Review Article

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Azospirillum sp. and mycorrhizal fungi: key microorganisms in sustainable agriculture

Abstract

The role and importance of two types of soil microorganisms are highlighted: the bacteria of the Azospirillum genus and mycorrhiza-forming fungi, which establish beneficial interactions with plants and the application of these generates great interest due to the potential and to be considered as these soil microorganisms as a tool in sustainable and agroecological agriculture. The importance of developing biofertilizers with native strains of plant-promoting microorganisms, such as Azospirillum brasilense and mycorrhizal fungi, is also highlighted as a strategy to control diseases and improve the agronomic performance of crops. The development and application of this type of biofertilizers can be considered an important alternative for the partial or total replacement of mineral fertilizers, which would generate great benefits without having a detrimental impact on the environment.

Keywords: PGPR; mycorrhizae; agroecology; biofertilizers

Volume 10 Issue 6 - 2023

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Received: December 15, 2023 | **Published:** Dcember 29, 2023

Introduction

The continuous increase in the world population, the progressive reduction of arable land due to the advance of urbanization, soil erosion and soil contamination due to the accumulation of toxic products, are aspects that make the application of strategies and biotechnologies essential to increase crop productivity. Among them, the use of soil microorganisms with the ability to promote plant growth stands out.

Due to the knowledge of the contribution of soil microorganisms in promoting plant growth, it is considered of interest to use them in crops of economic importance in the region. Among these microorganisms are the so-called PGPR, plant growth promoting rhizobacteria, which facilitate plant growth either directly by providing nitrogen, phosphorus, and essential minerals or by biosynthesis and regulation of hormone levels, or indirectly by the reduction of the inhibitory effects of various phytopathogens and the development of forms of biological control agents. All this due to its ability to biologically fix atmospheric nitrogen, the increase in nitrate reductase activity when plants grow endophytically, the production of hormones such as auxins, cytokinin's, gibberellins, ethylene and a variety of other molecules, the solubility of phosphate, by favoring mycorrhizal associations that are beneficial for plants and can act indirectly on growth by protecting plants from phytopathogenic microorganisms in the soil.[1–7]

Therefore, this work has as its main objective to contribute to the knowledge of microorganisms of the genus *Azospirillum* and mycorrhizal fungi as agents to improve the growth, development, and production of plants, by intervening in the nutrition and defense of phytopathogens through isolation, selection, inoculation, and evaluation of these soil microorganisms as promoters of plant growth and their potential when incorporated into the productive activity. Its use has the advantage of having practices that do not contaminate the environment and that at the same time are in balance with the ecological conditions of the soil.

The bacterium: *Azospirillum*

Azospirillum is a genus of bacteria belonging to the alpha subclass of proteobacteria, with *Azospirillum lipoferum* being identified as the type species. Its typical characteristics are vibrioid shape, pleomorphism and spiral mobility. Their cells contain high quantities, up to 50% of the cell dry weight, of poly-beta-hydroxybutyrate, observed microscopically as refringent granules in young cells. Old cultures frequently present ovoid-shaped, thick-walled, cyst-like refringent cells.[8]

The use of microorganisms that promote plant growth has been investigated for many years, the *Azospirillum* genus being one of the most prominent, mainly due to its ability to produce a wide range of active metabolites such as indole acetic acid, cytokinin's, gibberellins and siderophores,[8,9] which positively influence the growth and healthy development of plants. *Azospirillum sp*. as PGPRs, that is "Plant Growth Promoting Rhizobacteria" or "Plant Growth Promoting Microorganisms" (MPCV), non-specific providing varied contributions to the improvement of growth and productivity in many species of agricultural crops.[4,5,10,11]

The practice of inoculation with rhizosphere bacteria can provide different benefits to crops from the moment of germination and in the stages of their subsequent development.[10,12] *Azospirillum* is a rhizobacteria considered to promote plant growth due to its ability to fix atmospheric nitrogen, produce plant growth regulators and greater root development, which may involve other effects such as increased absorption of water and nutrients, greater tolerance to stress, such as salinity and drought, that result in a more vigorous and productive plant.[5,8,9] Probably due to increased root growth and better plant nutrition there is an increased tolerance to phytopathogenic agents[5,13–15] and by improving the gene expression of resistance to the disease of genes related to the disease.[16,17]

