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1 **Biofilms 2018: A diversity of microbes and mechanisms**

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12 **Abstract**

13 The 8th ASM Conference on Biofilms was held in Washington D.C. on October 7-11, 2018. This very
14 highly subscribed meeting represented a wide breadth of current research in biofilms, and included over
15 500 attendees, 12 sessions with 64 oral presentations, and four poster sessions with about 400 posters.

16
17 **Introduction**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

454 Given the established tolerance of biofilms towards antimicrobial treatments, one important area
455 is the development of rapid and accurate evaluation of drug concentrations necessary to effectively
456 eradicate biofilms. Jodi Connell (3M, Saint Paul, Minnesota) presented strategies from 3M to develop
457 fundamental research that will help take anti-biofilm therapies from the laboratory to the market. She
458 described the development of a rapid and more quantitative method using the MBECTM assay (66) and a
459 10 kDa Alexafluor-labelled dextran that incorporates into the biofilm matrix and provide a fast
460 quantification method, reducing processing time from ~5 days to only 30 hours.

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

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

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

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

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

496 Stuti Desai (laboratory of Linda Kenney, National University of Singapore, Singapore) used the
497 host nematode *Caenorhabditis elegans* to evaluate the role of regulators in host-associated biofilm
498 formation by *Salmonella* (Fig. 4). They discovered that *Salmonella* rapidly formed static biofilm clusters
499 that were dependent on the function of the response regulator SsrB. Whereas the phosphorylated form of
500 SsrB is associated with virulence in other models, it is the unphosphorylated form that promotes biofilms
501 (70), indicating that SsrB has different activities in promoting *Salmonella* interaction with distinct hosts
502 (71).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

641
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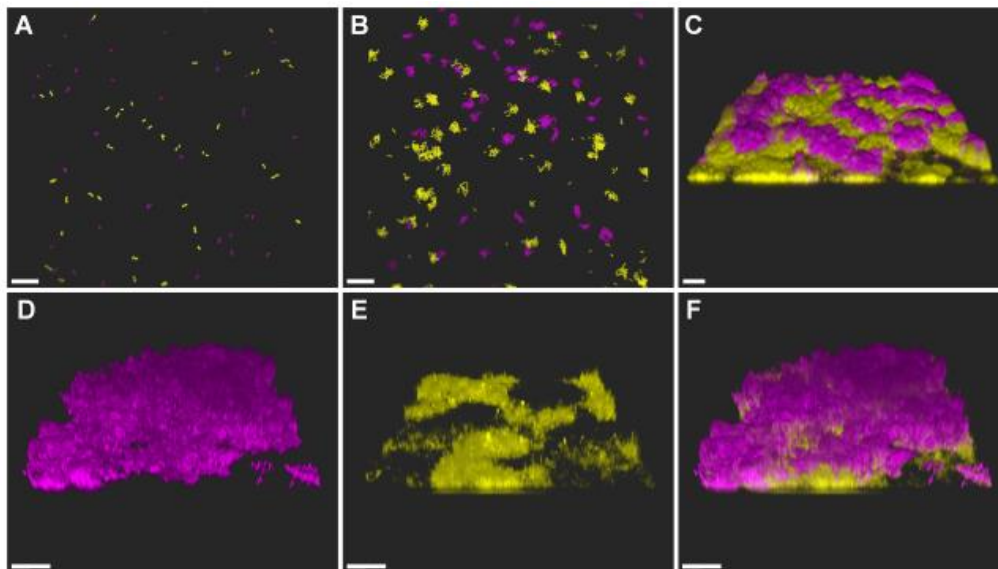
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948 **Figure legends**

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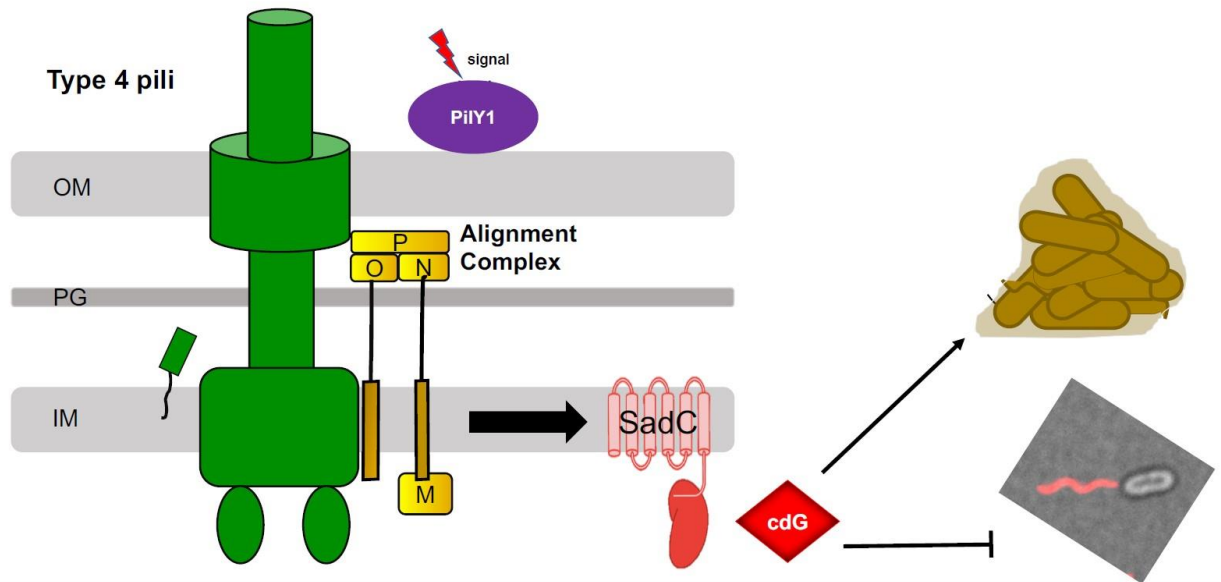
950 **Figure 1. The battle begins: competition within *Vibrio cholerae* biofilms.** For this experiment,
951 wildtype *V. cholerae* (yellow) and a *V. cholerae* mutant with increased intracellular c-di-GMP levels
952 (purple) were mixed at a 1:1 ratio, and directly injected into a flow cell chamber under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



