BACTERIOLOGY

New Technique Offers a Window On Bacteria's Secret Weapons

Researchers fighting bacterial diseases have a handicap: They can study bacteria in a test tube, but they can't see what weapons the organisms bring out when they get inside a living host. And those secret weapons may be the key to new forms of treatment or prevention. In the past, researchers have fiddled with laboratory conditions to try to trick the bacteria into revealing their deadly secrets. But in a paper in this issue of *Science* (page 686), John Mekalanos of Harvard Medical School, with postdocs Michael Mahan and James Slauch, offers an intriguing new option: a scheme that puts the bacteria right into an animal host and lets the host point out the genes related to disease. The scheme, says Stanford bacteriologist Stanley Falkow, is a "bellwether," signaling the arrival of a whole new wave in bacterial-disease research.

Falkow is not alone in his enthusiasm for this method—and its prospects for new antibiotics. "Genes that are turned on in vivo have been the Holy Grail of bacterial pathogenesis," says Dale Spriggs, enteric diseases program officer at the National Institute of Allergy and Infectious Diseases (NIAID). "When [Mekalanos'] proposal came through NIH [the National Institutes of Health], terms like 'revolutionary' were used [by reviewers] to describe it." Adds Barry Bloom, who studies tuberculosis (TB) at Albert Einstein College of Medicine: "It's brilliant. I'm champing at the bit until we are ready to do it in TB."

What Bloom and others are hoping to identify are the "virulence proteins" that are essential for bacteria to cause disease. Some proteins of this type—including diphtheria and cholera toxins—were discovered through analysis of the proteins bacteria make under laboratory conditions. While that approach has yielded many important genes, says Mekalanos, its drawback is that researchers may miss other genes, because they don't know the conditions that cause them to be expressed in the host, and therefore can't duplicate those conditions in the test tube. About 10 years ago, that realization triggered an "evolution" in the field, says Falkow, as researchers began to do such things as mixing host cells with bacteria to approximate host conditions.

Now Mekalanos and his group have taken that trend one step further, with a method that exploits the animal itself to select genes expressed in disease. As a test case for their method (which they call IVET, for in vivo expression technology), the team used *Salmonella typhimurium*, which causes a typhoid-like disease in mice. They began with a strain that can't grow in mice because it lacks a gene called *purA* that is necessary for purine metabolism. They took functional copies of a *purA* gene from a related bacterium and hooked them to *lacZ*, a gene that makes an enzyme easily detectable by a color assay. They then chopped up the entire *Salmonella* genome and inserted pieces from that mix into a spot directly in front of the two-gene combination. They reasoned that some of those DNA pieces must contain parts of *Salmonella* genes that would turn on *purA* and *lacZ* under certain conditions. All the DNA constructs were then inserted into mutant bacteria lacking *purA*, which were used to infect mice.

The investigators harvested bacteria that survived 3 days in the mice, reasoning that in order to survive, those bacteria must have had their foreign *purA* genes activated. The researchers then eliminated all the bacteria whose *purA* genes are also turned on in the laboratory, by plating the bacteria on special agar that changes color in response to *lacZ*. That weeded out 95% of the bacteria—leaving the 5% whose *purA* genes were turned on only in the host. That 5% must contain host-specific *Salmonella* gene fragments.

The genes corresponding to those fragments must be needed only during host infection, says Mekalanos, and that makes them good candidates for virulence genes. That hunch proved right: As the group began to fish out those genes, they found that mutating them rendered the *Salmonella* unable to cause disease. The group hasn't found new toxins yet among the group of host-specific genes, but they have turned up genes for enzymes essential to growth in the animal. Such genes, from *Salmonella* or other bacterial systems in which the method can be used, may be potential targets for new antibiotics.

Mekalanos expects to come upon other virulence genes that are not required for bacterial survival but that increase the deadliness of infection. Genes like that, he says, might be mutated to form an attenuated strain of bacteria that could serve as a live vaccine. New virulence genes could also be useful in designing multivalent vaccines, he adds, in which a single live vaccine is engineered to make several antigens, immunizing against several diseases at once.

The technique will also help ferret out genes that are turned on only in certain host tissues, or that aid a disease's progress from one tissue to another, says Einstein's Bloom. For example, both TB and cryptococcus (a fungal disease that kills 10% of AIDS patients) start in the lungs but turn deadly when they later invade the brain. A key question for both diseases, says Bloom, is, "What genes enable it to get from the lung to the brain?" Whatever they are, IVET could help track them down.

IVET could do for bacteriology what the all-purpose method, PCR, did for molecular biology, says NIAID's Spriggs. And as with PCR, a single version will not hold the spotlight long. Bacterial geneticist Roy Curtiss of Washington University says his lab and that of his wife, Josephine Clark-Curtiss, have independently developed similar schemes that are not yet published. Abstracts for an upcoming bacteriology meeting promise other variations. In the search for virulence genes, 1993 promises to be a bumper year.

–Marcia Barinaga

Mein host. To identify bacterial genes that are specifically expressed during infection, researchers took pieces of DNA from *Salmonella* bacteria (1) and linked them to genes called *purA* and *lacZ* (2) in positions where they would control *purA* and *lacZ* expression. These genes were inserted into mutant *Salmonella* lacking *purA*, which is needed for survival in the host (3). When the bacteria were injected into mice (4), some survived (5)—indicating that they were making *purA* under the control of the pieces of *Salmonella* DNA. When those bacteria were later cultured (6), most showed colors produced by the *lacZ* gene. Some 5%, however, did not—indicating that their pieces of DNA were expressed in the host but not in culture (7).