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# THE CARIBBEAN *Rhizobium* GROUP; APPLYING BIOTECHNOLOGY TO INCREASE BIOLOGICAL NITROGEN FIXATION

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## ABSTBACT

A few bacteria can use nitrogen from the atmosphere, but plants cannot. The successful transfer of the genes responsible for nitrogen fixation from *Klebsiella* to *Escherichia* raised the possibility of using gene manipulation to create nitrogen-fixing crops. Ecological factors affect the efficiency of nitrogen fixation and reduce the energy devoted to produce ammonia. Introduction of *hup* genes into deficient *Rhizobium* would create superior strains, and the potential for environmental damage is minimum. To reduce the isolation of scientists working in Biological Nitrogen Fixation, the Caribbean Rhizobium Group (CRG) was established in 1984. Similar networks, supported by UNESCO (MIRCEN's) already exist. The initiation of BNFNET, a computer linkage conference, should facilitate the exchange of information and resources in the region.

## GENETIC ENGINEERING OF NITROGEN FIXATION

Nitrogen Fixation is an essential component of the nitrogen cycle, but only a few microorganisms can use atmospheric $N^2$ and produce a reduced form of the element ($NH_3$). This is possible because they possess a unique enzyme: nitrogenase. This is an extremely complex enzyme, and is the result of at least 18 genes (see Figure 1, Postgate, 1990). The most important nitrogen-fixing systems are symbiotic, and of these, legumes contribute most of the biofixation in agricultural ecosystems. After the first successful transfer of the *nif* genes from *Klebsiella* to *Escherichia*, it was thought that transfer of genes to grasses would solve the need of adding N fertilizers. However, we are hardly starting to understand the complex process of nodulation, where at least 30 *nod* and *fix* genes have been found. Nowadays, more simple improvements to the symbiosis are being tested. Among them, increasing the efficiency of the nitrogenase reaction and finding mechanisms to overcome competition of less effective rhizobial strains are promising alternatives.

The greatest challenge to transfer nitrogen-fixing abilities to non-legumes, is to find, map and study the regulation of nodulation genes. Each *Rhizobium* species is restricted to its specific group of host plants, forming the

