Bacterial species impervious to antibiotics, such as *Escherichia coli* (shown here), are a health concern worldwide.

Breaking the Pattern of Antibiotic Resistance

WORLD health organizations have been concerned for the past few decades by the growing problem of antibiotic resistance in developing countries. This discussion grew louder when such bacteria reached the developed world, including the U.S., where for the first time since the discovery of antibiotics, patients have acquired bacterial infections that cannot be treated, putting lives at risk and increasing health-care costs.

The list of bacterial species impervious to antibiotics—*Escherichia coli*, *Salmonella*, *Campylobacter*, and at least 10 others—is growing, presenting significant health concerns worldwide. The rise of rapid, frequent, and relatively cheap international travel allows diseases to leap from continent to continent. Inadequate sanitation, lack of clean drinking water, and misuse of antibiotics contribute to the problem. And zoonotic sources—infectious diseases transmissible under natural conditions between vertebrate animals and human beings—pose more treatment challenges. In the past 20 years, new infectious diseases have appeared, including New Delhi metallo-beta-lactamase-1 and methicillin-resistant *Staphylococcus aureus*. Old ones, such as tuberculosis, are reemerging as serious health threats.

The conventional pharmaceutical response to antibiotic resistance has been to develop new drugs or combine established compounds to knock out multiple metabolic reactions within bacterial cells. Because the specific bacterium causing an infection is difficult to identify, broad-spectrum antibiotics are applied in a shotgun-blast approach to treat a range of disease-causing bacteria. This option, although effective in the short term, gives bacteria an opportunity to develop resistance to several antibiotic compounds.

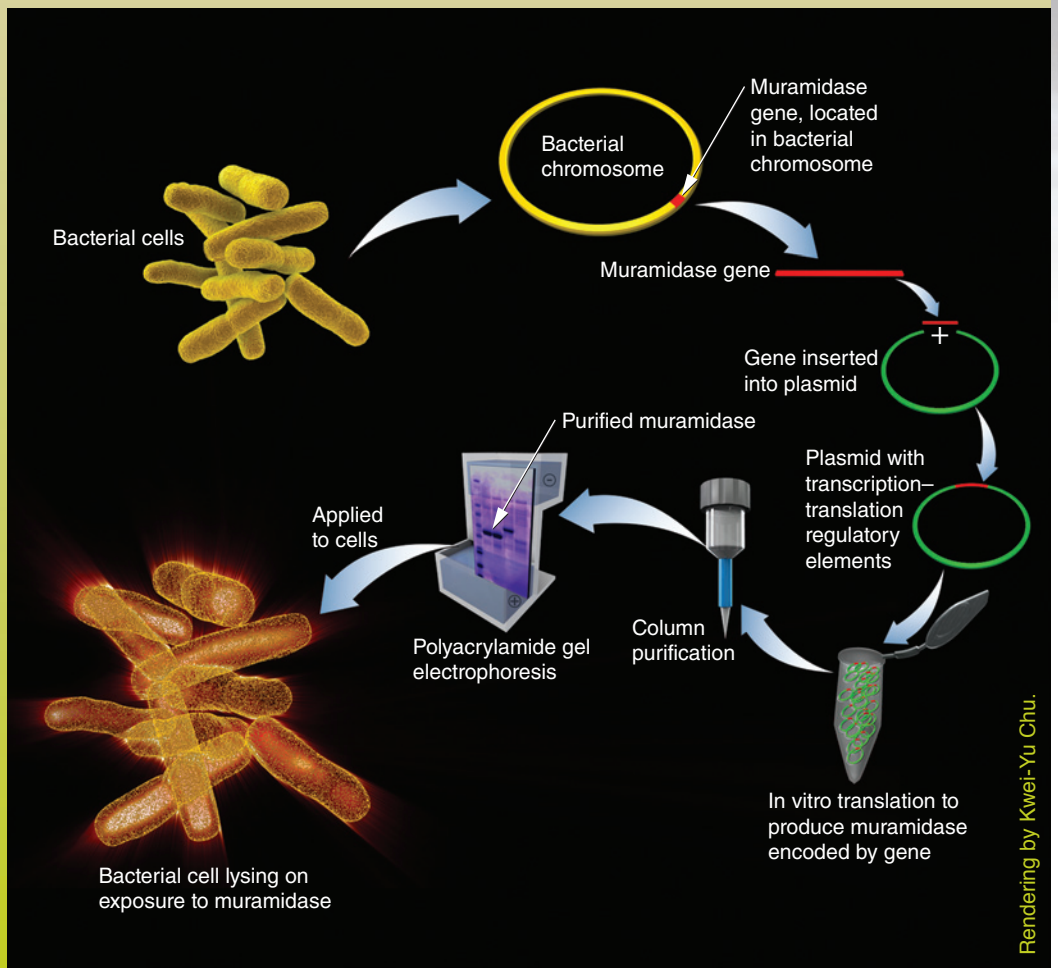
Resistance to antibiotics occurs when a bacterium mutates. "The bacterium modifies itself to prevent the drug from affecting its ability to grow," says Livermore scientist Paul Jackson, who works in the Physical and Life Sciences Directorate. "Microbes are always adapting, so some random mutation eventually protects a small number of them from a particular antibiotic. The protected microbes continue to grow in the presence of the drug."

