2022-A-290-ASM-BIO
Topic 1:	Host-associated Biofilms
Publishing Title:	Sodium salicylate decreases *Pseudomonas aeruginosa* virulence and modulates the host immune response
Author Block:	**P. Giraldo Osorno**, E. Gerner, A. Johansson Loo, R. Firdaus, H. Amara, M. Werthen, P. Thomsen, O. Omar, S. Almqvist, M. Trobos; University of Gothenburg, Göteborg, SWEDEN.
Abstract Body:	**Background:** Biomaterial-associated infection (BAI) is a major cause of implant failure. *Pseudomonas aeruginosa*, a common causative pathogen of BAI, produces numerous virulence factors regulated by quorum sensing (QS). *P. aeruginosa* dampens inflammation and promotes bacterial survival through the QS system. Therefore, QS-inhibition appears as a relevant strategy to diminish bacterial virulence and improve the immune response. This study explores how secreted factors from *P. aeruginosa* PAO1 wild-type (WT) and a QS mutant (PAO1 *ΔlasIΔrhlI*), treated with and without the QS inhibitor (QSI) sodium salicylate (NaSa), modulate the immune response *in vitro* in macrophages and neutrophils and *in vivo* in a subcutaneous rat infection model. **Methods:** The effect of virulence factors secreted by *P. aeruginosa* cultures with and without NaSa on the host immune response was evaluated *in vitro* and *in vivo*, by analysing cell viability, migration, phagocytosis, gene expression, cytokine

secretion and histology. *In vitro*, the experiments were conducted using THP-1 and HL-60 cells. *In vivo*, titanium discs were placed in dorsal subcutaneous pockets and bacterial supernatant was administrated, to simulate virulence factors secreted during BAI. **Results:** *In vitro*, PAO1 WT virulence factors from NaSa cultures significantly increased THP-1 macrophage phagocytic activity and HL-60 cell migration, as compared to factors from untreated cultures. *In vitro* stimulation with factors from PAO1 QS mutant strain did not cause cytotoxicity but increased cell migration and cytokine levels as compared to WT factors. *In vivo*, rats receiving NaSa-treated *P. aeruginosa* factors exhibited significantly increased cell infiltration and attachment to the implanted titanium discs, GRO-a secretion, and IL-8 and IL-10 gene expression, while reduced production of IL-1β, IL-6, IL-12 and IFN-g. **Conclusions:** NaSa-treatment of *P. aeruginosa* infection appears to reduce virulence factors and enhance the immune response, by promoting cell migration and bacterial clearance, likely via the disruption of the QS system. Further, NaSa-treatment was found to mitigate the increased secretion of pro-inflammatory cytokines, triggered by the administration of *P. aeruginosa* supernatant *in vivo*. These data represent an important initial step in the understanding of the potential role of sodium salicylate as a novel strategy for treating chronic infections, including BAI.

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Control Number: 2022-A-291-ASM-BIO

Topic 1: Control, Prevention and Elimination of Biofilms

Publishing Title: EVALUATION OF THE EFFECTS OF NANOBUBBLES ON BIOFILMS IN FLOW AND STATIC MODELS

Author Block: **T. Le**[1], J. Wu[1], H. Liu[2], W. Gong[2], C. Xi[1], Y. Dong[2];
[1]University of Michigan, Ann Arbor, MI, [2]Peking University School and Hospital of Stomatology, Beijing, CHINA.

Abstract Body: **Background** Nanobubbles (NBs) are gas bubbles less than one micrometer in diameter. Due to their unique properties, they have shown promise in various applications including wastewater treatment, algal bloom mitigation, and drug delivery. NBs also impact microbial communities, and a few studies have shown that NBs can remove some single-species biofilms. However, not much is known about how NBs perform against other bacterial species or multi-species biofilms, when other disinfectants are present, and under conditions representative of the industrial and medical settings where NBs may be used as a biofilm removal agent. This ongoing project aims to test how well NBs, in conjunction with various disinfectants, reduce colonization and biofilm formation of bacteria in flow and static models.

Methods NB water was generated using the Turbu-Flow device. In the flow model, *Escherichia coli* biofilm was grown for 2 days in a flow cell system and then treated with NB water. The numbers of viable cells in the biofilm and planktonic phases were monitored by plate culture. The biofilm was also imaged by fluorescence microscopy. In the static model, a time-kill assay was performed to

evaluate the disinfectant capacity of NBs together with different concentrations of sodium hypochlorite on *Enterococcus faecalis*.
Results NB water achieved a 2-log reduction of *E. coli* viable cells in both the biofilm and planktonic phases compared to the control. In the *E. faecalis* time-kill assay, while NBs alone had no effect on cell count, they were able to enhance the disinfectant efficacy of sodium hypochlorite.
Conclusions NBs can remove *E. coli* biofilms and enhance the effectiveness of sodium hypochlorite against *E. faecalis*. The antimicrobial and antibiofilm effects of NBs thus depend on the flow conditions, bacterial species, and concentration of other disinfectants.

