Symbiosis, 15 (1993) 281–288
Balaban, Philadelphia/Rehovot

Meeting Review

"Nitrogen-92", September 22–26, 1992

The 8th Eastern Europe Symposium on Biological Nitrogen Fixation "Nitrogenfix-92" was held in Saratov, Russia, September 22-26, 1992. It was organized by the Institute of Biochemistry and Physiology of Plants and Microorganisms (Russian Academy of Sciences, Saratov) and All-Russia Research Institute for Agricultural Microbiology (Russian Academy of Agricultural Sciences, St.-Petersburg).

In spite of the unstable political and difficult financial situation in Russia, 70 scientists from 12 countries took part in this meeting. The list of participants included: Prof. Y. Bashan (Mexico), Prof. H. Bothe (FRG), Dr. K. Goethals (Belgium), Prof. P. Gresshoff (USA), Dr. M. Keller (FRG), Dr. N. Kerby (Scotland), Prof. G. Kiss (Hungary), Prof. A. Legocki and Prof. Z. Lorkiewich (Poland), Prof. B. Lugtenberg (The Netherlands), and Dr. K. Michelis (Belgium). The delegation of Russian specialists, to whom the Symposium gave a real opportunity to present their results to the international scientific community included about 50 participants, among them Prof. B. Simarov, Prof. S. Shestakov, Prof. V. Emtsev.

The program was started after the opening speech of the Chairman of the Organizing Committee Prof. V. Ignatov. It included 12 plenary lectures, 17 oral communications and 44 poster communications. These presentations comprised 4 sections:

1. **Molecular genetics and biochemistry of nitrogen-fixing microorganisms**
   Organization and regulation of the genes controlling nitrogen fixation and interaction with higher plants in both symbiotic (*Rhizobium, Anabaena*) and freeliving (*Rhodobacter, Azospirillum, Agrobacterium*) microorganisms were presented in this section.

   In his lecture Prof. B. Simarov presented the results of isolation and analysis of *R. meliloti* Tn5 mutants with increased symbiotic efficiency (ability to promote plant growth). The obtained mutations were located and mapped on the megaplasmids and chromosome. The DNA fragments harbouring some of these Tn5 insertions were cloned and their restriction maps were constructed. This approach was demonstrated to provide an opportunity for generating commercially important rhizobia strains by use of Tn5 mutagenesis and isolating the genes controlling symbiotic efficiency for subsequent construction of improved strains.

The report of Dr. M. Keller was devoted to the *R. meliloti* genes controlling exopolysaccharide synthesis and infectivity. The author demonstrated that mutations in some of these genes fail to suppress the plant defense system and therefore to initiate the infection thread formation.

Great interest of the audience was raised by the report of Prof. S. Shestakov who presented the results of cloning and sequencing of the new regulatory genes involved in nitrogen fixation in the photosynthetic bacterium *Rhodobacter sphaeroides*.

2. **Genetics and biochemistry of symbiotic activity in legumes**

A broad spectrum of problems concerning the role of the legume host plant in the control of symbiosis development was discussed (isolation, mapping and functional analysis of the symbiotic genes; identification of the nodule-specific forms of aspartate amino transferases, peroxidases, ATP-ases; genetic polymorphism of legume crops for symbiotic activity). The major part of the discussion of this section was directed to the mutual regulation of the genetic systems of partners during formation of the legume-rhizobial symbiosis, and especially to the exchange of the specific regulatory signals between plant and bacteria.

The level of reports and discussions at this section has been set by the two lectures of Prof. P. Gresshoff "Nodulation does not require *Rhizobium*" and "Molecular mapping of nodulation genes in soybean". In the first one, the results of genetic and physiological analysis of the alfalfa genotypes able to form "spontaneous" pseudonodules in the absence of rhizobia were given. The author demonstrated that this property is connected with the autoregulatory mechanisms of nodulation and speculated that in the course of plant evolution the ability to form "spontaneous" nodules appeared much earlier than the ability to form nitrogen-fixing symbiosis. Possibly, the spontaneous nodules served as the organs for starch accumulation in the legume plants. From the viewpoint of molecular biology, these "spontaneous" nodules can serve as a useful tool for the elucidation of a range of fundamental problems, such as mechanisms of cell division. The second talk of Prof. G. Gresshoff showed the way to overcome the gap between formal genetical and molecular genetical analysis of symbiotic properties in legumes. It can be achieved using the method for mapping of nodulation mutations in soybeans by analysing the restriction fragments length polymorphism (RFLP). The successful application of this approach was demonstrated by Prof. G. Kiss, who presented the results on construction of the chromosomal maps in alfalfa. Data on the expression of the gene controlling synthesis of

the methionine-rich protein in the transgenic alfalfa plants were demonstrated in his lecture.

Prof. B. Lugtenberg summarized in his lecture the data on control of symbiotic specificity in both partners (legume and rhizobia) and demonstrated that the transfer of the lectin gene (*psl*) from pea to clover lead to the acquisition of the ability in the recipient to form nodules with *R. leguminosarum* bv. *viciae* strains. This specificity may be correlated with the heterogeneity of symbiotic signals excreted by the microsymbiont into the plant rhizosphere.