It is known that this bacterium, inoculated in cereal seeds, increases the percentage of germination and biomass,[18,19] since it produces substances that stimulate root growth, which allows the absorption potential of nutrients and water to be increased. elevate, a benefit that, in the case of crops in arid and semi-arid zones, constitutes an even greater advantage.[9]

Díaz-Zorita and Fernández-Caniggia[4] quantified the productivity of wheat (*Triticum aestivum* L.) inoculated with *Azospirillum brasilense* under normal dry farming conditions in localities of the Argentine Pampas region and determined that the inoculated plants showed a more vigorous vegetative growth, with greater number and yield of grains. In Brazil, Piccinin Gleberson et al.,[6] experimented with liquid and peat inoculants and evaluated the efficiency of seed inoculation with *A. brasilense* on agronomic performance and wheat yield. At harvest time, they evaluated the number of grains per spike, grain weight and yield, and concluded that *A. brasilense* is efficient in nitrogen fixation and that yield is positively influenced by nitrogen fertilization associated with inoculation, independently of the type of inoculant.

In addition to research on the efficiency of *Azospirillum* in promoting plant growth in different culture conditions, the compatibility of its inoculation with other PGPR microorganisms in various crops is also studied,[11,15,20–24] the influence of the plant genotype, the substances generated in the inoculated plants and their impact on the microbial communities of the soil.[25–27]

Garcia de Salamone et al.,[21] measured the response of three rice cultivars under field conditions inoculated with a commercial formulation of *A. brasilense* and Pseudomonas fluorescens and evaluated the influence on soil microbial communities through molecular analysis; and concluded that the combined inoculation with *A. brasilense* and *P. fluorescens* increased aerial biomass production and grain yield in the three rice cultivars and did not generate a significant impact on soil microbial communities. There are numerous investigations of bipartite inoculations with *Azospirillum* and rhizobia on leguminous plants with the aim of studying a strategy to improve the sustainability of agricultural production and even highlight the biotechnological potential of secondary metabolites of rhizobia together with inoculants containing rhizobia and *A. brasilense* to improve the growth and yield of legume and cereal crops such as maize.[22,23,28,29] There are also studies that determine that the genotypic variations of the bacteria and *Azospirillum* do not influence their ability to promote plant growth,[30] as well as the phenotypic variations of the colonies of different strains of *Azospirillum brasilense* in culture media.[31]

The combination of nitrogen fertilization and *Azospirillum* inoculation can ensure higher nutrient uptake and thus increase crop yields. Ferrera et al.,[32] evaluated the response of the maize crop to inoculation with *A. brasilense* and fertilization with macronutrients and micronutrients, conducting experiments in greenhouse and field conditions, in clayey and sandy soils. They obtained different responses, from significant increases in the growth and yield parameters evaluated with the inoculation of *A. brasilense* compared to the control and fertilized treatment, while in sandy soils and under greenhouse conditions there were no differences between treatments. They determined that the inoculation with *A. brasilense* gave a yield comparable to the fertilized treatment; that the combination of *A. brasilense* and fertilizer increased grain production by 29%; They also concluded that the response to inoculation and fertilization was dependent on the type of soil under greenhouse conditions. There are studies on the application in plants for reforestation of the combination of *A. brasilense* with organic residue, better results were obtained in the growth and establishment of the plants and an additive effect of improvement of the biochemical and microbiological quality of the soils, in comparison with the independent application of the microbial inoculum and the organic residue.[33] In this line of study, a saving of nitrogenous fertilizer was determined with the inoculation with *A. brasilense*, which made it an economically viable technology due to the reduction of the necessary chemical fertilizers, which improved the nitrogen content and counteracted the effects of salinity and increased salinity tolerance of plants.[5,34,35]

Some investigations within the field of molecular biology, aim to determine the substances generated in plants inoculated with *A. brasilense*, it has been possible to determine proteins that accumulate only in corn seedlings in response to inoculation;[36] as well as to determine the molecules biosynthesized by *A. brasilense* and those that influence its behavior.[37–44]

Larraburu et al.,[45] studied the changes in enzyme levels produced during in vitro rhizogenesis of pink lapacho (*Handroanthus impetiginosus*) by inoculation with *A. brasilense*, and determined the significant effect of the triple interaction between the composition of the culture medium, auxin concentration and bacterization in antioxidant enzyme activities, and analyzed the role of *A. brasilense* in rooting and stress *in vitro*. The biochemical determinations made in this study contribute to a better understanding of how auxin induction, culture media, and PGPR inoculation affect the physiology of woody plants. Research lines currently being carried out aim to study the diversity of new isolates of *A. brasilense* and select bacteria according to their ability to promote plant growth, from native forage,[46,47] cereals,[16,19,44,48–53] fruit trees,[54] microplants,[55] as well as the ability to promote the growth of microalgae.[56–58]