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Control Number: 2022-A-292-ASM-BIO
Topic 1: Synthesis, Assembly and Function of Extracellular Biofilm Determinants
Publishing Title: UNRAVELLING THE REGULATORY PATHWAY OF CELLULOSE BIOSYNTHESIS IN UROPATHOGENIC *Escherichia coli*
Author Block: **C. Ravi**, N. Nguyen, M. Phan, M. Schembri;
University of Queensland, Brisbane, AUSTRALIA.

Abstract Body:
Background: Uropathogenic *Escherichia coli* (UPEC) infection of the urinary tract is frequently associated with biofilms, necessitating an understanding of the genetic pathways that coordinate the formation of these resistant communities. A key component of the UPEC biofilm extracellular matrix is cellulose, a polysaccharide that provides structural integrity and stabilizes the biofilm. Here, we employed a genetic screen to identify genes required for cellulose biosynthesis.
Methods: We employed a combination of transposon mutagenesis and transposon-directed insertion site sequencing (TraDIS), together with a phenotypic screen to detect cellulose based colony on staining with Congo Red and Calcofluor White. The involvement of genes in cellulose production was validated by constructing defined mutants and complementation.
Results: Our genome-wide screen identified 96 genes that, when mutated, reduced cellulose production. These genes were involved in a range of cellular processes, and included genes from the co-located *bcsRQABZC* and *bcsEFG* operons that together encode the machinery required for cellulose biosynthesis, secretion and modification, as well as genes involved in regulation via the RpoS-CsgD cascade, metabolism, cell envelope biogenesis, purine/pyrimidine biosynthesis and energy production. A number of these genes were confirmed to have a significant contribution to cellulose production by targeted mutation and complementation. Genes previously linked to cellulose production included a diguanylate cyclase gene (*dgcC*) that synthesizes cyclic-di-GMP, as well as genes involved in carbon metabolism (*pgi, pgm* and *fbp*), purine *de novo* biosynthesis (*purD* and *purH*) and sodium transport (*nhaA*). New genes involved in cellulose biosynthesis were also identified and validated, comprising genes involved in pyrimidine *de novo* biosynthesis (*carB, pyrE* and *pyrB)*, lipid-A core biosynthesis (*waaD*, *waaC* and *waaG*) and ATP synthesis (*atpA* and *atpB*). We also identified a

unidirectional Tn*5* insertion upstream of the *pdeH* gene (phosphodiesterase that degrades cyclic-di-GMP) and showed that strong expression of PdeH induced by plasmid over-expression led to loss of cellulose production.
Conclusions: Overall, this study has revealed a complex network of genes involved in cellulose regulation and biosynthesis in UPEC, providing new insight to unravel the contribution of this bacterial polysaccharide in biofilm formation.

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Control Number: 2022-A-313-ASM-BIO
Topic 1: Signals and Mechanisms Promoting Biofilm Disassembly and Dispersal
Publishing Title: Role of polysaccharide intercellular adhesin in *Staphylococcus epidermidis* interactions with fibrin
Author Block: **S. VanAken**, J. VanEpps;
Michigan Medicine, ANN ARBOR, MI.
Abstract Body: **Background:** Staphylococcal bacteria and host proteins (*e.g.*, fibrin) routinely coexist on infected indwelling medical devices. IcaB, a cell surface-anchored protein, plays a key role in staphylococcal biofilm formation and immune evasion by deacetylating and introducing positive charge to polysaccharide intercellular adhesin (PIA). However, the role of IcaB in staphylococcal biofilm formation and persistence in the presence of host fibrin network remains unclear. Here we study the role of IcaB on an *in vitro* model of a *Staphylococcus epidermidis*-infected fibrin clot. **Methods:** Using an *icaB* deletion mutant, we evaluated the effects of PIA deacetylation on staphylococcal interaction with fibrin and the resulting changes in fibrin clot structure and expression of excreted proteases and cell surface binding proteins. Fibrin clot structure was evaluated using quantitative confocal laser scanning microscopy. Gene expression was evaluated by quantitative RT-PCR. **Results:** The ΔicaB mutant is more diffusive (*i.e.*, less likely to self-aggregate) than the WT strain and therefore more readily co-localizes with the fibrin network fibers. This is associated with increased *sdrG* (fibrin binding protein) expression. This co-localization of cells and fibrin is correlated with increased fibrin network degradation. This is reflected in increased expression of *sspA*, an extracellular protease thought to degrade fibrin. Addition of aprotinin, which inhibits *sspA*, restores fibrin structure as well as changes PIA structure from clumps on the bottom of the well to surrounding the fibrin network and cell structure. **Conclusions:** These results suggest a pivotal role for IcaB in modulating staphylococcal-fibrin interactions and resulting fibrin clot mechanical properties. **Keywords**: Intracellular adhesion, ica, biofilm, thromboembolism, bloodstream infection, fibrinolysis, confocal microscopy, qPCR, sdrG, sspA

