Biofilms are receiving attention as never before, reflecting our increasing awareness of their impact on health, the environment, industrial processes, and natural and manmade materials. Problems associated with biofilms cost the United States billions of dollars every year in energy losses, equipment damage, product contamination, and the management of infections. Biofilms are implicated in more than 80% of chronic inflammatory and infectious diseases caused by bacteria, including otitis media, endocarditis, gastrointestinal ulcers, infections of the urinary tract, and pulmonary infections in cystic fibrosis (CF) patients.

So, what are biofilms? In general, biofilms are composed of microorganisms attached to surfaces and encased in a hydrated polymeric matrix of their own synthesis. The matrix is composed of polysaccharides, proteins, and nucleic acids which are collectively termed “extracellular polymeric substances” (EPS). The EPS matrix enables cells in a biofilm to stick together and is a key element in the development of complex, three-dimensional, attached communities. Water channels are dispersed throughout biofilms, allowing the exchange of nutrients, metabolites, and waste products.

Where do biofilms form? Virtually anywhere. Sites include inorganic natural and manmade materials above and below ground, on minerals and metals, including medical implant materials, and on organic surfaces such as plant and body tissues. Biofilm growth surfaces may act as an energy source, a source of organic carbon, or simply a support material. One common feature of biofilm environments is that they are periodically or continuously suffused with water.

Biofilms Display Specialized Functions and Properties

Biofilms function in some respects like a tissue, possessing a primitive circulatory
system. In fact, some investigators contend that biofilms actively pump fluids through channels by changing the ionic strength of the extracellular milieu, causing periodic contraction of matrix polymers. Biofilms can be formed by a single bacterial species, but in nature biofilms typically consist of assemblages containing many species of bacteria as well as fungi, algae, yeasts, protozoa, and other microorganisms. For instance, dental plaque biofilms typically contain more than 500 bacterial species.

Biofilms are not merely microbial cells that become stranded at surfaces. We now know that bacteria in biofilms differ from their planktonic counterparts in several significant ways. For instance, bacteria grown in a biofilm can be up to 1,500 times more resistant to antibiotics, biocides, and immune chemicals compared to the same bacteria grown suspended in liquid culture.

No single explanation accounts for how biofilms arise and what advantage they provide to the resident microorganisms. For example, the extracellular polymeric matrix protects biofilm bacteria by acting as a buffer against changes in the physical environment. The extracellular matrix can also block at least some antimicrobial agents from entering the biofilm, with the EPS acting as an ion exchanger, restricting diffusion of certain compounds into the biofilm from the surrounding environment. Slow growth within a biofilm may also lead to increased resistance to chemical challenge.

Overall, the protective mechanisms at work in biofilms appear to be distinct from those that are responsible for antibiotic resistance in planktonic cells. Thus, poor antimicrobial penetration, oxygen and nutrient limitation, slow growth, and adaptive stress responses constitute a multilayered defense by biofilms to challenges from the outside.

Bacteria within biofilms diversify genetically, even during short-term growth. Such genetic changes influence multiple traits of bacteria growing in biofilms, which can be observed even after these bacteria are removed from a biofilm context. For instance, biofilm-derived genetic variants have been observed to exhibit an increased ability to disseminate, increased adhesiveness, increased antimicrobial resistance, and accelerated biofilm formation. The presence of these functionally diverse bacteria increases the ability of a biofilm to adapt to environmental stresses. Genetic diversity within biofilm clonal populations is presumed to provide a safeguard against changing environmental conditions, according to Pradeep Singh at the University of Washington in Seattle.

**Biofilm Formation Can Be Viewed as a Developmental Process**

Biofilm formation, like fruiting body formation in *Myxococcus xanthus* or sporulation in *Bacillus subtilis*, is yet another example of a bacterial developmental process (Fig. 1). Like other developmental systems, biofilms form following a series of discrete and well-regulated steps. While molecular mechanisms differ from species to species, the broad stages of biofilm development appear to be conserved among a wide range of genera. These stages include attachment to a substrate, the growth and aggregation of cells into microcolonies, and the maturation and maintenance of architecture.

The development of biofilm architecture, particularly the spatial arrangement of colonies within the matrix and the open areas surrounding the colonies, is fundamental to the function of these complex communities (*Microbe*, May 2007, p. 231). In the earliest stage, microcolonies form, often in response to intercellular messenger molecules such as acylated homoserine lactones (AHLs) that are used to detect a surface or a high density of cells in the local environment, according to David Davies at Binghamton University in Binghamton, N.Y. As the biofilm develops, its biomass and thickness are influenced through cross-species bacterial communication via the signal molecule autoinducer 2, according to Thomas Wood at Texas A & M University at College Station, Alexander Rickard at Binghamton University, and Paul Kolenbrander at the National Institutes for Health. Further, rhamnolipid surfactants apparently play a role in maintaining water channels by influencing cell-to-cell interactions and the attachment of bacterial cells to surfaces.

