

Challenges in the extensive use of bioinoculants for sustainable agriculture, from the perspective of Mexico, Venezuela, and Brazil

Retos en el uso extensivo de bioinoculantes para una agricultura sostenible, desde la perspectiva de México, Venezuela y Brasil

Blanco, E.L. ¹, Díaz-Rodríguez, A.M. ², Castro, Y. ^{3,4}, Chávez-Luzanía, R.A. ²,
Álvarez, L. ⁵ de los Santos-Villalobos, S. ^{2*} 

¹ Laboratorio de Investigación en Biotecnología y Química de Polímeros. Decanato de Investigación. Universidad Nacional Experimental del Táchira. 5001. San Cristóbal, Táchira, Venezuela.

² Laboratorio de Biotecnología del Recurso Microbiano. Instituto Tecnológico de Sonora. 5 de febrero 818 sur 85000, Cd. Obregón, Sonora, México.

³ Laboratorio de Fitobiotecnología. Departamento de Biología. Facultad de Ciencias. Universidad de Los Andes. 5101. Mérida, Venezuela.

⁴ Laboratório de Fermentações, Universidade Federal de Lavras. 37203-202, Lavras, Minas Gerais, Brasil.

⁵ Grupo de Investigación en Biotecnología Agrícola y Ambiental. Decanato de Investigación. Universidad Nacional Experimental del Táchira. Apdo. 5001. San Cristóbal, Táchira, Venezuela.

ABSTRACT

The growing need to reduce the negative environmental impact of agrochemicals in conventional agriculture has led to the development of innovative biotechnology products such as bioinoculants. These products are specifically designed to reduce the use of both chemical fertilizers and pesticides, promoting more sustainable agricultural practices. Bioinoculants are composed of soil microorganisms that have a high potential to stimulate plant growth through different mechanisms of action, such as nitrogen fixation, nutrient solubilization, and production of bioactive compounds. In this review, a representative analysis of research in the area of bioinoculants in Mexico, Venezuela, and Brazil is presented, to know the current situation in these Latin American countries. In addition, the perspectives of their large-scale application are discussed, thus contributing to the literature of our region, where especially there is no compilation of research carried out in Venezuela in this field.

KEY WORDS: Agrosustainability, biocontrollers, biostimulants, biofertilizers, microbial biotechnology, PGPM.



Please cite this article as/Como citar este artículo:

Blanco, E.L., Díaz-Rodríguez, A.M., Castro, Y., Chávez-Luzanía, R.A., Álvarez, L., de los Santos-Villalobos, S. (2025). Challenges in the extensive use of bioinoculants for sustainable agriculture, from the perspective of Mexico, Venezuela, and Brazil. *Revista Bio Ciencias*, 12, e1807. <https://doi.org/10.15741/revbio.12.e1807>

Article Info/Información del artículo

Received/Recibido: October 29th 2024.

Accepted/Aceptado: March 18th 2025.

Available on line/Publicado: April 03th 2025.

*Corresponding Author:

Sergio de los Santos-Villalobos. Laboratorio de Biotecnología del Recurso Microbiano. Instituto Tecnológico de Sonora. 5 de febrero, 818 sur, Cajeme. 85000, Ciudad Obregón, Sonora, México. Teléfono: (644) 4100900 Ext. 2124. E-mail:

sergio.delossantos@itson.edu.mx

RESUMEN

La creciente necesidad de reducir el impacto negativo que sobre el medio ambiente han tenido los agroquímicos en la agricultura convencional ha permitido el desarrollo de productos biotecnológicos innovadores como los bioinoculantes. Estos productos están diseñados específicamente para disminuir el uso tanto de fertilizantes químicos como de pesticidas, promoviendo prácticas agrícolas más sostenibles. Los bioinoculantes están compuestos por microorganismos del suelo que poseen un alto potencial para estimular el crecimiento de las plantas a través de diferentes mecanismos de acción, como la fijación de nitrógeno, la solubilización de nutrientes y la producción de compuestos bioactivos. En esta revisión, se presenta un análisis representativo de la investigación en el área de bioinoculantes en México, Venezuela y Brasil, con el objetivo de conocer la situación actual en estos países latinoamericanos. Además, se discuten las perspectivas de su aplicación a gran escala, contribuyendo así a la literatura de nuestra región, donde especialmente no existe una recopilación de investigaciones realizadas en Venezuela en este campo.

PALABRAS CLAVE: Agrosostenibilidad, biocontroladores, bioestimulantes, biofertilizantes, biotecnología microbiana, PGPM.

Introduction

Conventional agriculture and its intensification, through the use of agrochemical products and machinery, have allowed the productive potential of agroecosystems to increase worldwide. However, the indiscriminate use of these products has led to the degradation of resources such as soil and water, as well as environmental pollution and risks to public health.

In this sense, it is necessary to adopt eco-friendly measures to reduce the use of chemical products and thus conserve natural resources, through the implementation of agro-sustainable practices that increase the quality and health of the soil. This concept is based on a soil capable of continuously maintaining its functionality as a living system, preserving the quality of its components, and promoting environmental health, and it is at this point where microorganisms acquire a transcendental role (Creus, 2017).

Bioinoculants are agrobiotechnological products composed of live or dormant beneficial microorganisms with the potential to benefit the development of different plant species, which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant,

thus promoting its growth, increasing the availability of nutrients and the health of the host plant; In addition, they can be used strategically as an alternative to the use of chemical fertilizers and pesticides, contributing to sustainable agriculture that helps mitigate the environmental impacts caused by agrochemicals (Vessey, 2003; SAGARPA, 2017; Santos *et al.*, 2019). These bioproducts contain Plant Growth Promoting Microorganisms (PGPM), which is a group of microorganisms that grow associated with the roots of host plants and produce stimulation of plant growth associated with different mechanisms of action, such as biological nitrogen fixation, dissolution of inorganic phosphates, production of phytohormones, siderophores, and antibiotics, among others, and are part of the rhizobiome that offers beneficial services for the growth of the plants to which they are associated (Vessey, 2003; Orozco-Mosqueda *et al.*, 2022).

The wide use of these microorganisms is due to their effects as biofertilizers (they facilitate the availability of nutrients to plants), biostimulants (they stimulate plant growth), and biopesticides or biological control agents (BCA) (control of phytopathogens) (Mitra *et al.*, 2023). Currently, various microorganisms have been reported that have been used for this purpose, among which the genera *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Streptomyces* stand out (Creus, 2017). Recently, it has been estimated that PGPM can vary between 1 and 5 % of the total population of microorganisms that compete to colonize the rhizosphere (Antoun & Prevost, 2005; Bhattacharyya *et al.*, 2020), and therefore the importance of mass production and extensive use in agriculture.

Bioformulations created from indigenous microorganisms are currently booming worldwide (Soumare *et al.*, 2020), with a common goal which is the benefit of agriculture and food sovereignty of each country, by improving soil fertility to meet the food demand of the growing world population using quality standards, within the framework of compliance with FAO SDGs such as zero hunger and life of terrestrial ecosystems (FAO, 2021). However, the production of biofertilizers at an industrial level acts as a bottleneck because bacterial strains are generally developed and managed by research laboratories and not by sub-production units (Soumare *et al.*, 2020). For this reason, and taking into account the number of existing studies, few microorganisms have been marketed as biofertilizers, which is why an efficient transition is necessary from their isolation and characterization in laboratories to their commercialization and application in the field, through rigorous schemes for their responsible use, such as the one we propose in Figure 1.

In Mexico, Venezuela, and Brazil the strategy of isolating native microorganisms to increase the agricultural potential of the region prevails, and re-inoculating them either in the same soils or in soils with similar characteristics. In this process, biofertilizers must overcome several barriers to establish themselves in the soil after their application, since the inoculation of allochthonous microorganisms induces an adaptation in them to the nutritional conditions of the soil, to the rhizospheric environment of the plant, and they must also be competitive with the native microorganisms of the soil (Creus, 2017; Blanco and Reyes, 2018).

Additionally, there are important aspects that determine the success of the formulation of a bioinoculant, especially the selection of a vehicle for the microorganisms, which should ensure the maintenance of cell viability, be economical and easy to apply. The vehicles can be solid or

liquid for applications in seeds, plants, or in the soil (Khan *et al.*, 2023). Among these substrates, peat stands out, as it is a material with a high content of organic matter and physical protection properties for microorganisms due to the matrix it forms, which allows microorganisms to survive adverse environmental conditions (Santos *et al.*, 2019). While liquid vehicles can be water or the culture media themselves. Other means to transport microorganisms can be an agricultural or industrial waste, lyophilized bacteria, or polymers for cell encapsulation (Figure 1) (Suyal *et al.*, 2016; Sahu *et al.*, 2018; Santos *et al.*, 2019; Chaudhary *et al.*, 2020).

