Beneficial Soil Microorganisms: A Key for Sustainable Agriculture

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Abstract : Microorganisms play a major role in the plant-soil relationship, particularly in the rhizosphere, i.e. the interface between root and soil. Rhizosphere is strongly influenced by the root, through its productions (rhizodeposition) and its respiration, as well as uptake of ions and minerals. In this environment, plant beneficial microorganisms are of peculiar importance: they stimulate plant growth, help mineral nutrition and water uptake, fix nitrogen, protect plants against parasites, and may interact positively between themselves (e.g. bacteria / mycorrhiza interactions). Selection of active strains and development of soil management practices should be combined to help improving crop production in a sustainable manner.

Introduction

Whereas the vascular plants appeared on earth during the Silurian age, less than 500 millions year ago, bacteria exist since 3.8 billion years. They had already evolved most of the basic geochemically significant cellular functions we know today. Several of these functions are of great importance in favouring plant nutrition, such as nitrification, sulfide oxidation, nitrogen fixation, as well as the complexation and/or solubilization of minerals. Therefore, one can imagine that plants, upon their appearance on earth, found in their environment many types of pre-existing microorganisms ready to help them! In Precambrian times, there were probably no earthworms, no soil insects, and before the "invention" of lignin by vascular plants, no humic acids similar to the present ones. Soil conditions should have been very different. Since Silurian and Devonian times, a coevolution occurred, involving all partners in soil biocenosis, plants, bacteria, fungi, protozoa, invertebrates (particularly insects and earthworms) and, last but not least, soil enzymes. This coevolution led to the present status of the plant-soil biocenosis.

In nature, a plant is not, as many plant physiologists still consider, an organism just living in contact with air and with an aqueous solution of minerals. This classical view of an oversimplified ecosystem is that of conventional agriculture, culminating in the soil-free cultivation systems. Conventional, intensive agriculture, which makes use of large amounts of inorganic fertilizers, of pesticides and of heavy machines, gives quite high yields in the short term. But these technologies often lead to irreversible destruction of soil structures, and therefore, in the long term, to a loss of fertility.

Sustainable development in agriculture requires us to re-consider the functions of plantsoil natural ecosystems, including their whole biocenosis. This is the scientific basis of organic agriculture. The return to Nature of Jean-Jacques Rousseau is actually not only an empirical, philosophical, ethical or religious movement; it also has now strong scientific bases.

The Rhizosphere The rhizosphere may be defined as the region of soil under the influence of the rootî. This is not actually a defined region, but rather a gradient existing between distant soil and the root itself, which may be colonized by microorganisms (endorhizosphere). It is the privileged interface between plant roots and soil, where most exchange occurs. These exchanges are largely controlled by microorganisms, bacteria and fungi, which in turn are controlled by their predators, protozoa and small invertebrates. Primarily, rhizosphere conditions are defined by root activities, mainly production of organic materials, respiration, and uptake of water and minerals. The production of organic materials in the soil by living root (the rhizodeposition) is surprisingly high, in the same order as net biomass production or respiration. In other terms, the plant secretes to soil as much organic material as it accumulates. Rhizodeposition comprises detached cells, mucilaginous polysaccharides, lysates from cortical webs, and secretion of soluble organic molecules. Therefore, the rhizosphere is characterized by an important nutrient flow, strongly contrasting to more distant soil. This flow determines growth and activities of rhizosphere microorganisms. In turn, through their activity, these microorganisms will alter the conditions in their neighbourhood. Some of their activities may be beneficial to plants, other detrimental, other neutral. Through the co-evolution mentioned above, natural ecosystems evolved towards very effective mutualistic interactions between plants and microorganisms.

Mutualistic interactions imply two or more partners, which benefit from one another. In many cases, under natural conditions, these organisms could not survive alone. These interactions may be nutritive, but may also consist in the modification by a partner of some characteristics of the habitat, like pH, oxygen content, repressive effects against competitors, and so on. They may occur between very different organisms as between soil bacteria and plant roots, or the partners may be physically intimately linked to oneanother, as is the case of symbioses.

