Current Topics

Bacterial Flagellar Motor Analysis Provides Mechanical, Medical Insights

Bacterial flagellar motors are impressive devices that rotate at more than 100,000 rpm and have a power-to-weight ratio that is about 50 times that of a Porsche Carrera. An updated understanding of the flagellar motor switch—the part that changes the motor’s direction of rotation—“allows us to reach some really new and useful conclusions about [its] overall structure,” says David F. Blair of the University of Utah in Salt Lake City. He and his colleagues published their findings in _Journal of Bacteriology_ in January (189:305–312).

The flagellar motor spans the bacterial cell membrane, powering the bacterium with a propeller-like filament. The overall device has much in common with a mechanical or electric motor, says Blair, who tinkered with cars and motorcycles as a teenager. The modeling work not only provides insights about the mechanics of these devices, he points out. It also is medically relevant because flagellar motility plays a role in virulence, and “because the flagellum is related evolutionarily and structurally to systems used by gram-negative pathogens to inject virulence factors into host cells.”

Blair and his collaborators drew upon a large body of work to assemble their model. For example, several research groups, including Blair’s and Brian Crane’s at Cornell University in Ithaca, N.Y, obtained crystal structures that provide detailed views of individual pieces of the switch. Additionally, David DeRosiers of Brandeis University and his collaborators recently derived a high-resolution, partially three-dimensional structure of the motor that is based on electron microscopy. Their structural model depicts a rotor, drive shaft, universal joint, and bushing (_Microbe_, November 2006, p. 528).

One overarching question is how these component structures interact within the whole motor. In their current work, Blair and his collaborators selected for mutations in residues in the protein surfaces of the motor switch components to see how these affected function. They also performed biochemical studies to gain further insight into the protein-protein interactions within the motor’s switch complex.

On the level of nuts and bolts, interactions between the stator and the FliG proteins of the rotor (part of the switch complex) generate torque. In addition to about 25 copies of FliG, the rotor incorporates around 35 FliM and 140 FliN proteins. The FliG proteins have several conserved, charged residues that interact with similarly conserved but oppositely charged residues on the stator protein, MotA. The switch complex is also critical to chemotaxis, according to Blair. “A structure-based understanding of the switch complex will help to complete the picture of the signaling process,” he says.

“This model raises a number of questions,” the answers to which “must be found before we can say that we have attained an in-depth understanding of the relationship between flagellar structure and flagellar function,” says Michael D. Manson of Texas A&M University, College Sta-
tion, who wrote a guest commentary on the paper, enumerating seven of those questions. Nonetheless, he adds, “The solution offered by Brown [and his collaborators] is ingenious and compelling.”

**David Holzman**

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### Popular Stomach Acid-Blocking Drugs May Also Target Gingivitis

Proton pump-inhibiting drugs that take the bite out of stomach acidity also disarm bacteria associated with causing gingivitis, which can lead to tooth loss, and may provide a novel means for combating this condition, according to researchers at the University Rochester (UR) in Rochester, New York. They are focusing their investigations on the widely used family of antacid compounds called benzimidazoles that includes lansoprazole, which is marketed as Prevacid. Specifically, they find that lansoprazole disrupts *Fusobacterium nucleatum* and *Prevotella intermedia*, two oral anaerobes that help to trigger gingivitis, according to their report in the November 2006 issue of *Archives of Oral Biology*.

Among about 50 benzimidazoles with antacid activity, lansoprazole is readily available in pure form, according to study leader UR professor of microbiology and immunology Robert Marquis. Treating *F. nucleatum* cells with this agent reduces their production of glutamic acid, a key intermediate for forming ammonia, acetate, propionate, and butyrate, all of which can trigger inflammation in crevices between the teeth and gums, or gingiva. Much the same happens with *P. intermedia*, except that the affected metabolic intermediate is aspartic instead of glutamic acid.

Because these bacteria readily take up lansoprazole, incorporating benzimidazoles into toothpaste seems a feasible approach to target these or other anaerobic microbes that help to form dental plaque and give rise to gingivitis, according to Marquis. Several benzimidazoles also can block glycolysis and acid production in *Streptococcus mutans*, another bacterium found in the mouth, but one that is involved in causing dental caries instead of gingivitis. Details of those studies appeared in the April 2005 issue of *Oral Microbiology Immunology*.