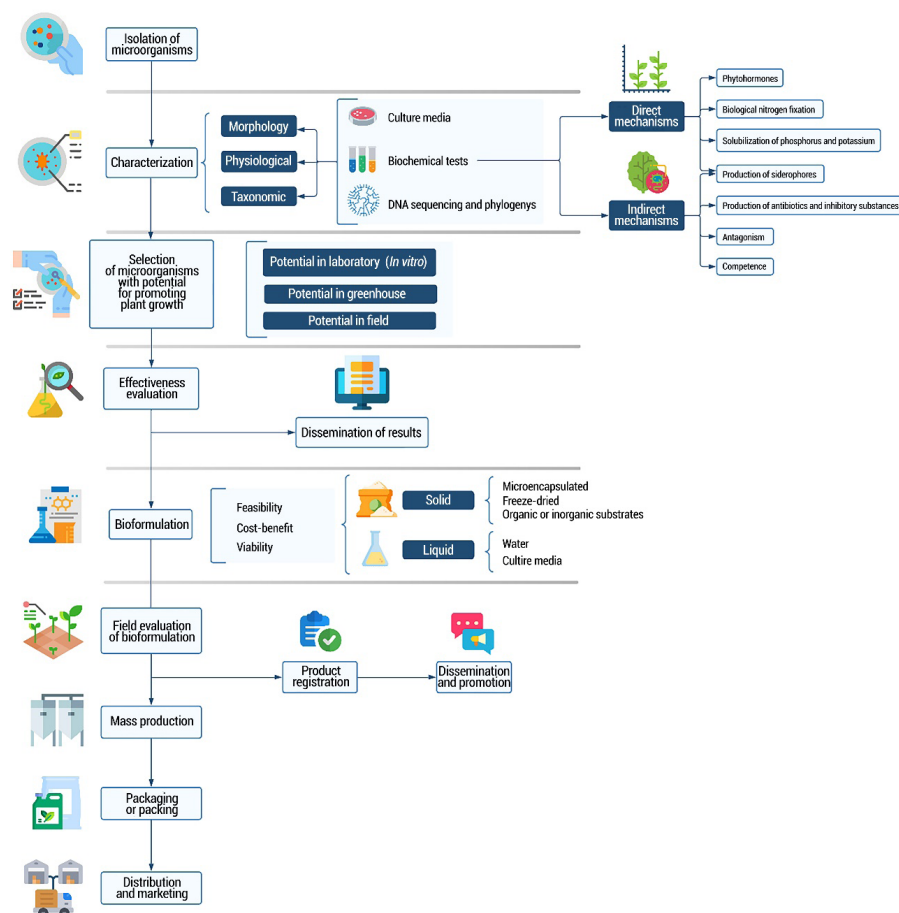


Figure 1. General scheme of bioprospecting of microorganisms for the production of bioinoculants.

Source: Own elaboration.

The bioinoculant industry has experienced significant global growth (Yadav & Yadav, 2024). The largest market for these bioinputs is in the Americas, where 2,264 patent applications were registered in 2023, either under state fertilizer regulations or biostimulant claims (BPIA, 2023). North America leads the global biofertilizer market, with key industry players in the U.S. including Kula Bio Inc., Novozymes, Rizobacter, Sustane Natural Fertilizer Inc., Symborg Inc., AgroLiquid, Indogulf BioAg LLC, Koppert Biological Systems Inc., Lallemand Inc., The Andersons Inc., UPL, and Syngenta (Sansinenea, 2021; Santos *et al.*, 2024). Europe ranks second in market size, with major contributions from Germany, the United Kingdom, Spain, Italy, Hungary, and France, followed by the Asia-Pacific region (China, Japan, India, Australia, New Zealand, and other Asian countries), South America, and Africa (Basu *et al.*, 2021). In quantitative terms, North America and Europe were the largest consumers of biofertilizers in 2022, accounting for 35.9% and 31.9% of global consumption, respectively (Mordor Intelligence, 2024). In Europe, the implementation of EU Regulation 2019/1009 introduced seven Product Function Categories (PFCs), which classify bioinputs—including bioinoculants, biofertilizers, and biocontrol agents—based on their specific functional purposes (FAO, 2019).

In Latin America, agrobiotechnology related to bioinoculants and other bio-inputs is well established, with a long-standing tradition, yet its large-scale implementation remains recent and dynamic. However, the sector operates at a relatively small scale, as reflected in the number of registered products, companies, and product categories (Aramendis *et al.*, 2023). According to these authors, biofertilizers predominate in Argentina, biological control agents in Colombia, plant extracts in Ecuador, and biological inoculants in Uruguay. Their study also provides an updated overview of the specific regulations governing the production and use of these products in each of these countries. Brazil stands out as a leader in the commercialization and application of biofertilizers. By 2018, approximately 45 million doses of liquid biofertilizers and 10 million doses of solid formulations were sold in the country (Santos *et al.*, 2019).

Regarding the studies carried out, the potential of these microorganisms has been evaluated both individually and in consortiums, to determine the potential in each of their presentations. Likewise, studies have been conducted that evaluate the compatibility between strains and the viability retained by the microorganisms in a given time using different types of substrates that serve as vehicles (Blanco *et al.*, 2018; Blanco *et al.*, 2021^a; Blanco & Reyes, 2018; Yahya *et al.*, 2022). For example, in Mexico, the development of biofertilizers has been the subject of growing interest in recent years, developing research to evaluate the potential of biofertilizers, both in terms of individual microorganisms and in consortia (Ibarra-Villarreal *et al.*, 2023; Guardiola-Márquez *et al.*, 2023). Recent studies have addressed the effectiveness of these products in different soil and crop conditions, highlighting the need to adapt and optimize their use in Mexican agricultural fields (Santillano-Cázares *et al.*, 2021). In this sense, the consolidation of an adequate legal framework is presented as a fundamental step to promote the massive adoption of biofertilizers in Mexican agriculture, thus contributing to the sustainability and competitiveness of the sector (Carrasco *et al.*, 2024).

Although this article contemplates a deep and critical review, it is not possible to mention all the work carried out in each country on this topic; however, a general consideration of the research

is provided, and a compilation of the existing products on the market, and the perspectives on the legal framework for the implementation of these bioproducts, as an approach to the potential of the region in this area, especially in Venezuela, of which there is little information in the available literature. Therefore, the objective of this review is to group a representative part of the research that has been carried out in recent decades in Mexico, Venezuela, and Brazil, and to list the products currently available, as well as to identify the challenges involved in the transition from research to the field to expand the use of biofertilizers in the region and to contribute ideas in favor of the standardization of registration and use standards for bioinoculants in Latin America.

Discussion

Bioinoculants in Mexico

History of the use of bioinoculants

Agriculture in Mexico has been one of the great economic pillars of the country. Three periods of development can be identified throughout history. During the growth period, between 1940 and 1957, agriculture became the main economic activity. In addition, during this period the green revolution took place, one of the great events in world agriculture, which focused on the monoculture of improved varieties and the increased use of fertilizers, practices that were translated into productive increases in rice, wheat, corn, among others (Diéguez *et al.*, 2010).

During the development period, between 1958 and 1981, agriculture played a fundamental role in the establishment of industry in Mexico. It was during these years that biofertilizers had a significant impact, especially in the biological fixation of nitrogen in crops such as soybeans and chickpeas. Furthermore, the widespread use of commercial *Rhizobium*-based inoculants was a prominent practice among agricultural producers, also recommended by research centers such as the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) (Armenta-Bojórquez *et al.*, 2010).

Finally, from 1982 to the present, agriculture has suffered a decline due to economic crises and environmental problems resulting from unsustainable conventional agricultural practices (Chávez-Díaz *et al.*, 2020). However, there has been an increase in the development of agrobiotechnologies for agricultural production, such as research on biofertilizers, based on the use of microbial resources with plant growth promotion functions, to contribute to the country's food security, reduce production costs and improve the quality and health of Mexican crops (Table 1).

Current status of research on bioinoculants

Unlike other Latin American countries such as Argentina, Brazil, and Uruguay, where bioinoculants have a long history of research and application, in Mexico this technology has been in the expansion and adoption phase by producers for more than two decades; however, there is a long history of scientific research on these topics. This delay was partly due to the abundance,

availability, and low cost of fertilizers until 2007 (Aguado-Santacruz and Moreno-Gómez *et al.*, 2012). Currently, this situation has changed and alternatives are being sought to increase agricultural productivity and reduce the use of synthetic agrochemicals. Various investigations have shown that the use of inoculants based on microorganisms with the capacity to promote plant growth and biological control agents represents an alternative to improve plant nutrition, tolerance to abiotic stress, control diseases and minimize environmental problems generated by the use of conventional synthetic fertilizers (García-Montelongo *et al.*, 2023; Nagrale *et al.*, 2023).

In Mexico, the current production of biofertilizers is carried out by companies, and educational and research institutions (Grageda-Cabrera *et al.*, 2012). Despite this development, large-scale distribution and application have had serious difficulties, mainly due to the lack of unified criteria for the selection and characterization of microorganisms, quality standards, and optimization in the production and bioformulation process, and the variability of results in the field (O'Callaghan *et al.*, 2022). In this sense, future research should consider the interaction of bioinoculants with the microbiome and the determining factors in the agroecosystem. On the other hand, the incorporation of ecological, metagenomic, population genetic, metabolomic, and transcriptomic studies is necessary for a holistic understanding of the complex network of interactions that constantly occur. This will bring us closer to an explanation of the variations in the results in the field and will improve the efficiencies in the application of bioinoculants.

Advances in field applications of bioinoculants

The development and trade of biofertilizers worldwide, as an alternative to traditional fertilizers, has seen an increase in its development and commercialization in recent years. In 2019, the market was valued at 4.27 billion dollars and is estimated to reach 11.81 billion in 2027, with an annual growth rate of 14.27% (Montoya-Martínez *et al.*, 2022). The panorama in Mexico, a country in which 1.52×10^7 ha are planted annually (SAGARPA, 2019), has seen prolific advances in the development of biofertilizers, obtaining technologies that allow increases in production and quality while reducing the use of synthetic fertilizers (Table 1).

Table 1. Use of biofertilizers in Mexican fields and their benefits.

