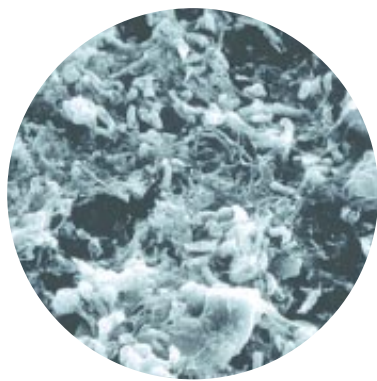




BY J. W. COSTERTON AND PHILIP S. STEWART
Photographs by Sam Ogden

Battling BIOFILMS

THE WAR IS AGAINST
BACTERIAL COLONIES THAT
CAUSE SOME OF THE MOST
TENACIOUS INFECTIONS KNOWN.
THE WEAPON IS KNOWLEDGE OF
THE ENEMY'S COMMUNICATION SYSTEM



CONTACT LENSES (*left*) are among the familiar surfaces that may be colonized by biofilms—slime-enclosed communities of microorganisms. The film shown above, from a contact lens case, presumably caused a corneal infection diagnosed in the lens wearer.

MICROGRAPH: LOUISE McLAUGHLIN-BORLACE, Institute of Ophthalmology, Department of Pathology, London; LICENSED FOR USE, ASM Microbelibrary (www.microbelibrary.org)

Pentagon planners concern themselves a great deal nowadays with information warfare. Why? Because interfering with a foe's ability to communicate can be far more effective than destroying its bunkers or factories. In the battle against harmful bacteria, some investigators are considering the same strategy.

The microbes that cause many stubborn infections organize themselves into complex and tenacious films—biofilms—that can be nearly impossible to eradicate with conventional antibiotics. In the past few years, medical researchers have discovered that the microorganisms in biofilms depend critically on their ability to signal one another. Drugs able to interfere with this transmission might then bar the microbes from establishing infections or undermine their well-fortified positions; such drugs might thus combat maladies ranging from the pneumonia that repeatedly afflicts people with cystic fibrosis to the slow-burning infections that often form around medical implants.

Signal-dampening compounds are currently being evaluated in animal studies, but why is it that such elegant weapons are only now being readied to enter the medical arsenal? The answer, in short, is that microbiologists took a very long time to size up the enemy. Ever since the late 19th century, when Robert Koch's laboratory studies in Germany validated the germ theory of disease, most people, scientists included, have envisioned

bacteria as single cells that float or swim through some kind of watery habitat, perhaps part of the human body. This picture emerged from the way investigators usually examine such organisms: by training their microscopes on cultured cells suspended in a fluid droplet. That procedure is convenient but not entirely appropriate, because these experimental conditions do not reflect actual microbial environments. As a result, the bacteria in typical laboratory cultures act nothing like the ones encountered in nature.

In recent years, we and other bacteriologists have gained important insights into how common disease-causing microbes actually live. Our work shows that many of these organisms do not, in fact, spend much time wafting about as isolated cells. Rather they adhere to various wetted surfaces in organized colonies that form amazingly diverse communities.

In retrospect, it is astonishing that investigators could overlook this microbial lifestyle for so long. After all, bacterial biofilms are ubiquitous—dental plaque (which most of us confront daily), the slippery coating on a rock in a stream, and the slime that inevitably materializes inside a flower vase after two or three days are but a few common examples. And bacteria, the focus of our studies, are not alone in the ability to create biofilms. Indeed, the genetic diversity of the microorganisms that can arrange themselves into living veneers and the breadth of environments they invade convince us that this ability must truly be an ancient strategy for microbial growth. Scientific appreciation and understanding of that strategy is, however, a modern phenomenon.

GermS in Flatland

SOME BIOLOGISTS had, in fact, attempted long ago to examine the bacteria living in biofilms using ordinary microscopes; a handful even employed electron microscopes. They always saw some bacteria, but being unable to obtain clear images from deep within living layers, they concluded that the cells inside were mostly dead and jumbled in random clumps. This view changed little until about a decade ago, when bacteriologists began employing a technique called laser scanning confocal microscopy. That technology enables investigators to view slices at different depths within a living biofilm and to stack these planes together to create a three-dimensional representation.

