Biofilms 2018: a Diversity of Microbes and Mechanisms

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Abstract

The 8th ASM Conference on Biofilms was held in Washington D.C. on October 7-11, 2018. This very highly subscribed meeting represented a wide breadth of current research in biofilms, and included over 500 attendees, 12 sessions with 64 oral presentations, and four poster sessions with about 400 posters.

Introduction

The 8th American Society for Microbiology (ASM) Conference on Biofilms was held in Washington, D.C. on October 7 to 11, 2018. The 2018 Biofilms meeting provided a forum for researchers from a diversity of workplaces, including academic institutions, industry, and government, to come together and share their understanding of biofilms and functions associated with the biofilm lifestyle, and to discuss ideas and approaches for the study and control of biofilms. Biofilms are communities of microorganisms that are typically embedded in a matrix and often attached to a surface. Biofilms can be beneficial or detrimental and can form in most wetted environments. Because biofilms are particularly problematic in medicine and industry, sharing knowledge about how different organisms form and

disperse from biofilms, and how biofilm microbes are distinct from planktonic ones, is critical for the next generation of creative solutions.

Attendees of the 8th ASM Conference on Biofilms had opportunities to enjoy three keynote addresses and twelve scientific sessions, including (Session 1) Biofilm: From Nature to Models, (Session 2) From Planktonic to Biofilm and Back, (Session 3) Grappling Hooks Involved in Biofilm Development, (Session 4) Regulation of Biofilm Development, (Session 5) Synthesis, Assembly and Function of the Biofilm Matrix, (Session 6) Biofilm Mechanics, (Session 7) Biofilm Antimicrobial Tolerance, (Session 8) Biofilms and Infections, (Session 9) Antibiofilm Strategies, (Session 10) Host-Microbe Biofilms, (Session 11) Biofilm Metabolism, and (Session 12) Social and Asocial Interactions in Biofilms. There were also four poster sessions comprising approximately 400 presentations. In addition to the exciting new research presented in the talks and the poster session, a unique aspect of this meeting was the opportunity for participants to enroll in one of two biofilm technical workshops that preceded the start of this conference, Basic Biofilm Methods, and Flow Cell Methods. These highly subscribed workshops were organized and staffed by Paul Stoodley (The Ohio State University, Columbus, Ohio) and Darla Goeres (Montana State University, Bozeman, Montana). Participants also learned about the new National Biofilms Innovation Centre in the U.K. designed to bring together researchers and industry to accelerate solutions to the problems posed by biofilms. Finally, the conference included a tribute to Dr. Mark Shirtliff, a workshop program leader and a professor at the University of Maryland, Baltimore, Maryland, who passed away in July, 2018. Dr. Shirtliff's contribution to the field was outstanding and he will be greatly missed.

Keynote address: Fitnat Yildiz. The meeting opened with a richly visual depiction of *Vibrio cholerae* biofilm formation by keynote speaker Fitnat Yildiz (University of California, Santa Cruz, CA) (Fig. 1). *V. cholerae* depends on Msh pili to attach to surfaces, and recent work from the Yildiz laboratory demonstrated the ability of this pilus to retract and to promote the ability of cells to spin orbitally, but not to move across surfaces, unlike other retractable pili. She presented striking high-resolution microscopic images of developing *V. cholerae* biofilms, dissecting the progression of biofilm formation in the context

of specific matrix components (1). Extending from this, Dr. Yildiz also described an in-depth study of the matrix protein RbmA, which is involved in cluster formation: this protein undergoes a dynamic structural switch between monomer and dimer form that is required for normal biofilm formation; mutationally locking the protein into a "closed" conformation results in a defect in biofilm formation that is more severe than that seen for an *rbmA* deletion mutant (2). Linking these *in vitro* studies to host interaction, an infant mouse model of infection was used to reveal that biofilm cells were hyper-infectious relative to planktonic cells. Furthermore, *V. cholerae* biofilms could be seen on microvilli surfaces within the small intestine using the recently reported MiPACT (Microbial Identification After Passive Clarity Technique) (3) with HCR-FISH (hybridization chain reaction-fluorescence *in situ* hybridization) (4). These elegant studies demonstrate the importance of biofilms in *V. cholerae* infections and implicate RbmA and other matrix components as potential targets for anti-microbials to treat cholera.

Biofilm: From Nature to Models. The first session, Biofilm: From Nature to Models, highlighted the importance of studying complex, multi-species biofilms, which likely represent the "norm" in many environments. Speakers described systems to investigate the complex interactions that are occurring in these more natural biofilms and how external perturbations affect community composition and function. Interactions within microbial communities such as biofilms are extremely complex, with potential interactions within clonal populations and between distantly related microbes (and other organisms) as well as with the environment.

Stefan Wuertz (Nanyang Technical University, Singapore, Singapore) described the study of how a complex biofilm community, namely activated sludge flocs formed during wastewater treatment, responds to perturbations in the environment, such as the addition of the common rubber industry chemical 3-chloroaniline. He assessed the contribution of stochastic community assembly mechanisms across different disturbance levels. Intermediately disturbed communities showed the highest levels of stochastic intensity in terms of diversity. He proposed the 'intermediate stochasticity hypothesis' to predict bacterial community shifts in diversity and ecosystem function, when given a range of possible disturbance types (5).

To understand the types of interactions that occur in complex ecosystems, Rachel Dutton (University of California, San Diego, California) has developed the cheese rind as a simple model system. Using randomly barcoded transposon mutants of *E. coli*, her lab has determined that amino acid auxotrophs frequently failed to grow as individuals in a protein-rich cheese medium, but were competent to grow within a cheese rind community, indicating that these organisms provide accessible nutrients to each other. Further, they found that close to 50% of genes involved with interactions in the community are part of "higher order" interactions (6). Similar experiments with natural cheese microbiota are also now underway. The cheese rind model thus provides a simple system to probe the dynamics of community assembly and how perturbations alter the stability and function of the community.

The next talk followed a similar theme: how does one strain affect another in the context of perturbation? In this case, the question was, when one strain is resistant and the other sensitive, how does the addition of an anti-microbial drug or a lytic phage influence the population dynamics? Sara Mitri (Université de Lausanne, Switzerland) described two studies in which sensitive and resistant strains of *Pseudomonas aeruginosa* were mixed and exposed to these agents. They found that resistant strains could in some cases protect sensitive cells against these anti-microbials, but that the outcome depended on the selective agent and the population structure of the bacteria ((7), Testa et al., in prep) Understanding these interactions and the dynamics they generate is critical to the design of effective therapeutics.

Concluding the session were two talks selected from the submitted abstracts. To address the question of which forces promote and maintain diversity in biofilms, Katrina Harris (laboratory of Vaughn Cooper, University of Pittsburgh, Pittsburgh, Pennsylvania) described an evolution study in which biofilm-grown *Pseudomonas* became highly diverse within 600 generations, driven at least partially by the high frequency of appearance of mutator strains (8). Carey Nadell (Dartmouth College, Hanover, New Hampshire) described the ability of some *V. cholerae* strains to form filamentous cells that were capable of wrapping around and colonizing chitin fragments, more efficiently than non-filamentous *V. cholerae* (9). This ability was independent of known biofilm factors such as the *vps* polysaccharide

locus, and may confer an advantage in the environment, permitting *V. cholerae* to colonize chitinous surfaces such as crustaceans.

From Planktonic to Biofilm and Back. The second session, From Planktonic to Biofilm and Back, highlighted transitions microorganisms make in forming and leaving from biofilms. Yves Brun (Indiana University, Bloomington, Indiana and Université de Montréal, Quebec, Canada) detailed the role of pili in surface sensing by *Caulobacter crescentus*, which binds to the surface using a holdfast that is rapidly synthesized (within 80 seconds) following contact with the surface. Mutants for Type IV pili (T4P) fail to stimulate holdfast synthesis, suggesting that pili are responsible for surface sensing (10). Specifically, it appears to be pilus retraction that is necessary, as providing physical resistance to pilus retraction independent of a surface similarly stimulated holdfast synthesis (11). Surface binding stimulates production of the second messenger cyclic diguanylate monophosphate (c-di-GMP), which in turn promotes holdfast synthesis, although the mechanism for this remains unknown.

c-di-GMP is also involved in attachment and biofilm formation by the plant pathogen *Agrobacterium tumefaciens*. This organism attaches to surfaces by a single pole using a unipolar adhesin called the unipolar polysaccharide UPP, analogous to the *Caulobacter* holdfast (10). Clay Fuqua (Indiana University, Bloomington, Indiana) described the role of small, self-produced metabolites called pterins in controlling c-di-GMP production by biasing the enzymatic activity of the dual diguanylate cyclase/phosphodiesterase protein DcpA from c-di-GMP synthesis to degradation, limiting UPP production and biofilm formation (12). The genetic components of this system are conserved among several different pathogenic bacteria.

Kelsey Hodge-Hanson (laboratory of Karen Visick, Loyola University Chicago, Maywood, Illinois) described processes and factors involved in attachment and dispersal in *Vibrio fischeri*, a marine microbe that uses those processes to colonize its symbiotic host, the Hawaiian squid *Euprymna scolopes* (13). Specifically, this organism uses a large adhesin for biofilm formation; removal of the adhesin from the surface by a homolog of the *Pseudomonas* cysteine protease LapG appears to permit this organism to disperse.

Nandhini Ashok (laboratory of Carl Bauer, Indiana University, Bloomington, Indiana) described how the photosynthetic bacterial species *Rhodospirillum centenum* uses light to control biofilm formation and dispersal. This organism can associate with roots and is suspected to be in the form of a cyst-containing biofilm there. When this biofilm is exposed to light in the far-red spectrum, cyst germination and biofilm disintegration occurs, resulting in free-living bacteria that can seek a new host.

Finally, this session also included an interesting talk by Clarissa Nobile (University of California, Merced, California), who described a survey of biofilm formation by clinical isolates of the yeast *Candida albicans*, and the discovery that some formed strikingly robust biofilms. The increased ability to form biofilms appears to be due to the presence of a bacterial endosymbiont in the yeast vacuole that somehow promotes biofilms and is in turn protected from antibiotics. The presence of the endosymbiont and its role in promoting biofilms has important implications for *C. albicans* infection.

Grappling Hooks Involved in Biofilm Development. Mark Schembri (University of Queensland, Brisbane, Australia) led off this third session by presenting work on the role of the Ag43 autotransporter protein in biofilm-associated urinary tract infections (UTIs) caused by uropathogenic *E. coli* (UPEC). Structure-function analysis of Ag43 demonstrated a mechanism whereby the head to tail interaction between Ag43 proteins found at the surface of two adjacent cells leads to bacterial aggregation (14). The concept that the UPEC capsule prevents aggregation and biofilm formation by shielding the function of Ag43 was investigated using an elegant approach involving Transposon Directed Insertion Sequencing (TraDIS) and capsule-dependent phage-mediated killing, which identified exciting new regulators for further investigation (15).