Crop	Microorganism	Benefit	Reference
<i>Phaseolus vulgaris</i>	<i>Rhizobium etli</i>	Increase in weight, thickness, width, length, and protein percentage, using reduced fertilization.	Lara-Capistrán <i>et al.</i> (2019)
<i>Solanum lycopersicum</i>	<i>Acinetobacter calcoaceticus</i> AcDB3, <i>Bacillus thuringiensis</i> BtMB9, <i>Bacillus subtilis</i> BsTA16, and <i>Bacillus amyloliquefaciens</i> BaMA26.	Inhibition of <i>Fusarium</i> spp.	Khalil <i>et al.</i> (2022)
<i>Zea mays</i>	<i>Trichoderma</i> sp. IE-978, <i>Trichoderma reesei</i> Simmons IE-639, <i>Trichoderma virens</i> Miller IE-996, and <i>Trichoderma harzianum</i> Rifai IE-980.	Yields higher than those obtained with native corn under similar growing conditions.	Vázquez-Martínez <i>et al.</i> (2019)
<i>Jatropha curcas</i> L.	<i>Azospirillum</i> (Azofer®), and <i>Trichoderma</i> (Tricovel®).	Inoculation of <i>Azospirillum</i> together with <i>Trichoderma</i> produced a greater number of mature fruits per plant and seed weight per plant.	Martiñón <i>et al.</i> (2017)
<i>Triticum turgidum</i> L. subsp. <i>durum</i>	<i>Bacillus megaterium</i> TRQ8, <i>Bacillus paralicheniformis</i> TRQ65 and <i>Bacillus cabrialesii</i> TE3 ^T .	Increase in aerial and root length, aerial and root dry weight, and bio-volume index.	Rojas-Padilla <i>et al.</i> (2020)
		Increased crop productivity and preservation of grain quality.	(Ayala-Zepeda <i>et al.</i> (2024)
<i>Zea mays</i>	<i>Bacillus cabrialesii</i> strain B25.	Inhibition of root and ear rot caused by <i>Fusarium verticillioides</i> .	Martínez-Álvarez <i>et al.</i> (2016)
<i>Arachis hypogaea</i>	<i>Trichoderma harzianum</i> T-H3, <i>Trichoderma asperellum</i> T-AS1, <i>Trichoderma hamatum</i> T-A12, <i>Trichoderma koningiopsis</i> T-K11, and <i>Trichoderma harzianum</i> T-Ah.	Greater production and lower incidence of rot caused by <i>Macrophomina phaseolina</i> .	Martínez-Salgado <i>et al.</i> (2021)
<i>Cicer arietinum</i>	<i>Trichoderma harzianum</i> T1 and T2.	Increase in pod production by 24%, root length by 40%, and yield by 23%.	Martínez-Martínez <i>et al.</i> (2020)
<i>Mangifera indica</i>	<i>Bacillus subtilis</i> 83 (Fungifree AB®).	Control of mango anthracnose.	Galindo <i>et al.</i> (2015)
<i>Cucurbita pepo</i>	<i>Bacillus subtilis</i> QST 713.	Control of <i>Cucurbita pepo</i> seedling drowning caused by phytopathogenic fungi.	Solano-Báez <i>et al.</i> (2021)
<i>Solanum lycopersicum</i> L. and <i>Capsicum annuum</i> L.	<i>Bacillus amyloliquefaciens</i> and <i>B. thuringiensis</i> .	Decreased severity of disease caused by <i>Phytophthora capsici</i> .	Ley-López <i>et al.</i> (2018)
<i>Solanum tuberosum</i> L.	<i>Bacillus cabrialesii</i> subsp. <i>tritici</i> TSO2 ^T and <i>Bacillus subtilis</i> TE3 ^T _UV25.	Increased productivity and decreased incidence of common scab.	Montoya-Martínez <i>et al.</i> (2024)

Source: own elaboration.

In this sense, the Mexican market for bioproducts based on microorganisms of agricultural interest has soil conditioners and improvers (2), biostimulants (3), biofortifiers (3), biopesticides and biopesticides (38), in various presentations, from the following brands: Zare Agrhos, Indebio, Agribest, Agrokenmyca, Activa, Biofábrica Siglo XXI, Biosustenta, BIOqualitum, Biokrone, Grupo Fagro, Altus Biopharm, Agro&Biotecnia, Biocampo, Bactiva, CIASA Agro and Certis Agro México (Cruz-Cárdenas *et al.*, 2021).

Experiences of producers

In Mexico, various investigations have been carried out on the development, innovation, and validation of biofertilizers. Various efforts have been consolidated in the field of dissemination of the use of microorganisms in agriculture in Mexico, such as the AgroEvento held in 2020, 2021, and 2022, organized by the Centro Nacional de Recursos Genéticos (CNGR) of INIFAP and the Instituto Tecnológico de Sonora (ITSON). This event aims to bring together academics, scientists, students, technicians, trainers, producers of agricultural inputs, and farmers to share their experiences, knowledge, and perspectives regarding the use of biological products or other agrobiotechnologies for sustainable agricultural innovation. Likewise, the Patronato para la Investigación y Experimentación Agrícola del Estado de Sonora A.C (PIEAES), the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and INIFAP, every year since 1955, the Day of the Farmer has been held as a space for the connection, exchange of knowledge and transfer of technologies between researchers, farmers and companies. On the other hand, the Laboratorio de Biotecnología del Recurso Microbiano (LBRM) of ITSON has organized the Regional Forum “Cutting-edge agro-biotechnological alternatives to contribute to food security in a sustainable way” which has been held in 2019 and 2020, where various technologies and strategies focused on the efficient use of nutrients by crops, and the increase in yield developed in said laboratory, have been disseminated with the productive sector.

Regulations or institutions that regulate the marketing and registration of microbial inoculants

Particularly, Mexico does not have regulations for the regulation of microbial inoculants, even though its industry is growing rapidly. The registration of microbial inoculants is currently controlled by COFEPRIS, however, for their registration, there is no distinction between bioinoculants, biofertilizers, biopesticides, biostimulants, and other variants, so all products registered with this body end up classified between pesticides and plant nutrients within its catalog. The registration of these products with COFEPRIS requires, among other requirements, the performance of biological effectiveness tests, laboratory analysis of the product to be marketed, and the presentation of a health license by the laboratory that formulates the microbial inoculant (Ramírez-Guzmán *et al.*, 2018).

In terms of biological effectiveness, in Mexico, SAGARPA, through the National Service for Health, Safety and Agri-Food Quality (SENASICA), is responsible for establishing the requirements and specifications for conducting biological effectiveness studies of plant nutrition inputs and agricultural pesticides and their technical opinion through the Mexican Official Standards NOM-

077-FITO-2000 and NOM-032-FITO-1995, respectively; however, the updating of this type of standards for technologies based on microorganisms is crucial since the NOM-077-FITO-2000 standard focuses mainly on chemical nutrition and both standards are not clear regarding the evaluation of biological effectiveness results for the use of inoculants (Carrasco *et al.*, 2024; Cruz-Cárdenas *et al.*, 2021).

Finally, concerning the labeling of microbial inoculants in Mexico, in addition to the general specifications in NOM-182-SSA1-1998, the only thing added to the labeling is the number of colony-forming units (CFU) per gram or milliliter of sample, which gives rise to the development of colonies in 72 hours at 37 °C, indicating the genus and species of the microorganism or microorganisms in question (Ramírez-Guzmán *et al.*, 2018).

Given the emergence of microbial inoculants as a viable alternative to increase agricultural productivity and the consequent appearance of a large number of products that do not contemplate the same minimum quality criteria, the Federal Government of Mexico, through SAGARPA and INIFAP, different institutions and specialists in the area, are working on the development of a specific official standard for the quality evaluation, marketing, and use of biofertilizers.

Bioinoculants in Venezuela

History and initial studies

According to López *et al.* (2010), the use of biofertilizers in Venezuela began in the late 1940s, with a work carried out by Savostin (1950), which dealt with research in the agricultural microbiology section of the agricultural department of the then Ministerio de Agricultura y Cría (MAC). This work was aimed at demonstrating the benefits of inoculation with native strains of *Rhizobium* in leguminous plants (beans, kidney beans, and pigeon peas). The Venezuelan Institute for Scientific Research has made a great push in the development of inoculants, achieving the production of Nitroba, a biofertilizer based on native strains of *Rhizobium* and useful for legumes for consumption such as soybeans, beans, and other forage legumes such as *Leucaena*, *Stylosanthes*, *Siratro* and *Centrosema*, which existed from 1977 to 1991 when they sold the patent to a private company, and after this sale the product disappeared from the market (López *et al.*, 2010).

In Venezuela, Dr. Isbelia Reyes began to investigate the biological activity of the soil of a coffee plantation subjected to different forms of management (Reyes, 1986; Reyes, 1992). She indicated that 43% of the fungi and 3% of the bacteria present in surface outcrops of phosphate rock are capable of solubilizing insoluble inorganic phosphates, such as tricalcium phosphate, calcium acid phosphate, and phosphate rocks, and that the greatest phospho-solubilizers in a phosphocompost from coffee pulp and phosphate rock were *Aspergillus*, *Mucor*, *Fusarium*, *Bacillus* and an *Enterobacteriaceae*. Later, at the end of the 1990s, important advances were made in the isolation, characterization, and selection of PGPM from different places in the country. For example, rhizobia was isolated and characterized from legumes from different regions of Venezuela and non-symbiotic nitrogen-fixing bacteria associated with grasses from the Mérida

moorland (Marquina *et al.*, 2011; Marquina *et al.*, 2001-2002); as well as Reyes *et al.* (1999^a; 1999^b) and Reyes *et al.* (2008) who tested the effect of isolates from different plants and locations in the state of Táchira on crops of agricultural importance, which allowed them to identify the microbial potential with plant growth-promoting characteristics, especially from the Andean region of the country. In other regions, notable studies have also emerged on the formulation of inoculants as a guide for their development in laboratories (Martínez-Viera *et al.*, 2006); as well as other experiments using nitrogen-fixing bacteria and phosphate solvents on corn crops in combination with inorganic fertilizers and in different types of soil (López *et al.*, 2008).

