Exploding Zoospores

Using biosurfactants to control plant pathogens

It began as many scientific breakthroughs do — it was an accident. In 1995, Michael Stanghellini, then a plant pathologist in the Department of Plant Pathology, was looking for ways to control fungal diseases in plants. In a greenhouse on campus, he injected hydroponic plant specimens with different pathogens and then added fungicides. During a routine check he noticed that in one unit inoculated with the pathogen, not a single plant had died. Even more odd, he saw that the solution in the unit was foaming extensively.

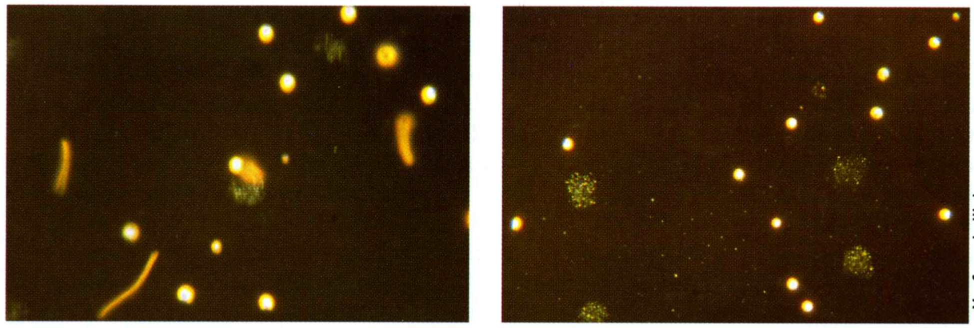
After considering and dismissing several causes, he contacted Raina Miller, a microbiologist in the Department of Soil, Water and Environmental Science. They had never worked together before. They isolated a bacterium from the recirculating nutrient solution of the hydroponic unit, and identified it as *Pseudomonas aeruginosa*. They found that the bacterium was synthesizing a biosurfactant that was responsible for the foaming in the nutrient solution.

"Biosurfactants are like a detergent or a soap, but are made naturally in the environment by microorganisms," Miller says. At the time, she had been studying the use of biosurfactants for waste management. "We were looking at the way biosurfactants could clean up soils by physically washing the contaminants from the soils and pulling heavy metals or toxic organics like petroleum from them."

Stanghellini's work focused on zoosporic plant pathogens, which cause the most economically damaging plant diseases in the world. He had been trying to find the most efficient way to control them using the only means then available: chemical fungicides.

These fungus diseases include the downy mildews, such as downy mildew of grape, cucumber, pumpkin, pepper and melons; root rots such as *Pythium spp.*; and the notorious late blight of potato, *Phytophthora infestans*, which destroyed the potato crop in Ireland during the last century. All of these diseases affect Arizona crops.

Why has it been so difficult to control zoosporic plant diseases? These fungi undergo a life stage called a zoospore, which has no cell wall and is very mobile. It has a membrane and two tails. The zoospore can encyst rapidly, developing cell walls within 60 seconds.



M. Stanghellini

Phytophthora bacteria one second after exposure to rhamnolipids (left) and about 30 seconds after (right). Note shattered areas.

The zoospore stage is therefore highly infectious and spreads quickly.

Miller and Stanghellini wanted to know just how the protective bacteria were killing the fungus, so they isolated the active material and tested it on a series of different zoosporic plant pathogens. They found that the biosurfactant produced by the bacteria was able to lyse, or destroy cell membranes, with dramatic results.

"We found that if you take zoospores and expose them to these rhamnolipids, which are a type of biosurfactant, they explode," Miller says. The rhamnolipids slice into the membrane, separating it enough to release the contents of the zoospores. Stanghellini and Miller also learned that the bacteria produce large amounts of rhamnolipid in the presence of a carbon source such as olive oil, glucose or soybean oil.

Their next step was to find out if the rhamnolipid-producing bacteria occurred naturally on field-grown plants. They located the bacteria on the roots and leaves of cucumber, tomato and cantaloupe. Then they conducted a series of greenhouse studies using pepper plants to determine where the biosurfactant could be applied on the plant, and which rhamnolipids worked best on each pathogen.

"We've added it to the roots and the leaves, and we've found that just the rhamnolipid by itself will provide control, or control can be achieved by adding the bacteria combined with a food source to allow rhamnolipid synthesis," Miller says. "We still have to figure out how to deliver it to the crop, though."

Miller says that potential application methods include 1) spraying the biosurfactant on leaf surfaces, or 2) spraying on the Pseudomonas bacteria in an emulsion (food source) to stimulate the production of the biosurfactant.

Some fungal pathogens strike during warm weather, others flourish during cool days and nights, and most prefer moist conditions. These factors would affect the timing of biosurfactant applications. "You could predict the times of year when these zoospores would be likely to spread — during wet or rainy weather, for example — and you could apply the appropriate biosurfactant maybe two or three times a year during that season," Miller suggests.

She and Stanghellini have applied for patents for this work, and they continue to study the way the rhamnolipids affect crop diseases in Arizona. The working association that began with an accident in a greenhouse has evolved into an ongoing research team. Others associated with this project include post-doctoral student Yimin Zhang, working with Miller; and Scott Rasmussen, D-H. Kim, and Pat Rorabaugh, technical staff working with Stanghellini.

Both scientists believe that the pathogens targeted by the rhamnolipids probably won't develop resistance to them because the pathogen would have to undergo a complete structural change in its plasma membrane, a major chemical alteration that differs from the way insects quickly develop resistance to pesticides.

Miller and Stanghellini admit that it's probably impossible for any single rhamnolipid producing strain of bacteria to control all zoosporic plant pathogens under all environmental conditions. Yet the prospect remains that biosurfactants produced by bacteria may eventually enable growers to control some of their worst fungus diseases without using chemical pesticides.

— Susan McGinley