“The advantage of benzimidazoles is that they become active when the pH drops,” says Marquis. The drugs work best below pH 5.0—the very conditions at which tooth enamel begins to erode and gums become inflamed. The capacities to produce acid and to operate at low pH are key virulence factors for microbes that incite gingivitis. Thus, benzimidazoles tend to concentrate in saliva and do some good,” Marquis says. However, benzimidazoles need to be delivered locally if they are to fight caries and gingivitis effectively. Meeting that challenge will entail convincing “someone to partner with us to develop an oral care product,” Marquis says. Fortunately, benzimidazoles tend to concentrate in dental plaque, he notes.

Likely vehicles for delivering benzimidazoles include conventional, albeit reformulated, versions of toothpaste and mouthwash. Many such commercial products now incorporate the antimicrobial agent triclosan, whose widespread use can help to select for antibiotic-resistant strains of bacteria. “If triclosan is banned, companies will need to look in other directions, and benzimidazoles are good candidates,” Marquis says.

“Inhibition of amino acid catabolism would be a worthwhile target, since many bacteria associated with gum disease depend on amino acids for energy and growth,” says Richard Ellen, a professor of dentistry at the University of Toronto in Ontario. Adding acid-loving benzimidazoles to toothpaste or mouthwash may prove particularly helpful for preventing...
bacterial growth before plaque forms, but less likely for treating existing gum disease, since the pH below the gumline tends to be alkaline, according to Ellen.

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Course Emulates, Updates van Niel Approach to Teaching Microbiology

About a generation ago, C. B. van Niel taught a special course in microbiology that reflected his interest in microbial diversity and his quest to identify fundamental biological principles (ASM News, August 2004, p. 258). In emulating van Niel’s approach, Alfred Spormann, Christopher Francis, Sebastian Behrens, and Paul Rainey are not claiming to duplicate his legendary course. But they surely invoked his spirit in their four-week summer course in microbiology at the Hopkins Marine Station in Pacific Grove, Calif.—taught last summer for the first time in 40 years, and scheduled for another round this coming July.

The students who once tromped through the marshes of Elkhorn Slough with van Niel as their teacher are now themselves close to retiring, says Spormann of Stanford University in Stanford, Calif. He spearheaded the 2006 version of the course along with Francis and Behrens, who are also from Stanford, and Rainey from the University of New Zealand, Auckland, in part to launch a successor generation along a modernized version of that pathway. When he taught the course, van Niel emphasized the unifying principles of physiology and biochemistry amid the microbial diversity that is abundant in marshes or other such environmental niches; he was then especially keen on investigating alternative photosynthetic processes.

“We’ve expanded this thinking...and taken elements that are reminiscent of van Niel, but would never dare to say we’re doing what he did,” Spormann says.

Thus, the updated course aims to “integrate physiology, ecology and evolution, and microbial population biology,” Spormann says. “The course is different from the van Niel course in terms of topic and in adding integrated physiological ecological and evolutionary thinking, [but] not so much in terms of our quest to find organizing principles in the microbial world.” For instance, students now conduct extensive genomic analyses on the microorganisms that they recover from their recent excursions into environments that van Niel and his students also once visited.

Moreover, amid a heavy schedule of classroom lectures and seminars delivered by a dozen guest lecturers, “the students do plenty of experimentation looking for mechanistic links to what causes and preserves diversity,” Spormann says. On a typical day, students appear at 8:30 AM and do not leave before 10 PM, if not later; often, the group ate dinner together to unwind before returning to the lab. “It’s a marathon,” he says.

Last summer, the overall approach included the students doing population genetics-based analyses of freshly isolated marine vibrios and then examining five genetic markers and doing “substantial sequence analysis,” Spormann continues. In analyzing the variability of those marker genes, the students discerned that there is “local population structure” within the microbial isolates along with evidence for “massive lateral gene transfers,” he notes. “That’s exciting and new, and helps them to put what they learn about the physiological function of these microorganisms into an environmental context.”

