INTERNATIONAL SYMPOSIUM

IDENTIFYING AND IMPROVING NITROGEN-FIXERS

JAKARTA, Indonesia—Brazilian scientists may have discovered a new, acid-tolerant, nitrogen-fixing bacterium associated with sugarcane—thereby adding to the growing body of evidence that nitrogen fixation occurs in grasses, as well as legumes.

Speaking at an international symposium on nitrogen fixation held in August at the Indonesian mountain resort of Cisarua, Johanna Dobereiner (a research scientist with Brazil's national agricultural research center, EMBRAPA) said that 20 strains of the suspected nitrogen-fixing bacterium have now been isolated from the roots and the stems of five sugarcane varieties from several locations. The isolates grow on a pure sugarcane juice medium containing as much as 30-percent sugar. They fix nitrogen at a pH as low as 2.8, which kills most other bacteria. Dobereiner says that the bacteria actually use the sugar to produce huge amounts of gluconic acid as a source of food.

There are only three known acid-loving bacteria—Frateuria, Gluconobacter, and Acetobacter—to which the new isolates could be related. None of these three, however, fixes nitrogen. The Brazilian scientists are now collaborating with a Belgian team to identify the new bacterium—preliminarily named *Saccharobacter nitrocaptans*—by DNA and ribosomal RNA sequencing. The assays have already determined that it is not Frateuria, the genus with which it shares the most phenotypic characteristics.

If confirmed, the research will have enormous implications for sugar producers around the world. In Brazil alone, sugarcane growers spend $250 million a year on nitrogen fertilizer. (EMBRAPA's entire research budget is only $200 million.) Brazilian sugarcane yields a net energy gain of 143 percent. If the initial indications about the nitrogen-fixing capabilities of the new bacterium prove true, Dobereiner says that this gain could increase to 250 percent.

New results in mapping the genome of a species of Rhizobium have implications for the organization of DNA in other nitrogen-fixing bacteria. To this end, research on *Rhizobium phaseoli*—the symbiont of the common bean, *Phaseolus vulgaris*—is being conducted by several scientists at Mexico's Centro de Investigacion sobre Fijacion de Nitrogeno (Center for the Investigation of Nitrogen Fixation). Rafael Palacios, who was the first to discover that *R. phaseoli* has several copies of nitrogen-fixing gene sequences, reported that this pattern of repeated gene sequences is common to several other species, as well. According to Palacios, the presence of repeated gene sequences is responsible for frequent genetic recombination which can alter and even eliminate the symbiotic capability of the bacterium. Palacios explained that the lack of stability has important implications for the preparation of nitrogen-fixing soil inoculants. He also stressed the necessity of gaining better insight into how much and in what way genetic information is transferred between strains in field environments.

To identify specific gene functions, Palacios has cut the bacterium's plasmid into several fragments, which he then reintroduced into *R. phaseoli* (cured of its plasmid) to determine the function of each fragment. To study the interaction of particular bacterial genes in the overall bacterial-plant symbiotic relationship, Palacio's colleague, Frederico Sanchez, has been introducing specific-gene-mutated bacteria into bean plants and a tree legume (*Leucaena esculenta*). This procedure should ultimately permit genetic engineering for improved characteristics. Nearer-term, a genome diagnostic kit is being developed, which will allow breeders and farmers to determine whether a given bacterium is a good nitrogen-fixer.

Another symbiotic relationship being dissected is that of an aquatic fern, Azolla, and its cyanobacterial symbiont, *Anabaena azollae*. Azolla is one of the most commonly used nitrogen-fixing plants; it is difficult to exploit commercially, however, because superior strains rarely survive transfer into a new environment. But the bacteria might. According to Jacek Plazinski (Australian National University), the difficulty with determining the success of such a transfer has been the phenotypic similarity among many different Anabaena genotypes. But, with the advent of DNA hybridization techniques, a research team of Australian, French, and Chinese scientists hopes to surmount this problem.

The group has developed three DNA probes to aid in the positive identification of Anabaena genotypes. The first probe—based on *nif*H and *nif*S nitrogenase genes isolated from a non-symbiotic Anabaena (sp. PCC 7120)—is able to differentiate between Anabaena species. And RuBisCo (ribulose-1,5-biphosphate carboxylase/oxygenase) and rRNA probes isolated from *Anacystis nidulans* can differentiate between different Anabaena isolated from the same Azolla plant (all would appear the same from a taxonomic point of view). The third probe—based on a native Anabaena plasmid subfragment—permits conclusive identification of the nine Anabaena genotypes known to exist.

—Mark Timm

RESEARCH PAPER ANALYSIS

SPEEDING TRANSGENIC PLANTS

In this issue of *Bio/Technology*, researchers at Rockefeller University's Laboratory of Plant Molecular Biology (New York, NY) report on the production of transgenic flowering plants in just six weeks, and the generation of seeds in only eight weeks. By contrast, approximately 12–14 weeks are required when tobacco plants are generated from leaf discs—one of the more prevalent techniques for plant transformation. As Nam-Hai Chua and co-workers point out in their research paper, "Rapid production of transgenic flowering shoots and F_1 progeny from epidermal peels of *Nicotiana plumbaginifolia*," such shortened time from inoculation to seeds can greatly hasten the pace of gene transfer and analysis.

The key here was the use of a technique developed by Tran Than Van and her colleagues at CNRS (Orsay, France) whereby thin layers of tissue are peeled from the surface of flowering branches and placed on specific media that can regenerate roots, vegetative shoots, or flowering shoots. There are many instances where vegetative shoots or roots can be regenerated in plant tissue culture, but it is only in this system where the cultural regimes have been so refined that flowering shoots can be routinely obtained as well. The flowering shoots complete their life cycle rapidly both in culture and after transfer to soil.

The technique, which also permits individual cells to be followed during morphogenesis, has been profitably used by the French workers for more than 15 years. In this paper, the approach is melded with the more recent and equally powerful technique of Agrobacterium-mediated plant gene transformation.

—Philip V. Ammirato