Current Topics

Biofilms Fail To Form when Subject to Good Vibrations

Low-energy surface acoustic waves in the form of ultrasonic vibrations can block bacterial biofilms from forming on medical devices, according to Gad Lavie and colleagues of the Sheba Medical Center, Tel-Hashomer, Israel. When rabbits are outfitted with catheters that are made to vibrate, the growth of biofilms is markedly inhibited on those devices. Moreover, the animals carrying those devices develop infections more slowly than do animals outfitted with ordinary catheters, the researchers report in the December *Antimicrobial Agents and Chemotherapy* (50:4144–4152).

Biofilms typically are resistant to antibiotics, in part because of exopolysaccharide coatings that they produce that keep such drugs from penetrating the interior of the biofilm. Efforts by other research groups to treat medical devices with mechanical energy have failed at driving antibiotics into biofilms. The research presented in this paper apparently represents the first time mechanical energy by itself prevented bacteria from attaching to a surface and forming biofilms.

Outfitting rabbits with catheters proved a formidable challenge, according to Lavie. It entailed dressing each rabbit in a coat-like harness, which was attached to an overhead wire that restricted each animal’s movement forward and backward, to prevent it from wriggling free of its catheter.

To generate the acoustic waves, collaborators at Nanovibronix Corp. in Nesher, Israel, devised special miniactuators that could be attached to the external portion of the catheters. These caused the entire surface of the catheters to vibrate longitudinally and transversely.

The idea behind this work sprang from an observation made by Lavie’s colleague Zadik Hazan, who had been using acoustic waves to try to germinate seeds. He noticed that biofilms developed on untreated seed plates but not on those treated with acoustic energy.

Those vibrations apparently prevent planktonic microbes from adhering to catheter surfaces. Additionally, the vibrations may affect “gene expression reprogramming, and synthesis of the corresponding protein products that transform the lifestyle of microorganisms from the planktonic to sessile form,” Lavie and his collaborators note. They further speculate that “chaotic microstreaming produced in fluids by the ongoing vibrations hampers the development of coherent concentration-dependent gradients of quorum-sensing molecules . . . interfering communications between microorganisms, virulence factor production, and other postattachment biofilm developmental processes.”

One complexity of this approach is that the vibrations must be maintained continuously to prevent biofilm formation. Another is that treating devices with too much acoustic energy can enhance cell adhesion instead of preventing it, possibly because high shear stress induces bacteria to firmly attach to nearby surfaces.

Bacterial biofilms, such as this *Staphylococcus aureus* biofilm on a plastic surface, can be significant problems in clinical settings. Researchers are finding ways to disrupt their formation by using ultrasonic vibrations as well as by chemical means. (Image copyright SciMAT/Photo Researchers, Inc.)
“One important advantage of this approach is that the use of antimicrobial agents is not required,” says Rodney M. Donlan of the Centers for Disease Control and Prevention in Atlanta, Ga., who notes that such indwelling devices are a major source of infections in clinical settings. “In the United States, there are approximately 80,000 central venous catheter-associated bloodstream infections in intensive care units each year,” he points out. By one estimate, biofilms in hospitals infect 2 million U.S. patients annually.

“This is very nice work,” says William Costerton of the University of Southern California in Los Angeles, referring to Lavie and his collaborators. “There are now at least two ways to make bugs uncomfortable on surfaces where we don’t want them: acoustic waves, and DC fields, and all can help with the serious problem of device-related infections. More physics, less chemistry!”

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

Disrupting Social Lives To Block Bacterial Biofilms

Perhaps some bacterial biofilms can be held in check with small molecules, some of which are now considered drug candidates, says Eric Olson of Vertex Pharmaceuticals in Cambridge, Mass. Alternatively, disrupting biofilm social activities, whether within or between bacterial species, may offer another means for quelling biofilms that prove pathogenic in humans, according to Peter Greenberg and Samuel Miller of the University of Washington (UW), Seattle, who also participated in the focus session, “Contribution of Biofilms in Infectious Diseases,” convened during the 46th annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held last September in San Francisco.