Several of the students from the 2006 session shared their enthusiasm for the intensive summer experience months later: “The course itself was a whirlwind tour of microbiology,” says Geoffrey Dilly, a graduate stu-
Spiky Polymers Puncture and Kill Bacteria, Viruses

Polymers that are highly effective at deactivating bacteria and viruses on surfaces might someday be used to make microbicidal “paints” for curbing transmission of infectious diseases in hospitals and other settings, according to Alexander Klibanov at the Massachusetts Institute of Technology in Cambridge, Mass. He and his collaborators recently developed a set of such polymers that they are continuing to evaluate for their microbicidal properties.

The polymers, collectively a series of N,N-dodecyl methylpolyethyleneimines, buckle and fold when painted onto smooth surfaces, forming what Klibanov calls a molecular cityscape, in which metaphorical “skyscrapers” protrude from surrounding “streets” and lower “buildings.” Those protruding spikes apparently account for the microbicidal effectiveness of the polymers, he says. “Spikes, whiskers, whatever they’re called, there are sharp segments that stick out and can damage bacterial cell membranes, and presumably this same mechanism works on viruses.”

Klibanov and his colleagues test these and other polymers by painting them onto glass slides, then placing droplets of suspended viruses or bacteria on the slides to mimic mucus-containing spray from infected individuals. After incubating the slides at room temperature for varying periods, the researchers rinse them and count the bacteria and viruses that are washed away. Remarkably, the best of the polymers kill virtually 100% of the bacteria and viruses after exposures as brief as five minutes.

The microorganisms aren’t just “tangled up” or “absorbed” by the polymers—they are killed or inactivated, according to Klibanov. Incubating slides coated with polymer and bacteria beneath a layer of agar growth medium revealed little to no growth. The same held true for the virus-treated slides.

Klibanov says the polymer-painted surfaces can eventually become overloaded. “At some point . . . dead bacteria and viruses will fill the surface,” he says. However, “if you take a soapy sponge and you wash it, it will be as good as new.” What’s more, the polymer paints remain effective for long periods. “For practical purposes, they behave the same way as oil paint,” he says. “[They don’t] wash off.”

Researchers Delve into Antibacterial Properties of Particular French Clays

The late Line Brunet de Courssou used clays imported from France to treat her West African patients who had Buruli ulcers, which is now considered “an emerging public health threat” by the World Health Organization. Like leprosy, such ulcers, which are caused by Mycobacteria ulcerans, destroy skin as well as tissues under it. To understand how clays may exert antibacterial activity, microbiologist Shelley Hayden and geochemist Lynda Williams of Arizona State University in Tempe are investigating the effects of such clays on this and other pathogens. For instance, they find that one such clay inhibits Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Mycobacterium marinum, and Staphylococcus aureus, albeit to a lesser extent. Clays have crystalline structures that make them “both flexible and fluid.” Typically, they have two-nanometer-thick, silicate layers with tetrahedral rings bounding an octahedral core that contains an “interlayer” of charged cations as well as uncharged organic compounds, Williams says. Although recent results suggest that the antibacterial activity is associated with the interlayer, crystal size and structure also seem to play a role, she notes.

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Positively charged sites on the polymers apparently play a significant role in their lethality, since similarly constructed neutral and negatively charged polymers are markedly less effective than positively charged and zwitterionic (mixed-charged) polymers. Moreover, some polymers are microbicidal in part because a fraction of the molecules dissolve in the bacteria- or virus-laden buffer applied to the slides. However, all the activity of the most promising polymer stems from molecules deposited on surfaces.

Although some of the solvents used to suspend and then apply these polymers may be toxic to humans, they evaporate soon after application—much like the solvents in regular paint, making them relatively safe, assuming adequate ventilation is available, Klibanov says. The polymers themselves are not toxic according to preliminary data, and further testing hopefully will bear this out, he adds.

Jonathan Dordick, the Howard P. Isermann Professor of Chemical and Biological Engineering at Rensselaer Polytechnic Institute in Troy, N.Y., says that Klibanov’s approach to creating a microbicidal surface is an elegant one. Although the polymer “paint” needs to be cleaned periodically, it “allows a surface to be self-cleaning for some percent of the time, which would be long enough to act as a preventive measure” against the transmission of infectious diseases, Dordick says. “From that perspective it can be very useful.”