Applying this approach in a concerted effort to study the structure of biofilms, John R. Lawrence of the Canadian National Water Research Institute, Douglas E. Caldwell of the University of

TROUBLE IN TUBES

Biofilms that form in urinary catheters are a common source of infection. When the tubes stay in only briefly, they pose little risk, but the danger increases with prolonged use.

A 1996 study found, for example, that after a week, infections strike 10 to 50 percent of catheterized patients; after a month, virtually all such patients are affected.





TROJAN HORSES

Despite elaborate precautions, biofilm bacteria sometimes get into biomedical products. In 1993 and 1994, 100 asthmatics died because the albuterol inhalants they were using contained the biofilm-forming bacterium *Pseudomonas aeruginosa*. The source was traced to a tank involved in the manufacture of this drug. In 1989 another well-known biofilm bacterium, *P. cepacia*, colonized bottles of a potent antiseptic (povidone-iodine solution), causing infections in patients at a children's hospital in Texas.

Saskatchewan and one of us (Costerton) demonstrated for the first time in 1991 that the bacteria grow in tiny enclaves, which we called microcolonies. Bacteria themselves generally constitute less than a third of what is there. The rest is a gooey substance the cells secrete, which invariably absorbs water and traps small particles.

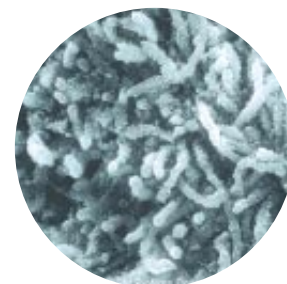
The goo—or, more formally, the extracellular matrix—holds each microcolony together. A biofilm is built of countless such groupings, separated by a network of open water channels. The fluid coursing through these tiny conduits bathes each congregation of microbes, providing dissolved nutrients and removing waste products. The cells situated on the outside of a microcolony are well served by this plumbing system, but those in the interior are largely cut off. The dense aggregation of cells surrounding them and the organic matrix that cements things together act as barriers to water flow. So the cells inside the colony must make do with the nutrients that can diffuse inward to them. Actually, the supply is not all that meager: because the glue is mostly just water,

small molecules can move through it freely—albeit with certain important exceptions. A substance will have a hard time diffusing to the center of a microcolony if it reacts with the cells or matrix material it encounters along the way.

Such chemical reactivity gives rise to small-scale environmental changes within a biofilm. These variations were recognized even before confocal microscopy revealed the cause. In 1985 our colleague Zbigniew Lewandowski began making direct measurements of chemical conditions in biofilms using needle-shaped microelectrodes with tips just one hundredth of a millimeter across. He found, among other things, that the oxygen concentration varies radically between locations as close as five hundredths of a millimeter apart—little more than the width of a human hair. Scientists often look at the amount of oxygen in a bacterial community because it can reflect the physiological status of the cells. For example, in a biofilm composed solely of *Pseudomonas aeruginosa* (the bacterium responsible for cystic fibrosis pneumonia), cellular activity and growth take place only where oxygen can penetrate—the outer two or three hundredths of a millimeter of each tiny colony. Deeper down, the cells are alive but dormant. This mix of metabolic states differs markedly from the uniformity typically found in laboratory cultures.

The variety of chemical environments that arise within a single biofilm means that one cell may look and act very different from the next even

DENTAL PLAQUE is a biofilm. Mounting evidence implicates it, surprisingly, in heart disease.



