Rhizobial Magic

Collection preserves valuable nitrogen-fixing bacteria.

The American Revolution was over by 1784, but John Binns was getting ready to fight another battle on the fading farms of northern Virginia.

Agriculture was in trouble on once-fertile soils in Loudoun County and other areas along the Potomac River. Decades of growing corn and wheat had depleted soil nutrients, forcing farmers to move in search of fresh land. Binns wanted to keep farming in his native county, so he had to find a way to rejuvenate the soil.

Binns had heard about a farmer who was spreading crushed chalk on his fields, planting clover, grazing cattle, then plowing the clover into the soil. Binns decided to try it himself. He got 15 pounds of chalk stone, sledgehammered it into powder, and spread it on his corn mounds. Later, he planted clover after spreading lime. Gradually, his soil improved.

After 8 years, his corn yields had doubled, his wheat yields quadrupled. By then, he had named his farm "Clover Hill."

In the late 1790's, he was buying one farm after another, bringing the soil back to life with what became known as the Loudoun system. In 1803, he wrote *A Treatise on Practical Farming,* describing his findings to other farmers so they could put the practices to use on their fields.

Binns' story, told in Frederick Gutheim's book *The Potomac,* illustrates the value of what today is called low-input, sustainable agriculture. The lime reduces soil acidity, keeping it near the ideal pH of 6 and providing a healthy environment for clover. Then, once the clover is plowed into the soil and decomposes, it provides a source of nitrogen and organic matter.

Clover gets its nitrogen by providing a home for one of nature's most valuable bacteria: rhizobia.

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Microbiologist Peter van Berkum compares growth of alfalfa plants (left) inoculated with *Rhizobium* with plants that haven't been given the bacteria. (K5801-14)

Low-input, sustainable agriculture would be hard to achieve without rhizobia, because they bridge the gap between nitrogen in the air and the soil.

About 80 percent of the air is nitrogen. An estimated 34,500 tons of nitrogen float above a single acre of cropland. But grasses and other plants that don't have rhizobia can't make use of that vast sea of nitrogen in the air. So they often suffer from a lack of nitrogen, a key component of proteins and other nutrients that are essential for plant growth.

But clover, alfalfa, soybeans, beans, peanuts, and other legumes provide a home for rhizobia, creating one of nature's most important landlord/tenant relationships.

The bacteria live in structures called nodules that they induce the plant to build on its roots. Inside these tiny homes, rhizobia draw nitrogen from air pores in the soil and chemically convert it to a form that the plant can absorb. The rhizobia are like miniature nitrogen fertilizer factories.

Today's farmers who rotate their crops know the value of rhizobia. Wheat and corn, for example, take nitrogen from the soil. So farmers often rotate these crops with alfalfa, clover, or another legume cover crop that, working with rhizobia, adds nitrogen back into the soil. By doing this, farmers can avoid using nitrogen fertilizer that can wash away and pollute water supplies. This isn't a problem with rhizobia, because more than 90 percent of the nitrogen that they fix in nodules goes directly into the plant.

"The only time the nitrogen is released is when the plant is plowed into the soil. Even then the nitrogen is bound to the organic matter and very little of it is leached away," explains ARS microbiologist Peter van Berkum.

The World's First Rhizobium Collection

Van Berkum has spent more than a decade studying nitrogen-fixing bacteria. He's curator of the National Rhizobium Culture Collection at Beltsville, Maryland. There are 3,900 accessions in the collection, representing the four known classes of rhizobia.

This, the largest collection of its type, started with two single rhizobium nodules from soybean plants growing in 1913 at the USDA research farm in Arlington, Virginia, where the Pentagon now stands.

In 1915, two more rhizobial strains, from soybeans, were added. The first rhizobium from alfalfa was added to the collection in 1919.

Over the years, the collection continued to grow, as USDA scientists amassed about 1,200 different rhizobia as part of their ongoing research to learn more about the role of these bacteria in nitrogen fixation. In the 1950's and 60's, it was known as "the Beltsville collection."

The formal collection was established in 1975, when the U.S. Agency for International Development began funding it. USAID recognized the importance of rhizobia and legumes in agriculture in developing countries. Rhizobia from other collections were added to the Beltsville collection, consolidating the rhizobia in one place and giving the collection international importance.

More accessions are added each year, as scientists discover new strains. This year, for example, van Berkum added 102 accessions from alfalfa-like legumes that ARS agronomist Austin Campbell collected from the Inner Mongolia region of China.

Van Berkum says there is a lot of genetic diversity in the collection. Several rhizobia can create nodules on plant stems and produce bacteriochlorophyll. This substance is involved in plants' capture of light energy, or photosynthesis.

The rhizobia factories have been hard at work for a long time. Van Berkum notes that the Romans knew the value of legumes, using clover as a fallow crop. "They didn't know why it worked but knew that it did."

It wasn't until the late 1880's, however, that German scientists noted that legumes seemed to have higher nitrogen levels than grassy plants. And it wasn't until 1901 that a Dutch scientist isolated the first rhizobium, from a fava bean root.

Since then, USDA scientists and others have been learning more and more about these nitrogen-fixing bacteria. It turns out that a rhizobium that lives within a soybean root nodule might not take up residence in clover or alfalfa roots.