More recent studies have also determined the potential of PGPM, specifically rhizobia, as soil bioremediation, by reporting strains that co-metabolize carbamates (Blanco *et al.*, 2013), and there are also works where it has been shown that the kinetics of production of indoleacetic acid (IAA) type phytohormones by PGPM shows a sigmoidal curve and even the growth-promoting effect of these microorganisms *in vitro* plant crops has been shown (Marquina *et al.*, 2018). It is important to highlight that the studies reported in Venezuela on PGPM have evaluated most of the mechanisms expressed by these microorganisms in laboratory, greenhouse, and field conditions to induce the growth and/or health of crops (Tables 2 and 3).

Advances in the application of bioinoculants in the laboratory, greenhouse, and field

In Venezuela, we highlight that several of their studies have shown a specificity between microorganisms, soil, and crops. In this sense, it has been shown that PGPM responds differently and specificities have been identified between strain-soil characteristics (Reyes & Valery, 2007), strain-crop (Blanco *et al.*, 2018), strain-variety of the same plant species (Blanco & Reyes, 2018), strain-strain (Castro & Blanco, 2018; Blanco *et al.*, 2021^a), in response to the plant-microorganism-soil interaction, thus indicating that the positive effect of an identified microorganism on a certain crop cannot be generalized to all crops, but rather studies must be done on several plant species and varieties, and soil types to determine a wide range of effectiveness, thus avoiding adverse effects on the crop to be evaluated. The development of dual cultures of microorganisms (*Enterobacter cloacae* and *Penicillium rugulosum*) has also been evaluated to determine the feasibility of joint production, the study of which indicated that, although it is better to grow them individually, their most favorable application is dually (Blanco and Reyes, 2022). In addition, the combination of *Trichoderma harzianum* and *Trichoderma asperellum* with organic fertilizers such as chicken manure, tomato stubble compost, and coconut fiber has been evaluated, obtaining increases in potato yield (Roa & Bautista, 2018; Montoya *et al.*, 2018). Research has also been carried out on the effect of biofertilizers and phosphate rock on forage grasses such as *Urochloa decumbens* with satisfactory results (Barbosa *et al.*, 2024). Most bioinoculant studies in Venezuela have been carried out in combination with inorganic fertilizers, however, they have also been combined with organic fertilization, for a more agroecological management of crops (Table 2).

Table 2. Greenhouse and field research carried out in Venezuela with native microorganisms isolated from different areas of the country with biofertilizer and biostimulant potential.

Plant species	Microorganisms	Benefits	Reference
<i>Capsicum annuum</i>	Consortium <i>Rhizobium tropici</i> and <i>Bradyrhizobium</i> sp.	Increase in chlorophyll and N content in plants under shade conditions (180 days), with 50% fertilizer.	Castro and Blanco (2018)
	Strains of the genera <i>Rhizobium tropici</i> , <i>Ochrobactrum</i> sp., <i>Bradyrhizobium japonicum</i> , <i>Bradyrhizobium</i> sp., and <i>Pseudomonas fluorescens</i> .	Increase in % germination, and increase in number of leaves, length, and dry weight of 60 das seedlings.	Blanco <i>et al.</i> (2018)
	Strains of the genera <i>Azotobacter</i> spp., <i>Azospirillum</i> spp., and <i>Rhizobium</i> spp.	Increased germination. Increased %N in plants under shade conditions (9 weeks). Increased germination. Increased length of the aerial and radical parts, and dry root biomass, under <i>in vitro</i> conditions.	Reyes <i>et al.</i> (2008)
	Strains of the genera <i>Rhizobium</i> spp. and <i>Azospirillum</i> spp.	Increase in the number of leaves, plant height, and number of flower buds with soluble P source and phosphate rock, under shade-house conditions. Increase in field yield without P application, compared to the full fertilization treatment.	Marquina <i>et al.</i> (2018)
	<i>Penicillium rugulosum</i> , <i>Trichoderma</i> sp., <i>Enterobacter</i> sp., <i>Microbacterium</i> sp., <i>A. brasilense</i> , and microbial consortia by pairs of microorganisms.	Increase of available P in the soil with the use of phosphate rock. Increase in the number of leaves in the field.	Sánchez and Reyes (2018)

Continuation

Table 2. Greenhouse and field research carried out in Venezuela with native microorganisms isolated from different areas of the country with biofertilizer and biostimulant potential.

Plant species	Microorganisms	Benefits	Reference
<i>Lactuca sativa</i>	Two strains of the genus <i>Rhizobium</i> spp. (individual and in consortium).	Increase in dry weight of seedlings at seedbed level (30 days).	Peña & Reyes (2007)
	Strains of the genera <i>Rhizobium tropici</i> , <i>Ochrobactrum</i> sp., <i>Bradyrhizobium japonicum</i> , <i>Bradyrhizobium</i> sp., and <i>Pseudomonas fluorescens</i> .	Increase in number of leaves, length, and dry weight of seedlings after 30 days.	Blanco et al. (2018)
	Consortio <i>Enterobacter cloacae</i> and <i>Penicillium rugulosum</i> .	Increase in number of leaves and %foliar P. Replacement of soluble P source with phosphate rock. Formulation of a solid bioinoculant (field).	Blanco & Reyes (2018)
		Increase in the % of germination. Increase in the vigor of seedlings of two varieties of lettuce (seedbed).	Blanco & Reyes (2022)
	Three strains of <i>Trichoderma</i> .	Increase in seedling growth parameters with the liquid and granulated application of the microorganism.	Roche et al. (2018)

Continuation

Table 2. Greenhouse and field research carried out in Venezuela with native microorganisms isolated from different areas of the country with biofertilizer and biostimulant potential.

Plant species	Microorganisms	Benefits	Reference
<i>Allium cepa</i>	Consortium of <i>Rhizobium tropici</i> , <i>Bradyrhizobium japonicum</i> .	Increase in seedling vigor and higher rate of CO ₂ assimilation in seedlings (seedbed) and plants (field).	Blanco <i>et al.</i> (2021 ^a); Blanco <i>et al.</i> (2023)
	Consortium of <i>Rhizobium tropici</i> , <i>Bradyrhizobium japonicum</i> .	Yield and %N are similar to production control with only 50% NPK fertilizer under 100% irrigation conditions and increases in %N in bulbs under water deficit conditions (field).	Blanco <i>et al.</i> (2021 ^b)
	Three strains of <i>Trichoderma</i> spp.	Increase in seedling growth parameters with the liquid and granulated application of the microorganism.	Roche <i>et al.</i> (2018)
<i>A. Oryza sativa</i>	Consortium of 10 strains of the following genera: <i>Pseudomonas</i> sp., <i>Aeromonas</i> sp., <i>A. veronii</i> , <i>Serratia</i> sp., <i>Bacillus</i> sp., <i>Rhizobium</i> sp., <i>Delftia</i> sp., <i>Pseudomonas</i> sp.	Increase in dry weight of seedlings 30 days after inoculation.	Moronta-Barrios <i>et al.</i> (2018)
<i>Zea mays</i>	Strains of the genera <i>Azotobacter</i> spp, <i>Azospirillum</i> spp, <i>Rhizobium</i> spp.	Increased germination. Increased %N and %P in leaf tissue in plants under shade conditions (6 weeks).	Reyes <i>et al.</i> (2008)
<i>Coffea arabica</i>	<i>Penicillium rugulosum</i> , <i>Trichoderma</i> sp., <i>Enterobacter</i> sp., <i>Microbacterium</i> sp., <i>A. brasilense</i> , and microbial consortia by pairs of microorganisms.	Increase in P available in the soil with the use of phosphate rock. Increase in the number of leaves in the field.	Sánchez <i>et al.</i> (2018)
<i>Solanum lycopersicum</i>	<i>Trichoderma asperellum</i> .	Increase in aerial and root dry weight, total dry matter, height, and stem diameter of seedlings.	Bautista & Córdón (2018)

Source: own elaboration.

Regarding the topic of biocontrol, a great diversity of the antagonist *Trichoderma* spp. has been reported in soils cultivated with cocoa and corn, including the species *T. brevicompactum*, *T. theobromicola*, *T. ovalisporum*, *T. asperellum*, *T. atroviride*, *T. erinaceum*, *T. harzianum*, *T. koningiopsis*, *T. pleurotum*, *T. reesei*, *T. spirale* and *T. virens*, with *T. harzianum* being the most abundant (Rivas & Pavone, 2010; Pavone & Dorta, 2015). Even the effect of non-pathogenic strains of microorganisms that are commonly phytopathogenic, such as *F. oxysporum*, has been investigated, and an increase in growth parameters of cucumber, onion, and tomato seedlings has been observed (Bautista & Granados, 2018). Some of the studies carried out in Venezuela on the biocontrol of PGPM on phytopathogens are shown in Table 3.

Table 3. Some studies in Venezuela of microorganisms with biocontrol potential.