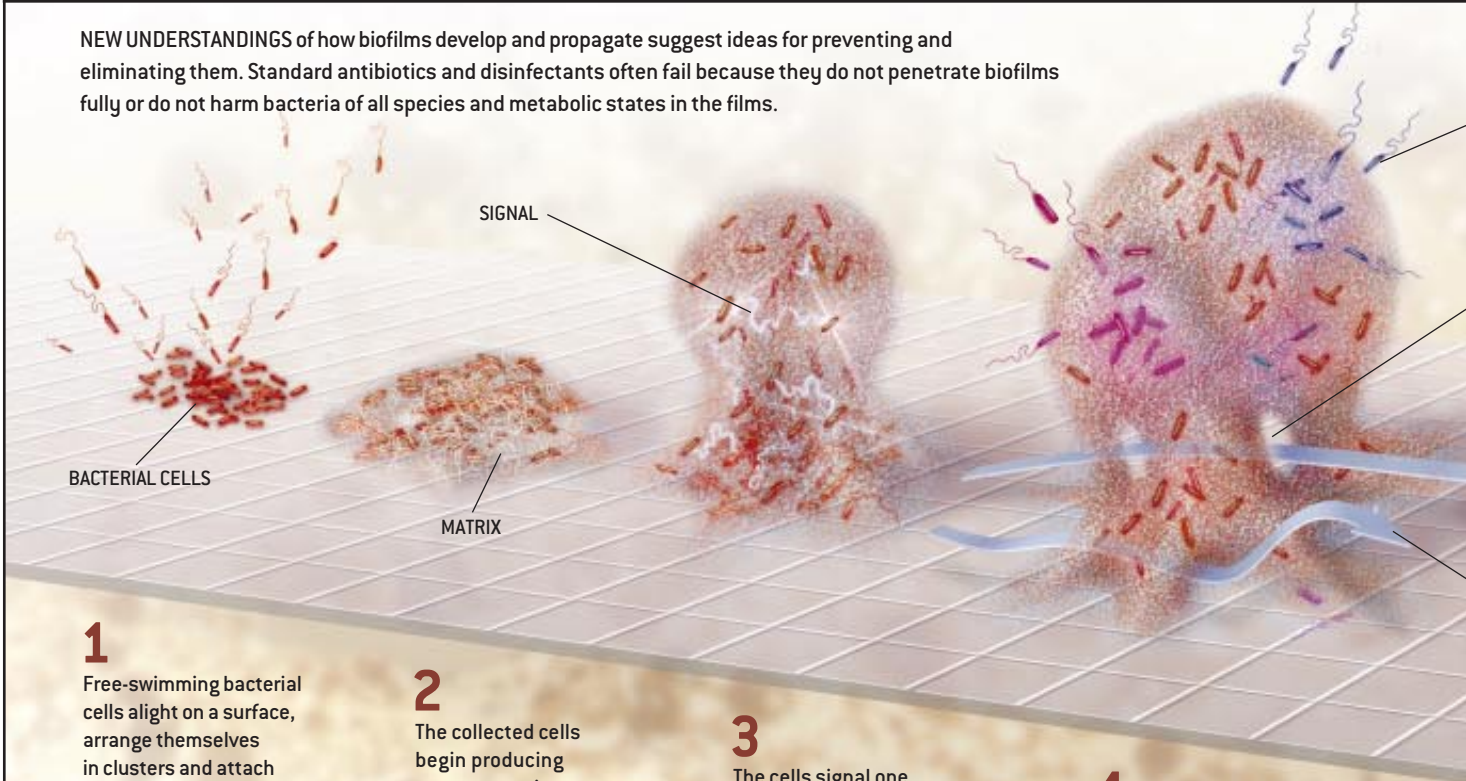
MICROGRAPH: R. BOS, H. J. BUSSCHER, W. L. JONGEBLOED AND H. C. VAN DER MEI Laboratory for Materia Technica, University of Groningen, The Netherlands; LICENSED FOR USE, ASM Microbelibrary (www.microbelibrary.org)

THE AUTHORS

J. W. ("BILL") COSTERTON and PHILIPS S. STEWART have worked together for almost 10 years. Costerton, who holds a Ph.D. in bacteriology, is head of the Center for Biofilm Engineering at Montana State University. Stewart, whose doctorate is in chemical engineering, is deputy director and research coordinator at the center.