Pili are also instrumental in attachment and biofilm formation, as well as in other functions such as motility or DNA uptake. Courtney Ellison (laboratories of Yves Brun and Ankur Dalia, Indiana University, Bloomington, Indiana) showed time-lapse fluorescence microscopy that revealed how bacterial cells bind to and pull in extracellular DNA (eDNA) using T4P (16). Several lines of evidence clearly suggested that the DNA is bound at the pilus tip including, for example, that mutations in positively charged residues of minor pilins found at the tip of the pilus result in diminished DNA binding.

These observations are groundbreaking and provide novel insights into the molecular mechanism underlying T4P function and how this may impact transformation and DNA uptake within biofilm.

Alexandre Persat (École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland) reported on mechanical interactions of single bacteria with their environment, and the concept of mechanosensing using *Pseudomonas*. He described a new microscopic method based on interference (iSCAT) that allows direct observation of native T4P in action without chemical labelling, as was used for *V. cholerae* (16). Combining different mutants impaired in retraction of the pilus (*e.g. pilT* or *pilU* genes encoding the ATPases) allowed the identification of key parameters for surface sensing, including retraction and physical tension on the pilus (17).

Enteroccocci also have adhesive pili, Ebp, that contribute to biofilm formation. Gary Dunny (University of Minnesota, Minneapolis, Minnesota) described the use of a Tn mutant library to confirm the *ebp* locus and identify transcriptional regulators of pili and other critical biofilm genes as well as virulence factors (18; 19). He also described a germ-free mouse model that could be used to monitor evolution of the pathogen in complex microbial communities. This model revealed that conjugative transfer of the *E. faecalis* antibiotic resistance plasmid, which is stimulated by the peptide pheromone cCF10, is enhanced in the gut (20). Overall, it is clear that the tools are now available to disentangle *Enterococcus* mechanisms of establishing biofilms, competing with the gut microbiota, and acquiring antibiotic resistance while maintaining diversity, which should facilitate major advances in the future.

Attachment to abiotic surfaces and biofilm formation by *Acinetobacter baumanii* depends on the Csu pili, which are thin and unusually long. Anton Zavialov (University of Turku, Turku, Finland) reported that the structure of the CsuE adhesin is now solved, revealing a 3 finger-like loop structure with a hydrophobic tip (21). Remarkably, decreasing the hydrophobicity by site-directed mutagenesis did not impact the formation of the pilus, but had dramatic consequences for biofilm formation on plastic. The novel concept was proposed in which the CsuE fingers represent the archaic form for general binding to abiotic surfaces, whereas other pili utilize specific recognition of a cell surface receptor utilizing a classical cavity binding mechanism.

Regulation of Biofilm Development. Oral session four focused on the regulation of biofilm development. It is clear that many regulatory mechanisms can converge during biofilm formation, from those responsive to environmental cues, to metabolic controls, to cell-cell communication. Although there is great variety in the specific mechanisms that orchestrate this complex process, there are emerging general themes as well. This session highlighted some of the diverse control pathways that can come into play during biofilm formation, with the prospects of manipulating these networks to inhibit or promote biofilms.

Kai Papenfort (Ludwig-Maximilians University of Munich, Germany), described the recent work of his group in defining a new quorum sensing signal, 3,5-dimethylpyrazin-2-ol (DPO), and its cognate pathway in *V. cholerae* (22). Derived from threonine, DPO is a potent inhibitor of biofilm formation and is sensed through its interaction with the VqmA transcription factor, which in turn regulates the small RNA VqmR. Biofilm inhibition seems to be mediated at least in part through translation inhibition by the *vqmR* RNA acting on the transcripts for *vpsT* and *aphA*, two important transcription factors (23; 24). The degree to which DPO is integrated with the multiple additional quorum sensing signals in *V. cholerae* is a topic of future research.

Kevin Mlynek (laboratory of Shaun Brinsmade, Georgetown University, Washington, D.C.) described his recent studies revealing that loss of *Staphylococcus aureus* regulator CodY results in hyper-biofilm formation with a matrix composed, in part, of eDNA. Polysaccharide Intercellular Adhesion (PIA) also contributes to biofilm formation in a number of clinical isolates devoid of CodY DNA-binding activity. The dual function toxin/DNA ligase Hlb (25) was interrogated for its role as a DNA scaffold in the *codY* mutant. Current work is focused on screening for factors that promote biofilm formation in the *codY* mutant, including possible factors involved in DNA release or extrusion in this organism.

Oxygen gradients have long been recognized as a common consequence of biofilm formation (26). Work from Maria Hadjifrangiskou (Vanderbilt University Medical Center, Nashville, Tennessee) with biofilms of uropathogenic *E. coli* (UPEC) has revealed a prominent role for the high affinity cytochrome bd in respiration under oxygen limitation in biofilms. Mutants lacking Cytochrome Bd-I

(disrupted for *cydAB*) are altered in biofilm structure and decreased total biomass of UPEC. Imaging of biofilms reveals the positions of the low affinity Cytochrome B0 at the periphery and Cyt Bd-I in the interior. Each Cyt-expressing sub-population manifests distinct proteome profiles within the biofilm, as determined through imaging mass spectrometry (27).

A very different form of regulation was described by Gürol Süel (University of California, San Diego, California) who has reported that electrical signaling similar to action potentials occurs during biofilm growth (28). These electrical pulses can be observed microscopically in biofilms using fluorescent dyes, and provide a mechanism by which physically separated regions of the biofilm can communicate. The Süel lab has proposed a percolation mechanism by which these signals are transmitted cost-effectively through a heterogeneous biofilm where not all cells participate in signal transmission (29). The fraction of signaling cells is thus poised at a tipping point to enable electrical transmission as evident by the observed power law distribution of the size of signaling cell clusters. The physiological roles for these action potentials and the general role of biofilm electrophysiology are currently under study.

One of the most striking applied examples of biofilm manipulation at the conference was presented by Ingmar Riedel-Kruse (Stanford University, Stanford, California). His group has engineered strains of *E. coli* that express heterophilic synthetic adhesins (*i.e.*, small antigen peptides and the corresponding nanobodies) and then display them on the cell surface (30). This allows the programmed self-assembly of multicellular morphologies and patterns. The Riedel-Kruse group also used an optogenetic approach with *E. coli* expressing the homophilic Ag43 adhesion molecule via a light sensitive promotor to drive biofilm formation (31). These programmed cellular deposits form stable patterns on surfaces dictated by the specific illumination (‘Biofilm Lithography’) and may represent rudimentary microbial circuit boards.

Synthesis, Assembly and Function of the Biofilm Matrix. Biofilms are comprised of cells and their contents, but are held together by extracellular materials that may be self-produced, or also provided by their environment. The extracellular biofilm matrix often defines many of the overall properties of the biofilm. Different microorganisms generate different types of biofilm matrix components, but the most

common constituents are polysaccharides, proteins and DNA. These components often interact, as is the case for DNA binding proteins that can coordinate nucleic acid fibers in the biofilm matrix (32), and lectins which bind polysaccharides. This session focused on several different biofilm matrices, produced by a range of microorganisms.

Polysaccharides are among the most common constituents within the biofilm matrix. Several of the presentations in this session reported new findings on polysaccharide matrix components. Iñigo Lasa (Navarrabiomed, Public University of Navarra, Pamplona, Spain) reported work analyzing the poly-N-acetylglucosamine (PNAG; also known as PIA) component produced by several gram-positive and gram-negative bacteria, including species of *Staphylococcus*, *Bacillus*, *Acinetobacter* and *E. coli* (33). Surprisingly, *Salmonella*, despite its close relationship to *E. coli*, does not produce PNAG. A *Salmonella* derivative engineered to express the PNAG biosynthesis (*pga*) genes makes the polysaccharide, but this augments the susceptibility to bile salts and oxygen radicals, reducing bacterial survival inside macrophages and rendering *Salmonella* avirulent (34). This raises the possibility that *Salmonella* may have lost this polysaccharide during its evolution from its common ancestor with *E. coli* as part of its pathoadaptation.

Several different members of the Alphaproteobacteria (APB) produce polysaccharides that stably localize to a single pole of the cell, and often act as adhesives that function in attachment to surfaces and cellular aggregate formation (35). Maureen Onyeziri (laboratory of Clay Fuqua, Indiana University, Bloomington, Indiana) presented findings that the plant pathogen *A. tumefaciens* produces two genetically and chemically separable unipolar polysaccharides (UPPs) that each can contribute to surface adhesion. A genetic approach has revealed independent but overlapping pathways. It is not yet clear how many other APBs produce multiple polar polysaccharides.

Fungi also utilize polysaccharides as matrix components. Natalie Bamford (laboratory of Lynne Howell, The Hospital for Sick Kids, Toronto, Ontario, Canada) presented her work on the galactosaminogalactan (GAG) polysaccharide of the opportunistic fungal pathogen *Aspergillus fumigatus* (36). The adhesiveness of this polysaccharide and the virulence of this pathogen is increased by the

activity of a secreted deacetylase enzyme Agd3, which removes a fraction of the acetyl groups from GAG, thereby increasing the range of surfaces to which *A. fumigatus* will attach (37). Patchy deacetylation is a common mechanism by which the adhesive character of acetylated polysaccharides can be modified (38). Agd3 is thus a promising target to reduce the virulence of *A. fumigatus* and possibly other pathogenic fungi.

Proteinaceous components of biofilm matrices also contribute significantly to their physical and chemical properties. Often, proteins form extended filaments or fibers that can provide tensile strength and elasticity to biofilms. In several cases, these proteins also interact with polysaccharide components of the matrix to further stabilize the biofilm. Matthew Parsek (University of Washington, Seattle, Washington) described an intriguing study of the CdrA matrix protein in *P. aeruginosa*. CdrA has been shown to interact with the PSL polysaccharide of *P. aeruginosa*, effectively tethering it to cells and fostering multicellular aggregates (39). In mutants that do not produce PSL, CdrA continues to drive aggregate formation by CdrA-CdrA interactions between cells. This intercellular coordination is susceptible to protease activity, but can be protected through the interaction of CdrA with PSL (40). These observations suggest that the interaction between CrdA and PSL may combine to form a protease-resistant biofilm matrix.

Fibers that adopt an amyloid conformation can also play structural roles in the biofilm matrix, as with curli produced by *E. coli*. Studies on the CsgA curlin component of the *E. coli* biofilm matrix have provided major insights into the controlled biogenesis of functional amyloids (41). Neha Jain (Ahmedabad University, Gujarat, India) has identified human TTR (transthyretin) protein as a potent CsgA amyloid and amyloid-dependent biofilm inhibitor (42). TTR is a structural homolog of CsgC (amyloid inhibitor from bacteria) enriched with β-strands. TTR derivatives and its homologs may represent broad spectrum amyloid inhibitors, with potential applications in controlling aberrant formation of the fibers and destabilizing biofilms.