Biocontrol microorganisms	Controlled phytopathogens	Benefit	Reference
Various <i>rhizobial</i> species and <i>Pseudomonas fluorescens</i> .	<i>Fusarium oxysporum</i> and <i>Colletotrichum gloeosporioides</i> .	Growth inhibition under <i>in vitro</i> conditions. Production of siderophores and hydrocyanic acid.	Blanco & Castro (2021)
<i>Bacillus licheniformis</i> and <i>Bacillus pumilus</i> .	<i>Botrytis cinerea</i> and <i>Fusarium solani</i> .	Growth inhibition under <i>in vitro</i> conditions Induction of systemic resistance in paprika (<i>C. annuum</i>) plants <i>in vivo</i> .	Márquez <i>et al.</i> (2020)
<i>Enterobacter</i> sp., <i>Penicillium rugulosum</i> , and <i>Trichoderma koningii</i> .	<i>Alternaria alternata</i> .	<i>In vitro</i> inhibition of <i>A. alternata</i> growth and <i>in vivo</i> control of infection in tomato (<i>Solanum lycopersicum</i> L.) seedlings.	Alcedo & Reyes (2018)
<i>Penicillium rugulosum</i> .	<i>Sclerotinia sclerotiorum</i> .	<i>In vitro</i> growth inhibition of <i>Sclerotinia sclerotiorum</i>	Blanco and Reyes (2022)
<i>Pseudomonas</i> sp., <i>Bacillus</i> sp. and <i>Delftia</i> sp.	<i>Dickeya zea</i> <i>Pseudomonas fuscovaginae</i> <i>Xanthomonas oryzae</i> .	<i>In vitro</i> antibacterial activity against phytopathogens.	Moronta-Barrios <i>et al.</i> (2018)
<i>Trichoderma harzianum</i> , <i>Trichoderma virens</i> and <i>Trichoderma theobromicola</i> .	<i>Crinipellis perniciosa</i> .	<i>In vitro</i> biocontrol capacity of <i>Trichoderma</i> on the phytopathogen.	Rivas & Pavone (2010)
<i>Trichoderma harzianum</i> and <i>Trichoderma asperellum</i> .	<i>Pyricularia grisea</i> .	Reduction of grain spotting in rice plants in the nursery and control of the <i>in vitro</i> growth of the phytopathogen.	Núñez & Pavone (2014)
<i>Trichoderma asperellum</i> .	<i>Rhizoctonia solani</i>	Control of phytopathogen infection in corn plants under nursery conditions.	Tortolero & Pavone (2012)

Source: own elaboration.

Experiences with the use of bioformulations in the field

At the Laboratorio de Fitobiotecnología of the Universidad de Los Andes en Mérida, Venezuela (previously known as Laboratorio de Fijación Biológica del Nitrógeno y Cultivo de Tejidos Vegetales *in vitro*) professors María Eugenia Marquina and Roberto Skwierinski[†] developed a bioinoculant with native strains of rhizobia, using ground charcoal as a substrate, which has been applied to inoculate legume seeds. Although it has not been possible to monitor it in the field with producers, the good impressions of the effect on bean and pea production by farmers are known (personal information from E.L. Blanco and Y. Castro). On the other hand, in other institutions such as UNET, localized application biosubstrates have been manufactured, composed of San Joaquín de Navay phosphate rock (RFSJN), sand, and an organic substrate composed of sugar-rice 50:50 inoculated with the consortium *E. cloacae* and *P. rugulosum*, and it was evaluated on the development of two varieties of lettuce Great Lakes (GL) and Black Seeded Simpson (BSS) in field conditions; The viability in storage was determined and it was observed that since its preparation it was reduced in two orders, that is, from 10^8 CFU mL⁻¹ to 10^6 CFU mL⁻¹ and in the latter, which was the value applied in the field, the product was effective (Blanco & Reyes, 2018). Also, the experience of the evaluation of dual growth for the production of this consortium was not favorable, but its joint application was (Blanco & Reyes, 2022). In other regions of the country, studies have recently been carried out to harvest *T. asperellum* spores through the flocculation process with promising results using the Urfos 44[®] fertilizer as a product (Pavone-Maniscalco *et al.*, 2020). Other granulated formulations of *T. asperellum* have also been standardized using defatted corn germ as a substrate, and liquid formulations using vegetable oil, lignosulfonates, and water as a base (Herrera *et al.*, 2020). Likewise, other abundant, economical, and eco-friendly substrates such as paper sludge, a waste product in paper manufacturing, have been found for the production of *T. asperellum*, thanks to the cellulolytic activity of this fungus (Dorta-Vásquez *et al.*, 2019).

Sulbarán *et al.* (2012) showed an experience carried out on a farm in the state of Guárico in Venezuela that was significant for the area of biofertilizers, since a group of researchers from the Instituto Nacional de Investigaciones Agrícolas (INIA) provided support to producers, farmers and technicians and demonstrated the beneficial results of bioinoculants in the field on onion crops, reproducing the test by Sulbarán *et al.* (2011) carried out in plots with producers on onion crops. There, biofertilizer products composed of phosphorus-dissolving microorganisms and non-symbiotic N fixers were applied, prepared in the production laboratories of the Instituto Nacional de Salud Agrícola Integral INSAI-Calabozo and the Referencia Nacional en Investigación e Innovación en Biofertilizantes attached to INIA CENIAP (National Center for Agricultural Research), using the methods and procedures described by Martínez-Viera *et al.* (2006). According to Sulbarán *et al.* (2012), the inoculants were applied in the field using a 600 L mechanical sprayer, and only 50% of the NPK fertilizer was applied. The production costs of the experience of Sulbarán *et al.*, (2012) indicated that in the field the reduction of the onion crop cycle was up to 29% shorter (35 days earlier) for the crop inoculated with biofertilizers, a saving of 57% in production costs for fertilization with the use of these bioinoculants, and an increase in yield of 24% bringing as benefits the saving of money and time, in other words, little investment and higher income in less time, something very

beneficial and profitable for farmers. This experience also highlights the importance of socializing the results through evaluation in demonstration plots, since with this practice there was a real approach from science in the laboratory to production in the field to answer questions such as: how to use biofertilizers? What dosage to apply? How to apply them? And, above all: where are they distributed and what is the cost of these? (Sulbarán *et al.*, 2012).

Current situation of bioinoculant availability in Venezuela

Despite the PGPM benefits, in Venezuela, the way of using these microorganisms has generally been at a local level and on a small scale in the field, which means that to date the application of these microorganisms on a large scale in the country for the benefit of agriculture has not been able to be established. This is because it has not been possible to mass-produce and bioformulate the majority of microbial cultures already tested in research for various reasons, such as lack of financial support to universities, lack of equipment, scarce specialized personnel, and little availability of qualified experimental labor due to the migration phenomenon of Venezuelans that has worsened in the last 10 years.

In Venezuela, some of the available liquid biofertilizers are produced by the network of semi-industrial production laboratories attached to the National Institute of Integral Agricultural Health (INSAI) (López *et al.*, 2010). However, there are more bioproducts available on the current market, but not all of them have proven provenance and results, nor are they registered with INSAI. Some of the bioinoculant products formulated with PGPM with biofertilizer, biostimulant, and disease biocontrol potential are *Bradyrhizobium*, *Rhizobium*, *Rhizofos*, *Azotobacter*, *Azotofos*, Phosphorus solubilizer, *Trichoderma* (INSAI product line), BIOFERMAX PM, TRICOMAX PM, SOILBIOMAX PM, SUNEMAX PM (Agrobiológico Montecarmelo C.A.), TrichoGreen (Tecnovita C.A.), BioMic (GIBAA-UNET), F1 and RF MAX (Biodelta C.A.) The current prices of these products vary for liquid presentations between 3 and 5 USD/L, while solid presentations vary between 2 USD and 6 USD for 50 g of product.

In this sense, it is necessary to work on completing the cycle of biofertilizer production in Venezuela on a large scale to make them available to the farming community in all regions of the country, in a responsible and biosafe manner, according to rigorous scientific studies carried out, and thus try to reduce the resistance that exists in conventional agriculture regarding the final acceptance of these bioproducts and cover the largest possible space within national production.

Legal framework

Previously, in Venezuela, registrations of products for livestock, agricultural, plant, domestic, public health, and industrial use were processed before the defunct Servicio Autónomo de Sanidad Agropecuaria (SASA). In Official Gazette No. 40,981 dated September 5, 2016, the Instituto Nacional de Salud Agrícola Integral (INSAI), published a Provision through which the requirements for granting these registrations are established to replace the SASA, and currently in Official Gazette No. 42,687 of August 8, 2023, INSAI published the Administrative Provision No. 007/2023 through which the Norms that regulate the Registration, Supervision and Control

of Agroecological or Organic Inputs and their inherent activities, in the Bolivarian Republic of Venezuela, are established. The permit that bioinoculants/biofertilizers/biocontrollers grouped within agrobiological inputs must have is the Registro Único de Salud Agrícola Integral (RUNSAI), which is granted by INSAI (INSAI, 2024). On the product label, it is appropriate to indicate the registration number, number of colony-forming units, or spore concentration depending on the nature of the product, the dose to be applied, as well as the viability time of the product.

Bioinoculants in Brazil

Background

Biological inputs began to be produced in Brazilian industries in 1956, with the pioneering work of agronomists Johanna Döbereiner (1924-2000) and João Ruy Jardim Freire (1923-2015), members of the scientific team of the Empresa Brasileira de Investigación Agropecuaria (Embrapa) (Bomfim *et al.*, 2021).

Brazil has a long history of research with inoculants. In 1956, the first inoculant factory in Brazil was founded in Pelotas, in the state of Rio Grande do Sul, to select strains for clover (*Trifolium* spp.) and alfalfa (*Medicago sativa*) crops for livestock production (Araujo, 2013). In the early 1960s, when soybean (*Glycine max* (L.) Merr) production began its expansion in Brazil, the rhizobia-based bioinoculant industry also followed the same trend, new inoculant factories were opened to supply the market with this input, which was already highly effective in increasing soybean productivity (Santos *et al.*, 2019). Subsequently, the expansion of soybean cultivation in the Cerrado area (savannah zone of Brazil) in the 1980s, began the search and selection of elite rhizobia strains capable of meeting plant nitrogen demand under low humidity conditions and in acidic soils (Mendes *et al.*, 2004; Araujo, 2013). The first strains selected for soybean inoculation in the Brazilian Cerrado were *Bradyrhizobium elkanii* (SEMIA 587 and SEMIA 5019) (Peres *et al.*, 1993).