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Outside-in surface sensing signaling pathway

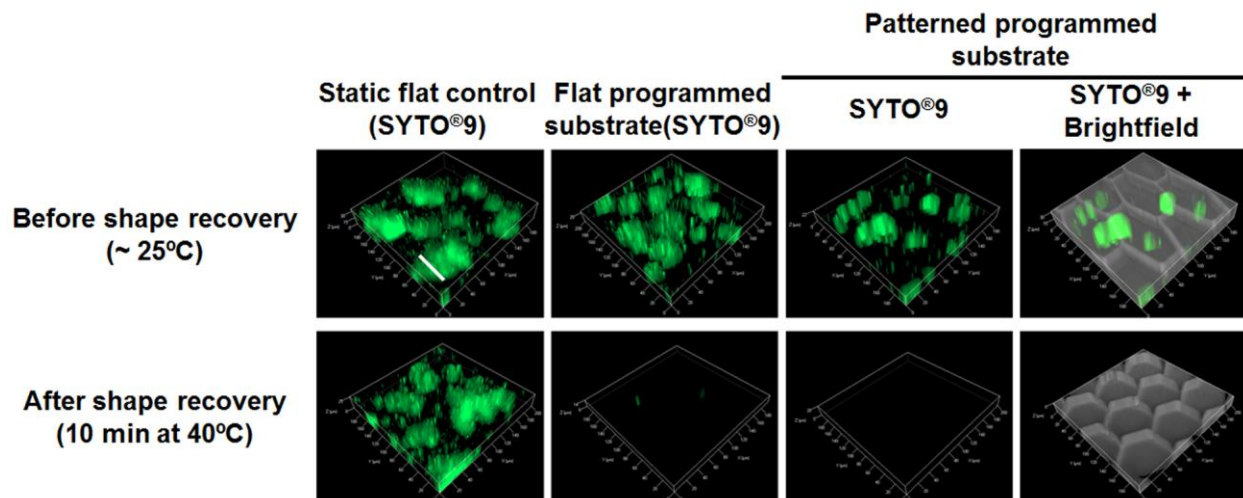


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965 **Figure 2. Outside-in signaling in the bacterial response to surfaces.** How is an external surface signal
966 transmitted intracellularly to increase levels of cyclic-di-GMP to promote biofilm formation? O'Toole
967 and colleagues propose a signaling 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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973 **Figure 3. Programmable, active surfaces to prevent biofilm formation.** *P. aeruginosa* PAO1 biofilms

974 stained with SYTO9 on different surfaces before and after triggering shape change (10 min incubation at

975 40 °C) (bar = 50 μm). Figure from (64). Reprinted with permission from American Chemical Society;

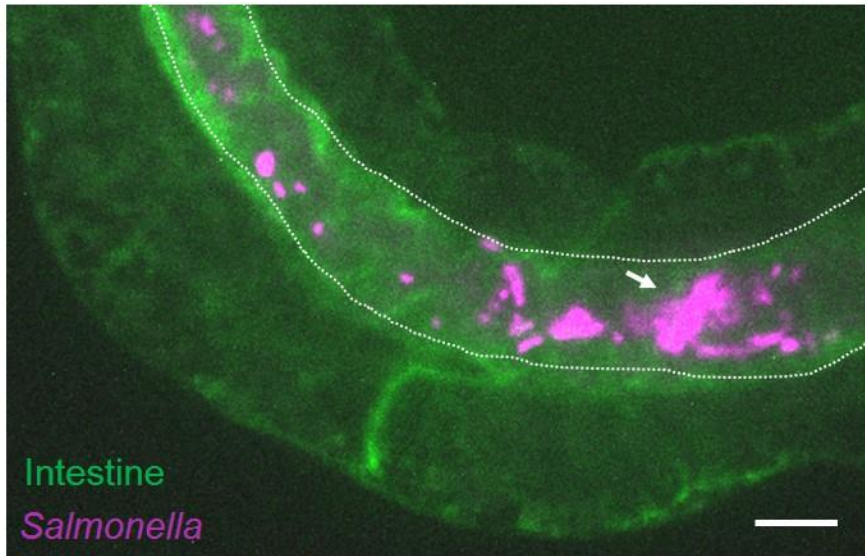
976 (<https://pubs.acs.org/doi/full/10.1021/acsami.6b06900>). Any further permissions related to the material

977 excerpted should be directed to the ACS.

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

985