Jackson is leading a team of Livermore biotechnology researchers who are investigating an alternative method for developing antimicrobial compounds: turning a pathogen's own genes against it. By taking advantage of bacterial genome sequences, the investigators identify which genes encode the proteins that cells require under normal conditions to survive. Then they apply those proteins in a manner not regulated by the cells, which damages the cells and rapidly kills the bacteria. "Because the protein we apply would normally be essential for bacterial

Finding the Right Proteins to Kill Bacteria

To evaluate candidate proteins for use as an antimicrobial treatment, Livermore scientists first select a bacterial pathogen, such as *Escherichia coli*, and from that cell's chromosomes, identify a gene likely involved in cell-wall metabolism or degradation. The selected gene is amplified using polymerase chain reaction, a technique designed to generate up to millions of copies of a particular DNA from a single sequence. The amplified gene is cloned into a plasmid that contains regulatory sequences to help control protein synthesis. DNA sequences encoding chemical (histidine) tags are included in the plasmid at this stage so researchers can rapidly purify the protein after it is synthesized.

Next, the plasmid is cloned into a laboratory strain of *E. coli* and purified to produce the required amount of plasmid for testing. An in vitro transcription–translation system uses the plasmid to direct the synthesis of large amounts of the lytic protein, which are then purified through columns that take advantage of the histidine tag. Finally, the protein is applied to a suspension or plate of cells, and lytic activity is measured.



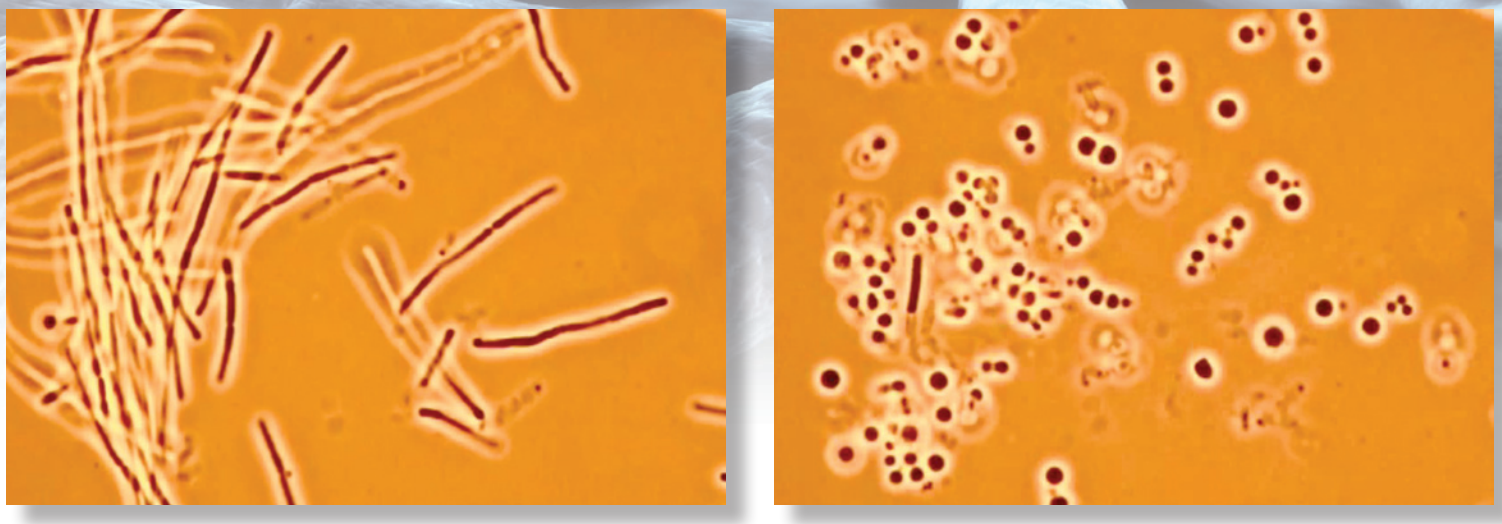
survival, we don't think the microbes can adapt to fend off the compound," says Jackson.

