Current Topics

*B. anthracis* and Its Phage: Surprising Dynamics In Soil

*Bacillus anthracis* in soil is nowhere near so quiescent as some scientists think, say Raymond Schuch and Vincent A. Fischetti of Rockefeller University in New York City, who challenge the long-held belief that *B. anthracis* safely sequester as spores outside their mammalian hosts. Their research on phage-mediated gene transfer reveals that this bacterium interacts far more dynamically with its neighbors in soil than previously assumed.

This startling discovery emerged from Schuch and Fischetti’s recent genomic and functional analysis of two historically important phages, *Wß* and *γ*. Their intent was to assess how lysogeny contributes to the phenotype of *B. anthracis*. They were particularly interested in whether the *Wß* and *γ* phage helped drive its ecological adaption. *B. anthracis* is a single, genetically homogeneous group in an otherwise heterogeneous *B. cereus* lineage that also includes *B. thuringiensis*. They reasoned that emergence of a fierce animal pathogen from this fairly benign family had to depend on more than its acquisition of the pXO1 and pXO2 plasmids, which encode antihost toxins and capsular structure.

Further, phage are known shapers of bacterial genomes, and both *B. anthracis* and *B. cereus* have more than their fair share of phage. Among the morphologically and genetically diverse phage that these bacilli harbor is a family of tailed, double-stranded members of the *Siphoviridae* that are very much like the *γ* lytic phage.

Schuch and Fischetti suspected that the *γ* phage they were investigating derived from a parental *Wß*, with its genetics reflecting a different lifestyle—lytic versus lysogenic, respectively. And *Wß* was conveniently encoded in *B. cereus* ATCC 11950, an “atypical” soil strain closely related to *B. anthracis*.

However, standard methods failed to induce this phage from its lysogenic state in the *B. cereus* chromosome. Fortuitously, a separate study of fosfomycin resistance (*Fos*) in *B. cereus* enabled them to induce, isolate, and purify *Wß*, which proved to be morphologically identical to *γ*. Additional investigation showed that the *γ* lytic phage evolved from the temperate *Wß* through mutations at key loci controlling host recognition, lysogenic growth and, perhaps, phenotypic host modification. Furthermore, the *γ* phage’s shift from a temperate to a lytic lifestyle was traced to a large 2,003-bp deletion in the *Wß* lysogeny module.

Other analysis shows that *Wß* is...
very similar in gene order and sequence to B. anthracis prophages that can recombine to create hybrid phage that are like γ. Additional molecular evidence revealed at least three phage proteins that appear to be part of the spore surface structure. And while genes encoding surface proteins and antibiotic resistance may not be virulence factors in the classic sense, they can help B. anthracis better survive within the highly competitive soil environment.

The fact that a phage encodes fosfomycin resistance has several important implications. For one thing, soil-dwelling bacteria produce and encounter a myriad of antibiotics, countering them with various sensing and evading strategies that amount to a huge reservoir of antibiotic resistance. Phage-mediated transfer of Fosr shows that the horizontal movement of antibiotic resistance is not restricted to plasmid, transposon, gene cassette, and integron vehicles or transduction and transformation—at least not in the soil environment. Furthermore, the induction of Wβ from its lysogenic state by fosfomycin appears ecologically important because its lytic pathway genes sit near a consensus antibiotic-inducible σW promoter.

But the pivotal finding of this investigation is that genes travel between infecting phage and chromosome in B. anthracis, and that spore antigens and resistance mechanisms move around in the process. Indeed, say Schuch and Fischetti, the very fact that phage can acquire and transmit growth-associated characteristics offers proof that B. anthracis undergoes vegetative growth outside mammalian hosts. Details of their findings appear in the April issue of the Journal of Bacteriology (188:3037–3051).