One of the best-known and most studied genera of PGPM is *Azospirillum*, and Brazil is a pioneer in studies with this genus (Tarrand *et al.*, 1978). *Azospirillum*, initially named *Spirillum* by Beijerinck (1925), modified its nomenclature after Brazilian researcher Johanna Döbereiner studied and described its ability to fix nitrogen when associated with grasses (Döbereiner, 1979). In 1978, the nomenclature “azo” was added as a prefix to the original name, of the term “azote” used by Lavoisier for the element nitrogen (Santos *et al.*, 2021). *Azospirillum brasilense* was first isolated in Brazil from the rhizosphere of *Digitaria decumbens* (forage grass) (Döbereiner & Day, 1976) and since then Brazil has maintained leadership in basic studies with *Azospirillum*, including taxonomy (Tarrand *et al.*, 1978; Ferreira *et al.*, 2019), ecology (Baldani & Döbereiner, 1980), quantification of BNF contribution (Day and Döbereiner, 1976; Döbereiner, 1979; Döbereiner and Pedrosa, 1987) and isolation of *Azospirillum* strains (Magalhães *et al.*, 1983; Santos *et al.*, 2021).

In the late 1990s, the benefits of annual reinoculation of soybean crops led to the demand for microbial inoculants for other crops grown in rotation or succession with soybean, especially corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.), which initiated an evaluation of *Azospirillum*

strains for these two cereals, resulting in the identification of six strains capable of promoting increases in grain yield (Hungria *et al.*, 2010; Moretti *et al.*, 2018). In Brazil, the first inoculant was registered by Stoller do Brasil SA (Campinas, São Paulo), paradoxically, 14 years after the first one was registered in Argentina (2009). It was named Masterfix L gramineas and was formulated with a combination of the *Azospirillum brasilense* Ab-V5 and Ab-V6 strains (Santos *et al.*, 2019; Cassán *et al.*, 2020).

In Brazil, the first liquid inoculant was approved by the Ministry of Agriculture, Livestock and Supply (MAPA) for commercial use in 2000, and a decade later almost 80% of the inoculants sold in the country were in liquid formulations (ANPII 2018). Particularly in the last decade, the use of mixed inoculants (co-inoculation) has expanded. In Brazil, the co-inoculation of *Azospirillum brasilense* with *Bradyrhizobium* spp. for soybean and *A. brasilense* with *Rhizobium tropici* for common bean was launched in 2014 and impressive increases in grain yield have been reported (Hungria *et al.* 2015; de Souza & Ferreira 2017; Nogueira *et al.* 2018).

The current state of production and use of Bioinoculants in Brazil

Brazil ranks fifth among countries with the largest land area devoted to agriculture, with about 7.8% of its territory used for agricultural practices (CONAB, 2021). According to the Asociación Nacional de Productores e Importadores de Inoculantes (ANPII, 2019), 20.2 million doses of inoculants were sold in Brazil in 2009, increasing to 73.5 million doses in 2018, representing an increase of 263%. Therefore, the bioinoculant market in Brazil has grown considerably, making it an excellent investment opportunity for national and international public and private companies (Oliveira *et al.*, 2022).

In 2019, it was estimated that 70 million doses of soybean inoculants were consumed, and used in more than 90% of the total area planted in Brazil (Hungria *et al.*, 2020). Soybean inoculation allows an average yield of 3.5 tons of grains ha⁻¹, without the need for nitrogen fertilizers. Similarly, in the cultivation of common beans, the sale of mixed inoculants between *Azospirillum brasilense* and *Bradyrhizobium* spp. increased by 85% between 2009 and 2013 (ANPII 2018), and in 2018 alone, approximately 280 thousand doses of bean inoculant in peat and liquid formulations were sold (Bomfim *et al.*, 2021).

Brazilian farmers have more than 70 years of tradition in using inoculants based on two strains of *Azospirillum brasilense* Ab-V5 and Ab-V6 (Hungary *et al.*, 1994; 2006; 2010). Since then, these strains have been increasingly evaluated in experiments with various crops including rice, sugarcane, and pastures, and for co-inoculation of legumes (Santos *et al.*, 2019; Rezende *et al.*, 2021). Therefore, the excellent results with these bioinoculants led to a series of new studies investigating the main mechanisms that could explain their good performance (Table 4).

Table 4. Use of bioinoculants in crops of interest in Brazil and their benefits.

Crop	Microorganisms	Benefit	Reference
<i>Phaseolus vulgaris</i>	<i>Rhizobium tropici</i> and <i>Herbaspirillum seropedicae</i> .	Relief from water stress increased dry biomass.	da Piedade Melo <i>et al.</i> (2017)
<i>Phaseolus vulgaris</i>	<i>Rhizobium tropici</i> , <i>Bradyrhizobium</i> spp. and <i>Azospirillum brasilense</i> .	Co-inoculations stimulated greater nodule mass and shoot biomass.	de Carvalho <i>et al.</i> (2020)
<i>Glycine max</i> (L.) Merr.	<i>Bradyrhizobium japonicum</i> and <i>B. diazoefficiens</i> .	Increase in nodulation and BNF capacity, increase in yield by 27 and 28%.	Moretti <i>et al.</i> (2018)
<i>Glycine max</i> (L.) Merr.	<i>Bradyrhizobium</i> spp. and <i>Azospirillum brasilense</i> .	Improvement of several morphological characteristics of the root, increasing the plant's capacity to overcome episodes of moderate drought stress, which allowed for higher yields to be achieved.	Rondina <i>et al.</i> (2020)
<i>Zea mays</i> L.	<i>Azospirillum brasilense</i> cepas Ab-V5 and Ab-V6.	Induction of acquired systemic resistance (SAR) and induced systemic resistance (ISR) mechanisms.	Fukami <i>et al.</i> (2018)
<i>Triticum aestivum</i> L.	<i>Azospirillum brasilense</i> and <i>Rhizobium pisi</i> .	Significant increase in wheat grain yield, number of grains per plant, and weight of 1000 grains by 36%, 11% and 17%.	Zaheer <i>et al.</i> (2019)
<i>Pennisetum glaucum</i> .	<i>Bacillus</i> sp.	Improvement of the dry weight of shoots and roots and nitrogen and phosphorus content.	Ribeiro <i>et al.</i> (2018)
<i>Zea mays</i> L.	<i>Azospirillum brasilense</i> Ab-V5.	Increased growth, yield, and nitrogen use efficiency.	Zeffa <i>et al.</i> (2019)

Source: own elaboration.

Legislation and Regulations

Brazil has a long tradition of research with inoculants containing strains of rhizobia and *Azospirillum*. According to the standards established by the Ministerio de Agricultura, Ganadería y Abastecimiento (MAPA), commercial inoculants must have a minimum concentration of 10^9 viable cells of *Bradyrhizobium* and 10^8 cells of *Azospirillum* per gram or milliliter of inoculant, without contaminants at 10^{-5} dilution, and must contain only elite strains with recognized agronomic efficiency (Embrapa, 2021).

Microorganisms such as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azospirillum*, *Bacillus*, and *Mesorhizobium*, used in inoculant formulations in patent applications, are included in the list of microorganisms authorized for use by the Ministry of Agriculture, Livestock and Supply of Brazil, according to Normative Instruction SDA No. 13 of March 24, 2011 (Oliveira et al., 2022). Currently, on December 23, 2024, a new specific regulation for bio-inputs (Law No. 15,070) was enacted, establishing criteria for the production, registration, use, and inspection of these products. It also addresses related areas such as research, packaging, advertising, transportation, storage, waste disposal, and incentives for the development of bio-inputs. With this measure, Brazil aims to establish a global reference for sustainable agricultural innovation (BiologicalsLatam, 2024).

Conclusions

Taking into account the scientific knowledge and experience that combine the biological, physiological, biochemical, molecular, and ecological bases in the plant-microorganism-soil interaction, it is important to evaluate the quality parameters of these products.

According to what has been described, for the success of bioinoculants, a thorough analysis of the factors that affect the efficiency of products based on these microorganisms is required, which will allow overcoming the barriers that reduce their effectiveness and, finally, impose the perception that those who apply them, the producers, have of them. Among these factors, different aspects stand out, such as the soil, the crop, the general process of bioprospecting of microorganisms for the production of bioinoculants, and the agricultural management practices carried out by the producer.

Considering the wide range of benefits that VMPs offer in an agroecosystem, it is important to determine and understand the role of bioinoculants through science, the scope of their use, and that their performance or function in various environments will help achieve a sustainable agricultural production system in a changing climate scenario.

In general, and depending on the state of development of the country, we can mention that the transition between research and the availability of bioinoculants to producers is the most important issue in countries. Convincing producers with demonstrable results that VMPs

improve crop health and productivity, and conserve natural resources is the desirable goal of researchers to facilitate their large-scale application. On the other hand, the adoption of strict, but non-bureaucratic registration regulations can facilitate the rapprochement between academia and farmers. In addition, taking into account the protection of the soil microbial resource and biosecurity considerations is the basis for the responsible massification of bioinoculants. Finally, support for universities and research institutes by governments and private companies in each country is essential for the development of infrastructure and training of qualified personnel for the production of bioinoculants and their availability to consumers, because of the great potential that exists in our region following the research reported today.

Authors' contribution

Conceptualization of work, ELB and SdISV; data management, ELB, AMD, YC, RAC, LA; writing and preparation of the manuscript, ELB, AMD, YC, RAC, LA.; writing, review, and editing, ELB, AMD, YC, RAC, LA, SdISV; project administrator, SdISV.; acquisition of funding, SdISV.

All authors of this manuscript have read and accepted the published version of the manuscript.

Funding

This research was funded by the Academia de Ciencias de América Latina (ACAL), and for the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) for a Master's scholarship (scholarship number: 1221581) and a PhD scholarship (scholarship number: 908966).