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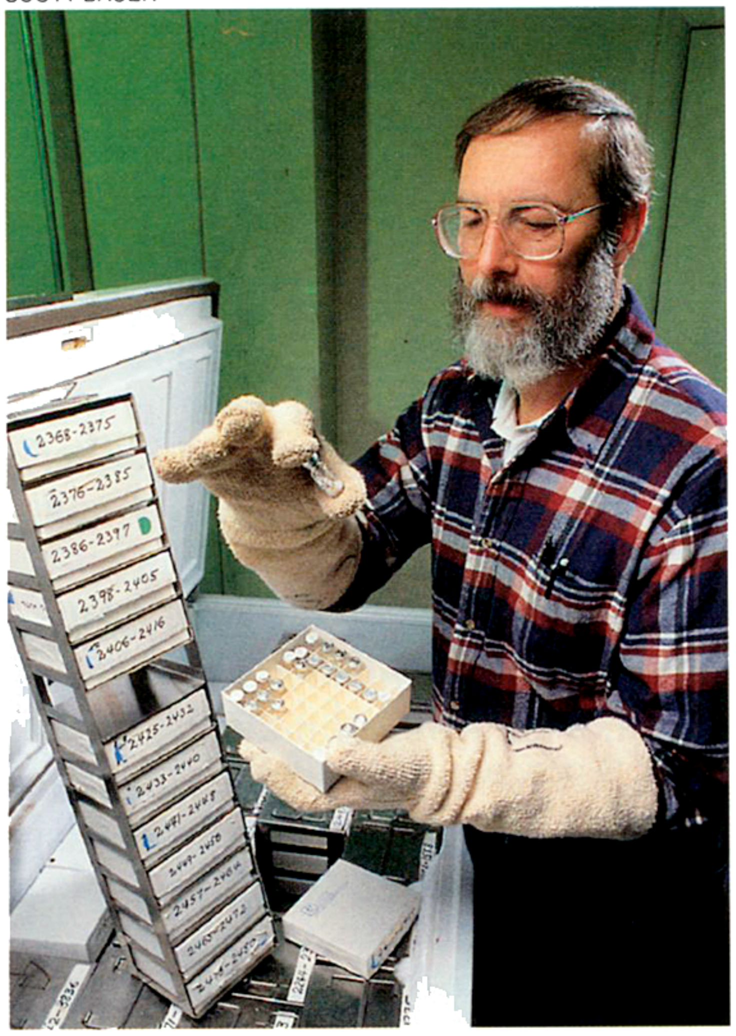

Mixed with dried skim milk for storage, rhizobia cultures are prepared for mailing by technician Lee Nash. (K5802-12)

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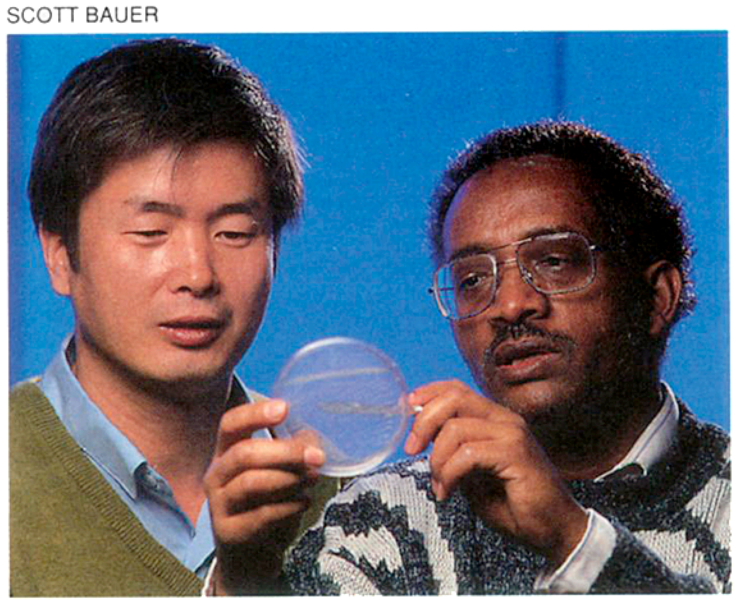

Visiting scientists Desta Beyene, from Ethiopia (right), and Bao Guiping, from the People's Republic of China, evaluate the growth of rhizobia in nutrient media. (K5802-9)

"It's very important to match the right rhizobium with a particular plant," says van Berkum. "That's one of the main purposes of the collection. We have genetically diverse types of rhizobia that researchers can use to improve nitrogen fixation and boost plant yields while enriching the soil."

Van Berkum says that as rain forests and other natural habitats are lost to development, it's important to preserve rhizobial genes. Some rhizobia, he notes, also live on the roots of leguminous trees.

"These genes will become valuable in the future, if microbiologists genetically engineer rhizobia and plants to more efficiently fix nitrogen," says van Berkum.

The collection itself is stored in modest surroundings, in a windowless basement room under a greenhouse. The decor is cinder block walls, concrete floor, heating and cooling ducts, and electrical boxes. Seven freezers, much like you'd find in the average home basement, hold the entire collection.

Each accession is preserved in glass vials inside metal boxes. The bacteria are kept at -70ºC in freeze-dried skim milk that provides a protein source and a visible substance for van Berkum to mail to people who request rhizobia for research.

"That's one of the things about rhizobia. They are largely invisible," he says. "They do their work in the soil, out of view, so it's easy to take them for granted. To most people, what they do is like magic."—By **Sean Adams,** ARS.

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