Olson and his collaborators screened some 200,000 compounds for their ability to prevent Staphylococcus cells from forming biofilms in vitro. More than 900 of them had some inhibitory effects, 73 proved specific for biofilms, while the others inhibited planktonic growth and were set aside. Within that biofilm-specific set, the Vertex group identified several “top compounds” to test further and to modify chemically as a way of enhancing their biofilm-inhibiting activities, he says.

Among the chemically modified compounds, one, called VRT-05, inhibits expression of RNA III from both Staphylococcus aureus and S. epidermidis and also blocks efflux pumps in such cells, according to Olson. Although it can prevent biofilms from forming, it does not disrupt those that are established. When used with other antibiotics, it lowers the amount needed to kill cells, but “we’re not sure exactly how it does that,” he says. Moreover, it is specific for staphylococci, and does not block Pseudomonas biofilms. Although promising as a candidate en route to becoming an antibiofilm drug, there are still “lots of challenges,” he notes.

Persistant Pseudomonas aeruginosa lung infections, typically as biofilms, are a major problem for cystic fibrosis (CF) patients, often leading to lung failure and early death (Microbe, December 2006, p. 571). Instead of containing only one pathogen, however, some biofilms in such patients may consist of symbiotic mixtures of P. aeruginosa with gram-positive pathogens such as S. aureus, according to Miller of UW. The balance between these two bacterial pathogens is affected not only by the antibiotics being used to treat CF patients but also by signal molecules such as 4-hydroxy, 2-heptyquinoline, N-oxide (HQNO) that biofilms of P. aeruginosa produce.

The interactions between these two bacterial species are complex, and key details escaped notice until recently, according to Miller. For instance, when P. aeruginosa biofilms produce HQNO, they help to protect S. aureus cells against the killing effects of antibiotics such as tobramycin. Moreover, the biofilms or HQNO by itself appa-
ently leads S. aureus to produce small-colony variants that “often are missed by clinical labs,” he says, adding: “There may be more coinfections in the airways of CF patients that are also underappreciated clinically.”

The term “sociomicrobiology” alludes to the highly “structured” communities that can form within biofilms that can prove “important to their success as pathogens,” says Greenberg of UW. One promising way to disrupt P. aeruginosa biofilms in the lungs of CF patients entails targeting iron metabolism in those bacteria and disrupting its important signaling role when forming biofilms. For instance, adding lactoferrin can block P. aeruginosa microcolony and biofilm formation, he says. “But that doesn’t help someone who already has a biofilm.”

Another approach is to substitute the metal gallium for iron, taking advantage of the structural similarities of these two metals plus gallium’s inability to function in the many redox reactions in which iron proves biologically crucial, according to Greenberg. Gallium by itself or in an adduct with deferoxamine (a chelating agent) not only kills planktonic cells but also proves “capable of blocking biofilm formation,” he says. Moreover, this adduct “kills bacteria in the internal portions of a biofilm,” at sites where ordinary antibiotics are only poorly active, if at all. Although this approach may not work so well in lungs and airways where iron is abundant, it might prove useful in augmenting antibiotics used for treating topical biofilms, such as those that sometimes form along the surface of the eye, he points out.

**Jeffrey L. Fox**

**Experts Recount Challenges in Seeking Drugs To Counter Resistance**

From the information overload that typifies the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)—and the September 2006 ICAAC in San Francisco met that standard—emerges a picture of shifting patterns for nosocomial infections and antibiotic resistance. Resistance development easily outpaces the appearance of new antimicrobial drugs, except perhaps in the case of fungal pathogens (see p. 7). This mismatch is a challenge that has some infectious disease experts urging novel policy strategies, such as establishing dedicated federal funding to support research on new antibiotics in an effort to reclaim momentum from the bacterial pathogens.