Marcia Stone
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Phage Provide Templates for Fashioning Conductive Nanowires

Angela M. Belcher and her collaborators at Massachusetts Institute of Technology (MIT) in Cambridge are using genetically engineered viruses to build durable nanowires for use in highly miniaturized electrodes. These wires could be used as key components in specialized lithium ion batteries to power a range of devices, including internally carried medical monitoring instruments and hearing aids or laptop computers and other battery-powered equipment considered vital for modern warfare. Details of the research appear in the April 6 online version of Science.

The M13 phage provides a template for building these nanowires. Unlike icosahedral phages, M13 is long and—at 880 by 6 nm—wiry. The first step in building such wires involves attaching cobalt oxide, an efficient conductor, uniformly to phage surfaces. To do this, Belcher needed to identify a peptide that binds the compound and could then be genetically engineered into the phage coat protein. Not taking any chances of missing good candidates, she used a technique called “phage display,” attaching many different peptides onto millions of phages and then testing their affinities for cobalt oxide, to find those that form the strongest bonds.

Belcher then established conditions under which wires self-assemble into an electrode on the surface of a polymer that serves as the electrolyte. “They self assemble naturally, based on packing,” she says.

Once Belcher made the electrodes, she ran them through many charge and discharge cycles to test their durability. After 30 cycles, their specific capacity had begun to fade. Not satisfied with this quick loss in performance, Belcher considered ways of boosting durability, figuring that, while cobalt oxide is a good ionic conductor, gold is a good electrical conductor. Perhaps the two would be complementary. Furthermore, because the MIT group already identified a virus-based peptide sequence that binds gold, she and her collaborators added gold to the wires and found that the gold-cobalt oxide combination in nanowires forestalls fade and enhances charging capacity. The
MIT group plans to determine how many cycles the gold-containing nanowires can endure as a critical step towards eventually commercializing such batteries.

Belcher's interest—and prowess—in making nanowires with phage stems from earlier efforts to learn how abalones grow shells, which in composition resemble pearls. Thus, abalone shells consist of very hard, highly uniform, lightweight materials. Critically, such shells include proteins, amounting to 2% of their mass, which is mainly calcium carbonate, and which she found are key to constructing the shell. When she put them in a beaker with calcium carbonate, they produced nanoscale shells. "I started thinking about how proteins in nature control inorganic materials, but the list is very short: calcium oxide, calcium phosphate, iron oxide, and silica," she says. "I thought, wouldn't it be great if organisms could work with the rest of the periodic table." Since then, she has worked on semiconductors and magnetic materials.

These disparate lines of research coalesced in part because the Department of Defense, which funds some of Belcher's research, is seeking new designs for batteries, which account for much of the weight that modern soldiers are required to shoulder. Belcher recognized that metal oxides, which are compatible with biological synthesis, would be good choices for use as lightweight electrode and wire materials in batteries.

"This paper establishes proof-of-principle for applying phage and nanoparticle assembly towards electric storage," says Renata Pasqualini of the University of Texas M.D. Anderson Cancer Center in Houston, who has used M13 virus and gold nanoparticles to develop devices that can home on specific tissues, or provide scaffolding to grow cells (Microbe, May 2006, p. 214). However, she adds, "One wonders whether comparable results might be obtained by a much simpler chemical method. Such an approach would eliminate the need for targeted assembly subsequent to a phage library selection aimed at isolating peptides that bind to gold nanoparticles. Side-by-side comparison will answer this question."

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

Delving into Traditional Chinese Medicine To Seek “New” Drug Structures

Traditional medicines from China and India deserve a careful review, particularly for their value as "scaffolds" for developing new drug products, including antimicrobial agents, according to Norton Peet, an international research and development consultant who is based in North Andover, Mass. The antimalarial drug artemisinin is perhaps the best example of an already useful product being examined and redesigned to enhance its activity, he says. Peet was one of several experts touting the value of this approach, who spoke during the PharmaDiscovery Conference, held in Bethesda, Md., last May.