Prof. I. Tikhonovich presented the results of isolation and characterization of the new Nod and Fix mutants in pea. Some of the plant mutations influenced the multiplication of bacteroids within the symbiosomes. They contained several bacteroid-like cells per envelope rather than bacteroids being packed individually, which is more typical for pea.

3. **Interaction of the associative microorganisms with higher plants**

The results of analysis of physiological and biochemical mechanisms of interaction between the associative nitrogen-fixers (*Azospirillum brasilense, Agrobacterium radiobacter, Pseudomonas spp.*) were discussed. Prof. Y. Bashan drew the audience's attention to the fact that the efficiency of this interaction depends not only on the intensity of nitrogen fixation but also on the ability of bacteria to produce plant growth promoting substances, to protect plant roots from pathogens, to intensify uptake of mineral nitrogen and water from the soil, etc. Prof. V. Emtsev and other participants demonstrated in their presentations the characteristics of new nitrogen-fixing bacteria isolated from the rhizoplane of different cereals and vegetable crops. A wide spectrum of such bacteria can be used as beneficial inoculants for various non-legume crops.

4. **Physiology and practical applications of nitrogen-fixing systems**

In this section a wide range of problems was discussed: technology of inoculation of higher plants with nitrogen-fixing bacteria, monitoring of these bacteria in the agrobiocenosis, the role of bacterial surface components in the interaction with plants, and construction of new nitrogen-fixing systems. It was stressed that application of associative microorganisms requires the development of methods for screening them both in soil and in the rhizoplane of plants. The approach to this problem was demonstrated by Prof. H. Bothe who presented the results of analysis of the distribution of diazotrophic and denitrifying bacteria in soils using DNA probes. Dr. N. Kerby demonstrated the results of experiments

on the construction of new highly-effective nitrogen-fixing associations between cereals and cyanobacteria.

During the discussion of this section, Dr. A. Kozhemyakov and Prof. V. Emtsev stressed the necessity of expanding the list of valuable species of agriculturally important microorganisms, as well as of using the new criteria for the selection of such microorganisms.

The round table organized by Prof. B. Lugtenberg and Prof. B. Simarov was devoted mainly to the practical aspects of biological nitrogen fixation. An intensive discussion concerning the problem of the carriers used for production of microbial preparations took place. The participants have demonstrated that the development of the appropriate carrier is a critical point upon which the practical application of the new active nitrogen-fixing microorganisms depends strongly. Although the use of the traditional carrier (peat) remains predominant in biopreparations, it has a number of disadvantages, and therefore in some cases vermiculate, different gels and other substrates have to be available.

As it was stressed in the discussion, the use of the preparations of nitrogen-fixing microorganisms involves the release of bacteria into the environment in extremely high quantities and therefore a problem of genetic safety of such strains is very actual. On the one hand, the risk of this release must not be overestimated because a more than 100 year use of nodule bacteria for legume inoculation did not lead to any dangerous genetic and ecological consequences. However, nowadays several laboratories have developed new highly active strains of these bacteria by means of genetical engineering methods. Therefore special rules for the release of such microorganisms should precede their large scale practical application.

The Symposium opened a good opportunity not only for the scientific but also for organizational activities of its participants. Several bilateral agreements for collaboration were established between Russian and Western scientific institutions. The Initiative Group of the Symposium (including Prof. P. Gresshoff, Prof. B. Lugtenberg, Prof. H. Bothe, Prof. Y. Bashan, Prof. I. Tikhonovich, Prof. B. Simarov) prepared the Resolution in which a successful work of the "Nitrogenfix" Symposia during the past 15 years was marked. The possibilities for stimulating the Russian-Western scientific contacts through the activities of these Symposia was discussed. The Initiative Group proposed to reorganize the "Nitrogenfix" into an All-European Symposium on Genetics of Nitrogen Fixation.

The Symposium participants gratefully accepted Prof. Kiss's invitation to call the next Symposium in Hungary (Szeged). The Initiative Group proposed to set up the Advisory Board of the 9th European Nitrogenfix Symposium

comprising 15 scientists, some of whom (Prof. Y. Okon, Prof. J. Sprent, Prof. A. Legocki, Prof. F. O'Gara) have already agreed to take part in this activity. Prof. A. Puhler (FRG) accepted the proposal to head the Advisory Board of the European "Nitrogenfix" Symposium. At present the Advisory Board has begun the activities on preparation for the next meeting and hopes for active participation of specialists in nitrogen fixation research in this meeting.

The Initiative Group emphasized the necessity for extending the financial support for the symposium from both Russian Government and European Community. They expressed their gratitude to the Local Organizing Committee not only for developing a fruitful scientific programme but also for the exciting cultural programme including sightseeing of the old part of Saratov city, trip by boat along the Volga, concerts of the local musical groups, etc. The marked increase of the scientific level and activity of the Russian scientists, working in the field of Biological Nitrogen Fixation was stressed in the Resolution of the Symposium.

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