Protozoa: The Inside Story

Are These Tiny Animals Accidental Allies of Salmonella?

PEGGY GREB (D402-2)

Microbiologist Maria Brandl views microscopic *Tetrahymena* protozoa growing in a liquid medium.

Salmonella enterica, one of the planet's most problematic food-poisoning bacteria, may have an inadvertent ally in the world of one-celled organisms known as "protozoa." Agricultural Research Service microbiologist Maria T. Brandl, based at the agency's Western Regional Research Center in Albany, California, leads a team that's delving into the mostly mysterious interaction between the bacterium and the protozoan. Brandl is part of the center's Produce Safety and Microbiology Research Unit.

Her team's discoveries may lead to new, more powerful, and more environmentally friendly ways to reduce the incidence of *Salmonella* associated with meat, poultry, and fresh produce at the market.

Swallowed Alive, *Salmonella* Survives

At some point in its life, *Salmonella* may encounter a commonplace protozoan known as a *Tetrahymena*. This transparent, nearly invisible animal lives in water—including the water in soil. It uses its hundreds of miniature, oarlike filaments, called cilia, to propel itself through the water, while grazing on any unwary bacteria it happens on.

For instance, it may gulp down *Salmonella*. But that entree choice is really a menu mistake.

Why?

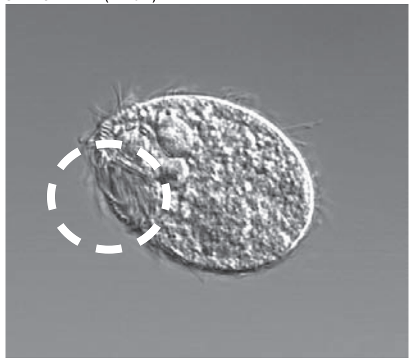
Brandl's laboratory tests showed that the protozoan apparently can't digest *Salmonella*. Instead, it expels *Salmonella* into the environment—alive, well, and fully encased in a miniature pouch. Called a "food vacuole," the pouch is made of an ultrathin, flexible membrane that, Brandl's findings suggest, may afford important protection to *Salmonella*.

But the digestive drama unfolds quite differently for another major foodborne pathogen, *Listeria monocytogenes,* Brandl's experiments show. Once gobbled up, *L. monocytogenes* is deftly digested, disabled, and destroyed by the protozoan.

What happens to *Salmonella* during its brief, gustatorial encounter with the protozoan's innards may strongly affect *Salmonella*'s later survival and success, Brandl says. In fact, *Salmonella* may emerge as more resistant than ever to our efforts to kill it.

Salmonella *emerges from the innards of the protozoan encased in a food vacuole, which may make it more resistant than ever to our efforts to kill it.*

SHARON BERK (D420-1)



A *Tetrahymena* protozoan. The small hairlike projections (cilia) are used by the protozoan to propel itself while grazing on bacteria in water. Note the *Tetrahymena's* mouth (circled), a broad spot covered by many cilia.

When Pathogen Meets Protozoan

Inside a *Tetrahymena, Salmonella* is apparently able to cluster, unharmed, within the food vacuole. After exposing *Salmonella* cells to a *Tetrahymena* for several hours, Brandl found that some vacuoles held as many as 50 *Salmonella* cells. This dense clustering might safeguard the innermost ones from environmental stresses such as ultraviolet rays or harmful temperatures.

Another Albany experiment showed that twice as many *Salmonella* cells stayed alive in water if they were encased in the expelled vacuoles than if they were not enclosed in them.

Sequestering the cells in the vacuoles also protected them against low doses of calcium hypochlorite. Similar to household bleach, it's often used to sanitize food-processing equipment and foods before they reach the supermarket. Brandl found that the encased cells were three times more likely than unenclosed cells to survive exposure to a 10-minute bath of 2 parts per million of calcium hypochlorite.

This study is the first to show that *Tetrahymena* expel living *S. enterica* bacteria encased in food vacuoles and that the still-encased, expelled bacteria can better resist food sanitation.

PEGGY GREB (D403-1)

Research assistant Aileen Haxo (left) and Maria Brandl examine confocal micrographs of green fluorescent-labeled *Salmonella* cells enclosed within food vacuoles of *Tetrahymena* protozoa.

Brandl did the work in collaboration with Sharon G. Berk of Tennessee Technological University, in Cookeville, and with zoologist Benjamin M. Rosenthal at ARS's Henry A. Wallace Beltsville (Maryland) Agricultural Research Center. They documented their findings in a 2005 issue of *Applied and Environmental Microbiology.*

The research exposes how much more there is to learn about *Salmonella's* survival tactics and what growers, food processors, and others can do to keep this microbial menace at bay.

Genes: What's Their Role?

Now, Brandl is taking a closer look at the genes involved in the hidden interaction between *Salmonella* and *Tetrahymena*. Scientists elsewhere are helping solve this puzzle by posting, on the World Wide Web, new information about the estimated 4,000 genes that make up *S. enterica*. Other researchers are now working to decipher all the genes in *Tetrahymena* to learn what jobs they perform inside the little animal.

Brandl's intent is to use microarray technology to capture a record of which genes are turned on or turned off as *S. enterica* is gobbled up by *Tetrahymena,* crammed into a food vacuole, then ejected from the protozoan. She is particularly interested in what genes *Salmonella* turns on "while within the food vacuole. Those genes could be the same ones *Salmonella* activates when inside a human."

Brandl continues, "We may discover new clues to how *Salmonella* behaves outside of hosts such as *Tetrahymena*—and what makes it such a powerful pathogen. Our explorations may also lead to new approaches for controlling *Salmonella."*

Exposing the hidden details of the encounters of *Salmonella* and its *Tetrahymena* ally may help knock *Salmonella* off the list of major foodborne pathogens.—By **Marcia Wood,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Maria T. Brandl is with the USDA-ARS Western Regional Research Center, Produce Safety and Microbiology Research Unit, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5885, fax (510) 559-6162, e-mail mbrandl@pw.usda.gov. ✷