Intensive care units are “toxic waste dumps” in terms of the promiscuous use there of antibiotics, says Louis Rice of the VA Medical Center in Cleveland, Ohio, who spoke during the symposium “Addressing the Challenge of Multidrug-Resistant Gram-Negative Infections.” He faults both physicians and drug companies for medical practices that contribute to the development of drug resistance. To counteract such forces, he calls on the federal government to impose a new tax on pharmaceutical companies that “made billions of dollars” by marketing antibiotics. He envisions using those proceeds, much like the federal Superfund is used to clean up environmental messes, to address pressing antibiotic needs by funding research and development efforts of companies that remain in this field.

However tantalizing they may be for those working on antibiotics, such schemes seem unlikely to be enacted during a period when new federal taxes are considered politically untouchable. The same may be true for another scheme to encourage drug development—namely, by providing pharmaceutical companies extensions on patents covering blockbuster drugs as an incentive to continue pursuing products with more modest monetary expectations, according to David Shlaes of Active-Infectives Consulting in Stonington, Conn.

Shlaes calls other federal initiatives to foster development of antibiotics as bioterrorist countermeasures “insufficient,” noting that BioShield resources are being channeled mainly into vaccines rather than into antimicrobial drugs. Although the federal government is positioned to “provide incentives, do-

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**Microbial Life 2.8 km Underground Is Extraordinarily Slow but Sustainable**

A subsurface microbial community that is 2.8 km below a South African gold mine is dominated by a single Firmicutes phylotype, according to Li-Hung Lin of National Taiwan University in Taipei, Taiwan, Tullis Onstott of Princeton University in Princeton, N.J., and their collaborators. Although the metabolism of these Firmicutes is remarkably lethargic, they are apparently hardy and sustainable, depending on hydrogen and sulfate but not on materials derived from photosynthesis. Moreover, their dominance at this underground site is “exceptional,” and their presence there could date from 3 million to 35 million years ago. Their cell turnover times are estimated at anywhere from 45 to 300 years. Thus, although this dark, dank niche is relatively rich in nutrients, it remains a mystery as to what restrains their metabolic activity, the researchers note. Details of their findings appear in the 20 October 2006 *Science.*
ing so will cost money, and I don’t think the government will provide it,” he says.

On a more pragmatic level, Rice calls for clinical research on antibiotic dosing to determine whether treatment courses could be reduced to lower the overall burden of antibiotics and thus perhaps reduce the selective pressures on pathogens to develop resistance. “We just don’t know how long it takes to get the burden of microorganisms down to let the immune system take over,” he says. Noting that merely suggesting that patients stop short of a full antibiotic treatment course is now considered “heresy,” he calls for a series of clinical trials to reevaluate fundamental antibiotic drug-dosing questions.

Other symposium participants, including Shlaes and Karen Bush of Johnson & Johnson (J&J) in Raritan, N.J., point out that some antibiotics are now being formulated and administered in ways to reduce the likelihood of resistance developing. Furthermore, economic pressures also are helping to dictate that patients receive shorter courses of antibiotics in hospital settings, Bush says.

In terms of meeting medical needs, the quest for new antibiotics to treat infections by gram-negative pathogens is “becoming extremely important,” Bush says. Some drugs or combinations of drugs that once were effective against such pathogens are “not working.” Although researchers are identifying a few promising new drugs that work against gram-positive pathogens, there is “very little” in the way of new candidate drugs that are effective against ordinary gram-negative pathogens, and even less for the more challenging but rarer gram-negative pathogens such as Pseudomonas and Acinetobacter spp., adds Jeffrey Edwards, a consultant based in Holywell, Flintshire, Wales. These latter microbes pose a “real challenge” in part because it is difficult to get drugs into them, they mutate readily, and they also “pump drugs back out.”