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Control Number: 2022-A-365-ASM-BIO
Topic 1: Innovative Approaches and New Technologies in Biofilm Research

Publishing Title: PHENOTYPES, TRANSCRIPTOME, AND BIOFILM FORMATION ANALYSIS OF *Geobacter sulfurreducens* GSU1771 DEFICIENT MUTANT STRAIN IN DIFFERENT SUPPORTS

Author Block: **K. Juarez**[1], B. Jaramillo-Rodríguez[1], G. Huerta-Miranda[2], L. M. Rodriguez[1], L. Vega[3], M. Miranda[2], A. Hernandez-Eligio[1];
[1]Instituto de Biotecnología, UNAM, Cuernavaca, MEXICO, [2]Instituto de Energías Renovables, UNAM, Cuernavaca, MEXICO, [3]Instituto de Ciencias Aplicadas y Tecnología, UNAM, Ciudad de Mexico, MEXICO.

Abstract Body: *Geobacter sulfurreducens* plays an important role in Fe(III) and Mn(IV) biogeochemical cycles in subsurface environments and is widely studied for its ability to acquire energy by coupling oxidation of organic compounds with extracellular electron transfer to different insoluble electron acceptors through *c*-type cytochromes and conductive pili. It forms electroactive biofilms, important in electricity production from waste organic matter in bioelectrochemical systems. Deleting the *gsu1771* gene in *G. sulfurreducens* (*Δgsu1771* strain) that codifies a SARP-like transcriptional regulator results in a fast Fe(III) reduction; the biofilm formed is three times thicker and 100 times more electroconductive than the wild-type strain. In this work, we studied the transcriptomic response of Δ*gsu1771* strain in biofilm using glass as support. Results showed 467 differentially expressed (DE) genes (167 over-expressed and 300 down-expressed) with respect to the WT strain. Among the DE genes are energy metabolism and electron transport, transmembrane transport, exopolysaccharide production, signal transduction, regulation, and others. RNA-seq assay was validated with RT-qPCR with selected genes (*epsH*, *gsu0972*, *hybA*, *pgcA*, *omcM*, *aroG*, *panC*, *gnfK*, *gsu2507*, *acnA*, *ato-I*, *gsu0810*, *dcuB*, *pilA*, *ppcD*, *csrA* and *gsu3356*). To explore whether GSU1771 directly regulates its target genes, we also performed electrophoretic mobility shift assays (EMSA) with the promotor regions of *pgcA*, *omcM*, *hybE*, *pulF*, *gsu3356*, *relA*, *acnA*, and *gsu1771* genes; demonstrating that this regulator binds directly to those promoter sequences. We also studied the biofilm formation in other conductive supports such as graphite, FTO, Fe_2O_3-glass, and Fe_2O_3-FTO by CLSM and performed electrochemical studies to elucidate their electron transfer properties. Altogether the results allow us to understand the role of GSU1771 as a global regulator in biofilm formation, exopolysaccharides production, transport, and energy metabolism. 1) Hernandez-Eligio et al. 2022. *Bioelectrochemistry*

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Control Number: 2022-A-371-ASM-BIO

Topic 1: Initiating Contact: Signals and Mechanisms Promoting Attachment

Publishing Title: Make It or Break It: Pterin-Dependent Control of a Dual Function Diguanylate Cyclase-Phosphodiesterase to Regulate Surface Attachment in *Agrobacterium tumefaciens*

Author Block: **J. Greenwich**[1], J. Eagan[1], K. Boswinkle[2], K. Allen[2], G. Minasov[3], K. Satchell[3], C. Fuqua[1];