Biofilm mechanics. The sixth session started with George O'Toole (Geisel School of Medicine at Dartmouth, Hanover, New Hampshire) asking a fundamental question, "Do bacteria know they are on a

surface?". For *P. aeruginosa*, the answer is yes. These cells sense the surface using a complex system that includes a chemosensor-like protein complex that upregulates cAMP production, which in turn induces production of T4P. The cell-surface-localized protein PilY1 is now able to interact with T4P alignment complex subunits, ultimately facilitating the interaction of one subunit, PilO, with the diguanylate cyclase SadC, resulting in a 20-fold increase in c-di-GMP production (Fig. 2). PilY1 thus can be considered to be a key part of the surface-sensing system (43; 44). Overall, these studies make it clear that there are hierarchical pathways involved in surface sensing, with cAMP at the top of the cascade and followed by c-di-GMP signaling, coordinating subsequent functions associated with attachment and biofilm formation, including T4P production and function.

Expanding on the concept of how mechanosensing is linked with bacterial physiology, Albert Siryaporn (University of California, Irvine, California) asked, "What determines biofilm organization and do universal principles guide biofilm shape?". Using a microfluidic device and *P. aeruginosa* as model, cyclical events of attachment, detachment and reattachment were observed, interspersed with periods of movement on the surface or within flow. This dynamic switching was proposed to maximize the spreading of the bacteria (45). Fluorescence Lifetime Imaging Microscopy (FLIM) and spatial and temporal resolution with NADH as a metabolic marker revealed that surface attachment increases free NADH. The observations generated from these very advanced methods were integrated into mathematical models, allowing prediction of bacterial behavior during processes such as colonization in the vasculature and the spread of an infection (46).

The ability of bacteria to sense mechanical force as a cue for biological activity is a concept that is gaining recognition. Vernita Gordon (University of Texas, Austin, Texas) reported studies examining the mechanosensing of shear force by bacteria as a cue to begin forming a biofilm. In *P. aeruginosa* PAO1, the factors that determine the mechanical and geometrical coupling to the surface are the extracellular polysaccharides Pel and Psl, with the latter responsible for stronger, more permanent adhesion. Gordon showed that loss of Pel impacts both the mechanics (the force needed to remove *P. aeruginosa* from the surface) and the geometry of the attachment, as a *pel* mutant is attached only by one

end rather than by the entire length as seen for the wild type (47). Loss of Pel also impacted the dynamics of c-di-GMP signaling: while the parental strain and the *pel* mutant had equivalent levels of c-di-GMP just after attachment, over time after attachment, the *pel* mutant exhibited decreased levels of c-di-GMP compared with its wild-type parent (48). This study raises the prospect that manipulation of the nature of the surface to reduce sensed shear forces may help the development of biofilm-resistant material.

Berenike Maier (University of Cologne, Köln, Germany) further linked molecular forces to the shape and dynamics of biofilms using the spherical bacterium *Neisseria gonorrhoeae*. Within *N. gonorrhoeae* colonies, cells show liquid-like order, dependent on T4P and their retraction capability (49). Analysis using dual laser trap methods revealed that both motor activity and pilin post-translational modification affect the fluidity of gonococcal colonies, with a small increase in pilus-pilus interaction strongly enhancing viscosity (49; 50). This represents another striking observation expanding the role of T4P retraction beyond motility and highlighting the idea that they function in the organizational dynamics of a microcolony, with implications for the resistance to various stresses.

To close the session, Tamara Rossy (laboratory of Alexandre Persat, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland) further elaborated on complex heterogenous and spatial organization of biofilms grown in flow, using *C. crescentus* as a model. Flow rate drastically influences surface coverage, with low flow resulting in uniformly mixed and dense colonization, and high flow resulting in decreased surface coverage, a slow rate of colonization, and patchy, clonally segregated patterns (51). Interference with swimming motility revealed that cell movement is also involved in surface coverage. Thus, what shapes a biofilm is not exclusively molecular determinants such as exopolysaccharide, but also the environment and the mechanics associated with that environment.

Keynote address: Pradeep Singh. The second keynote speaker, Pradeep Singh (University of Washington, Seattle, Washington) addressed the question of how bacterial aggregates form at chronic infection sites. He contrasted a model that postulates active mechanisms of biofilm formation driven by bacterial functions, with a model that suggests host-driven processes cause aggregation. In support of the second model, Singh highlighted research in which genes/processes required for biofilm formation in the

lab were not necessary in chronic infections and/or those processes were lost during the course of chronic infection. He then went on to describe two mechanisms by which the host environment can produce bacterial aggregation without contributions by bacterial processes, namely entrapment of replicating bacteria by viscous gels such as mucus (52), and aggregate-promoting forces produced by polymers abundant at infection sites (53). The latter mechanism can occur via electrostatic bridging of negatively charged bacteria with positively charged polymers, or via depletion aggregation, a reaction that is favored when like-charged molecules and bacteria are present in crowded environments. Laboratory-induced depletion aggregation of bacteria can produce aggregates with properties similar to biofilms, including antibiotic tolerance. This thought-provoking talk reminded us that the host environment is complex, and that bacterial aggregates in the host may derive from bacteria-driven and/or host-mediated events. While the resulting aggregates have similar properties, the development of successful therapeutics will need to take into account which of these two processes is dominant.

Biofilm Antimicrobial Tolerance. The 7th session focused on the problem of antimicrobial tolerance of biofilms. Christophe Beloin (laboratory of Jean-Marc Ghigo, Institut Pasteur, Paris, France) led off the session by asking whether persistence contributes to evolution of antibiotic resistance in biofilms. He described experiments in which biofilms displayed a rapid and high frequency emergence of antibiotic resistant mutants, while planktonic cells evolved resistance more slowly and at a low frequency (Usui et al. in prep). This rapid evolution to resistance of biofilm cells may be due to both the increased tolerance of cells in biofilms and the increased mutation rate.

Susanne Häußler (Helmholtz Center for Infection Research, Braunschweig, Germany) described the collection and sequencing of over 450 clinical isolates of *P. aeruginosa* (54). This group of strains exhibited diverse biofilm phenotypes with similar transcriptional profiles under rich medium conditions but more divergent transcriptional profiles when grown in infection-relevant biofilm growth conditions. The characterization of these strains highlights the genetic diversity of bacteria and their ability to adapt in different ways to a changing environment.

Liang Yang (Nanyang Technological University, Singapore, Singapore) described chemical biology approaches for developing antimicrobials effective against biofilm bacteria. This group has already developed a number of antimicrobials, including quorum sensing inhibitors and biofilm dispersal agents. In addition, Liang described recent work that uses cell permeabilizing compounds to promote uptake of antibiotics by Gram-negative pathogens, resulting in increased effectiveness of treatment.

Sophie Darch (laboratory of Marvin Whiteley, Georgia Institute of Technology, Atlanta, Georgia) described a powerful study system for examining the spatial requirements of *P. aeruginosa* for intra- and inter-aggregate communication and response to the acylhomoserine lactone quorum signal 3-oxo-dodecanoyl-HSL. Using a synthetic cystic fibrosis (CF) sputum media (SCFM2), which promotes natural *P. aeruginosa* aggregate formation, she combined this with a micro-3D printing technology to design aggregates of a specific shape and volume (55). This unique model revealed that quorum sensing appears to be primarily an intra-aggregate phenomenon in SCFM2, with aggregates having different sensitivities to signal. Understanding how auto-aggregation impacts signaling dynamics during infection will be an important direction of study as this ability of cells to form aggregates independent of classical biofilm factors becomes better appreciated.

In studying the impact of antibiotics on the spatial architecture of biofilms formed by *E. faecalis*, Kelsey Hallinen (laboratory of Kevin Wood, University of Michigan, Ann Arbor, Michigan) found that exposure to low doses of cell wall synthesis inhibitors, but not other antibiotics, induced cell lysis and eDNA release, increasing biofilm formation, and promoting bacterial cooperation (56). With high drug exposure, sensitive cells were more likely to have resistant neighbors. Remaining questions to address include determining how these dynamics change with different resistance mechanisms and different amounts of antibiotic.

Biofilms and Infections. Session 8 focused on biofilms and infections. The first speaker, Kimberly Kline (Nanyang Technological University, Singapore, Singapore), described a role for extracellular electron transfer (EET) in *E. faecalis* biofilm formation (57). Biofilms are enhanced in the presence of iron, which is bound by Ebp pili and serves as an electron acceptor that is necessary for

current production. Mutants defective for lactate dehydrogenase (required for redox balance and transport of electrons across the membrane), quinones, or a specialized NADH dehydrogenase that is part of a flavin-mediated EET system that is conserved in other Gram-positive bacteria (58), are attenuated for electric current production. Thus, the ability of bacteria to transfer electrons in biofilms is a common attribute in the environment. Indeed, EET and iron appear to promote *E. faecalis* growth in the gastrointestinal tract. Understanding the mechanisms involved in promoting EET and its consequences on biofilms in nature are important directions for the field.

Kendra Rumbaugh (Texas Tech University Health Sciences Center, Lubbock Texas) described the different outcomes that can occur during infection by *P. aeruginosa* in a mouse model, depending on the type of infection and the host environment, as well as the genetic make-up of the strain. For example, in a burn model, inoculation with as little as 100 CFU results in an acute, systemic infection with 100% mortality within 48 h, while a surgical excision model results in a biofilm-associated chronic infection that is highly recalcitrant to treatment. Treatment of the latter infection with glycoside hydrolases (GH), which target glycosidic linkages in polysaccharides of bacterial biofilms, caused *P. aeruginosa* to disperse, resulting in death of the mice (59). When used in combination with antibiotics, these enzymes reduced virulence. Ultimately, the therapeutic strategy will need to depend on the type of infection, and is complicated by polymicrobial infections, but these combination therapeutics show promise.

The ability of *S. aureus* to form biofilms is associated with its ability to cause chronic infections, and is particularly problematic in the context of orthopedic devices. Tammy Kielian described a mouse model for *S. aureus* orthopedic implant biofilm infection (60) that she used to probe why *S. aureus* infections result in an anti-inflammatory response in the host. Understanding this mechanism will contribute to the development of therapeutics to boost host immunity in the context of *S. aureus* infections.

Janette Harro (laboratory of the late Mark Shirtliff, University of Maryland, Baltimore, Maryland) discussed strategies for identifying appropriate antigens for vaccine development to prevent *S. aureus* infections, including peritoneal abscesses and osteomyelitis. A vaccine approach that included

four biofilm antigens was successful at preventing symptoms, but did not eliminate *S. aureus*. However, inclusion of an additional antigen from planktonic cells was successful: 65% of animals cleared the infection. Thus, this strategy holds promise for use of this type of vaccine, which prominently includes the targeting of biofilm-specific antigens, for application in human disease. Also working on *S. aureus,* Brian Pettygrove (laboratory of Phil Stewart, Montana State University, Bozeman, Montana) discussed a 2D model for probing the role of neutrophils in clearing nascent *S. aureus* biofilms. In this model, both a sufficient number of neutrophils and their rapid recruitment to the surface were necessary to control biofilm formation. This work has implications for the design of new strategies for preventing biomaterial infection (61).