Other lines of work study the mechanisms and molecules involved in the establishment of plant-microorganism interaction. In recent molecular genetic studies using immunomicroscopic techniques, they determined the cell surface proteins involved in the establishment of the plant- interaction *A. brasilense.*[59]

Mycorrhizal fungi

Mycorrhizae are mutualistic associations between soil fungi and roots of higher plants.[60,61] As in other symbiotic relationships, both participants benefit. These fungi depend on the plant for the supply of energy, carbohydrates, and vitamins, which the fungus itself is unable to synthesize while the plant can do so thanks to photosynthesis and other internal reactions. At the same time, they deliver mineral nutrients to the plant, especially those that are not very mobile, such as phosphorus and water.[62–65] In addition, fungi impart other benefits to plants such as: stimulation of growth-regulating substances, increased photosynthetic rate, osmotic adjustments when there is drought, increased nitrogen fixation by symbiotic or associated bacteria, increased resistance to pests and diseases, tolerance to environmental stress, improvement of soil aggregation and mediation in many interactions of microflora and microfauna that occur in the rhizosphere.[60] That is, mycorrhizal associations are the result of three interactive pathways between mycorrhizal fungi, plants, and the environment or soil conditions where they thrive.[61,66]

Factors that can influence the occurrence and effectiveness of mycorrhizal associations include root properties, edaphic and climatic factors, soil organisms, soil disturbance, and plant-fungus compatibility.[61] Therefore, its study is complex since it involves the plant, the environment and the fungus. It should be known phenology of mycorrhizae, factors responsible for greater or lesser mycorrhizal dependence on host plants (degrees of mycotrophy), the role of hyphae in the soil, competition for nutrients between mycorrhizal and non-mycorrhizal plants, and mycorrhizal interactions involving pollution and other stressors, rhizosphere, soil properties, and allelopathy.[61]

Mycorrhizae have been classified based on their structure, morphology, and mode of infection.[60,66] At least seven different types of mycorrhizal associations are recognized, involving different groups of fungi and host plants, and with different morphological patterns.[61,67] The most common associations are: vesicular-arbuscular mycorrhizae (VAM) in which fungi belonging to the Phylum Glomeromycota produce arbuscules, hyphae and, some of them, inter- and intracellular vesicles in the root cortex; ectomycorrhizae (ECM) where fungi belonging to the Phylum Basidiomycota and Ascomycota form a layer of hyphae, more or less compact above the surface of the root, called mantle, sheath or sleeve and the hyphae that penetrate intercellularly in the first layers of root cells form what is known as the Hartig network.[67] Orchid mycorrhizae or ball mycorrhizae, are characterized by forming coils of hyphae, called intracellular balls and are distributed in the canopy (spongy layer of the root of these epiphytic and terrestrial plant species) in plants of the Orchidaceae family.[66,67] Ericoid mycorrhizae penetrate cells and form hyphal tangles in members of the family Ericaceae; its roots are characterized by forming cell monolayers in the epidermis, bark, phloem and xylem; they do not form a mantle and interconnect intercellularly. Arbutoid mycorrhizae form an outer mantle, a well-developed Hartig network, and hyphae that penetrate cells where they form "curls" of coiled hyphae.[67] They occur in plants of the genera Arctostaphylos, Arbutus and Pyrola, members of the order Ericales. Generally, fungi that form arbutoid mycorrhizae can form ectomycorrhizae if they interact with plants of the genus Pinus.[67] Finally, monotropoid mycorrhizae are characterized by the way hyphal penetration is straight or at an angle of almost 90 degrees with respect to the root surface.[61,68,69]

Mycorrhizae play a key role in sustainable agriculture, since, if you want to reduce the use of agrochemicals for environmental reasons, production costs and health, then it is necessary to restore mycorrhizal fungi and other beneficial microorganisms, but it is essential a optimal and responsible use of microorganisms and other soil inhabitants.[60] The key function of the mycorrhiza lies in the fact that its intra and extra radical mycelium constitutes a link or bridge between the plants and the soil. When mycorrhizae form, root physiology and exudation are altered, which in turn changes the surrounding microbial population. It not only contributes to plant nutrition by exploring a larger volume of soil than the root alone, but also to soil nutrition by increasing microbial activity.[60,61]

