Staging Germ Warfare in Foods

Science harnesses bacteria to fend off food poisoning and spoilage

By JANET RALOFF

In the past few years, manufacturers have marketed a variety of products that contain germ-fighting chemical additives. First came cellulose sponges that incorporate chemicals to retard the growth of bacteria, then plastic cutting boards, brushes, and other housewares impregnated with pesticides. In Japan, consumers can even buy plastic bags that claim to bombard their contents with germ-fighting "rays."

Most of these products have been developed to kill or prevent the spread of foodborne pathogens. The U.S. General Accounting Office estimates the cost of treating food poisoning from *Salmonella* and other pathogens in the United States at up to $22 billion annually.

Not surprisingly, fighting these germs has become big business. Each year, U.S. restaurants alone spend some $55 million on disinfectants and cleansers. Manufacturers spend considerably more, not only to keep food free of disease-causing germs, but also to control microbes that can cause spoilage.

Traditionally, these companies have battled bacteria with chemicals such as trisodium phosphate, chlorine bleach, salt, and a variety of acids. Increasingly, however, many investigators are exploring another alternative—battling bacteria with bacteria.

Many bacteria generate small proteins known as bacteriocins. Some biologists suspect that bacteriocins are communications molecules, while others suggest that they developed as poisons to kill competing bacteria.

Whatever their original purpose, bacteriocins function as unusual, narrow-spectrum antibiotics. They tend to harm only microbes that closely resemble the bacteria that manufactured them. In many cases, bacteriocins attack potentially fatal food-poisoning germs, such as *Listeria monocytogenes* or the *Clostridium* responsible for botulism.

Though microbes have been impregnating the food supply with bacteriocins for millennia, the vast majority of these natural, nontoxic food preservatives functioned in obscurity until about a decade ago. Lately, food scientists have been scouting out these germ killers with the aim of enlisting their help. If these researchers get their way, a host of newfound biopreservatives may soon be blended into commercially prepared foods or incorporated into food packaging.

We live in a sea of bacteria. Though most people tend to think of these single-celled organisms as purveyors of disease, large numbers of them have proven to be allies rather than adversaries.

Consider yogurt. Each spoonful can contain some 10 million live, lactic-acid-producing bacteria, notes Peter M. Muriana, a microbiologist at Oklahoma State University in Stillwater. Yogurt manufacturers seed milk with starter cultures of these bacteria to initiate fermentation and the development of a tart, creamy product. In addition to eating the bacteria, yogurt consumers down any bacteriocins made by the microbes.

Over the past few decades, Muriana and his colleagues around the world have identified upward of 80 bacteriocins, most of them produced by fermentation microbes.

Nisin is such a bacteriocin. The microbiologists who isolated it in 1928 from a lactic acid–producing organism initially mistook it for a conventional antibiotic. Much later, it became clear that nisin is distinctive in several important ways. It is a protein, whereas most therapeutic antibiotics are not; it is far more discriminating than penicillin and other broad-spectrum drugs in what it kills; it is produced at a different time in a bacterium's life cycle; and it savages its targets through a different mechanism.

Some 30 years after it was discovered, nisin began being used commercially in England and Europe. In 1988, it won acceptance from the U.S. Food and Drug Administration for a narrow range of foods, including pasteurized egg products. Today, accepted by 45 countries, it is the most widely used commercial bacteriocin—and still the only one that may be added to U.S. foods.

The next wave of marketed antibacterial proteins is at hand, however. Some bacteriocins have been discovered in cured meats, such as bologna, pepperoni, and summer sausage. Others have turned up in milk and cheese. Scientists have even isolated bacteriocins from fruits, spoiled salad dressing, and soybean paste.

"Virtually all bacteria—probably 99 percent"—make at least one bacteriocin, says food microbiologist Todd R. Klaenhammer of North Carolina State University in Raleigh. That more such antimicrobial proteins aren't known, he says, reflects how few people have looked for them.

Muriana is one of those who have. About 8 years ago, he screened 20 different varieties of packaged hot dogs for *Listeria*. This bacterium, which neither refrigeration nor salt can check, kills up to one-third of the people it poisons. *Salmonella*, in contrast, causes a flulike illness in many more people each year, but most of its victims recover fully.

Muriana found *Listeria* in six different types of hot dogs. In fact, 75 percent of the packages of one variety he sampled over 18 months tested positive. However, some of the sampled hot dogs also contained nontoxic bacteria that produce bacteriocins. In subsequent tests, these bacteriocins proved effective at killing *Listeria*.

John B. Luchansky and his colleagues at the University of Wisconsin-Madison have investigated the value of pediocin, another bacteriocin made by a lactic acid–producing bacterium, in protecting frankfurters.

They encapsulated the bacteriocin in a gel and then sprayed it on the inside of hot dog packages. "That gelatin was kind of neat," Luchansky observes, because when the product was allowed to get too warm, "the gelatin melted, bathing the outside of the hot dogs with the bacteriocin—and reducing appreciably its *Listeria* contamination."

In a related project, his team added a strain of *Pediococcus* bacteria that generated pediocin to some batter for a poultry summer sausage. An identical strain of bacteria that did not make pediocin was mixed into a second batch. They then inoculated both batches with a known quantity of *Listeria*, allowed the batter to ferment, and tallied how many *Listeria* remained.

While there was very little reduction in the number of *Listeria* present in the second batch, the batch that had been seeded with the pediocin-producing bacteria contained fewer than one-ten-thousandth of the original number of food poisoning microbes. More importantly, Luchansky's team could find active pediocin in this batch of sausage even after an additional 2 months of refrigeration.