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Abstract Body: Agrobacterium tumefaciens is a pathogenic alphaproteobacterium capable of attachment to biotic and abiotic surfaces through production of a unipolar polysaccharide (UPP) adhesin. Surface contact stimulates UPP production and it is modulated by environmental conditions. The intracellular second messenger cyclic diguanylate monophosphate (cdGMP) controls UPP biosynthesis. cdGMP levels are tightly controlled, and the dual function diguanylate cyclase-phosphodiesterase (DGC-PDE) DcpA regulates UPP production and surface attachment. In planktonic laboratory culture, the PDE activity of DcpA is high, lowering cdGMP and UPP formation. While the DGC and PDE domains of DcpA are cytoplasmic, it also has a periplasmic domain. A small metabolite known as a pterin regulates the activity of DcpA. Pterins are similar to folates and derived from GTP. In A. tumefaciens, pterin biosynthesis is dependent upon PruA, an enzyme that catalyzes the reduction of 5,6-dihydromonapterin (H2MPt) to 5,6,7,8-tetrahydromonapterin (H4MPt). Null mutants of pruA show increased surface attachment due to elevated DGC activity from DcpA, which increases cdGMP levels and drives UPP production independent of surface contact. DcpA is regulated by a second protein, PruR, which represents a novel branch of the SUOX family of molybdopterin (MoCo) binding proteins. It is encoded directly upstream of dcpA and co-purifies with a monapterin-type small molecule. In vitro studies show PruR preferentially binds H4MPt over H2MPt. Structural studies reveal a pterin binding cleft on the PruR surface in a similar position to Moco binding on SUOX proteins, and co-crystals with a pterin confirm this prediction. Pterins are best known as cytoplasmic cofactors, but PruR is a periplasmic protein. Many bacteria excrete pterins and there is evidence that PruR and pterins interact in the periplasm. In vivo crosslinking shows that PruR interacts with the periplasmic domain of DcpA, with formation of the PruR-DcpA complex reduced in a pruA null mutant. Several conserved residues are required for stable PruR-DcpA complex formation and mutations at these positions disrupt regulatory control. Taken together, our findings suggest that H4MPt promotes PruR-DcpA complex formation and keeps DcpA as a PDE. Environmental control shifts thebalance towards DGC activity, stimulating UPP production and surface attachment.

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Control Number: 2022-A-382-ASM-BIO

Topic 1: Innovative Approaches and New Technologies in Biofilm Research

Publishing Title: BUILDING SYNTHETIC BIOFILM VIA 3D PRINTING

Author Block: **M. W. Fields**, I. Thornton, K. Zimlich, I. Miller, N. Bowman, K. Townsend, H. J. Smith, J. Wilking;
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Abstract Body: It is becoming increasingly clear that attached microbial growth (*i.e.*, biofilm) more closely resembles *in situ* conditions for many microorganisms in different

environments and is likely a universal feature with unique physiology, ecology, and evolution that requires deeper investigation to improve overall understanding of the microbial world. Moreover, microorganisms and the biofilms they form are known to play vital roles in ecosystem functions, including global biogeochemical cycling, industrial processes, and human health that have profound implications for the grand societal challenges of water, food, energy, and health (both human and environmental). The ubiquity and uniqueness of biofilms is commonly attributed to the distinct physiological state of cells existing and growing in the biofilm growth mode as well as to the secreted matrix components that interact with surfaces and the external environment. Microbial biofilms are spatially structured, and this structure often gives rise to emergent properties not observed in planktonic, free-living populations that was likely forged early in microbial evolution. However, biofilms are typically defined and studied as self-assembled bio-systems, and the underlying mechanisms that drive biofilm formation and behavior are difficult to manipulate experimentally. To understand these complex biological systems, methods of controlling and manipulating biofilm structure and composition are needed. Therefore, we developed 3D bioprinting methods with laser lithography as a rapid fabrication technique that could provide control over the structure and composition of living materials with thicknesses between 100 and 300 um. Using our developed technique, we 3D printed hydrogels with either an encapsulated bacterium (*Pseudomonas)* or green alga (*Chlorella*) and developed methods for spatially and temporally resolved growth quantification in the hydrogel matrix based upon red-fluorescent protein in *Pseudomonas* or chlorophyll in the green alga. We anticipate this technology will advance into a powerful method to identify key physical and chemical constraints that likely drive inherent structure-function relationships within microbial biofilms.