These exhortations to delve more deeply into medicines prized by traditional Eastern practitioners come as China is proving adept at adapting to very modern technologies and industries, according to Peet. For example, China is rapidly expanding its chemical industry, whose shipments more than doubled in value between 1993 and 2003, grew to more than $151 billion per year in 2005, and will likely more than double again during the next decade, he says. This growth in chemical exports is accompanied by steady productivity and increasing sophistication, as judged by the expanding volume of publishing by Chinese scientists, he points out.

The use of artemisinin, a sesquiterpene lactone that is isolated from the shrub Artemisia annua, also known as sweet wormwood, can be traced back some 1,500 years in traditional Chinese medicine, according to Peet. At one time used for treating hemorrhoids, artemisinin now is widely used to combat malaria, a disease that leads to millions of deaths per year, largely in sub-Saharan Africa. Although artemisinin is effective against drug-resistant forms of malaria, the search is on for improved versions, according to Jonathan Vennerstrom of the University of Nebraska Medical Center in Omaha. Several features of

Rice Gene Precariously Balances Fertility against Disease Resistance

Expression of a single disease resistance gene in rice, designated xa13, helps to tip the balance between fertility of the plants and their ability to withstand microbial pathogens, according to an international group of researchers led by Shiping Wang of Huazhong Agricultural University in China. Thus, the recessive xa13 allele provides resistance to bacterial leaf blight, which rice experts consider the most devastating bacterial plant disease in the world. However, although expression of the dominant Xa13 allele makes plants more susceptible to this and other diseases, it also promotes development of pollen. This positive effect of Xa13 on plant fertility likely explains why this allele remains in the rice gene pool, the researchers point out. Details of their findings appear in the May 15 issue of Genes & Development.
the molecule are important, particularly an internally stabilized peroxide. When used to treat patients with malaria, artemisinin appears to recognize heme, the oxygen-binding structure embedded in hemoglobin of red blood cells, a principal target of malaria parasites. The peroxide enables artemisinin to alkylate heme and thus interfere with its role in supporting parasites within such cells.

Several simpler, synthetic artemisinin-based knockoffs are being evaluated as alternatives to the natural product, including a trioxolane candidate that Vennerstorm and his collaborators described in 2004, he continues. The synthetic tetra-substituted ozonide has “high intrinsic activity against parasites” and, when tested in mice infected with parasites, leads to “some cures.” The candidate drug is in the midst of a phase 2 clinical trial that is being sponsored by Ranbaxy Laboratories Limited, a pharmaceutical company that is based in India. He expects that, if successful in clinical trials, the drug “will be used as a single dose over three days...[which] should allow shorter treatments.” It is active when administered orally and does not appear to trigger development of resistance.

Berberine, a five-membered ring alkaloid that is extracted from the roots of a variety of plants, including golden seal, Oregon grape, and tree turmeric, is another potentially promising traditional medicinal, according to Tim Birdsall, a practitioner of naturopathic medicine who is a vice president of integrative medicine for the Cancer Treatment Centers of America, which are located in several regions of the United States. Berberine, which was used in traditional Chinese, Indian, and Native American medicine, is being studied for a range of activities, including antimicrobial, antitumor, antidiarrheal, and cholesterol-lowering, he says. Of these, perhaps the most promising is its activity against *Giardia lamblia*, a parasite that causes diarrhea.

**Jeffrey L. Fox**

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**Oral, Plant-Based Vaccine against Shiga Toxin Effective in Mice**

An orally administered, plant-produced, inactivated version of the *Escherichia coli* Shiga toxin protects mice against hemolytic uremic syndrome, according to Alison O’Brien of the Uniformed Services University of the Health Sciences (USUHS) in Bethesda, Md., and her collaborators. They call these results an encouraging step toward developing an edible, plant-based vaccine to protect humans against the sometimes deadly hemolytic uremic syndrome.

