

Safer Sprouts

Microbiologist Amy Charkowski and graduate student Abdulah Harris observe the display of a confocal microscope being used to examine an alfalfa sprout root that has been experimentally contaminated with *Salmonella*. The microbes show up green or blue on the computer screen.

Fresh and colorful alfalfa sprouts, crisp and crunchy mung bean sprouts, or any of the half dozen other kinds of raw sprouts sold in America today add taste and texture to salads, sandwiches, soups, omelets, and other dishes. What's more, sprouts can give you plenty of protein, a lot of fiber, and a generous amount of antioxidants like vitamin C.

But those same sprouts—if not grown and processed under hygienic conditions—can also leave you with a nasty case of food poisoning caused by pathogenic microbes such as *Salmonella* or *E. coli* O157:H7.

Now, investigations by Agricultural Research Service microbiologist Amy O. Charkowski may help make sprouts safer. She is with the Food Safety and Health Research Unit at Albany, California.

Solutions for Small Farms

In the United States, the delicious, sprouted seeds of red clover, broccoli, wheat, radish, soybean, mung bean, alfalfa, and other vegetables or grains “are produced primarily by small operations,” says Charkowski. “Many are family-run. These ‘sprouters,’ as they are nicknamed in the industry, may not be able to afford expensive techniques to kill foodborne pathogens.

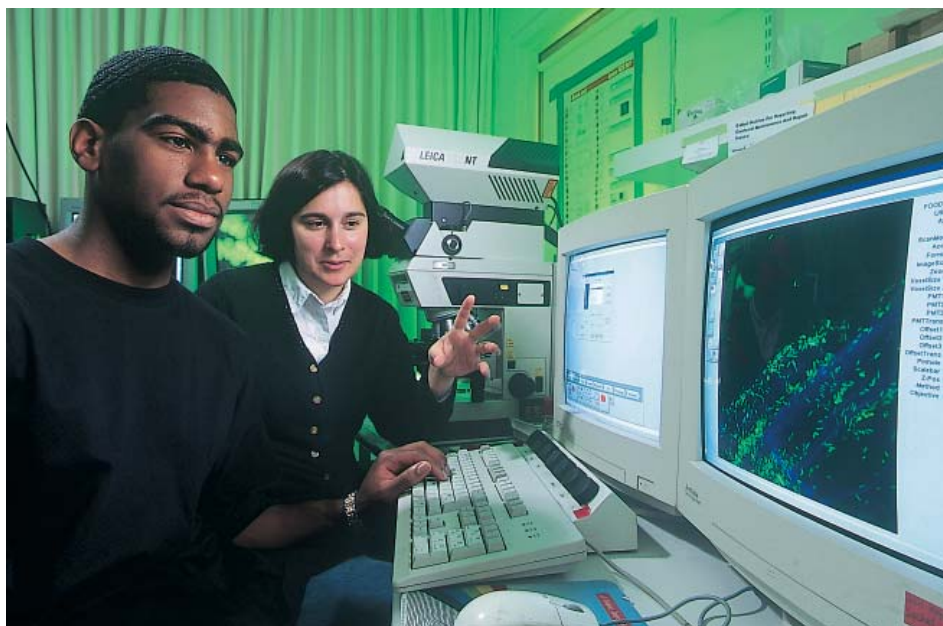
“Also,” notes Charkowski, “many sprouters run organic operations.” That means they simply won't use irradiation or another option—bathing sprouts in a chlorine-containing chemical called calcium hypochlorite. This compound—the same white powder that's used to disinfect swimming pools—is recommended by the Food and Drug Administration for sanitizing sprouts.

The FDA currently advises all consumers to cook sprouts before eating them. And it recommends that the young, the elderly, and those with compromised immune systems should not eat sprouts.

Salmonella and *E. coli* can flourish in the warm, moist indoor environment in which seeds are forced to sprout. Typically, unsprouted seeds are placed on trays or in rotating drums. There, they are kept warm and are periodically moistened with a fine mist of clean water.

There are many opportunities for the seeds to become contaminated with the microbes before they're even purchased by sprouters. *Salmonella* or *E. coli* could be harbored in bird droppings, in manure applied to fields as fertilizer, in contaminated water that's used to irrigate fields, or perhaps in dirt left over in improperly cleaned seed-sorting machinery. The pathogens might also live in droppings of rodents that eat seeds stored in bags, bins, or silos.

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Microbes' Preferred Attack Sites

Charkowski's studies with sprouted seeds of radish, alfalfa, broccoli, and mung bean may yield tactics that sprouters can use to ensure their products are free of *Salmonella* and *E. coli*. Experiments in her laboratory at the ARS Western Regional Research Center have revealed that these pathogens prefer to attack the roots and—secondarily—the seedcoats. Her studies, which required tracking the microbes' progress in thousands of sprouts, are among the first few to reveal the pathogens' priorities.

Related tests may indicate what sprout-produced compounds—such as amino acids—inadvertently nurture the attacking microbes. In addition, the experiments should show whether harmless bacteria could be applied to the sprouts to deprive the pathogens of the vital compounds.

"We might be able to undermine the harmful pathogens," says Charkowski, "if we can use beneficial bacteria to outcompete them in the race for essential compounds."

Salmonella Genes Scrutinized

In other approaches, Charkowski is probing the genetic makeup of *Salmonella*. Her intent: To discover which genes *Salmonella* "expresses"—that is, activates—when it contaminates sprouts.

For one group of experiments, she's producing genetically engineered lab strains of *Salmonella* by randomly knocking out genes. Then, she's determining whether removing those genes will reduce the microbe's ability to infect sprouts.

In a second set of genetic engineering investigations, Charkowski and post-doctoral fellow Jeri D. Barak will track the genes that *Salmonella* expresses as it colonizes the sprouts. They will do that with *Salmonella* that contain a gene borrowed from a jellyfish. The jellyfish gene causes *Salmonella* to fluoresce a bright green whenever a *Salmonella* gene turns on.

In a competition among ARS labs nationwide, Charkowski won special ARS funding that allows Barak to join in this genetic research effort.

Less Effective Salmonella Found

"So far," Charkowski reports, "one of our most interesting strains of genetically modified *Salmonella* is only about one-tenth as effective in colonizing sprouts as conventional *Salmonella*." But which of *Salmonella*'s 4,000 to 5,000 genes are missing or disabled in that strain? Help in identifying those genes—and their makeup, or sequence—may come from biotechnologists who are part of an international venture to sequence *Salmonella* genes.

In the meantime, Charkowski's quest to find out which *Salmonella* genes are crucial to successful attacks on sprouts is likely unique. "Once we know which *Salmonella* genes are critical in an invasion," she says, "we may be able to develop a strategy to activate and amplify the natural protective response by the sprouts.

"The genes that *Salmonella* activates when it invades sprouts," says Charkowski, "are likely the same as those it uses when it colonizes other fresh produce and perhaps meats and poultry. That means the food safety strategies developed from our genetic studies may help protect these other foods from *Salmonella*, as well."—By **Marcia Wood, ARS.**

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at <http://www.nps.ars.usda.gov/programs/appvs.htm>.

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ARS technician Chester Sarreal separates alfalfa seeds by color and shape to determine whether certain seed characteristics are correlated with higher bacterial contamination levels.