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Control Number: 2022-A-386-ASM-BIO

Topic 1: Control, Prevention and Elimination of Biofilms

Publishing Title: NATURAL INHIBITORS OF LISTERIAL EXOPOLYSACCHARIDE BIOFILMS

Author Block: A. M. Elbakush, A. M. Fulano, **M. Gomelsky**;
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Abstract Body: **Background.** Major listeriosis outbreaks have been caused by fresh produce contaminated with the foodborne pathogen *Listeria monocytogenes*. Listeria are expected to form biofilms on the surfaces of fresh produce. The listerial Pss exopolysaccharide (EPS) greatly enhances colonization of plant surfaces and bacterial resistance to desiccation and disinfectants. To enhance produce safety, antibiofilm compounds are needed that are safe for consumption and inexpensive. Here, we describe that maple sap and syrup, that are generally regarded as safe (GRAS), inhibit formation of the EPS-biofilms by *L. monocytogenes*, and identify the likely active ingredient. **Methods.** This study used the wild-type *L. monocytogenes* strain, EGDe, and its derivatives that synthesize high levels of the

Pss EPS (due to elevated intracellular c-di-GMP concentrations) or are impaired in EPS synthesis. Biofilm formation and dispersion were monitored by using wooden coupons and pieces of fresh produce inoculated in the minimal liquid medium. Antibiofilm activity of aqueous wood extracts, maple syrup and chemicals isolated from maple was tested and quantified. **Results.** The *L. monocytogenes* strain synthesizing high levels of EPS quickly (1-2 days) colonizes the surfaces of wooden coupons and fresh produce pieces. Unexpectedly, following extended incubation, preformed biofilms got spontaneously dislodged from the wooden coupons derived from maple (genus *Acer*), hickory (*Carya*), and jasmine (*Trachelospermum*), but not from oak, birch, apple or cherry trees. This observation suggested that a compound inhibiting biofilm formation or activating biofilm dispersal is present in the biomass of certain trees. Consistent with this assumption, aqueous extracts prepared from various components of the maple tree as well as commercially available maple syrup contained antibiofilm activity. To identify the active component, we tested major ingredients of the maple methanol extract reported in the literature. Nortrachelogenin-8'-O-β-D-glucopyranoside has emerged as the likely active component. The preliminary analysis suggests that it inhibits EPS synthesis, rather than activates EPS hydrolysis. **Conclusions.** This study has uncovered a chemical inhibitor of the EPS-biofilm formation by *L. monocytogenes* on plant surfaces. The relative abundance and GRAS status of maple sap and syrup and the ease of preparing aqueous wood extracts make them potential candidates for applications in enhancing fresh produce safety.

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Control Number: 2022-A-392-ASM-BIO

Topic 1: Metabolism, Physiology and Structure of Biofilms

Publishing Title: *Pseudomonas aeruginosa*induces aggregation and biofilm cell death in the co-infecting pathogen*Stenotrophomonas maltophilia*

Author Block: **S. Katharios-Lanwermeyer**, T. Zarrella, M. Godsil, A. Khare;
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Abstract Body: Cystic fibrosis (CF) is a genetic disease where persistent polymicrobial infections in the airway compromise quality of life and result in increased morbidity and mortality. However, the impact of polymicrobial dynamics and their effects on multicellular communities such as aggregates and biofilms are only beginning to be understood. Here, we investigated the interactions between *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*, two organisms often co-isolated from the lungs of CF patients. We observed that *P. aeruginosa* cell-free supernatant caused planktonic *S. maltophilia* to aggregate and was additionally markedly deleterious to *S. maltophilia* biofilm, where it caused cell death. We also found that high molecular weight (HMW) polymer could induce *S. maltophilia* aggregation, and that this was an active process mediated by viable cells. Finally, we found that aggregates caused by *P. aeruginosa* supernatant were protected from killing by the cationic antimicrobial Cetyltrimethylammonium Bromide.To identify the genetic determinants of aggregation in *S. maltophilia*, we exposed two independent

populations of *S. maltophilia* to *P. aeruginosa* supernatant over multiple rounds of selection. In both populations, we identified mutations in *smf-1,* a gene encoding a fimbrial adhesin required to form a biofilm. An allele-replacement strain of *S. maltophilia* carrying an evolved allele of *smf-1* exhibited enhanced motility, and failed aggregation when challenged with either *P. aeruginosa* supernatant or HMW polymer. These findings suggest that Smf-1 may be necessary for *S. maltophilia* to aggregate in response to unknown *P. aeruginosa*-secreted factors or host-produced polymer.Ongoing studies are focused on identifying the underlying *P. aeruginosa* determinants, as well as additional genes and pathways involved in the *S. maltophilia* response. This work thus identifies a novel interspecies interaction between the co-infecting pathogens *P. aeruginosa* and *S. maltophilia* and reveals a potential mechanism that mediates aggregation in *S. maltophilia*. These findings may provide further ecological insights into the long-term polymicrobial dynamics encountered in CF infections.

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