## PREFACE

The 12th International Congress on Nitrogen Fixation was held in Foz do Iguaçu, Paraná State, Brazil, from the 12th to 17th of September, 1999. This volume constitutes the proceedings of this Congress and represents a compilation of the presentations by scientists from 38 countries who came to discuss the progress made, to exchange views and to collaborate. Since the first meeting in Pullman, Washington, USA, in 1974, this series of Congresses has been held three times in the United States, four times in Western Europe, in Australia, Mexico and Russia, and now for the first time in South America. Brazil was a most appropriate choice because Brazilian agriculture is especially dependent on biological nitrogen fixation. An important example is the soybean crop. The 30 million metric tons of grain produced each year relies solely on symbiotic nitrogen fixation. N-fertilizers are expensive in Brazil and, in the absence of the biological process, this crop would require 4-5 M tonnes of N at an annual cost of almost 1 billion dollars. Crop rotation, soil reclamation, forestry, forage mixed cropping, intercropping with grasses and legumes, associative nitrogen fixation with non-legume crops, such as sugarcane, and other agricultural practices in Brazil are also dependent on biological nitrogen fixation. The Brazilian experience with biological nitrogen fixation is a paradigm to be followed by all countries where nitrogen fertiliser is expensive or where present day environmental concerns demand a feasible, efficient and inexpensive alternative to chemical N-fertilizers.

In the biochemistry section, the analysis of the structure of the MoFe protein of *Klebsiella pneumoniae* at a resolution of 1.6 angstroms was described as was the complex between the altered *Azotobacter vinelandii* Fe protein Δ127L, which remains locked in the MgATP conformation in the absence of MgATP, and the MoFe protein. The intriguing possibility was raised that the Fe protein can be reduced by two electrons and transfer them both during a single encounter with the MoFe protein thereby improving energy expenditure with only two MgATPs hydrolysed per two electrons transferred. Other advances include the study of the FeMoco in non-protein surroundings, involving the binding of inhibitors and the reduction of substrates. These studies are paralleled by FTIR analysis of CO binding to the MoFe protein. The regulation of *nif* gene expression and nitrogen metabolism attracted a large number of contributions. Major advances in regulation have centered on the activation of the *nifA* gene and the activity of the NifA protein. Regulation of activity by NifL involves the GlnK protein in either a positive or negative manner, depending on the organism. The N-terminus of the *Herbaspirillum* protein inhibits the binding of the central plus C-terminal domain to the *nifH* promotor, whereas *nifL* probably prevents NifA interacting with the RNA polymerase. PII of *Herbaspirillum,* which activates NifA, has a structure similar to the GlnK of *Escherichia coli*. Autoactivation of the *nifA* gene in *Herbaspirillum* is inhibited by IHF, which acts positively and negatively, to regulate *nifA* expression. The role of the $\sigma^N$ protein has been studied in depth.

A big shift has been made from genes to genomes. The sequence of the sym plasmid of NGR 234 has been completed as has the genome of *Rhodobacter capsulatus*. Other genomes under investigation are those of *Sinorhizobium meliloti*, *Bradyrhizobium japonicum*, the symbiosis island of a *Mesorhizobium loti* and the symbiotic plasmid of *Rhizobium etli*. Plant genomes that have been analyzed include *Medicago truncatula, Lotus japonicus* and soybean.

Interest persists in the associative and endophytic bacteria and evidence is growing that the endophytic bacteria, such as *Acetobacter diazotrophicus, Herbaspirillum* and *Azoarcus* species, colonize intercellular spaces in the cortex and also xylem vessels. The range of plant species hosting *Azospirillum*, *Herbaspirillum* and *Burkholderia*, as well as undefined species has increased. Clear evidence has been obtained that nitrogenase is expressed inside sugarcane plants harbouring *Acetobacter diazotrophicus* and inside rice harbouring *Azoarcus* spp. Because nitrogenase is expressed under these conditions, biological nitrogen fixation presumably contributes to bacterial growth and possibly to plant growth. However, the positive contribution of these organisms to plant growth goes beyond just nitrogen fixation.

Nod factors (LCO's) were the major focus concerning the control of nodulation in the legume symbiosis. Purified Nod factors, together with expression of the *enod40* gene, activate the inner cortical cells for the cell division necessary for nodule formation. Differentiation of meristematic cells involves arrest of cell division, an increase in cell size and infection by bacteroids. A plant cell regulator gene *ccs52* controls cell-division arrest and is essential for nodule formation. Lectin-like NTPases appear to be necessary for nodulation, possibly as Nod factor-binding proteins. A key issue in the session on metabolism of symbiosomes concerned alanine, rather than ammonium, as the major N exporter from the nodule to the plant. It is likely that either ammonium or alanine can be excreted, depending on the physiological state of the bacteroid. Interest continues to be high regarding both the nodule malic enzyme and the peribacteroid membrane.

New molecular tools are enabling progress in the study of molecular phylogeny and biological diversity. Several studies show similarities between $N_2$-fixing species and other species and genera not reported as $N_2$ fixers. Much effort is being expended in trying to unravel and comprehend the biodiversity of diazotrophic bacteria.

Agricultural practices emphasized the benefits of nitrogen fixation for improving soil fertility and increase yield, for playing an important role in the sustainable management of agricultural systems, and in soil reclamation. Phosphorus remains a key nutrient to improve the effectiveness of the nitrogen-fixation process and, therefore, the important role played by mycorrhizae was emphasized. Approaches using the inoculation of selected strains, improvement of inoculation technology, co-inoculation of microorganims and several other practices are being successfully used in several countries.

A final session was devoted to predictions of future research directions in nitrogen fixation for the 21st century. These included the possibility of establishing active nitrogen fixation in plants, the establishment of nitrogen-fixing symbioses with rice, and the use of transgenic legumes to combat environmental stress, including herbicide tolerance and resistance to pests and diseases.

We want to express our deep thanks to all those who worked hard and long to help us in the organization of this Congress. We want to emphasize our gratitude for the financial support that we received from all the institutions that believe in our work. Finally, we want to thank our families and friends for their moral support during the last two years.

Curitiba, September 1999

Fábio O. Pedrosa
Mariangela Hungria
M. Geoffrey Yates
William E. Newton