HOW BIOFILMS FORM AND HOW TO FIGHT THEM

NEW UNDERSTANDINGS of how biofilms develop and propagate suggest ideas for preventing and eliminating them. Standard antibiotics and disinfectants often fail because they do not penetrate biofilms fully or do not harm bacteria of all species and metabolic states in the films.



1

Free-swimming bacterial cells alight on a surface, arrange themselves in clusters and attach

ATTACK STRATEGY

Coat surfaces with molecules that block or disrupt microbial arrangement or attachment

2

The collected cells begin producing a gooey matrix

ATTACK STRATEGY

Coat surfaces with substances that interfere with matrix production

3

The cells signal one another to multiply and form a microcolony

ATTACK STRATEGY

Deliver signal blockers to threatened areas to abort biofilm formation

4

Chemical gradients arise and promote the coexistence of diverse species and metabolic states

ATTACK STRATEGY

Deliver multiple antibiotics or disinfectants to undermine the varied survival strategies of biofilm cells

when the two are genetically identical. Similarly, local conditions control the production of many toxins and other disease-causing substances by microbial cells in a biofilm; consequently, some cells may inflict little harm on a host, whereas others may be lethal. The wide range of conditions can also permit several bacterial species to live side by side and thrive. Sometimes one species feeds on the metabolic wastes of another, aiding them both.

An interesting case in point has been understood in a general way since the 1940s: the biofilms that form on fodder after cows or other ruminants eat it. These films are initially made up of organisms that digest the cellulose in plant matter and produce organic compounds called fatty acids. When these cellulose-eating bacteria have generated enough fatty acids to inhibit their own growth, mobile cells of *Treponema* and other species invade the biofilm and begin using these very substances to fuel their own metabolism. The forage material

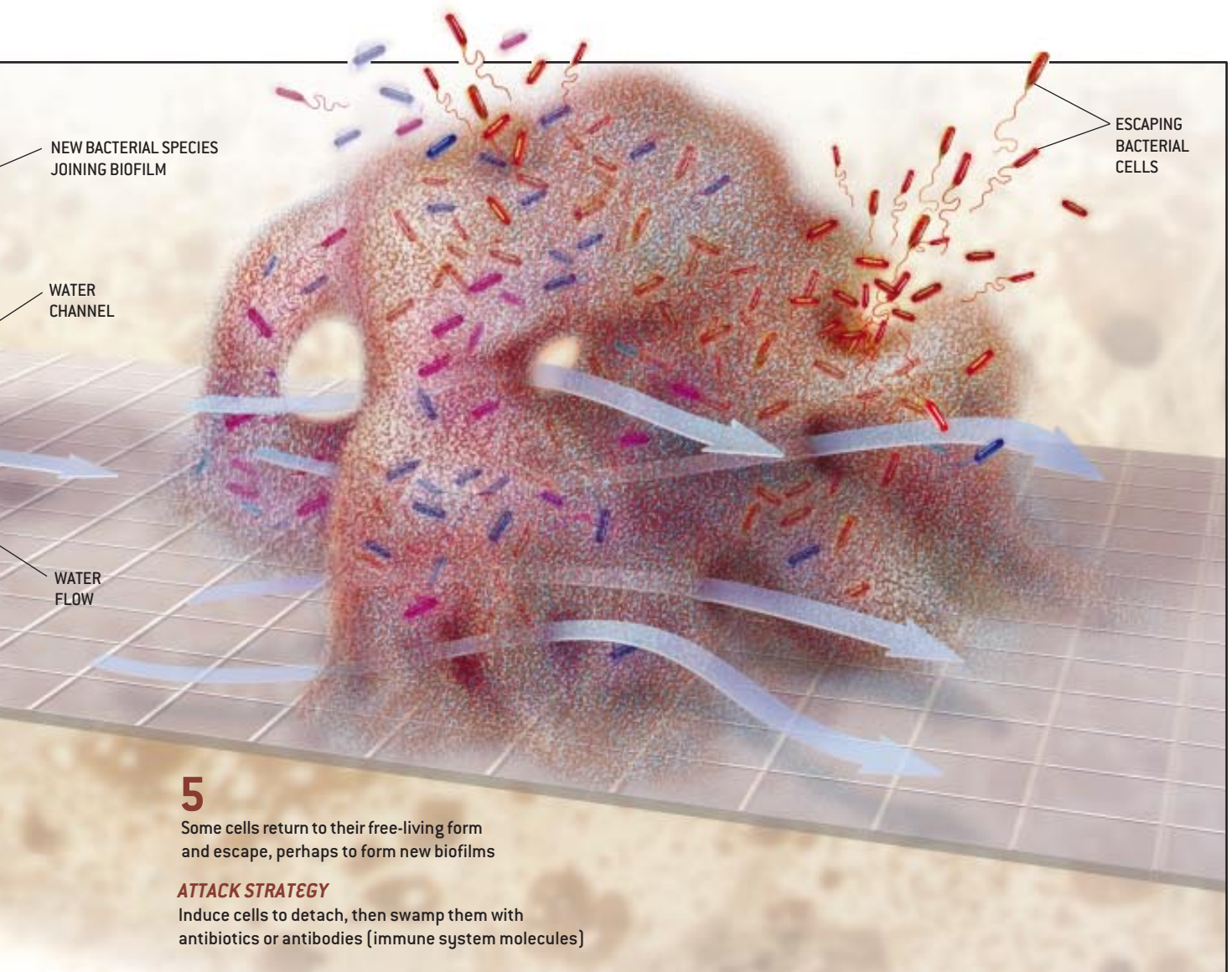
gradually disappears, being converted into a mass of bacteria that the animal digests later on. That is, cows subsist on bacterial biofilms, not hay.