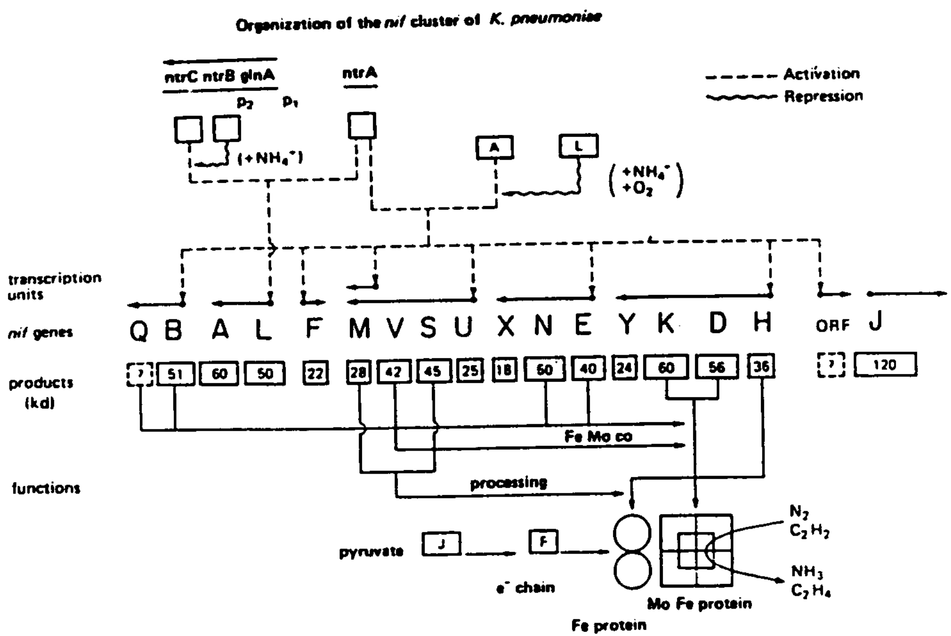


Fig. 1. Organization of nitrogen fixation genes

well known cross inoculation groups. For example, the *R. meliloti* host-specific nodulation genes (*nodE*, *nodF*, *nodG*, and *nodH*) allow it to form nodules on plants of the genera *Medicago*, *Melilotus*, and *Trigonella* but not on *Trifolium*, which is nodulated by *R. trifolii*. In contrast to these host-specific *nod* genes, "common" nod genes (*nodA*, *nodB*, *nodC*, and *nodD*) are conserved structurally and functionally among all *Rhizobium* species. It has been found that *nodABC* genes are induced by specific compounds exudated by the roots of the legume plants. The inducing compounds found in these exudates have been identified as flavones, flavanones or closely related compounds. This induction was shown to be dependent on the *nodD* gene. Recent genetic studies indicate that the determination of host specificity is a complex process involving many of the *nod* gene products (Surin and Downie, 1989). Therefore, the extension of the host range of symbiotic bacteria to new host species requires much more knowledge of basic biology. Techniques already developed for bacteria should be applied to the field of plant molecular biology.

Environmental factors also reduce the amount of biologically fixed dinitrogen. Those factors can be among the causes that affect the symbiotic response of a major grain legume such as *Phaseolus vulgaris*. It is evident that high temperatures and short days reduce the plant photosynthate supply necessary for the highly energy demanding reduction of $N_2$ by nitrogenase. Furthermore, unpublished results indicate that the *Rhizobium phaseoli*-*P. vulgaris* association lacks hydrogenase uptake (Hup), resulting in the loss of up to 50% of the ATP consumed in the nodule bacteroids. If functional *hup* genes were introduced into *R. phaseoli*, it would be possible to increase significantly the amount of symbiotically fixed nitrogen and raise the present level of protein yields. Since rhizobia have been used in agriculture for almost 100 years, the potential environmental impact of releasing recombinant bacteria would be minimized. Besides, assessment of the environmental safety of released recombinant bacteria is rapidly taking advantage of advances in the methodology to sensitively and accurately detect specific bacterial strains or their genes. Bacterial DNA can be extracted from the soil and identified and quantified by hybridization procedures, and the PCR method increases the sensitivity (Lindow et al., 1989).

## THE CARIBBEAN RHIZOBIUM GROUP

The Caribbean islands have many cultural and historic ties, as the Caribbean Food Crop Society shows; but they also share technological and economic problems. Scientists working in nitrogen fixation in the region tend to be few and isolated. The Caribbean Rhizobium Group was established in August, 1984, at the First Caribbean Nitrogen Fixation Conference, to overcome this isolation and share resources and information. Since then, a second meeting was held at Panama (1986), and a newsletter is published to exchange information. Several editions of the newsletter have appeared, and it is presently distributed to more than 60 scientists. They are located at Antigua, Barbados, Belize, Cuba, Dominican Republic, Guadalupe, Guyana, Haiti, Jamaica, Puerto Rico, St. Lucia, Surinam, Trinidad and the U.S. Virgin Islands. It is also distributed to other interested persons and institutions outside the region. If you are interested in joining or know of someone interested, please send a letter to:

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In the area of Agricultural Biotechnology, a collaborative research project is being carried out between the Secretaría de Estado de Agricultura of the Dominican Republic and the BNF Laboratory of the University of Puerto Rico at Mayaguez. The project started in 1988 and is funded by the Program in Science and Technology Cooperation (PSTC) of USAID. The purpose of the project is to control an important fungal disease in beans, caused by *Macrophomina phaseolina*, with the application of *Rhizobium* as a biopesticide. However, preliminary results indicate that the utilization of stronger antagonistic bacteria (such as *Pseudomonas*) could provide better biocontrol. The creation of a regional project to consider the application of these bacteria will be proposed during this meeting.

