Working Together on Nitrogen-Fixing Bacteria

Sugar cane plants don't normally make their own fertilizer. Yet that's what researchers in Brazil and the University of Arizona are investigating.

Special bacteria living within the leaves and stalks of certain sugar cane varieties in Brazil appear to convert nitrogen gas from the air into plant nitrogen, enabling the plants to thrive in poor soil.

Field experiments conducted by the Brazilian laboratory of Dr. Johanna Dobereiner have shown that up to 75 percent of the total nitrogen in sugar cane plants is coming from the nitrogen in the air, according to Christina Kennedy, a microbiologist working in the Department of Plant Pathology at the University of Arizona.

"The bacteria are getting sugar from the plants, and the plants get nitrogen from the bacteria," Kennedy said. It's a symbiotic, or mutually beneficial relationship between the plant and the bacteria living in it.

Called nitrogen fixation, the process usually occurs in bacteria within the roots of legumes, including beans and peas, rather than grass-type plants. Sugar cane, corn, rice and other cereals typically require extra nitrogen as fertilizer.

Kennedy is conducting laboratory experiments on the nitrogen-fixing bacteria from Brazil to find out how they work.

"First, we isolate the nitrogen fixation genes from the bacteria and make mutations in these genes," Kennedy said. "Then, we put the mutated genes back into the bacteria, take the mutated bacteria to Brazil, and do plant inoculation tests."

By observing which mutations still allow the bacteria to fix nitrogen and those that do not, the researchers will learn more about the genes that control the process. They'll also find out whether the bacteria are essential for plant growth in poor soils.

The other part of the study involves the propagation of sugar cane plants for laboratory use in Tucson.

"We will use sugar cane plants grown from tissue culture to generate sterile plants," Kennedy said. "Since bacteria are so intimately bound up inside plants in the field, it is difficult to isolate plants that do not contain them."

Because sterile plants would be bacteria-free, researchers would be able to identify the actions of the nitrogen fixing bacteria more easily after they were injected into the plant.

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"In previous work, we discovered how to make nitrogen-fixing bacteria that actually excrete the ammonia (nitrogen) they make into the environment," Kennedy said. "They normally don't do this. We figured out a way to disrupt the normal regulation of the nitrogen fixation process."

Kennedy and her co-workers also determined how some species of nitrogen fixing bacteria tolerate oxygen better than others. By injecting certain enzymes into the bacteria with a low tolerance for oxygen, Kennedy believes that they might fix more nitrogen inside the plant.

Using this knowledge, Kennedy plans to test the same interactions using *Azobacter diazotrophicus*, the bacteria extracted from the Brazilian sugar cane.

"Although this organism has been found inside the sugar cane, we still need to prove that it's the one causing the nitrogen fixation," Kennedy said.

Eventually, she hopes the information will be useful in finding ways to use nitrogen-fixing bacteria to promote growth of cereal crops. ❖

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