“We ran whole-cell assays on staph, E. coli, and Pseudomonas,” Bush says, describing how recent experience at J&J confirms Edwards’ generalization. “We got thousands of hits with staph, a few hundred with E. coli, but only five with Pseudomonas, and they were detergents, with nothing to follow up. Pseudomonas is a tough challenge.” Thus, although the challenge of identifying new candidate drugs active against gram-negative pathogens is now widely appreciated, the short-term prospects for meeting it are not at all promising.

Jeffrey L. Fox
Jeffrey L. Fox is the Microbe Current Topics and Features Editor.

Amid Antimicrobial Gloom, Antifungal Agents Shine Relatively Brightly

Although the outlook for those infected with fungi is brighter than a decade ago, such infections remain difficult to fend off or shed. Meanwhile puzzles, challenges, and controversies keep antifungal experts from complacency over recent progress, according to several participants who spoke during the symposium “Antifungal Therapy: New Drugs, New Choices,” convened during the 46th annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held last September in San Francisco.

“Fifteen years ago, all we had was amphotericin B, which is toxic and not easy to give,” says Elias Anaissie of the University of Arkansas for Medical Sciences in Little Rock. “Now we have at least nine antifungal agents in three different classes, along with better diagnostic tests. But we still face challenges, including which drugs to use and in what settings, when to use drugs prophylactically, and what to do about resistance.”

Antifungal drug resistance follows distinctive patterns compared to those associated with antibacterial drugs, according to Anaissie and others. For example, resistance to antifungal drugs tends to be “primary” rather than “developed,” meaning fungal pathogens tend to be intrinsically resistant to specific drugs but not to gain resistance through exchanges of genes between fungal strains or species.

“Amphotericin B has been in use for about 50 years, but without development of secondary resistance,” Anaissie says. Fluconazole, which belongs to the azole class of antifungal agents, has “done pretty well over about 15 years” without resistance developing, although “some resistance with third-generation azoles” is being seen, he adds. And, despite “occasional reports,” there is “nothing on a large scale” in terms of resistance to the more recently available candins, which make up the third class of agents used for treating systemic fungal infections in humans.

The candins target fungal cell wall synthesis, and three members of this family are licensed for U.S. clinical use, according to Annette Reboli of Cooper Hospital University Medical Center in Camden, N.J. “They have an excellent safety profile,” she says.

Meanwhile, the azoles interfere with specific steps in synthesis and thus disrupt fungal membranes. Last September, officials in the Food and Drug Administration (FDA) approved the latest member of the azole family—specifically, posaconazole, which is manufactured by Schering Corporation, Kenilworth, N.J., and marketed as Noxafil, according to symposium participant Andrew Ullmann of Klinikum Johannes Gutenberg-Universitaet in Mainz, Germany. The drug is licensed for use in preventing infections caused by Aspergillus and Candida among patients who have weakened immune systems following bone marrow transplants or chemotherapy.

“Despite having a number of new agents, the mortality rate for patients with fungal infections remains high,”
Staph Vaccine Proves Effective in Mice; Role of PVL Toxin Downgraded

An experimental vaccine containing four immunogenic surface proteins from *Staphylococcus aureus* protects mice from diverse strains of this bacterial pathogen that cause disease in humans, according to Olaf Schneewind of the University of Chicago in Chicago, Ill., and his collaborators. When administered separately, however, those four proteins afford only modest or no protection to the mice, the researchers note. Details of their findings appear in *Proceedings of the National Academy of Sciences* DOI:10.1073/PNAS.0606863103, 2006.

says Johan Mouton of Canisius Wilhelmina Hospital in Nijmegen, the Netherlands. That clinical fact has led some experts into thinking that “if one antifungal drug is good, two must be better,” he continues. “But that’s not always true.”

For instance, when tested against *Aspergillus*, combinations of amphotericin B and azoles sometimes appear to be “antagonistic” instead of synergistic, depending on how those tests are conducted in vitro or in animals, he points out. However, in patients, that same combination sometimes proves effective, and possibly synergistic, both he and Anaissie say.