Acknowledgments

E.L. Blanco thanks the Academia de Ciencias de América Latina (ACAL) for the scholarship awarded to carry out a short research internship at the Laboratorio de Biotecnología del Recurso Microbiano in the Instituto Tecnológico de Sonora (ITSON) in México. Y. Castro thanks the Coordination of Higher Education Personnel Training (CAPES) in Brazil for its financial support. All authors thank the Instituto Tecnológico de Sonora, Universidad Nacional Experimental del Táchira, Universidad de Los Andes, and the Universidad Federal de Lavras for the support provided.

Conflict of interest

The authors declare no conflict of interest.

References

- Aguado-Santacruz, G. A., & Moreno-Gómez, B. (2012). Biofertilizantes bacterianos desarrollados por el INIFAP. In G. A. Aguado-Santacruz (Ed.), *Introducción al uso y manejo de los biofertilizantes en la agricultura* (pp. 151-170). INIFAP/SAGARPA.
- Alcedo, Y., & Reyes, I. (2018). Microorganismos promotores de crecimiento en el biocontrol de *Alternaria alternata* en tomate (*Solanum lycopersicum* L.). *Bioagro*, 30(1), 59-66.
- Associação Nacional dos Produtores e Importadores de Inoculantes [ANPII]. (2018). Estadísticas. <http://www.anpii.org.br/estatisticas>
- Associação Nacional dos Produtores e Importadores de Inoculantes [ANPII]. (2019). Estadísticas. <http://www.anpii.org.br/estatisticas>. East/freeway.technologies
- Antoun, H., & Prévost, D. (2005). Ecology of plant growth promoting rhizobacteria. In Z. A. Siddiqui (Ed.), *PGPM: Biocontrol and biofertilization* (pp. 1-39). Springer. https://doi.org/10.1007/1-4020-4152-7_1
- Araújo Solon, C. (2013). A evolução da produção de inoculantes no Brasil. Associação Nacional dos Produtores e Importadores de Inoculantes (ANPII).
- Aramendis, R., Mondaini, A., & Rodríguez, A. (2023). Bioinsumos de uso agrícola: situación y perspectivas en América Latina y el Caribe. Documentos de Proyectos (LC/TS.2023/149). Comisión Económica para América Latina y el Caribe (CEPAL).
- Armenta-Bojórquez, A. D., García-Gutiérrez, C., Camacho-Báez, J. R., Apodaca-Sánchez, M. Á., Gerardo-Montoya, L., & Nava-Pérez, E. (2010). Biofertilizantes en el desarrollo agrícola de México. *Ra Ximhai*, 6(1), 51-56.
- Ayala-Zepeda, M., Valenzuela-Ruiz, V., Parra-Cota, F. I., Chinchilla-Soto, C., de la Cruz-Torres, E., Ibba, M. I., Estrada- Alvarado, M. I., & de los Santos-Villalobos, S. (2024). Genomic insights of a native bacterial consortium for wheat production sustainability. *Current Research in Microbial Sciences*, 6, Article 100230. <https://doi.org/10.1016/j.crmicr.2024.100230>
- Baldani, V. L. D., & Döbereiner, J. (1980). Host-plant specificity in the infection of cereals with *Azospirillum* spp. *Soil biology and biochemistry*, 12(4), 433-439. [https://doi.org/10.1016/0038-0717\(80\)90021-8](https://doi.org/10.1016/0038-0717(80)90021-8)
- Barbosa, A., Reyes, I., Valery, A., Chacón Labrador, C., Martínez, O., & Alonso, M. F. (2024). The use of phosphate rock and plant growth promoting microorganisms for the management of *Urochloa decumbens* (Stapf.) R.D. Webster in acidic soils. *PeerJ*, 12, Article e18610. <https://doi.org/10.7717/peerj.18610>
- Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPM) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability*, 13(3), Article 1140. <https://doi.org/10.3390/su13031140>
- Bautista, L., & Córdón, E. (2018). Evaluación de *Trichoderma asperellum*, *Metarhizium anisopliae* y *Bacillus subtilis* en la promoción del crecimiento de plántulas de tomate y pimentón. *Revista Científica UNET*, 30(2), 425-434.
- Bautista, L., & Granados, L. (2018). Efecto de cepas no patogénicas de *Fusarium oxysporum* (Ascomycota: Nectriaceae) en plántulas de tomate, pepino y cebolla. *Revista Científica UNET*, 30(1), 113-121.

- Bhattacharyya, C., Roy, R., Tribedi, P., Ghosh, A., & Ghosh, A. (2020). Biofertilizers as substitutes for commercial agrochemicals. In M. N. Vara Prasad (Ed.), *Agrochemicals detection, treatment and remediation* (pp. 263-290). Butterworth-Heinemann. <https://doi.org/10.1016/B978-0-08-103017-2.00011-8>
- Biological Products Industry Alliance [BPIA]. (2023). The listening session report: identifying ambiguities, gaps, inefficiencies, and uncertainties in the coordinated framework for the regulation of biotechnology. <https://www.bpia.org/wp-content/uploads/2023/02/BPIA-Biostimulant-Listening-Session-Report-copy-2.pdf>
- Beijerinck, M. W. (1925). Über ein Spirillum, welches frei in Stick-stoff binden kann?. *Zentralbl. Bakteriol*, 63, 353-359.
- BiologicalsLatam. (2024). Brasil ya cuenta con una regulación específica de bioinsumos. RedAgricola. <https://biologicalslatam.com/brasil-ya-cuenta-con-una-regulacion-especifica-de-bioinsumos/>
- Blanco, E. L., & Castro, Y. (2021). Antagonismo de rizobacterias sobre hongos fitopatógenos y su actividad microbiana con potencial biofertilizante, bioestimulante y biocontrolador. *Revista Colombiana de Biotecnología*, 23(1), 6-16. <https://doi.org/10.15446/rev.colomb.biote.v23n1.84808>
- Blanco, E. L., Castro, Y., Olivo, A., Skwierinski, R., & Moronta Barrios, F. (2018). Germinación y crecimiento de plántulas de pimentón y lechuga inoculadas con rizobios e identificación molecular de las cepas. *Bioagro*, 30(3), 207-218.
- Blanco, E. L., Marquina, M. E., & Castro, Y. (2013). Respuestas a la aplicación de carbamatos en dos aislados rizobianos provenientes de Mucuchíes, estado Mérida, Venezuela. *Bioagro*, 25(2), 117-128.
- Blanco, E.L., Rada, F., Castro, Y., & Paolini, J. (2021a). Selección de un consorcio microbiano promotor del crecimiento de plántulas de cebolla en condiciones de umbráculo. *Revista de la Facultad de Agronomía de la Universidad del Zulia* (LUZ), 38(2), 301-321.
- Blanco, E.L., Rada, F., & Paolini, J. (2023). The role of a microbial consortium on gas exchange and water relations in *Allium cepa* L. under water and nutritional deficit conditions. *Archives of Microbiology*, 205, 105. <https://doi.org/10.1007/s00203-023-03449-4>
- Blanco, E. L., Rada, F., Paolini, J., & Guerrero, J. A. (2021b). Effects of induced water deficit and biofertilization on growth dynamics and bulb yield of onion (*Allium cepa* L.) in a neotropical semi-arid environment. *Canadian Journal of Soil Science*, 101(3), 494-506. <https://doi.org/10.1139/cjss-2021-0011>
- Blanco, E.L., & Reyes, I. (2018). Aplicación de un biosustrato compuesto por microorganismos y roca fosfórica sobre el cultivo de dos variedades de lechuga (*Lactuca sativa* L.). *Revista de la Facultad de Agronomía de la Universidad del Zulia* (LUZ), 35, 408-434.
- Blanco, E.L., & Reyes, I. (2022). Caracterización de bioinoculantes y su efecto sobre el crecimiento de plántulas de dos variedades de lechuga. *Revista Científica UNET*, 34(1), 129-144.
- Bomfim, C. A., Coelho, L. G. F., do Vale, H. M. M., Carvalho, M., Megías, M., Ollero, & Bueno, F.(2021). Brief history of biofertilizers in Brazil: from conventional approaches to new biotechnological solutions. *Brazilian Journal of Microbiology*, 52, 2215-2232. <https://doi.org/10.1007/s42770-021-00618-9>
- Carrasco, A. M., Díaz-Rodríguez, A. M., Cota, F. I. P., & de los Santos-Villalobos, S. (2024).