## NETWORK OF MICROBIOLOGICAL RESOURCES

Microorganisms are part of the gene pool that can be utilized through genetic engineering. Therefore, conservation of their biodiversity should also be considered in the programs presently under development. Sufficient economic resources should be allocated for their preservation. UNESCO and UNDP have initiated a worldwide programme focussing on efforts to preserve microbial gene pools and making them accessible through the establishment of a network of Microbiological Resources Centers (MIRCENs). Their objectives are:

a) provide the infrastructure for a worldwide network which would incorporate regional and interregional cooperating laboratories geared to the management, distribution and utilization of the microbial gene pool;

b) reinforce efforts relating to the conservation of microorganisms, with emphasis on *Rhizobium* gene pools, in developing countries with an agrarian base;

c) foster the development of new inexpensive technologies native to the region;

d) promote the applications of microbiology in order to strengthen rural economies; and

e) serve as focal centers for the training of manpower and the diffusion of microbiological knowledge.

Presently, 19 Centers compose the World Network (Table 1) (MIRCEN News No. 11).

We believe that Caribbean interregional linkages will be stronger in the future. This undoubtedly will be caused by the arrival of computer communications to each researcher. The initiation of an electronic network (BNFNET) was announced during the last International Nitrogen Fixation Meeting (May 20-26, Knoxville, TN). It was introduced by the UNEP/UNESCO/ICRO MIRCEN at Stockholm. The Caribbean area scientists that have communication capabilities and access to BITNET can join BNFNET. The address is:

Eng-leong_Foo_@KOM.KOMINITY.SE

Local messages can be placed to:

BITNET address: E_Schroder@RUMAC.UPR.CLU.EDU

Furthermore, access to GenBank and other on-line databases should be available for storing and comparing gene sequences of agriculturally important bacteria and fungi. The recent proposal for the creation of the National Digital Library of unclassified federal data banks and software libraries should be supported by all scientists.

Table 1. List and location of the World Network of Microbiological Resources Centers (MIRCEN).

| Speciality | Location | Place and Country |
|---|---|---|
| Rhizobium | Department of Soil Science and Botany | University of Nairobi, Kenya |
| Rhizobium | Instituto de Pesquisas Agronomicas | Porto Alegre, Brazil |
| Fermentation, Food and Waste Recycling | Thailand Institute of Scientific and Technological Research | Bangkok, Thailand |
| Biotechnology | Ain Shams University | Cairo, Arab Rep. of Egypt |
| Biotechnology | Central American Research Institute for Industries | Guatemala City, Guatemala |
| Rhizobium | NifTAL Project, College of Tropical Agriculture | University of Hawaii, U.S.A. |
| Biotechnology | Karolinska Institute | Stockholm, Sweden |
| World Data Centre | Life Science Research Information Section, RIKEN | Saitama, Japan |
| Rhizobium | Centre National de Recherches Agronomiques | Bambey, Senegal |
| Biotechnology | Planta Piloto de Procesos Industriales Microbiologicos (PROIMI) | Tucuman, Argentina |
| Rhizobium | Soybean and Alfalfa Genetics Laboratory | Maryland, U.S.A. |
| Fermentation Technology | Institute of Biotechnology | University of Osaka, Japan |
| Biotechnology | Biological Laboratory | University of Kent and Canterbury, Kent, U.K. |
| Biotechnology | University of Waterloo and University of Guelph | Waterloo/Guelph, Ontario, Canada |
| Marine Biotechnology | Department of Microbiology | University of Maryland, U.S.A. |
| Biotechnology | Centre de Transfert | Toulouse, France |
| Biotechnology | University of Queensland | Brisbane, Australia |
| Microbial Technology | Institute of Microbiology, Academia Sinica | Beijing, China |
| Biotechnology | Caribbean Industrial Research Institute | Tunapuna, Trinidad and Tobago |

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