The extent to which biofilms form and whether a biofilm is composed of only a few or hundreds of layers of cells also depends on environmental conditions. The biofilm developmental cycle comes full circle when biofilms disperse (Fig. 1), allowing cells to spawn new biofilm communities elsewhere.
When Karin Sauer left Germany in 2000 for Montana, she had no idea what she was getting into. “I knew there were more trout than people inhabiting Montana, and, according to some maps I found on the Internet, there were lots of places with no two-legged creatures,” she says. “Quite a scary thought, coming from Germany.” Nevertheless, she packed her bags and left for a postdoctoral research position at the Center for Biofilm Engineering at Montana State University in Bozeman. It turned out great.

“Living in Montana was sometimes like being on vacation, with Yellowstone [National Park], great ski resorts—although I am a terrible skier—and fantastic scenery nearby,” she says. “It is also the perfect place to take pictures, one of my passions.” She also met her future husband, David, while attending an ASM-sponsored conference on biofilms in Big Sky, Mont. He had recently been appointed assistant professor at Binghamton University in upstate New York. In 2003, she became an assistant professor in the same department.

“It’s funny how things turn out,” Sauer says. “I was supposed to return to Germany and establish my own lab. I already had lab space and everything—and then I met David. As you can see, I never returned to Germany.”

The focus of her research group is the development of biofilms, or surface-associated bacterial communities. Her lab focuses on two microorganisms, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Her interest in biofilms stems from a summer course that she took at the Woods Hole Marine Biology Laboratory (MBL) in Woods Hole, Mass. during the year that she completed her Ph.D.

“What fascinated me most was the ability of bacteria to utilize insoluble metals for energy generation, forcing the bacteria to a lifestyle in association to surfaces,” she says. “And so it began—a fascination for biofilms and surface-associated growth.”

Sauer, 35, was born in Goettingen, in Lower Saxony, but her home was in Hessen. “I grew up seeing the border to East Germany from my parents’ kitchen window,” she recalls. She has two older brothers—one, a computer software engineer, and the other, an employee of the phone company. She is the only scientist in her extended family.

Sauer says that studying genetically engineered bacteria in high school biology hooked her on microbiology. “The bacteria were engineered to express a luciferase gene, and were used in waste water treatment plants to determine when the water was clean enough to be released into the river,” she recalls. “When the water was clean enough, the bacteria started to glow or produce light, which eliminated the need for extensive water testing. I was so fascinated by this that it sparked my interest in microbiology.”

She studied microbiology and eventually received her doctorate from Philipps-University in Marburg. She loved Marburg for its ancient architecture, including a castle dating from the 11th Century, its river, and its bars. “I suppose that most of my social skills and beer-drinking habits were perfected there—I’m kidding,” she says. “It was a very quaint town—I love old buildings and antiques, so I felt right at home. The only problem was that downtown was located on a hill. But the residents figured out a solution, an elevator going from uptown to downtown—kind of fun.”

While her current research was shaped through her experience at MBL in Woods Hole, her experimental approaches go back earlier. “While my degree is in microbiology, the lab I worked at was more or less a biochemistry lab,” she says. “We purified proteins and, in part, used molecular biology techniques for downstream approaches. I still do this. Can’t get it out of my system.”

Sauer keeps up her German and continues to do photography, a hobby she combines with hiking. “I love it,” she says. “I am not an expert, but I love capturing the moment, nature, the effect of light, laughter, a good time. I love landscape pictures, especially in black and white with the right light. It is just a shame that it is not possible to capture the smell with a camera.”

She took up gardening after moving to Binghamton. “I never thought I would like it—I certainly never liked it as a child,” she says. “But I found it brings balance to my professional life. Research is slow in producing results and, even then, the accomplishments are mostly on paper. Gardening, on the other hand, comes with very visible and fragrant results. It helps me to get over frustration—and to keep my head clear.”

