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Emerging Enemy of Veggies Unmasked


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Emerging Enemy of Veggies Unmasked

Chic, trendy veggies like arugula and baby broccoli, and familiar stand-bys like Brussels sprouts and cauliflower, are vulnerable to attack by a once-puzzling pathogen. Agricultural Research Service plant pathologist Carolee T. Bull and colleagues have, in laboratory, greenhouse, and field research, detected, identified, renamed, and classified the plant-killing microbe that's now officially known as *Pseudomonas cannabina* pv. *alisalensis*. "Pv." stands for "pathovar" and indicates that the microbe is a specific pathogenic form, or strain, of a species.

Their work has sorted out some of the taxonomic confusion surrounding classification of the large, complex group of harmful bacteria in the genus *Pseudomonas*, to which *P. cannabina* pv. *alisalensis* properly belongs.

In so doing, the team has helped growers, vegetable processors, fellow scientists, and anyone who enjoys eating good-for-you cruciferous veggies.

Bull is in the ARS Crop Improvement and Protection Research Unit in Salinas, California. The lab is located in the Salinas Valley, a region nicknamed "The Salad Bowl of the World" because of the quality, quantity, and diversity of vegetables grown there.

The pathovar was named for the Alisal district of Salinas, a neighborhood that's home to many ag workers. The district is also near the site where, in 1995, the microbe was collected—for laboratory analysis—from a commercial crop of broccoli raab, a leafy veggie with an intense, mustardy flavor.

Bull's detective work with the microbe dates back to 1998, when colleagues

ARS plant pathologist Carolee Bull examines the bacterium that causes bacterial blight of cruciferous crops such as arugula, broccoli, Brussels sprouts, and cauliflower.

Steve Koike, a University of California Cooperative Extension farm advisor; and Ron Bunch and the late Jim Mannasero, both with D'Arrigo Brothers Company of California, a major developer and producer of fresh vegetables, came to her for her input.

With funding from the company and ARS, Bull and coworkers began investigating what was, at the time, an unidentified pathogen affecting broccoli raab.

In the years that followed, the microbe was detected not just on that vegetable, but also on several other cruciferous crops in the region, including arugula, broccoli, Brussels sprouts, cauliflower, and rutabaga.

In addition to showing up in California vegetable fields, the pathogen has, to date, been found on crops in Nevada, New Jersey, Oklahoma, South Carolina, and Wisconsin, and overseas in Australia, France, Germany, and Greece.

Unightly Spots Ruin Crops

P. cannabina pv. *alisalensis* causes a disease known as “bacterial blight of crucifers.” Symptoms on afflicted leaves include water-soaked spots that develop a bright-yellow border. Eventually, the spots coalesce and turn brown, giving the leaves a burnt, unattractive appearance that makes the harvest from the plants unmarketable.

Besides ruining a harvest, bacterial blight can rack up other costs. For instance, it can cause delays in planting of previously infested fields. A study by Bull and colleagues showed that tilling, after harvest of broccoli raab plants infected with the pathogen, contaminated the soil and led to infection of the next crop.

“Allowing more time to elapse after tilling reduces incidence of the disease,” she says. But such delays can disrupt growers’ tight schedules for rotating fields from one crop to the next. Losses attributable to the bacterial blight pathogen affect consumers,

Brussels sprouts, a cruciferous crop that is vulnerable to bacterial blight.

too. Shortages of a popular specialty veggie at the supermarket or local produce stand, for example, can drive up prices.

Sorting Out Who’s Who Among *Pseudomonas* Species

The blight pathogen is among a number of pseudomonads that fluoresce when, in the lab, scientists expose them to ultraviolet (UV) light. This trait, and others, may have contributed to confusion surrounding *P. cannabina* pv. *alisalensis* and another microbe, *Pseudomonas syringae* pv. *maculicola*, best known as the culprit behind pepper spot disease—first known as “pepper spot disease of cauliflower.”

Not only do both pathogens fluoresce under UV light, but they also perform similarly in several physiological tests typically administered if microbes glow in the UV assay. Also, the host ranges of these two microbes overlap to some extent. An example: broccoli, cabbage, and cauliflower are hosts to both of the pathogens.

“In the past, these shared traits contributed to the inability to separate these pathogens from one another,” Bull says. “For



decades, any fluorescent pseudomonad causing disease on a crucifer was almost automatically identified as *Pseudomonas syringae* pv. *maculicola*, regardless of the severity of the symptoms.