Mycorrhizae have an advantage over the non-mycorrhizal root because the external mycelium extends further than the root hairs, which, from a nutritional point of view, the benefit is the greater growth of the plants due to an increase in the absorption of phosphorus when this element is limiting, when P is not limiting the benefit can be null or reduced, depending on the degree of mycorrhizal dependence of the plant. In addition, it directly or indirectly influences the absorption of other minerals (N, K, Ca, Mg, Fe, Mn).[60,61]

Mycotrophic plants have different degrees of mycorrhizal dependence. The obligate ones cannot grow without mycorrhiza even in fertile soils; facultative ones benefit from increased growth when P levels are low and can survive and grow without mycorrhizae in fertile soil conditions.[60] In addition, mycorrhizae present interspecific differences in effectiveness to absorb P and other nutrients and translocate them to the plant, and therefore confer different physiological benefits to the same plant species.[60,61,66]

Soil microorganisms present complex interactions that affect soil fertility and plant development. Mycorrhizal fungi, in addition to their direct effect on plant nutrition, induce physiological changes that include an increase in the photosynthetic rate and redistribution of fixed carbon in a greater proportion towards the roots, which causes a notable increase in carbon available for microbial activity.[60] Inhibitory and stimulatory effects of the processes of spore germination and growth of mycorrhizal fungi by different soil microorganisms have been verified.[60] Earthworms and nematodes have a positive effect on the populations of mycorrhizal fungi and contribute to their spatial distribution, while springtails feed on extraradical hyphae causing reductions in their effectiveness and reduce the length of the colonized root.[60,61] Numerous investigations studied the effects of the mutualistic association between mycorrhizae and plants, which have concluded in:

- The hyphae of mycorrhizal fungi intervene in the aggregation of soil particles, since they prolong the root system of plants, and this facilitates greater physical retention of soil particles, limiting the damaging effects of erosion caused by water.[61]

- They improve water and nutrient intake, which influences nutritional capacity, increased production and higher biological quality.[15,60,61,70,71]

- They give plants greater tolerance to many stress factors such as: drought, salinity, pH imbalances, among others.[61,71,72]

- They provide protection against herbivory and the attack of phytopathogenic agents from the soil.[73,74]

- They reduce the environmental stress that predisposes the plant to diseases.[61,66]

- Modify the endogenous level of phytohormones related to plant growth.[72]

- They generate a better adaptation to the photosynthetic capacity of the plant to satisfy the carbon demand that exists when associated with mycorrhizal fungi. (Obligatory mycotrophic plants depend on the supply of carbohydrates derived from photosynthesis to maintain an appropriate mycorrhizal symbiosis).[60,61,70,71]

- They function as a phytostabilizing system, because mycorrhizal plants grow in contaminated soils, which allows their use in the phytoremediation of contaminated soils.[75]

For all these reasons, mycorrhizae constitute an ecological alternative for sustainable agriculture and open new horizons not only in the field of agricultural production but also in reforestation, the cultivation of ornamental plants, etc.[60,61,68,76–78]

There are numerous investigations that have the objective of evaluating the colonization and the effect of associated native mycorrhizal fungi on different plant species.[15,47,79–91] Urgilés Gómez et al.,[92] evaluated the effect of inoculation with native mycorrhizal fungi on the propagation of *Alnus acuminata* and *Morella pubescens*. The two species propagated in the greenhouse showed an improvement in growth when inoculated with propagules of mycorrhizal fungi, compared to the controls, and concluded that the symbiotic relationship between plant-fungus ensured their survival in the nursery and later in plantations for afforestation purposes, reforestation or restoration of ecosystems.[93]

Conclusion

Biofertilizers emerged as the solution for organic and sustainable agriculture, since they allow to reduce production costs and reduce the use of agrochemicals, so it is important to increase their application to achieve sustainable agricultural practices, due to their benefits in agriculture and positive effects on soil fertility. It is important to develop biofertilizers with native strains of plant-promoting microorganisms, such as *Azospirillum brasilense* and mycorrhizal fungi, as a strategy to control diseases and improve the agronomic performance of crops.

The development and use of biofertilizers is seen as an important alternative for the partial or total replacement of mineral fertilizers. The large-scale application of biofertilizers in any agricultural production system would bring great benefits without having a detrimental impact on the environment.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

None.

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