Marlene Cimons

Marlene Cimons is a freelance writer in Bethesda, Md.
Looking at biofilm formation as a developmental process, it is not surprising that biofilm cells differ phenotypically from their planktonic counterparts in the genes and proteins that they express. For instance, between 2.9 and 17.1% of \( P. \) aeruginosa genes are differentially expressed between planktonic cells and biofilms, depending on the growth phase of planktonic cells used as a reference point, according to Michael Givskov of the Technical University of Denmark, Lyngby, and his collaborators. Similar findings are reported for several other species, including Escherichia coli, Vibrio cholerae, Streptococcus pneumoniae, Staphylococcus aureus, and Bacillus subtilis.

Meanwhile, Peter Greenberg and his colleagues at the University of Washington found that approximately 1% of the \( P. \) aeruginosa genome, or approximately 70 genes, show a change in regulation during the transition from exponentially growing planktonic cells to 5-day old biofilms. Their study was conducted at high cell densities to ensure that quorum sensing was activated in both planktonic and biofilm cells. Using in vivo expression technology (IVET) followed by insertional mutational analysis, Lori Burrows and her collaborators at the University of Toronto in Canada identified five genes that are essential for \( P. \) aeruginosa to form biofilms or that affect fitness.

With proteomic analyses, we find that up to 50% of the proteome in \( P. \) aeruginosa is modified during biofilm development. Furthermore, protein expression in biofilms has been shown in our laboratories to indicate distinct phases in the developmental process. For instance, specific early, middle, and late biofilm proteins are detectable in cultures of \( P. \) aeruginosa as the biofilm matures. Suites of proteins, which relate to virulence, antibiotic resistance, and quorum

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**FIGURE 1**

The biofilm developmental process in stages. (i) reversible attachment, (ii) irreversible attachment, (iii) maturation-1, (iv) maturation-2, and (v) dispersion.
sensing, are produced progressively. A similar protein production pattern occurs in *Bacillus cereus*, with distinct patterns of proteins appearing in biofilms at different stages of development, according to Volker Brozel and his collaborators at South Dakota State University in Brookings.

**Search for Common Features of Biofilm Development Proves Challenging**

It is intriguing that while numerous research groups observe genome- or proteome-wide expression patterns during biofilm formation, there is limited consensus linking the sets of genes identified by different laboratories. This has led some researchers to question the existence of a biofilm phenotype. However, as suggested by George O’Toole of Dartmouth Medical School in Hanover, N.H., bacteria have more than one way to make a biofilm. Thus, the availability of a particular carbon source or other nutrients or a particular environmental stimulus may trigger alternative developmental pathways toward the same end point.

While a comprehensive understanding of the genetic basis of biofilm formation remains elusive, several protein-encoding genes play key roles, based on mutations that either impair biofilm formation or alter biofilm structure. For instance, several proteins play a role in the transition from a planktonic to an attached mode of growth, including those involved in swimming and twitching motility, and chemotaxis. However, strains lacking a functional flagellum or pilus eventually form biofilms if kept in flowing fluids for an extended time. In such cases, the structure of the biofilm differs markedly from that observed for the wild-type strain.

Chemotaxis plays a stage-specific role in biofilm formation—in particular, for the *V. cholerae* CheY-3 mutant that is defective in forming monolayers but not biofilms, according to Paula Watnick and her collaborators at the Tufts-New England Medical Center in Boston, Mass. Other mutations that block biofilm formation at various stages include FleR, which controls production of the flagellum, RpoN, membrane proteins LapA and LapB, and several other gene products.

Like other pathways, biofilm development is controlled by a number of different regulators. In *P. aeruginosa* these regulators include LasR and RhlR (which are involved in quorum sensing), RpoS, Crc, and PvrR. The protein kinase PrkC, which is similar to the eukaryotic sensor Ser/Thr and to the Tyr kinases from *Bacillus subtilis*, also appears to regulate biofilms, according to Simone Seror and coworkers at the Université Paris-Sud, France. Mutants with prkC deletions have decreased sporulation efficiency and a reduced capacity to form biofilms. Furthermore, the gene prpC, which encodes a PPM phosphatase that is cotranscribed with prkC, is also required for normal biofilm and endospore formation.

AlgR is a response regulator protein that is required for synthesizing alginate, a major matrix component of biofilms that form in the lungs of CF patients. AlgR also appears to play a role in controlling biofilm formation. For instance, mutations that prevent phosphorylation of AlgR decrease biofilm maturation without affecting alginate. Meanwhile, an algR null mutant shows severe defects in biofilm initiation, suggesting that AlgR regulates additional functions.

The transcriptional regulator MvaT represses expression of cup genes, which are involved in the chaperone-usher fimbrial assembly pathway in *P. aeruginosa*, according to Alain Filloux and his colleagues at the Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM) in Marseille, France. MvaT mutants exhibit enhanced biofilm formation, indicating that various stages of biofilm formation and maturation are mediated by extracellular appendages, such as type IV pili and flagella.