Although it is unlikely that such polymers will ever be stocked at stores, there is a need for such microbicidal products in hospitals, where controlling patient-to-patient transmission of bacteria and viruses is a serious challenge. It may even be possible to use polymer paints in other highly trafficked public areas such as airplanes, Klibanov says. Additional details about this research can be found in the November 21, 2006 issue of the Proceedings of the National Academy of Sciences.

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Experts Urge—and Relish—Studying Microbes from Sea to Shining Sea

“Microorganisms are a major biological force influencing the biogeochemical state of the oceans,” says Farooq Azam of the Scripps Institution of Oceanography at the University of California, San Diego. They are also “a major part of the global food web, and their dynamics . . . with other organisms are highly variable.” Acknowledging that a wholesale effort to study microorganisms on the scale at which they influence oceans and global nutrient cycles is “a major challenge,” he calls for a spending initiative on the same order of “what we spend for research on human health.”

Azam spoke along with several other experts in environmental microbiology and related topics during a seminar, “Microbes, Minerals, and the Environment,” sponsored by the American Association for the Advancement of Science, and held in Washington, D.C., last October. The series honors the late Philip Abelson, who was editor of Science for several decades.

Delving into the dynamics of oceanic microbial life could prove critical in the face of plans to use the oceans for sopping up excess atmospheric carbon dioxide as one way to fend off global warming and climate change. One such plan calls for enriching the oceans with iron to foster blooms of photosynthetic plankton that would absorb carbon dioxide while producing biomass.

However, Azam cautions, “We have a poor ability to prognosticate what happens if we enrich the oceans with iron. Would we create a useful bloom or a polluted ocean?” He cites several microbial “surprises” when open waters in the eastern Mediterranean and Adriatic Seas were enriched with particular chemicals. Thus, instead of an expected bloom of phytoplankton when the Mediterranean was enriched with phosphorous, there was a 50% reduction in chlorophyll, he says. And, although large patches of organic matter accumulate in the north Adriatic every few years, microbes there fail to use that material.

Phytoplankton Blooms May Promote Cloud Formation

Phytoplankton blooms appear to promote cloud formation over the Southern Ocean, according to Nicholas Meskhidze and Athanasios Nenes of the Georgia Institute of Technology in Atlanta, who report their findings in the 1 December 2006 Science. Using remotely sensed data, they determined that cloud droplet number concentrations are twice as high over phytoplankton blooms as they are at sites away from those blooms, and they attributed these and other changes to the size distribution and chemical composition of cloud condensation nuclei that form. The researchers further propose that secondary organic aerosols, which form when phytoplankton-produced molecules of isoprene are oxidized, “affect chemical composition of marine cloud condensation nuclei and influence cloud droplet number.”
A recently identified deep-sea acidophile, a member of the deep-sea hydrothermal vent euryarchaeotic group 2 (DHVE2), nicknamed “little devil blob” for the horn-like protuberances among their outer surfaces (provisional name, Aciduliprofundum boonei). (Image courtesy of Terry Beveridge, University of Guelph, Guelph, Ontario, Canada.)

“Could this happen to large regions if we fertilized the oceans?” he asks.

Such notions of engineering the oceans with iron to capture excess atmospheric carbon dioxide, although fraught with uncertainties and far too premature to implement, are leading researchers to learn much more about the environmental science that underlies such schemes, according to seminar participant David Keith, a policy analyst from the University of Calgary in Calgary, Alberta, Canada. Indeed, short of the global analytic effort that Azam recommends, smaller-scale research efforts are plentiful, and they are furnishing plenty of fundamental surprises, albeit on a more modest scale.

Some of those surprises have to do with the unusual nature of some oceanic microorganisms. For instance, although in general the diversity of microorganisms in the oceans is “incredible,” the same cannot be said for marine-dwelling, nitrogen-fixing cyanobacteria, says Jonathan Zehr of the University of California, Santa Cruz. They are not only “much less abundant” than other microbes but also a good deal less diverse in terms of their genetic composition. He says that their relative uniformity suggests that they were subject to “a new mode of genetic evolution,” that apparently depends on rearrangements of genetic material. An analysis of genomic fragments from mixtures of such microorganisms reveals that they are “99% identical, even though we expected a lot of genetic diversity.” This relative uniformity extends to similar samples that were collected more than 20 years ago.