For ruminant animals, these films are clearly indispensable. But for the rest of us, they are a nuisance or, sometimes, a serious threat to health. They can survive most chemical treatments used to control bacteria in medicine and industry, treatments that would quickly eradicate free-floating cells. They can also evade the molecules and cells that the immune system unleashes. Biofilm infections thus tend to be quite persistent.

Tough Bugs

WHY, EXACTLY, are these biofilms so resilient? At times, antibiotics and germ-fighting cleansers may fail to pierce the film. Penicillin antibiotics, for instance, have great difficulty penetrating biofilms containing cells that produce enzymes known as beta-lactamases. These enzymes degrade the an-

ILLUSTRATION BY KEITH KASNOT



5

Some cells return to their free-living form and escape, perhaps to form new biofilms

ATTACK STRATEGY

Induce cells to detach, then swamp them with antibiotics or antibodies (immune system molecules)

MICROGRAPH: GORDON MCFETERS Center for Biofilm Engineering, Department of Microbiology, Montana State University; LICENSED FOR USE: ASM MICROBELIBRARY (www.microbellibrary.org)

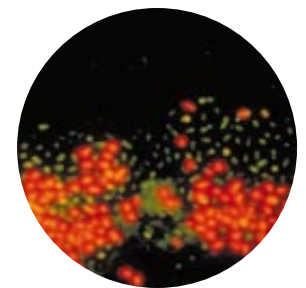
tibiotic faster than it can diffuse inward, so that it never reaches the deeper layers of a biofilm. Even chlorine bleach, a favorite of home and industry, has a hard time eradicating biofilms. This reactive oxidant will eventually burn its way in, but first it must deplete, layer by layer, the neutralizing capacity of the film. That process takes more time and bleach than one might expect. It is easy, therefore, to be lulled into thinking that all bacteria must be dead when many are still alive.

Other factors enhance tenacity as well. Even where an antimicrobial agent penetrates biofilms easily, the microorganisms often still survive aggressive treatment that would eradicate free-floating cells. This ability had long mystified biologists, but lately they have learned that the variety of conditions and bacterial types in a biofilm confers protection against antibacterial agents.