Their research is part of a broader effort to make vaccines to protect against toxigenic forms of *E. coli*, particularly *E. coli* O157:H7, which was the culprit pathogen that caused a deadly outbreak among adults and children in several states during the early 1990s after consumers ate contaminated, undercooked hamburgers from fast-food restaurants. Shiga toxin--producing *E. coli* including O157: H7, sicken some 100,000 Americans annually. Approximately 6,000 of these cases develop into hemolytic uremic syndrome, the main cause of life-threatening kidney failure in children.

Cattle are the natural reservoir for some of these toxigenic serotypes. O’Brien and her collaborators earlier developed a vaccine that interferes with bacterial adherence to host cells. Aimed at blocking colonization of reservoir animals and, therefore, reducing their capacity to transmit strains such as *E. coli* O157:H7 to humans, that vaccine reduced *E. coli* colonization of mice.

Meanwhile, the newer vaccine aims directly at protecting recipients against the ill effects of consuming and becoming infected with such toxigenic strains. This experimental vaccine uses a nicotine-deficient and, thus, nontoxic tobacco cell line that is engineered to produce a genetically “crippled,” or toxoid, version of Shiga toxin type 2, the more dangerous of the two types of Shiga toxin.

Although not particularly palatable, the vaccine was fed to mice “with the help of a little sucrose,” according to Sharon Wen of the USUHS group, whose findings appear in the May 2 issue of the *Proceedings of the National Academy of Science*. One group of mice was fed tobacco cells containing the Shiga toxoid, while members of another group were
IOM Panel Sees No Easy Way To Reuse Face Masks If Flu Pandemic Hits

There is no simple, reliable way to decontaminate face masks, meaning these devices cannot readily be worn more than once in the event of a major influenza outbreak or pandemic, according to a panel convened by the Institute of Medicine (IOM), part of the National Academies in Washington, D.C. (Microbe, April 2006, p. 165). “Even the best respirator or surgical mask will do little to protect a person who uses it incorrectly, and we know relatively little about how effective these devices will be against flu even when they are used correctly,” says IOM panel cochair Donald S. Burke, professor of international health and epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Md. “Substantial research must be done to increase our understanding of how flu spreads, develop better masks and respirators, and make it easier to decontaminate them.”

When the mice were challenged by drinking a liquid that contained the mouse-lethal *E. coli* serotype O91: H21, all 10 mice that received the five-day oral course of the toxoid-based vaccine and also 8 of the 10 mice that were inoculated and then orally boosted lived for up to 12 days, whereas all the mice that received only sucrose were dead within a week. Two of the mice that received toxoid-free tobacco cells survived a bit longer, possibly because *E. coli* are part of their gut flora, according to Wen. In any case, only those mice that received the experimental vaccine contained Shiga toxoid-specific antibodies in their feces.

“We feel that the systematic immunity against Shiga toxin type 2 that we observed indicates that an oral plant-based vaccine shows promise for protection against the severe, sometimes life-threatening, consequences of Shiga toxin-producing *E. coli* infection, against which no other suitable treatments or therapies are currently available,” O’Brien, Wen, and their collaborators conclude.

“This paper is significant,” says Charles Arntzen, codirector of the Center for Infectious Diseases and Vaccinology at Arizona State University, Tempe. The strategy of using purified components of plant tissue that includes an expressed vaccine “is good for oral delivery.” However, he says about the experimental vaccine, “Just because it’s technically capable doesn’t mean it will lead to a vaccine. Somebody has to cover the [estimated $50–$100 million] cost” of full commercial development.

“Because we are a nonprofit research institute, we are limited in our ability to manufacture such a vaccine,” Wen says. Nonetheless, O’Brien and colleagues plan to continue studying the plant-based vaccine approach for other antigens, including Shiga toxin type 1. Tailoring the tobacco-based vaccines for humans would entail extracting the active ingredient from tobacco cells, a strategy Arntzen now favors (Microbe, June 2006, p. 265) for plant-based vaccines, or perhaps engineering a more palatable fruit or vegetable to produce the toxoid version of Shiga toxin.