Antibiofilm Strategies. Session 9 was opened by Thomas Webster (Northeastern University, Boston, Massachusetts). He highlighted that an acute problem affecting populations worldwide is the bacterial contamination of implants and medical devices combined with the rise of antimicrobial resistance and the drought of the drug pipeline. Projections suggest that by 2050 the number of deaths associated with infectious diseases will reach 10 million/year, exceeding mortality from all cancer combined (8.2 million). He then described an alternative to antibiotics, the use of nanotechnology (mimicking nature) to change the energy of surfaces to prevent or reduce bacterial colonization (62). For example, changing a nanostructured silicon nitride from nano-rough to smooth was shown to dramatically impact bacterial coverage. Even though the effect of the nanotexture is inherently short term, since once it is colonized, the surface changes and energy would be different, these approaches are providing new and promising ways to fight biofilm formation and infection.

Ehud Banin (Bar-Ilan University, Ramat-Gan, Israel) also spoke about the concept of changing surface properties to fight biofilm formation and repel bacteria, but in this case using chemically active surfaces. Surfaces were designed such that they can release a halogen biocide, but could be recharged once the biocide was exhausted. The technology is based on *N*-halamine nanoparticles, which can covalently bind to a halogen and can be recharged by reexposure to a halogen. He also presented findings on the antimicrobial activity of nanoparticles which is based on production of ROS and their ability to

target bacteria (63). Finally, the ability to utilize these nanoparticles to functionalize irrigation drippers and reduce biofouling for several months was discussed.

Huan Gu (Laboratory of Dacheng Ren, Syracuse University, Syracuse, New York) presented the next generation of smart antifouling surfaces inspired from natural, actively self-cleaning surfaces such as the shark skin or the lotus leaf (64; 65). The use of "shape memory polymers", whose configuration could be modulated (for example, by temperature or other physical/chemical factors), could dislodge bacteria from biofilms (Fig. 3) and/or make them more susceptible to conventional antibiotics. The development of surface topographies with controllable or programmed motions is a novel and promising prospect for both biofilm inhibition and dispersal.

Sarah Tursi (laboratory of Çagla Tükel, Temple University, Philadelphia, Pennsylvania) focused on a strategy to eradicate *Salmonella Typhimurium* biofilms dependent on curli fibers. She described the use of an anti-host amyloid monoclonal antibody (mAb) that could bind curli and alter biofilm rigidity such that that beads and macrophages could penetrate the layers within the biofilm. The use of this mAb may be an effective strategy to treat *Salmonella* biofilm infections.

Given the established tolerance of biofilms towards antimicrobial treatments, one important area is the development of rapid and accurate evaluation of drug concentrations necessary to effectively eradicate biofilms. Jodi Connell (3M, Saint Paul, Minnesota) presented strategies from 3M to develop fundamental research that will help take anti-biofilm therapies from the laboratory to the market. She described the development of a rapid and more quantitative method using the MBEC^{TM} assay (66) and a 10 kDa Alexafluor-labelled dextran that incorporates into the biofilm matrix and provide a fast quantification method, reducing processing time from ~5 days to only 30 hours.

Host-microbe biofilms. Session 10 led off with a talk from Cynthia Sears (John Hopkins University, Baltimore, Maryland) on the involvement of biofilms in human colon cancer. About 50% of human sporadic colon cancer, particularly in the right colon, display biofilms and a marked infiltration of bacteria whereas ~15 percent of normal colonoscopy biopsies reveal polymicrobial biofilms (typically composed of *Bacteroidetes* and *Lachnospiraceae* with a subset of tumors, but not biopsies, also showing

Fusobacterium). Furthermore, samples from biofilm-positive human colon cancer could induce assembly of biofilms in distal germ-free mouse colon within a week following inoculation (Sears et al., submitted). One of the common hereditary human colon cancers (familial adenomatous polyposis; $APC^{+/-}$) also display biofilms but these are dominated by two bacteria, (pks^{+}) *E. coli* and enterotoxigenic *B. fragilis*; a mouse model to mimic this exhibited accelerated tumor formation and mortality when co-colonized with the cancer-associated bacteria, potentially by fostering increased adherence of the problematic bacteria to the mucosa (67). Current directions include continuing to test the hypothesis that human colon biofilm formation is associated with colon polyp formation.

With the goal of identifying new and better therapeutic strategies to treat pathogens in the lung, Jennifer Bomberger (University of Pittsburgh, Pennsylvania, USA) described the use of a primary human epithelial cell model as a proxy for CF lung conditions to determine that disparate respiratory viruses enhance *P. aeruginosa* biofilm growth and that anti-viral interferon signaling stimulates production of the biofilm (68). Virus infected cells secrete more iron, which in turn promoted biofilm growth. This turned out to be true *in vivo* as well: iron was increased in bronchoalveolar lavage fluid during RSV infection in a mouse model, and during viral infection, human patients were found to have higher levels of iron in their sinuses. Together, these data highlight the importance of co-resident microorganisms, including viruses in influencing the host environment, which in turn impacts biofilm formation and bacterial pathogenicity.

Lauren Bakaletz (The Ohio State University, Columbus, Ohio) focused on therapeutic strategies that target eDNA, which is abundant in the biofilm matrix where it plays a protective role. The bacterial DNA-binding protein DNABII family is readily observed at key structural junctions in the eDNA scaffold, where they may function to stabilize the matrix (32). Indeed, antibodies against DNABII significantly disrupt biofilms *in vitro* and render the pathogens more susceptible to antibiotics (69). DNABII is completely conserved amongst eubacteria, and thus this approach may provide a therapeutic for multiple and diverse human pathogens. Using the chinchilla middle ear model of infectious biofilms, Fab fragments against the DNA-binding Tip region of DNABII cleared biofilms formed by non-typeable

Haemophilus influenzae (nTHI) within 8 days. Because the natural adaptive response to DNABII bound to eDNA is directed against the non-protective tail regions of the DNABII protein, redirection of the response towards protective tip epitopes promoted biofilm clearance in experimental models and thus may similarly do so clinically.

Stuti Desai (laboratory of Linda Kenney, National University of Singapore, Singapore) used the host nematode *Caenorhabditis elegans* to evaluate the role of regulators in host-associated biofilm formation by *Salmonella* (Fig. 4). They discovered that *Salmonella* rapidly formed static biofilm clusters that were dependent on the function of the response regulator SsrB. Whereas the phosphorylated form of SsrB is associated with virulence in other models, it is the unphosphorylated form that promotes biofilms (70), indicating that SsrB has different activities in promoting *Salmonella* interaction with distinct hosts (71).

Alex Valm (University at Albany, State University of New York, Albany, New York) described the development of technology to permit imaging and spatial analysis of multiple species within oral biofilms using CLASI-FISH (Combinatorial Labeling and Spectral Imaging - Fluorescence In Situ Hybridization) (72), which permitted the identification of each of 15 cell types within a mixture of cells (73). When expanded with computer programming to impose a binary constraint (74), it was possible to resolve 120 *E. coli* strains in culture. Furthermore an *in vitro* oral biofilm model was developed with over 30 genera at 1% abundance or higher. These advances in imaging will permit better probing of biofilm structure and thus a deeper understanding of obligate and facultative taxa structure *in vivo*.

Biofilm Metabolism. It is well established that formation of biofilms can have a dramatic impact on the metabolism of cells that reside within them. Profound changes in nutrient and effluent gradients, access to oxygen or other terminal electron acceptors, and interaction between different species are some of the factors which can influence overall bacterial metabolism. This session focused on the metabolic activities of biofilms at several different scales. Trent Northen (Lawrence Berkeley National Laboratory, Berkeley, California) provided examples of the specialized metabolic activities that can be observed

across a range of scales within biofilms. One particularly fascinating example are biological soil crusts (biocrusts) that form in arid environments and are some of the largest natural biofilms. These crusts are held together through specific cyanobacteria of the genus *Microcoleus*, which produce copious polysaccharides that support the diverse microorganisms within the crusts (75). Biocrusts are largely inactive under dry conditions, but can rapidly activate their metabolism with the addition of water. Exometabolite profiling in biocrusts suggests that there are extensive metabolic interactions between biocrust constituents during these large-scale activation events (76).

Decreasing the biofilm scale from huge biocrusts to colony biofilms enables more fine scale analyses of metabolic phenomena. Lars Dietrich (Columbia University, New York, New York) presented recent findings on redox homeostasis within *P. aeruginosa* colony biofilms. Phenazines released from cells can function as soluble electron carriers, which Dietrich describes as a "snorkel" for cells at the base of the biofilm that are oxygen limited. Mutants for phenazine synthesis overproduce the PEL polysaccharide and form wrinkled colonies, perhaps to minimize anoxic zones. Use of micro electrodes that enable fine-scale monitoring of biofilm redox potential, coupled with isotopic labeling and Raman spectroscopy, is providing evidence for direct links between phenazine reduction, electron transport, and metabolic activity (77; 78). The altered metabolic state within phenazine-producing biofilms induces changes to a wide range of intracellular pathways, including protein synthesis.

Michael Franklin (Montana State University, Bozeman, Montana) presented findings from his group on the role of hibernation promoting factors (Hpf). Hpf is widely conserved among bacteria and associates with ribosomes, maintaining them in an inactive, protected state (79). In *P. aeruginosa*, in biofilms or under starvation, Hpf enables effective resuscitation of cells with low metabolic activity. Starved *hpf* mutants are greatly diminished in their ability to recover from these conditions and lose their ribosomes. Given the conservation of Hpf, it seems likely that many bacteria employ a ribosome hibernation mechanism in subpopulations within biofilms.

Metabolic changes also occur during migration across surfaces. Fata Moradali (laboratory of Mary Ellen Davey, University of Florida, Gainesville, Florida) described studies of the Gram-negative

oral pathogen *Porphyromonas gingivalis*. A type IX secretion system is involved in pathogenesis, and also is required for modification of the environment, enabling *P. gingivalis* to surface translocate between surfaces in a sandwich model. Metabolomic studies revealed genetic and metabolic adaptation of migrating populations through multiple pathways including folate biosynthesis and electron transport systems (80).