At the University of Melbourne in Australia, Barrie Davidson has been targeting *Listeria* with piscicolin, a bacteriocin from another lactic acid–producing bacterium. It appears promising for use in meat products, such as ham paste, and even as a rinse for fresh salad greens or chicken parts, he says.

Already patented, piscicolin could be ready for introduction to commercial food processing "within 12 to 18 months," says Davidson.

Most of these antimicrobial proteins are quite stable. Many remain biologically active after having been boiled or freeze-dried. Though the majority work best in acid—some in acid with a pH as high as the stomach's—others function at a nearly neutral pH.

Michael Johnson and his coworkers at the University of Arkansas in Fayetteville have been exploring the possibility of commercializing the newer antimicrobials. They're incorporating these proteins—sometimes together with their parent bacteria—in products ranging from the breading and marinades used for poultry to an experimental film that might coat foods inside plastic packages.

Why not just add the bacteriocin?

"Anytime you introduce a purified material like that, it becomes a new food additive and needs federal regulatory approval," Johnson observes. "That's very costly and time-consuming," especially for something that was already in the foods as a by-product of a bacterium that is generally recognized as safe by the FDA.

However, he notes, "you don't have to register a new process if you use the bacteriocin producer." One could think of it as a legal "end run around the additives rule," he says.

Johnson and his team have been growing a batch of the parent bacteria, extracting the bacteriocin, sterilizing the remaining broth and altering its pH, then returning the bacteriocin to the mix. If the process is done correctly, the antimicrobial protein will stick to the outside of its dead parent. The researchers then freeze-dry the whole mix.

The inactivated parent bacteria, which are generally recognized as a safe—and therefore legal—food additive, serve as carriers for the protein, Johnson explains. As long as the pair are delivered together, they don't represent a new product, at least to the FDA.

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"We've used this technique with a pediocin and showed that it inhibited the growth of *Listeria* in refrigerated chicken for 28 days."

One thing that Johnson and others have been learning is how tricky it can be to maintain the function of bacteriocins added to foods. Luchansky has shown that in some cases, bacteriocins will bind to food particles and become inactivated or will encounter enzymes that break them down. At a conference sponsored by the Institute of Food Technology last summer, the Arkansas team showed how a bacteriocin's activity can plummet when it's added to very salty foods yet skyrocket when incorporated into quite acidic ones.

Some explanations for the finicky nature of these proteins are emerging from studies by Thomas J. Montville of Rutgers University in New Brunswick, N.J. The proteins seem to enter the outer membrane of a susceptible bacterium, congregate in groups, and then form pores. "All bacteriocins appear to work by pore formation," Montville says, though they may not all form pores in the same way.

What does seem clear, he says, is that all bacteriocins deplete the electric charge stored across their victims' membrane. Energy-driven processes in bacterial cells can be powered by this electric charge or by an energy-storing chemical known as adenosine triphosphate (ATP).

"There's a kind of currency exchange between the two," Montville explains. As the bacteriocin runs down the electric charge, its embattled victim begins breaking down ATP to produce new protons and recharge the membrane. Montville has found that "this creates a futile cycle, because eventually the cell has no more ATP to maintain its other activities—and dies."

Since the electrochemistry of an environment can affect the extent to which bacteriocins can create holes that allow charged molecules to leak from a cell and deplete its stored electric charge, Montville says, these findings may explain why salt and acid concentrations in Johnson's studies affect a bacteriocin's cell-killing prowess.

Ironically, some of the factors that make bacteriocins so appealing as potential food additives also threaten to limit their usefulness.

For instance, because these antimicrobial proteins are highly selective, a bacteriocin that acts against one target pathogen probably won't sicken beneficial bacteria—or people. However, bacteriocins are so selective that those being considered for protection of food against *Listeria* have proven all but useless against *Salmonella* and many other common foodborne germs, such as the particularly virulent *E. coli* O157:H7.

The reason, Klaenhammer explains, is that most bacteriocins of interest to food scientists work well only against gram-positive bacteria, such as *Listeria* and *Clostridium*. Gram-positive microbes are enclosed in what he describes as a balloonlike outer membrane.

An additional outer coating shields this vulnerable membrane in *Salmonella*, *E. coli*, and other gram-negative bacteria. Without significant modifications, bacteriocins from gram-positive bacteria—the only ones now being explored for use in food—usually cannot breach a gram-negative bacterium's outer coat.

One approach would be to prospect for bacteriocins in fecally transmitted germs, including *E. coli* and *Salmonella*. Because their bacteriocins should be able to kill other gram-negative bacteria, food scientists could either add them directly to foods or engineer generally recognized as safe gram-negative bacteria to manufacture them.

What's possible and what's palatable, however, may be two entirely different things, Davidson observes. Not only are fecally derived proteins not likely to appeal to the public or to regulators, they have not been a common part of the healthful food supply, as have bacteriocins from gram-positive fermenters.

For now, he and most other bacteriocin researchers are investigating how best to harness antimicrobial proteins from the gram-positive microbes.

While these bacteriocins "are not a silver bullet," Klaenhammer argues, they may prove quite useful if carefully tailored to the precise food and target pathogen. Indeed, with a growing interest in natural foods, increasing demand for natural forms of food preservation, and a proliferation of minimally processed foods, he believes "we will need some novel preservation methods."

Bacteriocins could prove important here, he says, and not only as a backup for more conventional food protection processes. "In some cases, they might even become a first line of defense. I wouldn't preclude that possibility." □