BANKING ON Bt

Germplasm collection helps secure potent future for natural insecticide.

Instead of documents and other paperwork, Phyllis Martin's office cabinet is rife with bacteria—exactly the way she wants it.

A microbiologist with the Agricultural Research Service in Beltsville, Maryland, Martin has populated her cabinet with rows of petri dishes, many housing the naturally occurring bacterium *Bacillus thuringiensis* (Bt)—agriculture's oft-recruited microbial ally against insect pests.

Resembling hues of yellowish-green crust, spores of some Bt strains have lain dormant and dehydrated in their petri homes for 12 years, says Martin, who is with ARS' Insect Biocontrol Laboratory.

And yet, a single drop of water can awaken them in moments, notes Martin, who studies the bacterium for new, insect-killing proteins.

Over the past decade, Martin and her Beltsville colleagues have amassed, categorized, and maintained a Bt germplasm bank. This living collection currently numbers 12,145 Bt strains and isolates obtained from samples from around the world. In 1983, when the researchers first began their Bt work, that number was only 500.

Using biochemical tests and other lab techniques, they've since garnered many more new Bt strains—including some from such intriguing locales as the Himalaya Mountains at 18,000 feet, a cat's paws, human hands, and even freshly fallen snow. "Snowflakes," Martin notes, "form around dust particles, which often contain bacteria."

But why go to such lengths for the microbe? Martin likens their efforts to plant explorers who pursue exotic seeds, cuttings, and other plant germplasm to ensure genetic diversity in domestic crops.

Up until the last 5 or so years, commercial use of Bt had been limited to a handful of strains—HD-1 and *Israelensis* among them. Today, more than 20 may be in use in commercial products, Martin estimates. One reason is greater awareness of the microbe's ubiquitous nature in the environment—whether it be on soil, dust particles, grains, or plant leaves.

Martin says scrutinizing Bt from such diverse environs can unearth strains that harbor genes for new pest-killing proteins, or protoxins. Bt's protoxins are harmless to people, livestock, and most beneficial insects such as bees. But they rupture cell membranes in the midguts of caterpillars and other insect pests that chew on Bt-treated plants, causing them to starve and die.

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Reviving Bt spores is as easy as dropping sterile water onto a dot of dried agar. (K7096-14)

For over 30 years, the agricultural community has had a large stake in Bt in commercial sprays and, more recently, in transgenic corn, cotton, and potatoes. So new toxin-producing genes are a crucial safeguard against pests that might otherwise adapt to Bt and escape unharmed.

For example, "The diamondback moth, which attacks cabbage, watercress, and other vegetable crops, has developed resistance to the HD-1 strain," says Martin. "The collected Bt germplasm can help us find alternative toxins."

Much of her germplasm work centers on computer databases of more than 13,000 Bt strains or isolates—including the 12,145 "on file" in her cabinet. These databases store information on the origins of new Bt, their biochemical profile, frequency in soils, crystal structure, and insect-killing potential.

In one database project, Martin is searching for Bt strains more potent than those now available against the notorious Colorado potato beetle. As larvae and adults, the pest costs growers of tomatoes, potatoes, and eggplants over $150 million annually in chemical control expenses and lost yield.

One problem in combating this insect with Bt is its irksome tendency to shrug off Bt toxins—compliments of adaptable receptors on cells lining the pest's midgut.

"The more multiple-toxins you hit the insect with, the less able it is to develop resistance," Martin says. By adding several Bt toxin genes to the genetic material of a transgenic potato plant, for example, it may be possible to overcome the insect's resistance mechanism.

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Microbiologist Phyllis Martin checks some of the 12,145 living *Bacillus thuringiensis* strains and isolates collected from around the world. (K7094-2)

How Bt Does Its Thing

In other work, Martin is looking at why some Bt products work better than others—even though the strains are the same. One key factor is understanding how Bt and its environment interact.

Applied as an insecticidal spray, for example, Bt generally lasts in soils from 1 to 4 days, sometimes even longer. But that can hinge on several factors—including the medium on which it is grown, its stickiness on plant leaves, its protection from ultraviolet light, and competition by other microbes.

Conversely, Martin reports, substances called allelochemicals in certain plants and trees, like oak, can prolong Bt's longevity. That means bad news for gypsy moth larvae that munch on Bt-treated oak leaves—good news for park managers, tree doctors, and municipality workers who enlist Bt against the pest.

Much of the biocontrol lab's successful pursuit of Bt can be credited to a series of biochemical tests and a technique called sodium acetate selection.

After acquiring a soil sample, the researchers must first eliminate unwanted microbes, such as fungi and other bacterial species. To do this, they bathe the sample in acetate, a salt solution. Most Bt in culture will not germinate in acetate, though other microorganisms will. Once they do, the researchers can then kill them with a heat treatment.

Next, they coax the Bt spores to germinate on a nutrient-based growth medium in petri dishes. Germinated spores are transferred to dots of agar, so a single dish can hold up to 32 different isolates from samples. The researchers then use anywhere from 8 to 14 biochemical tests to identify different Bt samples on the agar.

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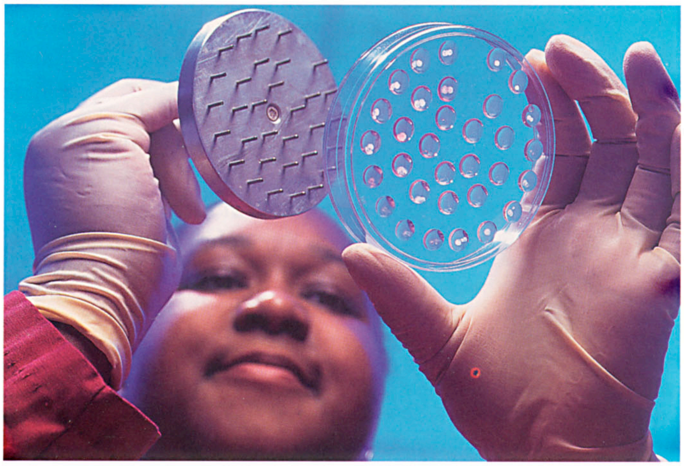

Using a pin inoculator, technician Ashaki Shropshire can simultaneously test 32 separate isolates. (K7095-17)

The tests work by causing the bacterium to excrete an acid that colors a buffer solution specific hues of yellow—acting as a "calling-card" for each Bt isolate. Martin says an overabundance of sugar used in the tests makes the bacterium's digestive system work harder—excreting the acid "much like humans produce lactic acid during a jog."

Using this approach, Martin, lab technician Ashaki Shropshire, and Cornell University entomologist David W. Watson recently identified Bt isolates that kill both house and stable flies.

As adult flies, these barnyard pests can cause serious stress and reduced weight gain in calves that are being weaned from their mothers in calf hutches, or pens. The stable flies also draw blood meals from the animals.

Martin says they isolated the fly-killing Bt from oak, maple, pine, tulip poplar, and straw used as pen bedding. "We were kind of surprised at the types of Bt we got," she recalls. "Out of about 4,000 Bt isolates, we tested 200 and found 49 that have activity against flies."

Watson, who is with Cornell's Entomology Department at Ithaca, New York, plans studies to further assess the Bt's biocontrol potential against fly pests. "We'll also go back over the data and see if a similar portion of those Bt isolates are toxic to caterpillars," says Martin.—By **Jan Suszkiw**, ARS.

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