Interactions between different bacterial taxa can radically change the structure and physiology of biofilms. Elizabeth Shank and colleagues (University of North Carolina at Chapel Hill, North Carolina) analyzed a dual-species colony biofilm of the soil microbes *Pantoea agglomerans* and *Bacillus subtilis* (81). The properties of this biofilm are distinct from either species on its own, and result in the formation of dramatic multicellular towers, the height of which depends on the initial proportion of each species. The bacteria also spatially partition themselves, with *B. subtilis* forming a top layer while the center of the tower is composed predominantly of *P. agglomerans*. *P. agglomerans* mutants that abolish this structure have defects in production of an extracellular polysaccharide. This structure leads to extensive metabolic interactions and enhanced antibiotic resistance of the *P. agglomerans* constituents.

Social and Asocial Interactions in Biofilms. Marvin Whiteley (Georgia Institute of Technology, Atlanta, Georgia) led off session 12 with the question of whether it is possible to use gene expression patterns to distinguish *in vitro*-grown *P. aeruginosa* from samples derived from the same organism collected from different contexts in a human host. The corollaries to this question were, can you "train" a computer through machine learning approaches to distinguish these different samples and, can the information of what gene expression patterns represent human association permit us to better understand the meaning of data derived from an animal model? He went on to describe a set of 19 genes that could be used to distinguish human and *in vitro* transcriptomes, and their use in the analysis of different mouse models of infection. Whereas transcriptomes from burn and pneumoniae models appeared to be more representative of *in vitro*-grown bacteria, the chronic infection model patterns were more consistent with human infection (82). These analyses provide researchers with a framework for choosing model systems that best represent the human condition—not just for *P. aeruginosa*, but for any organism. Future work

will determine if, for *P. aeruginosa*, the identity of the 19 genes provides significant insights into the human infection.

Microbe-microbe interactions were the topic of the next talk by Karine Gibbs (Harvard University, Cambridge, Massachusetts). *Proteus mirabilis*, a pathogen that causes persistent and recurrent infections, encodes receptors that permit it to distinguish "self" from "non-self" (83). Receptor mutants that cannot distinguish self from non-self exhibit, among other things, decreased flagellar transcription and increased stress response, including increased tolerance to antibiotics (84). The dynamics of the interactions between self and non-self are readily apparent in the oscillatory (bulls-eye) patterns of swarmer cell migration, where mixtures of strains eventually result in self-only cells on the outer edges of the swarm. These patterns and local dynamics change as the agar concentration increases, indicating that these cells integrate cues both from their cellular neighbors as well as environmental conditions (85).

Steve Diggle (Georgia Institute of Technology, Atlanta, Georgia) described antagonistic interactions between strains of *P. aeruginosa* through production of R-pyocin bacteriocins also known as tailocins. When directly applied to biofilms, R-pyocins have sufficient antimicrobial activity to cause significant killing of cells within about four hours. In addition to potential applications, this lethality may account for the observation that certain strains and lineages of *P. aeruginosa* dominate during CF lung infections (86).

Using *V. cholerae* as a model, recent studies from Knut Drescher (Max Planck Institute for Terrestrial Microbiology, Marburg, Germany) have used high-resolution optical dissection and image analysis to provide a complete accounting of individual cellular positioning within living biofilms, and their mechanical interactions (87). This imaging approach was applied to evaluate mechanisms of phage-biofilm interactions, and it was determined that phage could eliminate small biofilms, but larger and older biofilms became tolerant to phage. This phenomenon depended on curli, as mutation of the curli genes rendered biofilms susceptible to phage and exogenously added curli bound to the phage, preventing them from adhering to and lysing the bacteria (88).

In *Bacillus*, pellicle formation requires an EPS component and a protein (TasA) component (89), but these two components need not be produced by the same cell. Ákos T. Kovács (Technical University of Denmark, Kgs Lyngby, Denmark) described experiments in which mixtures of mutants lacking one or the other component could successfully form biofilms and improve productivity if the strains were present at the right ratios (30% protein-producing, 70% EPS producing) (89). In subsequent evolution experiments, *tasA* mutants began to dominate in the mixed biofilm, resulting in altered biofilm structure (90). The evolved *eps* mutant acquired mutations in *tasA*, resulting in introduction of cysteine residues that improved pellicle strength, while evolved *tasA* mutants acquired mutations that increased *eps* production. These studies nicely highlight the diversity of mechanisms that can be evolved to promote biofilm formation, indicating the relative importance of this lifestyle to bacteria in nature.

Keynote address: Paul Rainey. The meeting concluded with the third keynote speaker, Paul Rainey (Max Planck Institute for Evolutionary Biology, Plon, Germany and École Supérieure de Physique et Chimie Industrielle de la Ville (ESPCI), Paris, France), who discussed concepts of multi-cellularity with respect to bacteria within a biofilm and how multi-cellularity could evolve. He indicated that, from a "parts" perspective, biofilms are not analogous to true multi-cellular organisms: while different cell types exist, they can be homogenized (e.g., in a blender), and the constituent parts reassembled to produce a similar multicellular structure. From an evolutionary perspective, what matters is that collectives of cells participate as discrete groups in the process of evolution by natural selection. This requires that the collective state manifests heritable variance in fitness. In its absence, it is difficult to see how traits adaptive at the level of a multicellular organism, such as development, can ever evolve. The central issue in deciding whether or not biofilms are truly equivalent to multicellular organisms, is whether, and under what circumstances, biofilms ever give rise to biofilm offspring with offspring biofilms resembling parental types. In thinking about how biofilms might ever become truly multicellular, Rainey pointed out the need to explain the origins of Darwinian properties including the origins of collective level reproduction. This, he argued, presents a dilemma that can be solved by recognizing that

Darwinian properties can be scaffolded by the environment. Ongoing studies involving theory (91; 92) and experiment (93), are evaluating whether this can happen, and over what time scales (94).

Summary. The 8th ASM Conference on Biofilms was, overall, a tremendous success with a great deal of new and exciting findings and ideas exchanged between members of the community. The oral sessions described above were supported and expanded in the presentation of >400 posters, by scientists at all stages. It is clear that the advent of new technologies continues to propel novel observations and perspectives. Specifically, high resolution imaging such as the label-free iSCAT approach for visualizing extracellular pili in real time (described by A. Persat), and the ability to track diverse bacterial lineages in growing biofilms (described by both K. Drescher and A. Valm), provide new insights for biofilm formation and composition. Genomic, proteomic, metabolic and other systems-level approaches also continue to accelerate the pace of biofilm research. Amalgamated approaches that combine the power of microbial genetics with high-throughput sequencing (*e.g.*, TnSeq and TraDIS) are identifying new networks of biofilm-relevant functions that may provide targets for new therapies. Fine-scale material science both in using biofilms to construct programmed structures such as the optogenetic deposition approaches, and fabrication of dynamic surfaces to inhibit biofilm formation are examples of applications which are beginning to utilize the knowledge of biofilm formation and function, to develop real-world solutions for biotechnology. As judged by the record attendance and enthusiastic participation of the conference attendees, it is clear that biofilm research continues to be a growing and dynamic area within the microbial sciences.

The conference ended with the announcement that ASM will support a next iteration of the biofilm meeting, to be held in 2021, with Karen Visick and Clay Fuqua as co-chairs.

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References:

1. Berk, V., J. C. Fong, G. T. Dempsey, O. N. Develioglu, X. Zhuang, J. Liphardt, F. H. Yildiz and S. Chu. 2012. Molecular architecture and assembly principles of *Vibrio cholerae* biofilms. Science 337:236-239.

2. Fong, J. C., A. Rogers, A. K. Michael, N. C. Parsley, W. C. Cornell, Y. C. Lin, P. K. Singh, R. Hartmann, K. Drescher, E. Vinogradov, L. E. Dietrich, C. L. Partch and F. H. Yildiz. 2017. Structural dynamics of RbmA governs plasticity of *Vibrio cholerae* biofilms. Elife 6:e26163.

3. DePas, W. H., R. Starwalt-Lee, L. Van Sambeek, S. Ravindra Kumar, V. Gradinaru and D. K. Newman. 2016. Exposing the three-dimensional biogeography and metabolic states of pathogens in cystic fibrosis sputum via hydrogel embedding, clearing, and rRNA labeling. MBio 7:e00796-00716.

4. Choi, H. M., J. Y. Chang, A. Trinh le, J. E. Padilla, S. E. Fraser and N. A. Pierce. 2010. Programmable *in situ* amplification for multiplexed imaging of mRNA expression. Nat Biotechnol 28:1208-1212.

5. Santillan, E., H. Seshan and S. Wuertz. 2019. Frequency of disturbance alters diversity, function, and underlying assembly mechanisms of complex bacterial communities. npj Biofilms and Microbiomes, in press.

6. Morin, M., E. C. Pierce and R. J. Dutton. 2018. Changes in the genetic requirements for microbial interactions with increasing community complexity. Elife 7:e37072.

7. Frost, I., W. P. J. Smith, S. Mitri, A. S. Millan, Y. Davit, J. M. Osborne, J. M. Pitt-Francis, R. C. MacLean and K. R. Foster. 2018. Cooperation, competition and antibiotic resistance in bacterial colonies. ISME J 12:1582-1593.

8. Flynn, K. M., G. Dowell, T. M. Johnson, B. J. Koestler, C. M. Waters and V. S. Cooper. 2016. Evolution of ecological diversity in biofilms of *Pseudomonas aeruginosa* by altered cyclic diguanylate signaling. J Bacteriol 198:2608-2618.

9. Wucher, B. R., T. M. Bartlett, A. Persat and C. D. Nadell. 2018. Filamentation of *Vibrio cholerae* is an adaptation for surface attachment and biofilm architecture. bioRxiv: 470815.

10. Li, G., P. J. Brown, J. X. Tang, J. Xu, E. M. Quardokus, C. Fuqua and Y. V. Brun. 2012. Surface contact stimulates the just-in-time deployment of bacterial adhesins. Mol Microbiol 83:41-51.

11. Ellison, C. K., J. Kan, R. S. Dillard, D. T. Kysela, A. Ducret, C. Berne, C. M. Hampton, Z. Ke, E. R. Wright, N. Biais, A. B. Dalia and Y. V. Brun. 2017. Obstruction of pilus retraction stimulates bacterial surface sensing. Science 358:535-538.

12. Feirer, N., J. Xu, K. D. Allen, B. J. Koestler, E. L. Bruger, C. M. Waters, R. H. White and C. Fuqua. 2015. A pterin-dependent signaling pathway regulates a dual-function diguanylate cyclase-phosphodiesterase controlling surface attachment in *Agrobacterium tumefaciens*. MBio 6:e00156.

13. Norsworthy, A. N. and K. L. Visick. 2013. Gimme shelter: how *Vibrio fischeri* successfully navigates an animal's multiple environments. Front Microbiol 4:356.

14. Heras, B., M. Totsika, K. M. Peters, J. J. Paxman, C. L. Gee, R. J. Jarrott, M. A. Perugini, A. E. Whitten and M. A. Schembri. 2014. The antigen 43 structure reveals a molecular Velcro-like mechanism of autotransporter-mediated bacterial clumping. Proc Natl Acad Sci U S A 111:457-462.