Beating Back the Bugs

With funding from the Laboratory Directed Research and Development Program, the research team, which includes Livermore scientists Feliza Bourguet, Matt Coleman, and Brian Souza, investigated endolysins, a family of lytic enzymes that cells produce in a controlled manner. Normally, lytic enzymes introduce nicks in cell walls that permit cells to divide and propagate. However, in high concentrations, endolysins cause rapid cell-wall degradation and cell rupture, a process termed cell lysis.

To determine which genes encode these proteins, the Livermore scientists used computational tools to search DNA databases of bacterial genomes. (See the box above.) For example, they identified several such genes in *Bacillus anthracis*, the bacterium that causes anthrax. Then they produced a large amount of the protein, purified it, and introduced it to the exterior of the cells. Experimental results showed total destruction of the pathogen's cell wall and, most importantly, rapid cell death. "When applied to the outside, the protein completely destroyed the integrity of the cell wall, quickly killing the bacterium," Jackson says.

Current technologies allow investigators to sequence genomes within a few weeks, providing information on newly identified pathogens, unknown bacterial species, or even species that have yet to be sequenced. With that information, the Livermore team can determine which genes encode lytic proteins in the new sequences and then produce those proteins in the quantities needed



Before the antimicrobial treatment process (left), bacterial cells on the slide are whole and complete, as indicated by the long strands. Seconds after the purified form of the bacteria's own protein is introduced to its exterior, the cell walls are destroyed (right). The fragments on the slide appear as mere dots.

to destroy the identified pathogens. For help with this sequencing effort, the team works with molecular biologist Crystal Jaing and bioinformatics experts Adam Zemla, Kevin Mcloughlin, and Tom Slezak.

Jackson acknowledges that the team's work would be extremely difficult without all of the Laboratory's resources. "We benefit not only from the multidisciplinary expertise but also from the different way each person thinks," he says. "The simple idea we started with really grew through this collaboration."

From the Lab to the World

In tests by the Centers for Disease Control and Prevention (CDC), Livermore's lytic protein killed 100 percent of *B. anthracis* cells, meeting the CDC's strict assay standard for rapid and complete lysis. Experiments with large batches produced at the center's core production facility showed that the protein is fully functional and stable after freeze-drying, and it has a long shelf life. Because of this success, CDC is including the protein in a new assay—a procedure to determine which bacteria resist a particular antibiotic—for use by the center's Laboratory Response Network, which was established to respond to biological and chemical terrorism and other public health emergencies. Livermore investigators are also providing the lytic protein to colleagues at the U.S. Army Medical Research Institute for Infectious Diseases.

The class of lytic proteins identified by the Livermore team shows promise for a number of topical treatments: to disinfect surfaces, which may be effective in combating hospital-acquired infections; to sanitize a patient's skin or wounds; and to reduce biofilms, an aggregate of microorganisms in which cells adhere to each other on a surface. Future tests will determine whether the proteins can be used for intravenous therapy.

The *B. anthracis* lytic protein may also prove effective in combination with procedures that force anthrax spores to germinate, allowing remediation teams to clean spore-contaminated equipment and facilities without using toxic or caustic materials. This class of protein may even be applied to foods to remove listeria—bacteria responsible for listeriosis, a rare but potentially lethal food-borne infection—and other food pathogens such as *E. coli* serotypes O157 and O104. "The Food and Drug Administration has already approved a cocktail of six different bacteriophage to spray on meat to reduce or eliminate all listeria," says Jackson. "The targeted proteins we're developing could provide a more effective approach for that purpose and for removing other harmful bacteria."

The team continues to test its technique on additional pathogens. According to Jackson, technological advances will eventually lead to quick identification of specific bacteria, and treatments for bacterial infections will become more focused. Because the Livermore process identifies a lytic protein unique to each bacterial species, the purified protein will target that specific species plus a few other closely related ones, leaving beneficial bacteria unharmed. Different lytic proteins could then be combined when a more broad-spectrum treatment is needed.

—Kris Fury

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