In addition, several multicomponent systems help to control biofilm formation, including the three-component regulator system SadARS. Deletion in any of the sadARS genes results in a failure to progress to biofilm maturation in *P. aeruginosa*, according to O’Toole at Dartmouth Medical School.

Similarly, disrupting GacA, the response regulator of the GacA/GacS two-component regulatory system in *P. aeruginosa*, leads to a 10-fold reduction in biofilm formation capacity, according to Douglas Storey at the University of Calgary in Alberta, Canada. The GacA/S system also regulates secondary metabolism, including exoenzymes, alginate, and siderophore biosynthesis; the system also exerts posttranscriptional regulatory control of genes within the Gac regulon. Two additional hybrid sensor kinases...
response regulators, RetS and LadS, signal through the GacA/S/RsmZ pathway, exerting opposite effects.

Thus, inactivating RetS in *P. aeruginosa* attenuates virulence by prematurely activating genes involved in biofilm formation and coordinate repression of genes required for initial colonization, according to Steven Lory of Harvard Medical School in Boston, Mass. Meanwhile, mutants in *ladS* remain planktonic under conditions in which the parental strain transitions to biofilm growth, according to Filloux at LISM in France. Furthermore, the regulators control the reciprocal expression of genes for type III secretion, biofilm-promoting polysaccharides, and the small regulatory RNA *rsmZ*. Thus, LadS and RetS may mediate a switch between these bacterial lifestyles via *rsmZ*. Furthermore, the findings suggest that biofilm formation may be regulated via protein phosphorylation, a common modification used in signal transduction in microorganisms in response to stimuli such as changes in nutrient concentrations or iron availability.

**Even Greater Complexity May Underlie Biofilm Formation**

As complex as the catalogue of factors involved in controlling biofilm formation has become, it is apparently far from complete. For example, the bacterial intracellular signaling molecule cyclic-di-GMP is implicated as a global intracellular messenger in bacterial biofilm formation—modulating, among other functions, the production of exopolysaccharides. Furthermore, c-di-GMP is implicated as a signal controlling biofilm dispersion, the transitioning between sessile and motile lifestyles. For instance, high c-di-GMP concentrations in several bacterial species stimulate biofilm formation and EPS production (and thus, adhesiveness), but suppress motility. However, low concentrations inhibit biofilm formation, repress EPS production, and stimulate swimming and swarming motilities.

This involvement of c-di-GMP in forming or dispersing biofilm occurs in several bacterial species, including *Salmonella typhimurium*, *P. aeruginosa*, *P. putida*, and *Shewanella oneidensis*. We recently learned that c-di-GMP levels are regulated in response to environmental cues mediated by the sensory protein BdlA in a signaling cascade that links sensing, c-di-GMP levels, and detachment.

However, c-di-GMP appears not to be universal in signaling biofilm developmental processes and regulating adhesiveness of cells. For example, in *Escherichia coli*, the RNA-binding protein CsrA, which helps to regulate central carbon flux, apparently plays a role in forming and dispersing biofilms, according to Tony Romeo at Emory University School of Medicine in Atlanta, Ga. The effects of CsrA are mediated through the collective regulation of glycogen biosynthesis, carbon metabolism, and of flagellum biosynthesis and motility. Further, CsrA may play a role in producing an adhesin.

In summary, biofilm formation is a complex process requiring the coordinated action of multiple regulatory proteins, many of them two-component regulatory systems, to complete the developmental cycle. Bacterial signaling plays a role throughout this cycle, with quorum sensing signaling molecules affecting biofilm architecture. Meanwhile, c-di-GMP levels play a part in regulating the transitioning between sessile and motile lifestyles, probably in response to environmental signaling.

Environmental sensing and the existence of multiple pathways for the stage-specific progression of biofilm development may explain some of the differences that researchers studying biofilm genomics and proteomics observe. Further, biofilm development appears to be different from bacterial developmental processes such as sporulation and fruiting body formation. These developmental processes rely primarily on sigma factor regulation, whereas biofilm formation apparently does not. To date, no core regulator for biofilm formation has been identified. Instead, several two-component regulatory systems are essential for biofilm formation, much as they are involved in eukaryotic cells and tissues during crucial developmental processes such as embryogenesis. This fascinating similarity could mean that regulatory signaling cascades found in eukaryotic cells originated among bacterial cells living within biofilms.
SUGGESTED READING