**Brian Hoyle**
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**C. crescentus Cells Produce an Extraordinarily Potent Adhesive**

*Caulobacter crescentus*, a gram-negative bacterium that is widely distributed in aquatic environments, makes an extraordinary adhesive—for now, considered the strongest of biological origin and also exceeding the shear strength of some commercial super glues, according to bacteriologist Yves Brun at Indiana University in Bloomington. The polysaccharide-based cement is secreted through a holdfast region at the far end of each cell’s long, slender stalk, he says. “We weren’t looking for a glue; it just came out of basic research into the mechanisms of cell differentiation and adhesion.”

When Brun affixed single cells to glass coverslips, he found that they cling so tightly that high-pressure water cannot knock them off. “A major challenge was finding a way to measure the force of attachment because it was so strong,” he says. So he teamed up with physicist Jay Tang at Brown University in Providence, R.I.

One relatively new and accurate means for measuring the binding strengths of objects on a microscopic level uses laser tweezers (see page 330). Their upper limit is about 100 picoNewtons (pN), which typically is more than adequate for releasing bacteria from surfaces to which they adhere.

However, when laser tweezers failed to budge *C. crescentus* cells, the team devised a clever micromanipulation method. They first attach bacterial cells to a thin flexible pipette through their stalks. Then the re-
In contrast, a shear force of 68 N/mm² greatly reduces the adhesive property of the bacterial glue, which is required to detach *C. crescentus* from pipette surfaces, according to Tang and Brun, whose findings are detailed in the April 11, 2006 issue of the Proceedings of the National Academy of Sciences.

The *C. crescentus* glue is even stronger than the adhesive force of setae, or bristles, protruding from the toes of geckos that enable them to run upside down across ceilings. Setae depend on strong van der Waal interactions, and are considered one of the strongest biological adhesive mechanisms. Nonetheless, gecko adhesion fails when subjected to a shear force of a mere 10 N/mm², making it nearly sevenfold weaker than that of *C. crescentus*. Other sticky microbes, such as sulfate-reducing bacteria and *Bacillus mycoides* spores, produce adhesive forces of less than 1 N/mm². Commercial dental cement bonds at strengths of up to 30 n/mm².

Thus, the strength of *C. crescentus* exceeds all of these. “Given microbial diversity, I doubt this is the strongest glue out there,” Brun says. He plans to isolate other surface-adhering Caulobacter species to evaluate the relative strength of their glues.

Brun also is learning more about the biophysical mechanisms that give the *C. crescentus* adhesive its strength. He already knows that one critical component is N-acetyl glucosamine. Indeed, treating *C. crescentus* with lysozyme, which degrades polymers containing N-acetyl glucosamine, greatly reduces the adhesive properties of the cells. Brun suspects that polysaccharides containing N-acetyl glucosamine serve as support structures for other adhesive molecules, which he is seeking to identify and characterize. However, he says, “We’re having trouble getting glue off surfaces to analyze it.”

Potential commercial applications of this bacterial adhesive, which works on wet surfaces, include surgical glue or as a coating to prevent fouling by biofilms on surfaces, such as ship hulls. Although polyethylene glycol (PEG) coatings can prevent biofouling, PEG does not adhere well to surfaces. Possibly the bacterial glue may be formulated with PEG to improve its ability to coat surfaces and, in turn, better prevent biofilms from forming.

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**Environments Profoundly Shape Bacterial Gene Expression Patterns**

Bacterial species adapt to new environments by changing gene expression much more than by changing the genes themselves, according to L. Aravind of the National Center for Biotechnology (NCB), National Institutes of Health, Bethesda, Md.; M. Madan Babu, who is visiting the NCB, and Sarah Teichmann of the MRC Laboratory of Molecular Biology at the University of Cambridge in Cambridge, United Kingdom. Based on data from 174 microbial genomes containing about 500,000 protein sequences, they and their collaborators conclude that regulatory networks of different bacterial species converge when those species occupy similar environments.