“Combination therapy is very controversial right now,” says John Graybill of the University of Texas Health Science Center in San Antonio. He attributes some of this controversy to inconsistencies and other shortcomings of in vitro antifungal drug-testing methods. “I don’t think we can predict squat from fungicidal tests about how drugs will act in patients,” he says. “There is no good standardization, and a lot of disagreement over those tests.”

Mouton suggests that this and similar questions are probably best resolved by conducting controlled clinical trials instead of relying on animal experiments and anecdotal evidence from clinical settings. However, mustering adequate numbers of patients and working out agreements between competing drug companies on how to conduct those trials have proved difficult obstacles, he and Ullmann point out.

Another matter of recent concern comes from dealing with patients who are receiving monoclonal antibodies for therapy, in some cases as anti-inflammatory agents, according to Anaissie. Such patients belong to “a totally new population that was not at risk but now are developing significant fungal infections,” he says. Adds Reboli, “This is a disease of medical progress.”

Jeffrey L. Fox

Microfossil Data Show Yucatan Impact Did Not Wipe out Dinosaurs

Recent microfossil evidence casts fresh doubt as to whether an asteroid wiped out the dinosaurs, according to micropaleontologist Gerta Keller of Princeton University in Princeton, N.J., and her collaborators. Their analysis of ancient marine-dwelling microorganisms, called foraminifera, suggests that global warming caused by massive volcanism in India “led to dwindling of all species and a gradual decrease in their diversity beginning 400,000 years prior to the mass extinction,” she says.

This analysis challenges a hypothesis formulated more than 25 years ago by geologist Walter Alvarez of the University of California, Berkeley, his father Luis, a physicist who had won the Nobel Prize, and their collaborators. They argued that a catastrophic cosmic event, often called the Chicxulub impact, on the northern Yucatan Peninsula of Mexico led to the demise of the dinosaurs. They traced that impact to the Cretaceous Tertiary (KT) boundary period 65.5 million years ago and claimed it caused extinction of 60% of all life on Earth from dinosaurs to microscopic marine organisms such as the foraminifera.

“We now have evidence that the Chicxulub impact occurred about 300,000 years before the end of the Cretaceous and thus didn’t cause the mass extinction and, in fact, didn’t cause any species to go extinct,” Keller says, who presented her findings during the 2006 meeting of the Geological Society of America, held last October in Philadelphia, Pa. Thus, not only the foraminifera, but also amphibians, birds, insects, and dinosaurs apparently survived that impact.

Paleontologists analyze the foraminifera to infer and decipher ancient oxygen levels, salinity, nutrient conditions, and sea level changes in the oceans. Moreover, because marine microorganisms are sensitive to environmental stress, the foraminifera are good indicators for extinction events since they were likely the first to succumb to damaging environmental changes.

Keller and her collaborators obtained drill core samples from Chicxulub sediments along the Brazos River in Falls County, Tex., the same region where other researchers believe that the KT impact generated tsunami deposits. “Our recent work shows that the KT boundary... is up to 1.0 meter above the top of those storm events,” she says. Another 45–60 cm lower lies “the original Chicxulub impact glass layer...” Thus, these Brazos River sediments...
core samples contain evidence of “three distinct events well separated in time and apparently unrelated.”

Those drill core samples were crushed, analyzed, and examined for foraminifera. The findings suggest that global cooling led to a sea level drop from about 80 m to 30 m that apparently was more detrimental to foraminifera than was the Chicxulub impact, which occurred during the preceding warming. However, Keller says, it remains to be seen what really caused the mass extinction at the end of the Cretaceous period. It might have been another cosmic impact or the cumulative effects of rapid climatic and environmental changes.

Geologist Donald R. Prothero at the Occidental College in Los Angeles, Calif., is not surprised that new evidence leans away from the dinosaur impact extinction theory. “There is almost no further doubt among vertebrate paleontologists that birds are descended from dinosaurs,” he says. “If that is the case, then dinosaurs did not die out at the end of the Cretaceous after all. . . . Dinosaurs [as birds] are all around you!”