At least until recently, another surprise was the seeming absence of acidophiles near deep-sea vents, according to Anna-Louise Reysenbach of Portland State University in Portland, Oregon. However, she and her collaborators identified what she calls the “first true acidophile” from this niche, namely, a member of the deep-sea hydrothermal vent euryarchaeotic group 2 (DHVE2), whose provisional name is Aciduliprofundum boonei—or, more affectionately, “little devil blob.” This nickname reflects the distinctive, horn-like protuberances that appear along the outer surface of such cells (see micrograph).

Like many other archaea, this species is “enveloped by a plasma membrane and a single S-layer [that] is unusually thick, about 40nm,” Reysenbach says. Unlike S-layers in other microorganisms, “this S-layer bends into small, highly curved structures. . . It is possible that these vesicles . . . anneal to adjacent cells to convey cell substances to them, a process that is common among gram-negative bacterial counterparts.” Because this species accounts for about 15% of the archael population around such sea vents, it could well be a “key player” in the iron and sulfur cycling that occurs in this environmental niche.

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U.S.-Russian Group Eyes Bacteriocins for Protecting Poultry

With antibiotic supplements in animal feed under increasing scrutiny, researchers and other officials of the U.S. Department of Agriculture (USDA) are actively pursuing bacteriocins as alternative means for attaining fast and healthy growth of chickens and other birds for U.S. poultry producers. These research and development efforts include an active collaboration with Russian scientists, who are involved in discovering and screening these polypeptides that are active against several key bacterial pathogens associated with poultry, according to USDA officials.

Several specific bacterial species, including Salmonella enterica, various strains of Escherichia coli and Campylobacter, and Clostridium perfringens, are responsible for ongoing problems at poultry operations in the United States and elsewhere, according to Bruce Seal of the Poultry Microbiological Safety Research Unit at the USDA Russell Research Center in Athens, Ga. He spoke late in 2006 during one in a series of International Research Seminars sponsored by the Agricultural Research Service that are held at its facilities in Beltsville, Md.

Although levels of such bacteria are controlled in part by antibiotic supplements in feeds, increases in drug resistance put that practice under heightened scrutiny and make it a “driving force” behind the search for alternative antibacterial products such as bacteriocins, Seal says. The volume of U.S. poultry operations—for example, more than 9 billion broiler chickens were processed during 2005, yielding more than 35 billion pounds
of meat—makes microbial control a substantial challenge for producers working in this sector.

Bacteriocins, which work by punching holes in the membranes of target bacteria, are antimicrobial polypeptides ranging in size from about 2,000 to 6,000 in molecular weight. They tend to be hardy molecules, and thus resist heat and detergents but are susceptible to the hydrolytic effects of proteases, according to Seal’s colleague Norman Stern.

With their Russian collaborators, the USDA researchers identified several new class 2 bacteriocins that are highly active against bacteria that contaminate poultry, according to Stern. One of these bacteriocins is particularly effective and “can kill highly refractile [Campylobacter] bacteria when tested in live birds,” he says. These treatments spare other commensals such as lactic acid-producing bacteria. In tests involving the pathogen Salmonella enteritidis, treatments with other bacteriocins could “control high levels of [these bacteria that] colonize the liver and spleen,” he points out. The bacteriocins are “lethal to target bacteria,” including those that are arrayed on mucosal surfaces and some that are resistant to antibiotics.

One market sector where bacteriocins might prove useful is for treating otherwise “drug-free” birds, according to Greg Siragusa, who also is from the USDA Russell Research Center in Georgia. Withdrawing antibiotic growth promoters from the feed of broiler chickens can lead to sharp increases in colonization by C. perfringens, outbreaks of necrotic enteritis, and skyrocketing mortality rates, he says. But treating such birds with antibiotics means that they no longer can be marketed as “drug-free,” and that can lead to economic losses for producers.

Efforts are under way to scale up production of these bacteriocins for field trials, including at poultry facilities in Obolensk in Russia, according to Eric Line, another collaborator with the USDA group. “The poultry house environment is not clean, and birds peck at each others’ droppings and are exposed to wild birds, rodents, and insects,” he says. Nonetheless, such settings are the “real world” of large-scale poultry production—and establishing bacteriocin effectiveness there will be a real test of whether they can prove useful for promoting the growth of healthy chickens for consumer markets.

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