15. Goh, K. G. K., M. D. Phan, B. M. Forde, T. M. Chong, W. F. Yin, K. G. Chan, G. C. Ulett, M. J. Sweet, S. A. Beatson and M. A. Schembri. 2017. Genome-wide discovery of genes required for capsule production by uropathogenic *Escherichia coli*. MBio 8:e01558-01517.

16. Ellison, C. K., T. N. Dalia, A. Vidal Ceballos, J. C. Wang, N. Biais, Y. V. Brun and A. B. Dalia. 2018. Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural transformation in *Vibrio cholerae*. Nat Microbiol 3:773-780.

17. Tala, L., A. Fineberg, P. Kukura and A. Persat. 2018. Label-free visualization of type IV pili dynamics by interferometric scattering microscopy. bioRxiv: 298562.

18. Dale, J. L., K. B. Beckman, J. L. E. Willett, J. L. Nilson, N. P. Palani, J. A. Baller, A. Hauge, D. M. Gohl, R. Erickson, D. A. Manias, M. J. Sadowsky and G. M. Dunny. 2018. Comprehensive functional analysis of the *Enterococcus faecalis* core genome using an ordered, sequence-defined collection of insertional mutations in strain OG1RF. mSystems 3.

19. Manias, D. A. and G. M. Dunny. 2018. Expression of adhesive pili and the collagen-binding adhesin Ace Is activated by ArgR family transcription factors in *Enterococcus faecalis*. J Bacteriol 200:e00269-00218.

20. Hirt, H., K. E. Greenwood-Quaintance, M. J. Karau, L. M. Till, P. C. Kashyap, R. Patel and G. M. Dunny. 2018. *Enterococcus faecalis* sex pheromone cCF10 enhances conjugative plasmid transfer *in vivo*. MBio 9:e00037-00018.

21. Pakharukova, N., M. Tuittila, S. Paavilainen, H. Malmi, O. Parilova, S. Teneberg, S. D. Knight and A. V. Zavialov. 2018. Structural basis for *Acinetobacter baumannii* biofilm formation. Proc Natl Acad Sci U S A 115:5558-5563.

22. Papenfort, K., J. E. Silpe, K. R. Schramma, J. P. Cong, M. R. Seyedsayamdost and B. L. Bassler. 2017. A *Vibrio cholerae* autoinducer-receptor pair that controls biofilm formation. Nat Chem Biol 13:551-557.

23. Papenfort, K., K. U. Forstner, J. P. Cong, C. M. Sharma and B. L. Bassler. 2015. Differential RNA-seq of *Vibrio cholerae* identifies the VqmR small RNA as a regulator of biofilm formation. Proc Natl Acad Sci U S A 112:E766-775.

24. Herzog, R., N. Peschek, K. S. Frohlich, K. Schumacher and K. Papenfort. 2019. Three autoinducer molecules act in concert to control virulence gene expression in *Vibrio cholerae*. Nucleic Acids Res.

25. Huseby, M. J., A. C. Kruse, J. Digre, P. L. Kohler, J. A. Vocke, E. E. Mann, K. W. Bayles, G. A. Bohach, P. M. Schlievert, D. H. Ohlendorf and C. A. Earhart. 2010. Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. Proc Natl Acad Sci U S A 107:14407-14412.

26. Stewart, P. S. and M. J. Franklin. 2008. Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199-210.

27. Floyd, K. A., J. L. Moore, A. R. Eberly, J. A. Good, C. L. Shaffer, H. Zaver, F. Almqvist, E. P. Skaar, R. M. Caprioli and M. Hadjifrangiskou. 2015. Adhesive fiber stratification in uropathogenic *Escherichia coli* biofilms unveils oxygen-mediated control of type 1 pili. PLoS Pathog 11:e1004697.

28. Prindle, A., J. Liu, M. Asally, S. Ly, J. Garcia-Ojalvo and G. M. Suel. 2015. Ion channels enable electrical communication in bacterial communities. Nature 527:59-63.

29. Larkin, J. W., X. Zhai, K. Kikuchi, S. E. Redford, A. Prindle, J. Liu, S. Greenfield, A. M. Walczak, J. Garcia-Ojalvo, A. Mugler and G. M. Süel. 2018. Signal percolation within a bacterial community. Cell Systems 7:137-145.e133.

30. Glass, D. S. and I. H. Riedel-Kruse. 2018. A synthetic bacterial cell-cell adhesion toolbox for programming multicellular morphologies and patterns. Cell 174:649-658 e616.

31. Jin, X. and I. H. Riedel-Kruse. 2018. High-resolution patterned biofilm deposition using pDawn-Ag43. J Vis Exp 140:58625.

32. Devaraj, A., S. S. Justice, L. O. Bakaletz and S. D. Goodman. 2015. DNABII proteins play a central role in UPEC biofilm structure. Mol Microbiol 96:1119-1135.

33. Arciola, C. R., D. Campoccia, S. Ravaioli and L. Montanaro. 2015. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. Front Cell Infect Microbiol 5:7.

34. Echeverz, M., B. Garcia, A. Sabalza, J. Valle, T. Gabaldon, C. Solano and I. Lasa. 2017. Lack of the PGA exopolysaccharide in *Salmonella* as an adaptive trait for survival in the host. PLoS Genet 13:e1006816.

35. Fritts, R. K., B. LaSarre, A. M. Stoner, A. L. Posto and J. B. McKinlay. 2017. A *Rhizobiales*-specific unipolar polysaccharide adhesin contributes to *Rhodopseudomonas palustris* biofilm formation across diverse photoheterotrophic conditions. Appl Environ Microbiol 83:03035-03016.

36. Briard, B., L. Muszkieta, J. P. Latge and T. Fontaine. 2016. Galactosaminogalactan of *Aspergillus fumigatus*, a bioactive fungal polymer. Mycologia 108:572-580.

37. Lee, M. J., A. M. Geller, N. C. Bamford, H. Liu, F. N. Gravelat, B. D. Snarr, F. Le Mauff, J. Chabot, B. Ralph, H. Ostapska, M. Lehoux, R. P. Cerone, S. D. Baptista, E. Vinogradov, J. E. Stajich, S. G. Filler, P. L. Howell and D. C. Sheppard. 2016. Deacetylation of fungal exopolysaccharide mediates adhesion and biofilm formation. MBio 7:e00252-00216.

38. Ostapska, H., P. L. Howell and D. C. Sheppard. 2018. Deacetylated microbial biofilm exopolysaccharides: It pays to be positive. PLoS Pathog 14:e1007411.

39. Borlee, B. R., A. D. Goldman, K. Murakami, R. Samudrala, D. J. Wozniak and M. R. Parsek. 2010. *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Mol Microbiol 75:827-842.

40. Reichhardt, C., C. Wong, D. Passos da Silva, D. J. Wozniak and M. R. Parsek. 2018. CdrA interactions within the *Pseudomonas aeruginosa* biofilm matrix safeguard it from proteolysis and promote cellular packing. MBio 9:e01376-01318.

41. Evans, M. L., E. Chorell, J. D. Taylor, J. Aden, A. Gotheson, F. Li, M. Koch, L. Sefer, S. J. Matthews, P. Wittung-Stafshede, F. Almqvist and M. R. Chapman. 2015. The bacterial curli system possesses a potent and selective inhibitor of amyloid formation. Mol Cell 57:445-455.

42. Jain, N., J. Aden, K. Nagamatsu, M. L. Evans, X. Li, B. McMichael, M. I. Ivanova, F. Almqvist, J. N. Buxbaum and M. R. Chapman. 2017. Inhibition of curli assembly and *Escherichia coli* biofilm formation by the human systemic amyloid precursor transthyretin. Proc Natl Acad Sci U S A 114:12184-12189.

43. Luo, Y., K. Zhao, A. E. Baker, S. L. Kuchma, K. A. Coggan, M. C. Wolfgang, G. C. Wong and G. A. O'Toole. 2015. A hierarchical cascade of second messengers regulates *Pseudomonas aeruginosa* surface behaviors. MBio 6:e02456-02414.

44. Lee, C. K., J. de Anda, A. E. Baker, R. R. Bennett, Y. Luo, E. Y. Lee, J. A. Keefe, J. S. Helali, J. Ma, K. Zhao, R. Golestanian, G. A. O'Toole and G. C. L. Wong. 2018. Multigenerational memory and adaptive adhesion in early bacterial biofilm communities. Proc Natl Acad Sci U S A 115:4471-4476.

45. Kannan, A., Z. Yang, M. K. Kim, H. A. Stone and A. Siryaporn. 2018. Dynamic switching enables efficient bacterial colonization in flow. Proc Natl Acad Sci U S A 115:5438-5443.

46. Perinbam, K., J. V. Chacko, A. Kannan, M. Digman and A. Siryaporn. 2019 in revision. A shift in central metabolism accompanies virulence activation in *Pseudomonas aeruginosa*.

47. Cooley, B. J., T. W. Thatcher, S. M. Hashmi, G. L'Her, H. H. Le, D. A. Hurwitz, D. Provenzano, A. Touhami and V. D. Gordon. 2013. The extracellular polysaccharide Pel makes the attachment of *P. aeruginosa* to surfaces symmetric and short-ranged. Soft Matter 9:3871-3876.

48. Rodesney, C. A., B. Roman, N. Dhamani, B. J. Cooley, P. Katira, A. Touhami and V. D. Gordon. 2017. Mechanosensing of shear by *Pseudomonas aeruginosa* leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. Proc Natl Acad Sci U S A 114:5906-5911.

49. Welker, A., T. Cronenberg, R. Zollner, C. Meel, K. Siewering, N. Bender, M. Hennes, E. R. Oldewurtel and B. Maier. 2018. Molecular motors govern liquidlike ordering and fusion dynamics of bacterial colonies. Phys Rev Lett 121:118102.

812 50. Zöllner, R., T. Cronenberg, N. Kouzel, A. Welker, M. Koomey and B. Maier. 2019. Type
813 IV pilin post-translational modifications modulate materials properties of bacterial
814 colonies. Biophys. J., in press.

815 51. Rossy, T., C. D. Nadell and A. Persat. 2018. Cellular advective-diffusion drives the
816 emergence of bacterial surface colonization patterns and heterogeneity. bioRxiv: 434167.

817 52. Staudinger, B. J., J. F. Muller, S. Halldorsson, B. Boles, A. Angermeyer, D. Nguyen, H.
818 Rosen, O. Baldursson, M. Gottfreethsson, G. H. Guethmundsson and P. K. Singh. 2014.
819 Conditions associated with the cystic fibrosis defect promote chronic *Pseudomonas*
820 *aeruginosa* infection. Am J Respir Crit Care Med 189:812-824.

821 53. Secor, P. R., L. A. Michaels, A. Ratjen, L. K. Jennings and P. K. Singh. 2018.
822 Entropically driven aggregation of bacteria by host polymers promotes antibiotic
823 tolerance in *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 115:10780-10785.