Barry E. DiGregorio
Barry E. DiGregorio is a science writer in Middleport, N.Y.

**Lactobacillus Engineered To Make, Deliver Microbicides In Situ**

Genetically modified commensal bacteria offer an unusual means for producing and delivering antiviral microbicides near or along specific organs where they are needed, according to Peter P. Lee at Stanford University in Stanford, Calif., and his collaborators. They engineered *Lactobacillus jensenii*, a commensal species that inhabits the vaginal tract of healthy women, to express cyanovirin-N (CV-N), a protein that can inhibit HIV. The idea is to use this novel microbiocide-producing commensal to protect women, who account for half of new cases, against becoming infected with HIV.

Researchers at the National Cancer Institute identified CV-N about a decade ago while screening natural substances for antiviral activity. Its source is the cyanobacterium *Nostoc ellipsosporum*. In their U.S. patent, which issued earlier this year, they describe CV-N as belonging to a class of proteins that they call cyanovirins. Within that class, CV-N potently inhibits HIV, according to Lee and his collaborators.

Although their bioengineered bac-
terium expresses CV-N at levels high enough to block HIV infections, it took five years before L. jensenii would do so. “We spent a lot of time understanding the vaginal microflora as well as how to genetically manipulate these human strains,” Lee says.

After building an efficient CV-N expression cassette, Lee and his collaborators inserted a single copy into the L. jensenii chromosome in a manner that stabilizes the gene, yet does not disrupt normal functions of the microbe. An endogenous promoter directs expression of the CV-N gene, while another endogenous signal sequence directs the bacterium to secrete the antiviral protein. Details describing their efforts appear in the October 2006 issue of Antimicrobial Agents and Chemotherapy (50: 3250–3259).

When this modified Lactobacillus colonizes mouse vaginas, it produces active CV-N, according to Lee. Additional experiments indicate that the engineered bacterium can also grow and release CV-N in the vaginal tracts of macaques.

Lee proposes using this or other bacteria to deliver therapeutic products to specific sites in the body. After colonizing a specific anatomic site, such engineered, commensal bacteria then would behave like local drug factories. In addition to expressing CV-N, the Lactobacillus system can be used to produce other peptides, antibodies, and vaccine antigens.

The vaginal delivery method could be used to target not only HIV, but other pathogens of this site, such as the virus responsible for herpes or the bacteria responsible for syphilis or gonorrhea. Or microbes that ordinarily inhabit the gastrointestinal tract could be engineered, for instance, to produce antibodies that bind inflammatory agents as a means for treating either irritable bowel syndrome or Crohn’s disease. “It’s a revolutionary way to think about the delivery of biologics,” says Lee, who with Gary Schoolnik, an infectious disease expert at Stanford, and HIV expert David Ho, cofounded the biotechnology company Osel, Inc., in Santa Clara, Calif., to develop such products.

This approach of using an engineered commensal to colonize the vaginal mucosa and produce an antiviral product locally would be self-renewing, relatively less expensive to manufacture, and thus possibly useful for treating women in developing as well as developed countries, according to Lee. He envisions formulating the engineered commensals in tablet form for shipping purposes. Once such a tablet is inserted into the vagina, engineered commensals would dissolve, grow, and produce CV-N.

Lee’s approach is “very promising in a field that desperately needs new strategies to zap HIV before it can be transmitted,” says David Katz, a professor of obstetrics and gynecology at Duke University in Durham, North Carolina. However, inserting a genetically modified organism into the human body is new territory, raising questions about safety and dosage as well as issues about removing the engineered commensals once they establish themselves in this niche. Moreover, political issues might become as important as those having to do with the safety of this drug-delivery approach, he says, noting: “We have trouble selling genetically engineered corn.” Lee counters, “Genetically engineered corn is not being used to stop HIV.”

Carol Potera
Carol Potera is a freelance writer in Great Falls, Mont.