Consider again the action of penicillin, which attacks replicating bacterial cells of many species.

If a biofilm contains regions that are, say, starved of an essential nutrient, the cells in those areas, which are alive but not replicating, will survive exposure to penicillin. Because active and inactive microbes are closely juxtaposed in a biofilm, and because surviving bacteria can use dead ones as nutrients, the few cells remaining after the antibiotic therapy ends can restore the biofilm to its original state in a matter of hours.

Such abilities explain why antimicrobials that work fine on cultured cells often do not yield results that are useful to people doing battle with biofilms. Most of these people are physicians and patients, but a large number are engineers who have to contend with the ruinous effects of biofilms in industry, where bacteria often foul machinery and speed the corrosion of metal pipes. To aid both groups, in 1990 the National Science Foundation established the Engineering Research Center (now called the Center for Biofilm Engi-



AFTER 60 MINUTES of exposure to bleach, many cells in this biofilm were dying (green), but many others, especially in the interior, still remained active (red).

neering) at Montana State University, where the two of us have collaborated for nearly a decade.

Research here has revealed, among other things, that as bacteria adhere to a surface and form a biofilm, they manufacture hundreds of proteins not found in free-floating cells. Some of these proteins are involved in a curious shuffle that the cells carry out just after they settle on a surface but before they fix their positions, as Roberto Kolter and his colleagues at Harvard Medical School have shown by deleting certain genes (the blueprints for proteins) from various bacteria. Using *Staphylococcus epidermidis*, which is responsible for common staph infections, other researchers have identified genes that govern the next step in the development of a biofilm: the synthesis of the extracellular matrix. With these genes inactivated, the bacterium loses its ability to form a biofilm in the test tube and, apparently, in the tissues of laboratory animals.

Recent experiments have revealed similar genetic control centers in other species as well. For example, *P. aeruginosa* contains several genes that are, in essence, turned on within 15 minutes of this bacterium's attachment to a surface. One of these genes, *algC*, is needed to synthesize alginate, the gelatinous polymer that makes up much of the extracellular matrix.

How is it that the cells coming together to form a biofilm know to turn on certain genes in the first place? The answer is that these seemingly simple, autonomous microbes regularly com-

municate with one another. In *P. aeruginosa* and a broad class of similar bacteria, the relevant signaling molecules are acylated homoserine lactones, which each cell produces at a low level. When enough cells assemble, the concentration of these compounds increases, which in turn triggers changes in the activity of dozens of genes. David G. Davies of Binghamton University has shown that this mechanism, called quorum sensing, is critical for the development of biofilms. Indeed, laboratory strains of *P. aeruginosa* that lack the gene for a particular acylated homoserine lactone fail to build normal biofilms and instead pile up in a disorganized heap.

Investigators have now identified signaling molecules used by biofilms that grow, among other places, on urinary catheters. These films and the films that thrive on permanent medical implants cause the most worrisome types of biofilm infections, affecting perhaps 10 million people in the U.S. every year. Despite their being typically slow to develop, such smoldering infections lead to repeated flare-ups and are extraordinarily difficult to eradicate. Biofilms have also been implicated in periodontal disease, prostate infections, kidney stones, tuberculosis, Legionnaire's disease and some infections of the middle ear.

Now that biologists understand how bacterial biofilms form, controlling them with drugs able to target their unique properties should be possible. One could, for example, smother the sticky appendages on the surface of the cells with a molecule that



LUSH BIOFILM appeared on an industrial heat exchanger. Such contamination can reduce efficiency.

CAUSE OF CORROSION

Some biofilms cause serious trouble for industry when they establish colonies inside metal piping and hasten corrosion, a process that accounts for half of the forced outages at steam-driven electric power plants. Companies spend billions of dollars every year combating such problems.



readily attaches to them, reducing their ability to bind to surfaces and form a biofilm in the first place. Another option is to interfere with the synthesis of the extracellular matrix, such as by coating medical implants with chemicals that switch off the genes responsible for matrix production. One might also target the molecules that biofilm bacteria use to communicate, thereby halting biofilm formation or suppressing toxin production or other equally invidious activities. That is, instead of trying to overwhelm the offending organisms with poisons (and accidentally killing many more harmless or beneficial bacteria in the process), scientists will soon be able to manipulate the cells in more subtle ways to block their damaging activity.