Agricultural ecosystems, even the most natural ones, represent a considerable stress in the plant-soil ecosystem. Most crop plants are annual, cultivated as monocultures in soils with crop rotations, tilling, fertilization and so on. This differs much from natural soils, not tilled, limited in mineral nutrients, covered by a mixed vegetation of mostly perennial plants. Agricultural practices may induce higher susceptibility towards pathogens, higher needs in nutrients, and so on. Moreover, crop plants are in general fast-growing, r-strategists (ruderal plants) in terms of their general ecology.

With organic (natural) farming in mind, it is therefore interesting to know more about the beneficial rhizosphere microorganisms, resulting from a long co-evolution with plants, but which could also favour crop plants.

There are indeed two approaches in applying this knowledge to farming:

- selecting appropriate rhizosphere microorganisms (pure cultures or consortia), cultivating them in masses and seeding them in soil
- favouring, by soil practices, the maintenance and development of beneficial microorganisms in the rhizosphere

Indeed, these two approaches may be complementary, rather than concurrent. In both cases, the microbial ecologist will play a central role, working in synergy with plant physiologists, biotechnologists, soils scientists and agronomists.

Among the microorganisms beneficial to plants, we will mention here particularly the "plant growth promoting rhizobacteria" (PGPR) and the mycorrhizae.

Plant GrowthThe term 'PGPR' encompasses a wide variety of bacteria whose functions and
properties favour, at least in certain conditions, plant growth and survival. Several
functions may be related to such a beneficial effect:(PGPR)

- Stimulation and control of root growth by the production or degradation of phytohormones.
- Facilitation of root water uptake by mucigel secretion (polysaccharides) in the root hair zone.
- Improvement of plant nutrition, for example by: concentration of mineral nutriments, due to the high concentration capabilities of the bacteria solubilization of minerals, e.g. phosphates mineralization of organic, N-, P- and S-compounds secretion of plant-compatible siderophores for iron nutrition associative dinitrogen fixation.
- Protection of roots against parasites, by: antibiosis competition with parasites (e.g. for iron, again through siderophores production) stimulation (induction) of plant resistance
- Positive interaction with mycorrhiza ("mycorrhiza helper bacteria", MHB)

As an example, I can mention the research performed in my laboratory by Hamelin on the presence and diversity of nitrogen-fixing bacteria associated with *Molinia coerulea* roots. *Molinia coerulea* is a grass which can grow in soils with extremely low nitrogen content. Therefore, we postulated that rhizospheric, associative nitrogen fixation could play a major role in the survival strategy of this perennial grass. Indeed, significant N₂-fixing activity was measured in Molinia roots, whereas the occurrence of nitrogenfixing bacteria was detected by cloning of a gene characteristic of nitrogen fixation, the nifH gene, from the total environmental DNA. There was a diversity of nifH clones found in the different fractions of Molinia soil, rhizosphere and roots. However, most (about 60%) of all clones characterized were shown to be narrowly related phylogenetically. A comparison with published nifH sequences has shown that this major phylum did not correspond to any of the known cultivable nitrogen fixers. Similarities were shown only with environmental clones from the rice rhizosphere. Therefore, these not yet cultivated microorganisms could play an important beneficial role in bringing a complement of nitrogen to grasses growing in nitrogen-poor soils.

Mycorrhizae Mycorrhizae are mutualistic symbioses between fungi and plant roots. About 90% of all plant species harbour this type of symbiont, which are in general considered as beneficial. However, the mutualistic character of the symbiosis may depend on the conditions, so that the benefit can be unilateral in some cases. There are several types of mycorrhizae, the most important for crop plants being the **arbuscular mycorrhizae**, where the fungal partner is a zygomycete, member of the order Glomales. These fungi are not cultivable in the absence of plant roots. In most cases, the symbiosis is mutualistic, the plant bringing organic compounds as carbon and an energy source to the fungus, whereas the latter, through its mycelium extending in the surrounding soil, takes up water and minerals and transfers them to the root. They are particularly important in orthophosphate-deficient soils. However, fungal partners can also bestow other advantages to their host, like protection against root parasites. They also allow nutrient transfers between different plants harbouring common mycorrhizae, e.g. in prairie ecosystems. In this manner, mycorrhizal plants may outcompete non-mycorrhizal plants by forming successful guilds sharing the same mycorrhizal partners.