“We found that at every level of organization, the shape of the transcription network structure has been determined primarily by the environment in which the organism lives,” says Madan Babu. Thus, seemingly similar bacteria are finely tuned for the very specialized niches that they occupy, even when those niches all happen to be within a single species, such as humans.

For example, Madan Babu points out, “*E. coli* normally lives in a relatively stable environment, where it makes sense not to switch to anaerobic respiration in response to small

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**Distinctive Quorum Sensing in Yeast**

Like many types of bacteria, yeast cells communicate among themselves through quorum sensing, according to Hao Chen and Gerald Fink of the Massachusetts Institute of Technology in Cambridge, Mass. However, the mechanistic details of this chemically mediated self-control pathway in *Saccharomyces cerevisiae* not only are distinct from those in bacteria but also differ in some ways from those in the yeast *Candida albicans*, they report in the April 17 issue of *Genes & Development*. For instance, *S. cerevisiae* cells use aromatic alcohols as signals to stimulate filamentous growth when they are starved for nitrogen. However, these alcohols do not elicit a morphologic shift in *C. albicans*, indicating that these fungal quorum-sensing signals are species-specific. “The ability of these quorum-sensing molecules to stimulate growth or alter morphology could be important in pathogen virulence where the infecting organism is initially present in only small numbers of cells,” Fink points out.

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changes in oxygen concentration.” Meanwhile, *Haemophilus influenzae* travels through arterial and venous blood when it infects a human, and adapts quickly to changes in oxygen levels. Analysis indicates that the *E. coli* transcription network is insensitive to small fluxes, whereas the *H. influenzae* network is hypersensitive and has a single transcription factor that controls multiple genes.

“The evidence for convergent evolution towards similar networks is particularly strong evidence that the networks are highly flexible and strongly selected for optimal network structures,” says Eric Harvill of the Pennsylvania State University, University Park.

Transcription factors appear to be “less conserved than their target genes” and “evolve independently of them,” note Aravind and Madan Babu. Moreover, different microbial species contain “distinct repertoires of transcription factors responding to specific signals.” They infer that microbial transcriptional regulatory networks arose “principally through widespread tinkering with transcriptional interactions at the local level by embedding orthologous genes in different types of regulatory motifs.” Details of their findings appear in the April 28 *Journal of Molecular Biology*. In earlier work, Teichmann and Madan Babu analyzed protein sequences in the *E. coli* and yeast transcriptional networks. They concluded that various features of these networks, such as network motifs, evolved convergently rather than by duplication of existing regulatory circuits.

The recent study at NCB involved developing computational procedures to reconstruct and compare these many networks, to predict what the transcription factors are and, in some cases, what genes they regulate, for the 174 prokaryotes, according to Aravind and Madan Babu. The *E. coli* network, which was already mapped, served as a reference. Additional information about this computational approach is available at http://www.mrc-lmb.cam.ac.uk/genomes/madanm/evdy/.

This computational approach, if augmented with experiments using chromatin immunoprecipitation chips to map protein-DNA contacts on a genomic scale, could be used to improve understanding of virulence gene regulation, according to Madan Babu. “If you can identify groups of genes that are regulated by the same transcription factors, you know they are going to be used together against a particular host, or in a particular stage during the infection,” he says. “Our computational study on understanding the evolution of transcription networks resulted in several meaningful predictions which can be used to carry out these experiments in a more rational way.”

This approach also might be valuable in biotechnology when applied to microbial metabolite production systems, according to Madan Babu. Thus, someday it could be possible “to engineer regulatory networks so you don’t disturb existing systems.” Such engineering could provide an alternative means, for example, “to increase expression of a molecule of interest,” he says.

David Holzman