824 54. Hornischer, K., A. Khaledi, S. Pohl, M. Schniederjans, L. Pezoldt, F. Casilag, U.
825 Muthukumarasamy, S. Bruchmann, J. Thoming, A. Kordes and S. Haussler. 2019.
826 BACTOME-a reference database to explore the sequence- and gene expression-variation
827 landscape of *Pseudomonas aeruginosa* clinical isolates. Nucleic Acids Res 47:D716-
828 D720.

829 55. Darch, S. E., O. Simoska, M. Fitzpatrick, J. P. Barraza, K. J. Stevenson, R. T. Bonnecaze,
830 J. B. Shear and M. Whiteley. 2018. Spatial determinants of quorum signaling in a
831 *Pseudomonas aeruginosa* infection model. Proc Natl Acad Sci U S A 115:4779-4784.

832 56. Yu, W., K. M. Hallinen and K. B. Wood. 2018. Interplay between antibiotic efficacy and
833 drug-induced lysis underlies enhanced biofilm formation at subinhibitory drug
834 concentrations. Antimicrobial agents and chemotherapy 62:e01603-01617.

835 57. Keogh, D., L. N. Lam, L. E. Doyle, A. Matysik, S. Pavagadhi, S. Umashankar, P. M.
836 Low, J. L. Dale, Y. Song, S. P. Ng, C. B. Boothroyd, G. M. Dunny, S. Swarup, R. B. H.
837 Williams, E. Marsili and K. A. Kline. 2018. Extracellular electron transfer powers
838 *Enterococcus faecalis* biofilm metabolism. MBio 9:e00626-00617.

839 58. Light, S. H., L. Su, R. Rivera-Lugo, J. A. Cornejo, A. Louie, A. T. Iavarone, C. M. Ajo-
840 Franklin and D. A. Portnoy. 2018. A flavin-based extracellular electron transfer
841 mechanism in diverse Gram-positive bacteria. Nature 562:140-144.

842 59. Fleming, D. and K. Rumbaugh. 2018. The consequences of biofilm dispersal on the host.
843 Sci Rep 8:10738.

844 60. Heim, C. E., D. Vidlak, T. D. Scherr, J. A. Kozel, M. Holzapfel, D. E. Muirhead and T.
845 Kielian. 2014. Myeloid-derived suppressor cells contribute to *Staphylococcus aureus*
846 orthopedic biofilm infection. J Immunol 192:3778-3792.

847 61. Stewart, P. S. 2014. Biophysics of biofilm infection. Pathogens and Disease 70:212-218.

848 62. Seil, J. T. and T. J. Webster. 2012. Antimicrobial applications of nanotechnology:
849 methods and literature. Int J Nanomedicine 7:2767-2781.

850 63. Natan, M., O. Gutman, R. Lavi, S. Margel and E. Banin. 2015. Killing mechanism of
851 stable N-halamine cross-linked polymethacrylamide nanoparticles that selectively target
852 bacteria. ACS Nano 9:1175-1188.

853 64. Gu, H., S. W. Lee, S. L. Buffington, J. H. Henderson and D. Ren. 2016. On-demand
854 removal of bacterial biofilms via shape memory activation. ACS Appl Mater Interfaces
855 8:21140-21144.

856 65. Lee, S. W., H. Gu, J. B. Kilberg and D. Ren. 2018. Sensitizing bacterial cells to
857 antibiotics by shape recovery triggered biofilm dispersion. Acta Biomater 81:93-102.

858 66. Harrison, J. J., C. A. Stremick, R. J. Turner, N. D. Allan, M. E. Olson and H. Ceri. 2010.
859 Microtiter susceptibility testing of microbes growing on peg lids: a miniaturized biofilm
860 model for high-throughput screening. Nat Protoc 5:1236-1254.

861 67. Dejea, C. M., P. Fathi, J. M. Craig, A. Boleij, R. Taddese, A. L. Geis, X. Wu, C. E.
862 DeStefano Shields, E. M. Hechenbleikner, D. L. Huso, R. A. Anders, F. M. Giardiello, E.
863 C. Wick, H. Wang, S. Wu, D. M. Pardoll, F. Housseau and C. L. Sears. 2018. Patients
864 with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic
865 bacteria. Science 359:592-597.

866 68. Hendricks, M. R., L. P. Lashua, D. K. Fischer, B. A. Flitter, K. M. Eichinger, J. E.
867 Durbin, S. N. Sarkar, C. B. Coyne, K. M. Empey and J. M. Bomberger. 2016. Respiratory
868 syncytial virus infection enhances *Pseudomonas aeruginosa* biofilm growth through
869 dysregulation of nutritional immunity. Proc Natl Acad Sci U S A 113:1642-1647.

870 69. Novotny, L. A., J. A. Jurcisek, S. D. Goodman and L. O. Bakaletz. 2016. Monoclonal
871 antibodies against DNA-binding tips of DNABII proteins disrupt biofilms *in vitro* and
872 induce bacterial clearance *in vivo*. EBioMedicine 10:33-44.

873 70. Desai, S. K., R. S. Winardhi, S. Periasamy, M. M. Dykas, Y. Jie and L. J. Kenney. 2016.
874 The horizontally-acquired response regulator SsrB drives a *Salmonella* lifestyle switch by
875 relieving biofilm silencing. Elife 5:e10747.

876 71. Desai, S. K. and L. J. Kenney. 2017. To ~P or Not to ~P? Non-canonical activation by
877 two-component response regulators. Mol Microbiol 103:203-213.

878 72. Valm, A. M., J. L. Mark Welch and G. G. Borisy. 2012. CLASI-FISH: principles of
879 combinatorial labeling and spectral imaging. Syst Appl Microbiol 35:496-502.

73. Valm, A. M., J. L. Mark Welch, C. W. Rieken, Y. Hasegawa, M. L. Sogin, R. Oldenbourg, F. E. Dewhirst and G. G. Borisy. 2011. Systems-level analysis of microbial community organization through combinatorial labeling and spectral imaging. Proc Natl Acad Sci U S A 108:4152-4157.

74. Valm, A. M., R. Oldenbourg and G. G. Borisy. 2016. Multiplexed spectral imaging of 120 different fluorescent labels. PLoS One 11:e0158495.

75. Baran, R., E. L. Brodie, J. Mayberry-Lewis, E. Hummel, U. N. Da Rocha, R. Chakraborty, B. P. Bowen, U. Karaoz, H. Cadillo-Quiroz, F. Garcia-Pichel and T. R. Northen. 2015. Exometabolite niche partitioning among sympatric soil bacteria. Nat Commun 6:8289.

76. Swenson, T. L., U. Karaoz, J. M. Swenson, B. P. Bowen and T. R. Northen. 2018. Linking soil biology and chemistry in biological soil crust using isolate exometabolomics. Nat Commun 9:19.

77. Jo, J., K. L. Cortez, W. C. Cornell, A. Price-Whelan and L. E. Dietrich. 2017. An orphan cbb_3-type cytochrome oxidase subunit supports *Pseudomonas aeruginosa* biofilm growth and virulence. Elife 6:e30205.

78. Schiessl, K., F. Hu, J. Jo, S. Nazia, B. Wang, A. Price-Whelan, W. Min and L. E. Dietrich. 2019 in press. Phenazine production promotes antibiotic tolerance and metabolic heterogeneity in *Pseudomonas aeruginosa* biofilms. Nat Commun.

79. Akiyama, T., K. S. Williamson, R. Schaefer, S. Pratt, C. B. Chang and M. J. Franklin. 2017. Resuscitation of *Pseudomonas aeruginosa* from dormancy requires hibernation promoting factor (PA4463) for ribosome preservation. Proc Natl Acad Sci U S A 114:3204-3209.

80. Moradali, M. F., S. Ghods, T. E. Angelini and M. E. Davey. 2019. Amino acids as wetting agents: surface translocation by *Porphyromonas gingivalis*. The ISME Journal. in press.

81. Yannarell, S. M., G. M. Grandchamp, S.-Y. Chen, K. E. Daniels and E. A. Shank. 2019. A dual-species biofilm with emergent mechanical and protective properties. J Bacteriol Submitted to special issue of J. Bacteriology.

82. Cornforth, D. M., J. L. Dees, C. B. Ibberson, H. K. Huse, I. H. Mathiesen, K. Kirketerp-Moller, R. D. Wolcott, K. P. Rumbaugh, T. Bjarnsholt and M. Whiteley. 2018. *Pseudomonas aeruginosa* transcriptome during human infection. Proc Natl Acad Sci U S A 115:E5125-E5134.

83. Saak, C. C. and K. A. Gibbs. 2016. The self-identity protein IdsD is communicated between cells in swarming *Proteus mirabilis* colonies. J Bacteriol 198:3278-3286.

84. Tipping, M. J. and K. A. Gibbs. 2018. Peer pressure from a *Proteus mirabilis* self-recognition system controls participation in cooperative swarm motility. bioRxiv: 490771.

85. Little, K., J. L. Austerman, J. Zheng and K. A. Gibbs. 2018. Swarming bacteria respond to increasing barriers to motility by increasing cell length and modifying colony structure. bioRxiv: 398321.

86. Oluyombo, O., C. N. Penfold and S. P. Diggle. 2018. Competition in biofilms between cystic fibrosis isolates of *Pseudomonas aeruginosa* is influenced by R-pyocins. bioRxiv: 264580.

87. Hartmann, R., P. K. Singh, P. Pearce, R. Mok, B. Song, F. Díaz-Pascual, J. Dunkel and K. Drescher. 2018. Emergence of three-dimensional order and structure in growing biofilms. Nature Physics.

88. Vidakovic, L., P. K. Singh, R. Hartmann, C. D. Nadell and K. Drescher. 2018. Dynamic biofilm architecture confers individual and collective mechanisms of viral protection. Nat Microbiol 3:26-31.

89. Dragos, A., H. Kiesewalter, M. Martin, C. Y. Hsu, R. Hartmann, T. Wechsler, C. Eriksen, S. Brix, K. Drescher, N. Stanley-Wall, R. Kummerli and A. T. Kovacs. 2018. Division of labor during biofilm matrix production. Curr Biol 28:1903-1913 e1905.

90. Dragos, A., M. Martin, C. Falcon Garcia, L. Kricks, P. Pausch, T. Heimerl, B. Balint, G. Maroti, G. Bange, D. Lopez, O. Lieleg and A. T. Kovacs. 2018. Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms. Nat Microbiol 3:1451-1460.

91. Rainey, P. B., P. Remigi, A. D. Farr and P. A. Lind. 2017. Darwin was right: where now for experimental evolution? Curr Opin Genet Dev 47:102-109.