“Pepper spot disease is mild compared to bacterial blight. Bacterial blight can lead to complete crop loss, while pepper spot disease does not.”

Why is it so important to know precisely “who’s who” when identifying a plant pathogen?

“Correct identification and classification is the first step toward controlling plant diseases,” explains Bull, whose research includes finding environmentally friendly, affordable ways to undermine costly plant pathogens.



Carolee Bull and University of California Cooperative Extension farm advisor Steve Koike scout for bacterial blight in a field of broccoli raab.

There are other compelling reasons for precise identification of microbes that make plants ill. Growers who are correctly informed about the cause of a disease can make better decisions about which crops to plant—or not plant.

“We showed that the host range of the blight pathogen is broader than the host range of the pepper spot pathovar,” says Bull. “Our host-range data helps growers plan strategic crop rotations, such as planting lettuce, a nonhost of the blight pathogen, in blighted fields.”

Correct identification is also essential to plant scientists worldwide who detect, diagnose, report, and track outbreaks of plant diseases. These reports provide vital profiles of the behavior of disease organisms in various climates and soils and help delineate the pathogens’ host ranges.

Modern Tactics Used for Reliable Identification

Bull’s group used what she describes as “a suite of assays” to quickly and reliably differentiate the blight and pepper spot pathogens from each other—as well as from other microbial species and strains.

Some of the tests, such as rep-PCR (repetitive polymerase chain reaction), focus on the microbes’ genetic makeup.

Another approach relies on a virus, PBS1. Referred to as a “bacteriophage” in its bacteria-killing role, the virus is “a pathogen that kills a pathogen,” says Bull.

Her team isolated PBS1 from cells of *P. cannabina* pv. *alisalensis* found in soil in a broccoli raab field. Tests in her lab showed that although PBS1 is capable of killing the blight pathogen, it does not kill the pepper spot pathovar, meaning that it can be used to differentiate one from the other.

“This bacteriophage-based assay is a fast, reliable laboratory tool for preliminary diagnosis,” she says. “We’ve shown that the bacteriophage can be modified to detect and quantify *P. cannabina* pv. *alisalensis* infection of plants that have yet to develop any external symptoms.”

Online Database Speeds Identification

In related work, Bull and other researchers have contributed key information about the genetic makeup of *P. cannabina* pv. *alisalensis*, *P. syringae* pv. *maculicola*, and more than 100 other *P. syringae* pathovars, to a new database called “PAMDB” (Plant-Associated and Environmental Microbes Data Base).

Intended for use by plant disease specialists at universities, plant health labs, and elsewhere, this online compendium, publicly available at www.PAMDB.org, displays the sequence, or makeup, of DNA fragments of genes common to bacteria.

“These ‘housekeeping genes,’” Bull notes, “are essential to the survival of all known bacteria. The sequences of these DNA fragments are used in sequence analyses to get a quick, tentative identifi-

cation of a microbe and to help determine taxonomic relationships among microbes.

“Labs can use ordinary protocols to amplify and sequence DNA from the microbe they’re trying to identify and then compare those sequences to ones posted at PAMDB to find a match.”

The database includes sequences from not only *Pseudomonas* species and strains, but also from other kinds of plant-dwelling microbes (“from A to X—*Acidovorax* to *Xanthomonas*,” Bull says), and encompasses pathogenic and nonpathogenic bacteria alike.

Plant Doctors of Tomorrow?

In unmasking the blight pathogen, Bull and colleagues worked with more than two dozen undergraduate students funded by grants to the nearby Hartnell College and by California State University-Monterey Bay. Bull became the university’s first “Mentor of the Year” in May 2012.

“Our students use their education in authentic research experiences,” she says. “Many of our alums are in demand as ‘budding’ plant pathologists in M.S. and Ph.D. programs. Two of our students have won 3-year, \$90,000 fellowships from the National Science Foundation for graduate work in plant pathology.”

The scientists—and many of the students—have documented their pepper spot and bacterial blight findings in peer-reviewed scientific articles in *Phytopathology*, *Plant Disease*, and *Systematic and Applied Microbiology*.

Cruciferous vegetables are a \$1.9 billion crop in the United States. Many are an excellent source of vitamin C and also provide dietary fiber, calcium, and iron.—
By **Marcia Wood**, ARS.

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Technician Polly Goldman (left) observes bacterial characteristics while biological science aide Teresa Jardini isolates DNA from bacteria for pathogen identification.



PEGGY GREB (D2745-1)