Recently, it was shown that bacterial populations can be associated with mycorrhization, and help establishment and functioning of the symbiosis. These "mycorrhizae helper bacteria" realize therefore, together with plants and fungi, a three-membered mutualistic plant beneficial association.

Two strategies may be called upon for this purpose:

How to Favour Plant - Beneficial Micro - Organisms Protective in Natural Agriculture

* selection of microbial strains presenting efficient plant growth promotion or traits, for inoculating seeds or soils

* development of soil management practices which favour the diversity, development and activity of PGPR and mycorrhizal fungi.

The first strategy was often developed in recent years, and several PGPR strains are commercially available as green manure. They are most often applied within an appropriate coating to seeds. However, in most cases, the results are inconsistent in the field, whereas the strains were shown to be very effective in greenhouse assays. Indeed, important aspects may not have been taken into consideration, which could be decisive for the success of acclimation of these new strains in the field. These aspects include soil characteristics, ability to colonize roots and ability to compete with the indigenous root microflora.

The second strategy will be illustrated by a research performed in the framework of Indo-Swiss Collaboration in Biotechnology (ISCB), a programme between the Swiss Agency of Development and Cooperation and of Indian Department of Biotechnology. Our research deals with the diversity and functions of rhizobacteria and mycorrhizae associated with wheat rhizosphere and their influence on soil quality and productivity. Two Swiss universities (Neuchâtel and Basle) are associated with Pantnagar University and Tata Environment Research Institute (TERI) in Delhi.

Large surfaces in the Ganga plain, on loess soils low in phosphate and in organic matter, are cultivated with a rotation of rice (during the monsoon season, May to September) and wheat (from November to March). In the classical practice, fields are covered with water during rice cultivation, so that the soil becomes largely anoxic, inactivating or even killing the aerobic organisms. Different assays were performed by Dr. Alok Adholeya, head of the mycorrhiza group at TERI, to change this practice. One appears particularly promising: cultivation of rice and wheat on elevated beds without tilling. Water flows to the gaps between the beds during rice cultivation, so that rice roots will be underwater, whereas the top of the bed will remain aerated. It functions therefore as a reservoir for aerobic microorganisms, in particular mycorrhizal fungi and PGPR bacteria. At the end of the rice season, after water depletion, the soil becomes oxic again. Wheat is then planted on the same beds in the rice straw, and the aerobic microorganisms in the top of beds will be able to recolonize the soil. This practice has been shown to decrease rice yields by 10%, but to increase wheat yields by 25%, using 25% less seeds and fertilizer, and 50% less water. It was shown that the number of mycorrhizal spores increased tenfold compared to the classic practice, whereas the diversity of species was also considerably increased. The effect of this elevated bed system on bacterial diversity and occurrence of PGPR in the wheat rhizosphere is at present under investigation by Pantnagar and Neuchâtel microbiologists.

- **Conclusion** Plant beneficial microorganisms are inherent to the main strategies of plant survival in natural environments. Therefore, an important issue in natural agriculture should be to profit from the important functions played by these plant partners. This requires a much better understanding of the subtle relationships between the plant and its soil partners. Apart of the development and commercialization of effective microbial strains, a less commercially fruitful approach must be attempted, i.e. the development of agricultural practices favouring colonization, activity and maintenance of plant beneficial microorganisms. Such practices should also allow poor farmers the possibility of increasing in a sustainable way and at low cost the fertility of their fields, respecting natural and man made ecosystems.
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