Tactical Warfare

INDEED, COMMERCIAL development of at least one novel drug has already begun. Staffan Kjelleberg and Peter Steinberg of the University of New South Wales in Sydney, Australia, noted in 1995 that the fronds of a red alga (*Delisea pulchra*) growing in Botany Bay are rarely covered with biofilms. Despite the thousands of bacterial species thriving in these waters, the algal specimens all remain immaculate. How do they do it? Kjelleberg and Steinberg have determined that *D. pulchra* uses chemicals called substituted furanones to keep free of biofilms. The researchers and their university have now launched a company, Biosignal, to produce protective coatings that incorporate substituted furanones, for application to ship hulls and aquaculture equipment.

In the past few years, researchers have gained exciting insights into how the substituted furanones isolated by Kjelleberg and Steinberg work. These substances turn out to be similar to two classes of bacterial molecules: to the acylated homoserine lactones that many biofilm-making bacteria use for quorum sensing and to a class of molecules, newly described by Bonnie L. Bassler of Princeton University, that virtually all bacteria emit to convey signals between different species. Apparently the substituted furanones bind to bacterial cells at the sites normally used by the other signals and thus block the signaling molecules from delivering biofilm-promoting messages.

Indications are that substituted furanones can both prevent biofilm formation and help to break up existing films. They also seem ideal for medical use because they are nontoxic and relatively stable in the body. Moreover, furanones have been present in oceans for millions of years without inducing bacteria to become resistant to their effects—which raises hope that they will be unable



WATER WASTERS

The safety of drinking-water supplies can be compromised by biofilms, which often grow inside distribution pipes. Protected by a gooey film, disease-causing microorganisms can proliferate despite chlorination. Researchers at Stanford University have shown, for example, that by forming itself into a biofilm, the organism responsible for outbreaks of cholera, *Vibrio cholerae*, can survive chlorine concentrations 10 to 20 times higher than are normally used to treat drinking water. In 1996 biofilms repeatedly caused the water supply of Washington, D.C., to violate federal standards for bacterial contamination.

to engender resistance in bacteria that colonize medical devices and human tissues.

This line of research is also providing what is perhaps a less practical benefit but one that may in the end prove equally important because it revolutionizes conceptions of bacteria. Biologists are now beginning to speak of the formation of bacterial biofilms as a developmental process, borrowing language normally used to describe a growing embryo. Just as a fertilized egg gives rise to varied cell types during fetal development, bacteria, too, differentiate after they alight on a surface. They synthesize communication molecules, reminiscent of the pheromones and hormones of insects and animals, to coordinate the building of microcolonies within a sophisticated architecture. The design allows nutrients to flow in and wastes to flow out, inviting comparison to the circulatory systems of higher organisms. In some biofilms, bacteria of many species cooperate to digest nutrients that a single type cannot fully exploit. These observations suggest that what most biologists had long viewed as the lowly bacterium may, in fact, occupy a much higher rank in the scheme of life than was ever imagined. SA

MORE TO EXPLORE

Bacterial Biofilms: A Common Cause of Persistent Infections. J. W. Costerton, Philip S. Stewart and E. P. Greenberg in *Science*, Vol. 284, pages 1318–1322; May 21, 1999.

Community Structure and Co-operation in Biofilms. Edited by D. G. Allison, P. Gilbert, H. M. Lappin-Scott and M. Wilson. Cambridge University Press, 2001.

Images and information about biofilms can be found at the Center for Biofilm Engineering at Montana State University at www.erc.montana.edu, the American Society for Microbiology at <http://dev.asmusa.org/edusrc/biofilms/> and at the MicrobeLibrary at www.microbelibrary.org (search for “biofilm”).

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