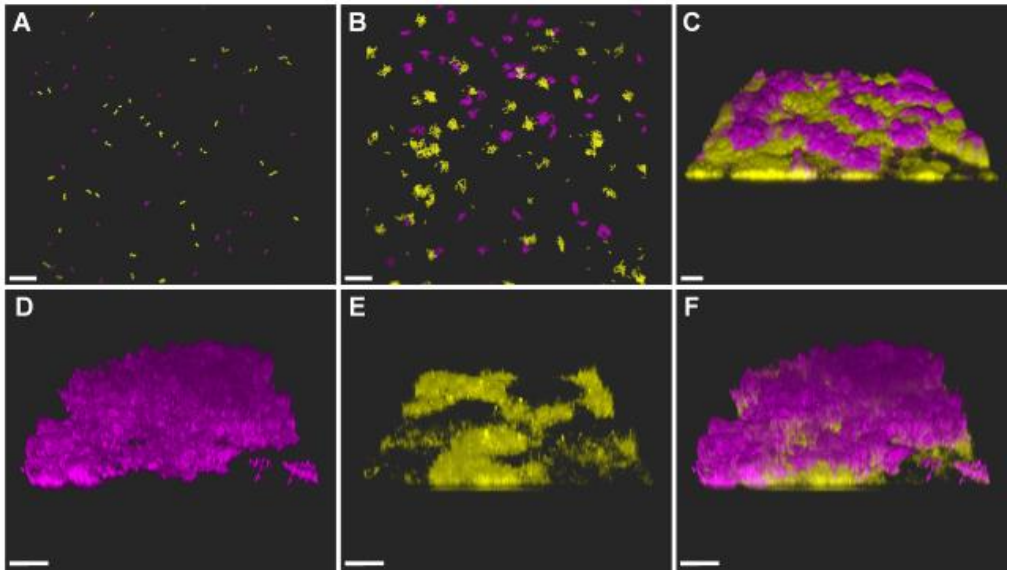
92. Black, A., P. Bouratt and P. B. Rainey. Submitted. Ecological scaffolding and the evolution of individuality: the transition from cells to multicellular life.

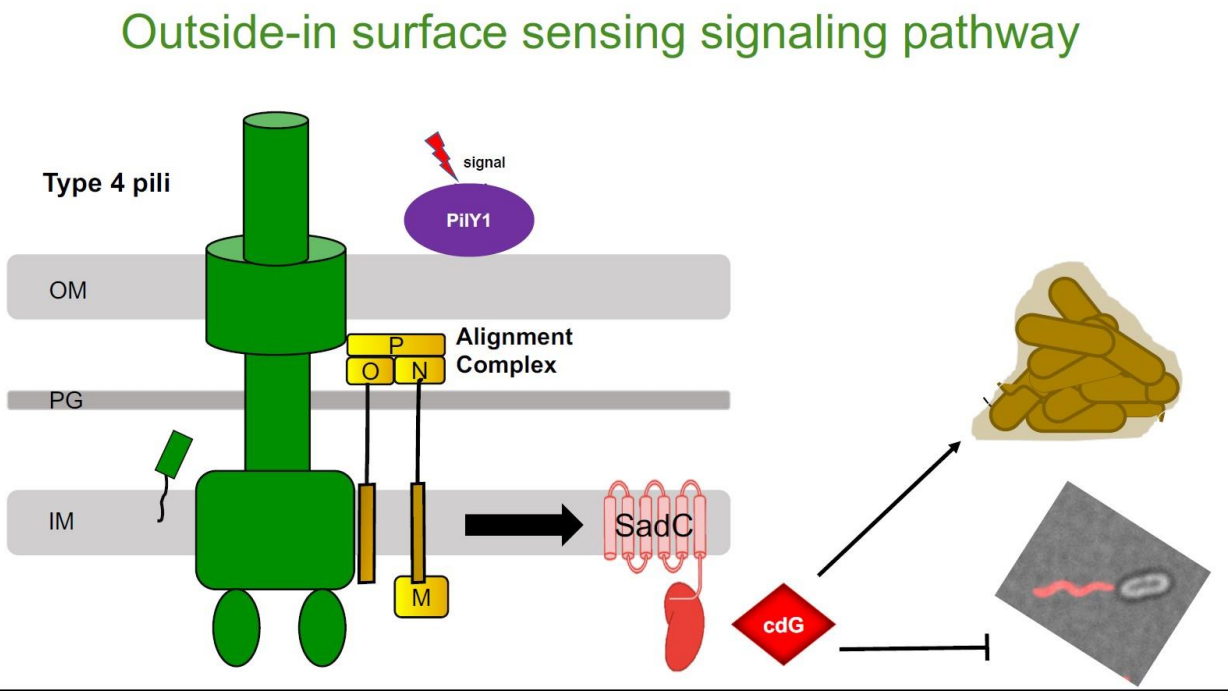
93. Hammerschmidt, K., C. J. Rose, B. Kerr and P. B. Rainey. 2014. Life cycles, fitness decoupling and the evolution of multicellularity. Nature 515:75-79.

94. Rose, C. J., K. Hammerschmidt and P. B. Rainey. 2018. Meta-population structure and the evolutionary transition to multicellularity. bioRxiv: 407163.

Figure legends

Figure 1. The battle begins: competition within *Vibrio cholerae* biofilms. For this experiment, wildtype *V. cholerae* (yellow) and a *V. cholerae* mutant with increased intracellular c-di-GMP levels (purple) were mixed at a 1:1 ratio, and directly injected into a flow cell chamber under flow conditions. Images were then obtained at 1 (A), 4 (B), and 24(B) hours post-injection to visualize surface colonization and biofilm formation. The high c-di-GMP strain is able to initially colonize (A), and grow on the surface (B) at levels similar to the wildtype strain. However, by 24 hours post-injection (C) the high c-di-GMP strain displays biofilms larger than those formed by the wildtype strain. Examining the competition biofilm more closely with high-resolution Airyscan imaging (D-F), demonstrates that the biofilms formed by both the high c-di-GMP (D) and wildtype (E) strains are largely monoclonal. While not only forming larger biofilms than wildtype, biofilms formed by the high c-di-GMP strain can also overgrow and dominate portions of the wildtype biofilms (F). Scale bar for all images is 20 microns. Photo credit: Kyle Floyd and Fitnat Yildiz.





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965 **Figure 2. Outside-in signaling in the bacterial response to surfaces.** How is an external surface signal
966 transmitted intracellularly to increase levels of cyclic-di-GMP to promote biofilm formation? O'Toole
967 and colleagues propose a signaling pathway which requires a cell-surface-associated protein, components
968 of the type 4 pili machinery and a cyclic-di-GMP synthesis enzyme that promote a robust switch to a
969 sessile lifestyle.

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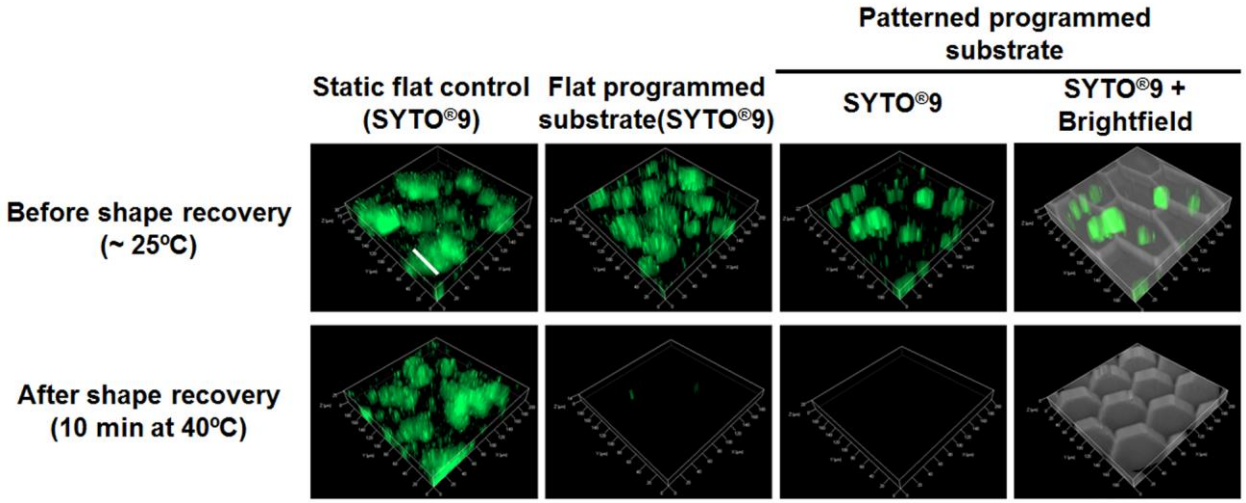

Figure 3. Programmable, active surfaces to prevent biofilm formation. *P. aeruginosa* PAO1 biofilms stained with SYTO9 on different surfaces before and after triggering shape change (10 min incubation at 40 °C) (bar = 50 μm). Figure from (64). Reprinted with permission from American Chemical Society; (https://pubs.acs.org/doi/full/10.1021/acsami.6b06900). Any further permissions related to the material excerpted should be directed to the ACS.

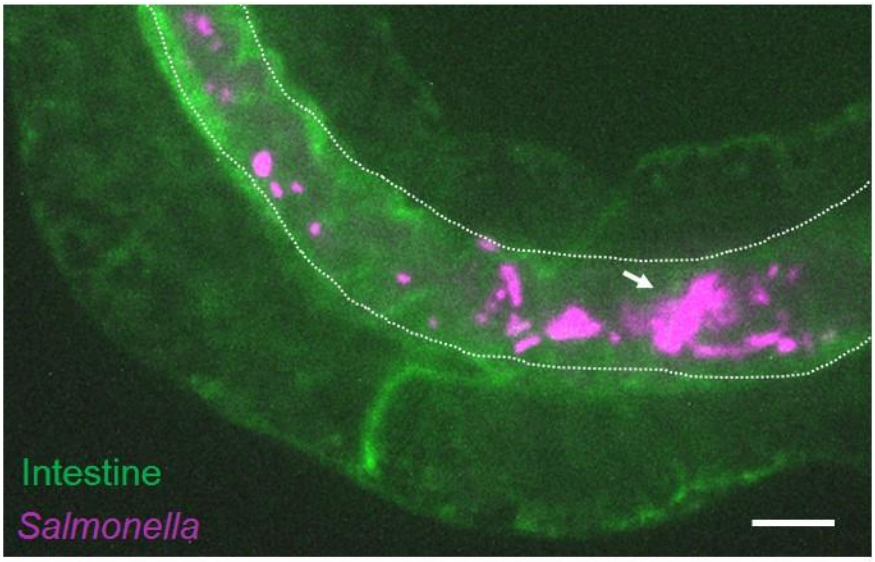

Figure 4. *In vivo* biofilms in an animal model. *Salmonella Typhimurium* forms biofilms in the intestinal lumen of persistently infected *C. elegans* (Scale bar = 10 µm). Photo credit: S. Harshe, S. K. Desai and L. J. Kenney.