

Agricultural Application of Non-Symbiotic Nitrogen-Fixing Bacteria

E. N. MISHUSTIN, T. A. KALININSKAYA and T. V. RED'KINA

Institute of Microbiology, USSR Academy of Sciences, Moscow /USSR/

Enhancing the role of biological nitrogen in agriculture is a very important problem at present. On the one hand, nitrogen fertilizer production is a very energy-consuming process; on the other hand, there are problems with the food products and environmental pollution when high doses of nitrogen fertilizers are used.

Besides the activity of Rhizobia, it is very important to enhance the activity of non-symbiotic nitrogen-fixers with the aim of increasing the yields of non-leguminous cultures and reducing the doses of nitrogen fertilizers.

Research in this direction has advanced considerably over the past 15 years, since DÖBEREINER and DAY /1976/ discovered associations between bacteria of the Azospirillum genus and the roots of tropical cereals. The greatest part of these investigations is devoted to the study of associative nitrogen-fixers, which can actively develop in the root zone of plants and perform nitrogen fixation there. This group of microorganisms comprises a great number of species. Azospirillum is one of the best studied associative nitrogen-fixers. The mass development of this microorganism was observed in the root zone of tropical grasses, and in the rhizosphere of rice, corn, barley and other grain cultures. It usually occurs in the rhizosphere of plants growing in southern latitudes, though Azospirillum is sometimes found in the soils of the temperate zone. In the Soviet Union Azospirillum was discovered in the rhizosphere of wheat, barley, corn and fodder grasses in Ukraine, Belorussia, West Siberia and in the Moscow and Seratov regions.

In regions with colder climates, enterobacteria play the most important role. In some cases such classic nitrogen-fixers as Azotobacter and Clostridia may function as associative diazotrophs.

Current studies have proved that the infection of the root system with Azospirilla often occurs through microorganisms found on grains /KALININSKAYA and RED'KINA, 1981/. The soil may also be a source of Azospirilla developing in the rhizosphere. In this case the interaction of Azospirilla with the roots is facilitated thanks to their ability for chemotaxis and to interaction with lectine receptors on the plant roots. Most of the studies revealed that Azospirilla are usually concentrated in the rhizoplane of the plants. This localization is connected with the active metabolite exchange between the plants and the bacteria. It has been demonstrated that in gnotobiotic

systems, rice seedlings assimilated 30 to 70% of the isotope nitrogen fixed by *Azospirilla* /VASYUK, 1988/.

Under temperate climatic conditions inoculation with *Azospirilla* does not always result in their mass development in the rhizosphere, so the increases in yield cannot be explained by the nitrogen-fixing activity of *Azospirilla* alone. It has been shown that *Azospirilla* primarily promotes the synthesis of growth substances stimulating the development of plants and the root system. The absorption of water and the assimilation of nitrogen and fertilizers increase, while the loss of nitrogen from the soil lessens.

The other reason for yield increases after *Azospirilla* inoculation may be its antagonistic influence on the development of phytopathogenic and especially fungal microflora /RED'KINA, 1988/. *Azospirilla* have been shown to synthesize fungistatic compounds, which suppress the growth of phytopathogenic fungi. Inoculation with *Azospirilla* considerably improved the growth of plants from grains with fungal infection /RED'KINA, 1988/.

The interrelations of *Azospirilla* with plants are very specific and depend to a great extent on the peculiarities of different plant cultivars.

The problem is thus to discover and select plant cultivars capable of ensuring the highest rate of associative nitrogen fixation.

A large number of associative diazotrophs have been isolated in the USSR and their effect on plants has been thoroughly tested /VASYUK, 1988/. Apart from *Azospirilla*, a favourable effect on the growth of plants is produced by *Aquaspirilla*, *Agrobacteria*, *Enterobacteria* and some other groups of nitrogen fixers. At the Scientific Research Institute for Agricultural Microbiology /Leningrad/ the production of industrial preparations is carried out, including *Azospirillum*, *Flavobacterium* and *Arthrobacter*, which proved to be the most effective under various conditions /VASYUK, 1988/.

Good results have been obtained by the use of diazotroph preparations to inoculate grasses in Karelia, barley and grasses in Belorussia, winter rye crops, buckweed and grasses in the Ukraine, sorghum in the Rostov region, and rice in the Crimea and Kuban.

As a result of inoculation, the yield surpluses varied from 10-12 to 20-25%. The effect observed was equivalent to the application of 30-40 kg/ha of nitrogen fertilizers.

The use of optimum doses of nitrogen fertilizers and the maintenance of optimum soil humidity must be regarded as the most important factors for increasing the effectiveness of associative nitrogen fixation.

Another way of using nitrogen fixers for increasing agricultural yields is the stimulation of their life activity in the soil itself. In this case it is essential to enrich the soil with available organic compounds /plant residues may serve as sources of these/.

In laboratory experiments straw application methods have been developed to increase soil fertility. It was demonstrated that the preliminary addition of straw into the most active surface soil layer prevented the appearance of unfavourable effects connected with the accumulation of phytotoxic substances and with the immobilization of soil nitrogen /MISHUSTIN et al., 1970/.

The best effect of straw application was obtained in soils under rice; this was connected with the stimulation of nitrogen fixation /the nitrogen fixation rate usually amounted to 7-10 kg N for each ton of straw/.

Experiments with isotopic nitrogen have shown that during one vegetation season rice could assimilate up to 30% of the biological nitrogen from the soil. During the first year other plants /wheat, maize, oats/ assimilated 10-15% of the nitrogen fixed by diazotrophs. Nearly 50% of the fixed nitrogen was incorporated into the soil humus, contributing to the stabilization of the nitrogen reserves of the soil.

At present scientists in England and Australia are working on effective methods of straw application for increasing the rate of nitrogen fixation /GIBSON et al., 1988/. Investigations conducted in Australia revealed a considerable increase in nitrogenase activity in soils with straw added. The effectiveness of the straw increased markedly after inoculation with a mixture of cellulose-decomposing and nitrogen-fixing microorganisms. This mixture will be used to sprinkle straw residues after harvesting, to be followed by ploughing into the soil /GIBSON et al., 1988/.

References

- DÖBEREINER, J. and DAY, J. M., 1976. In: Proc. 1st Int. Symp. on N₂ fixation /Eds.: NEWTON, W. E. and NYMAN, C. V./ 518-537. Pullman. Washington.
- GIBSON, A. H., HALSALL, D. M. and ROPER, M. M., 1988. In: Nitrogen fixation: hundred years after. /Eds.: BOTHE, H., DE BRUIJN, F. E. and NEWTON, W. E./ 753-758. Gustav Fischer, Stuttgart-New York.
- KALININSKAYA, T. A. and RED'KINA, T. V., 1981. Izvestiya AN SSSR, Ser. Biol. /4/ 617-620.
- MISHUSTIN, E. N., VOSTROV, I. S. and PETROVA, A. N., 1970. Izvestiya AN SSSR. Ser. Biol. /2/ 291-296.
- RED'KINA, T. V., 1988. In: Proc. 6th Congress of the FEPPS. Yugoslavia.
- VASYUK, L. F., 1983. Biological nitrogen in the agriculture of the USSR. Nauka. Moscow. 88-98.