- Legal framework for the development of microbial inoculants. In *New Insights, Trends, and Challenges in the Development and Applications of Microbial Inoculants in Agriculture* (pp. 143-151). Academic Press.
- Cassán, F., Coniglio, A., López, G., Molina, R., Nievas, S., Le Noir, C., Donaido, F., Torres, D., Rosas, S., Olivera, P., De Souza, E., Díaz, M., Basán, L., & Mora, V. (2020). Everything you must know about Azospirillum and its impact on agriculture and beyond. *Biology and Fertility of Soils*, 56, 461–479. <https://doi.org/10.1007/s00374-020-01463-y>
- Castro, Y., & Blanco, E. L. (2018). Estimación del contenido de clorofila y nitrógeno en plantas de pimentón inoculadas con bacterias rizosféricas. *Revista Científica UNET*, 30(1), 105-112.
- Chaudhary, T., Dixit, M., Gera, R., Kumar, S., Prakash, A., Gupta, G., Shilka, P. (2020). Techniques for improving formulations of bioinoculants. *3 Biotech*, 10, 199. <https://doi.org/10.1007/s13205-020-02182-9>
- Chávez-Díaz, I. F., Zelaya Molina, L. X., Cruz Cárdenas, C. I., Rojas Anaya, E., Ruiz Ramírez, S., & de los Santos-Villalobos, S. (2020). Consideraciones sobre el uso de biofertilizantes como alternativa agro-biotecnológica sostenible para la seguridad alimentaria en México. *Revista Mexicana de Ciencias Agrícolas*, 11(6), 1423-1436. <https://doi.org/10.29312/remexca.v11i6.2492>
- Companhia Nacional de Abastecimento [CONAB]. (2021). Acompanhamento da safra de grãos, Safra 2020/21 – Décimo levantamento. *Brasília*, 1-68.
- Creus, C. M. (2017). Inoculantes microbianos: piezas de un rompecabezas que aún requiere ser ensamblado. *Revista Argentina de Microbiología*, 49(3), 207-209. <https://dx.doi.org/10.1016/j.ram.2017.07.001>
- Cruz-Cárdenas, C. I., Zelaya-Molina, L. X., Sandoval-Cancino, G., de los Santos-Villalobos, S., Rojas-Anaya, E., Chávez-Díaz, I. F., & Ramírez, S. R. (2021). Utilización de microorganismos para una agricultura sostenible en México: consideraciones y retos. *Revista Mexicana de Ciencias Agrícolas*, 12(5), 899–913. <https://doi.org/10.29312/remexca.v12i5.2905>
- Da Piedade Melo, A., Olivares, F. L., Médici, L. O. et al. (2017). Mixed rhizobia and Herbaspirillum seropedicae inoculations with humic acid-like substances improve water-stress recovery in common beans. *Chemical and Biological Technologies in Agriculture*, 4, Article 6. <https://doi.org/10.1186/s40538-017-0090-z>
- Day, J. M., & Döbereiner, J. (1976). Physiological aspects of N₂-fixation by a Spirillum from Digitaria roots. *Soil Biology and Biochemistry*, 8(1), 45-50.
- De Carvalho, R. H., da Conceição Jesus, E., Favero, V. O. et al. (2020). The co-inoculation of Rhizobium and Bradyrhizobium increases the early nodulation and development of common beans. *Journal of Soil Science and Plant Nutrition*, 20, Article 860-864. <https://doi.org/10.1007/s42729-020-00171-8>
- De Souza, J. E. B., & de Brito Ferreira, E. P. (2017). Improving sustainability of common bean production systems by co-inoculating rhizobia and azospirilla. *Agriculture, Ecosystems & Environment*, 237, 250-257. <https://doi.org/10.1016/j.agee.2016.12.040>
- Diéguez, T., Cruz-Falcón, E., Norzagaray-Campos, A., Beltrán-Morales, M., Murillo-Amador, L., Beltrán-Morales, B., Félix, A., García-Hernández, J. L., & Valdez-Cepeda, R. (2010). Agotamiento hidro-agrícola a partir de la Revolución Verde: extracción de agua y gestión de la tecnología de riego en Baja California Sur, México. *Estudios Sociales*, 18(36), 177-201.
- Döbereiner, J., & Baldani, V. L. D. (1979). Selective infection of maize roots by streptomycin-

- resistant *Azospirillum lipoferum* and other bacteria. *Canadian Journal of Microbiology*, 25(11), 1264-1269.
- Döbereiner, J., & Day, J. M. (1976). Associative symbioses in tropical grasses: characterization of microorganisms and dinitrogen-fixing sites. SCISPACE. <https://scispace.com/papers/associative-symbioses-in-tropical-grasses-characterization-117fehasb9>
- Döbereiner, J., & Pedrosa, F. O. (1987). Nitrogen-fixing bacteria in nonleguminous crop plants (Vol. 154, No. 1). Madison: Science Tech Publishers.
- Dorta-Vásquez, R., Valbuena, O., & Pavone-Maniscalco, D. (2019). Solid-state fermentation of paper sludge to obtain spores of the fungus *Trichoderma asperellum*. *The EuroBiotech Journal*, 3(2), 71-77. <https://doi.org/10.2478/ebtj-2019-0008>
- Embrapa Notícias. (2021). Brasil tem o primeiro laboratório público de pesquisa acreditado para análise de inoculantes. <https://www.embrapa.br/busca-de-noticias/-/noticia/60586241/brasil-tem-o-primeiro-laboratorio-publico-de-pesquisa-acreditado-para-analise-de-inoculantes>
- Ferreira, M. J., Silva, H., & Cunha, A. (2019). Siderophore-producing rhizobacteria as a promising tool for empowering plants to cope with iron limitation in saline soils: a review. *Pedosphere*, 29(4), 409-420. [https://doi.org/10.1016/S1002-0160\(19\)60810-6](https://doi.org/10.1016/S1002-0160(19)60810-6)
- Food and Agriculture Organization [FAO]. (2019). Regulation (EU) 2019/1009 of the European Parliament and of the Council laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No. 1069/2009 and (EC) No. 1107/2009 and repealing Regulation (EC) No. 2003/2003. <https://eur-lex.europa.eu/oj/direct-access.html.cessed>
- Fukami, J., Cerezini, P., & Hungria, M. (2018). *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *Amb Express*, 8(1), 73. <https://doi.org/10.1186/s13568-018-0608-1>
- Galindo, E., Serrano-Carreón, L., Gutiérrez, C. R., Balderas-Ruiz, K. A., Muñoz-Celaya, A. L., Mezo-Villalobos, M., & Arroyo-Colín, J. (2015). Desarrollo histórico y los retos tecnológicos y legales para comercializar Fungifree AB®, el primer biofungicida 100% mexicano. *TIP. Revista especializada en ciencias químico-biológicas*, 18(1), 52-60. <https://doi.org/10.1016/j.recqb.2015.05.005>
- García-Montelongo, A. M., Montoya-Martínez, A. C., Morales-Sandoval, P. H., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2023). Beneficial microorganisms as a sustainable alternative for mitigating biotic stresses in crops. *Stresses*, 3(1), 210-228. <https://doi.org/10.3390/stresses3010016>
- Grageda-Cabrera, O. A., Díaz-Franco, A., Peña-Cabriaes, J. J., & Vera-Núñez, J. A. (2012). Impacto de los biofertilizantes en la agricultura. *Revista Mexicana de Ciencias Agrícolas*, 3(6), 1261-1274. <https://doi.org/10.29312/remexca.v3i6.1376>
- Guardiola-Márquez, C. E., Santos-Ramírez, M. T., Figueroa-Montes, M. L., Valencia-de Los Cobos, E. O., Stamatis-Félix, I. J., Navarro-López, D. E., & Jacobo-Velázquez, D. A. (2023). Identification and characterization of beneficial soil microbial strains for the formulation of biofertilizers based on native plant growth-promoting microorganisms isolated from northern Mexico. *Plants*, 12(18), 3262. <https://doi.org/10.3390/plants12183262>
- Herrera, W., Valbuena, O., & Pavone-Maniscalco, D. (2020). Formulation of *Trichoderma asperellum* TV190 for biological control of *Rhizoctonia solani* on corn seedlings. *Egyptian Journal of Biological Pest Control*, 30, Article 44. <https://doi.org/10.1186/s41938-020-00246-9>

- Hungría, M., Campo, R. J., Souza, E. M., & Pedrosa, F. O. (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil*, 331(1), 413-425. <https://doi.org/10.1007/s11104-009-0262-0>
- Hungría, M., Nogueira, M. A., Campos, L. J. M., Menna, P., Brandi, F., & Ramos, Y. G. (2020). Seed pre-inoculation with *Bradyrhizobium* as a time-optimizing option for large-scale soybean cropping systems. *Agronomy Journal*, 112(6), 5222–5236. <https://doi.org/10.1002/agj2.20392>
- Hungría, M., Franchini, J. C., Campo, R. J., Crispino, C. C., Moraes, J. Z., Sibaldelli, R. N., Mendes, L. C., & Arihara, J. (2006). Nitrogen nutrition of soybean in Brazil: Contributions of biological N_2 fixation and N fertilizer to grain yield. *Canadian Journal of Plant Science*, 86(4), 927-939.
- Hungría, M. (1994). Manual de métodos empregados em estudos de microbiologia agrícola. EMBRAPA-CNPAP. Documentos, 46.
- Hungría, M., Nogueira, M. A., & Araujo, R. S. (2015). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. *American Journal of Plant Sciences*, 6(6), 811-817. <https://doi.org/10.4236/ajps.2015.66087>
- Ibarra-Villarreal, A. L., Villarreal-Delgado, M. F., Parra-Cota, F. I., Yépez, E. A., Guzmán, C., Gutiérrez-Coronado, M. A., Valdez, L. C., Saint-Pierre, C., & de los Santos-Villalobos, S. (2023). Effect of a native bacterial consortium on growth, yield, and grain quality of durum wheat (*Triticum turgidum* L. subsp. durum) under different nitrogen rates in the Yaqui Valley, Mexico. *Plant Signaling & Behavior*, 18(1), 2219837. <https://doi.org/10.1080/15592324.2023.2219837>
- INSAI [Instituto Nacional de Salud Agrícola Integral]. (2024). Registro Único de Salud Agrícola Integral (RUNSAI). <http://www.insai.gob.pe/>
- Khalil, M. M. R., Félix-Gastélum, R., Peñuelas-Rubio, O., Argentel-Martínez, L., & Maldonado-Mendoza, I. E. (2022). Rhizospheric bacteria for use in preventing Fusarium wilt and crown root rot of tomato under natural field conditions. *Canadian Journal of Plant Pathology*, 1-13. <https://doi.org/10.1080/07060661.2022.2087104>
- Khan, A., Singh, A. V., Gautam, S. S., Agarwal, A., Punetha, A., Upadhayay, V. K., Kukreti, B., Bundela, V., Jugran, A. K., & Goel, R. (2023). Microbial bioformulation: A microbial-assisted biostimulating fertilization technique for sustainable agriculture. *Plant Science*, 14, 1270039. <https://doi.org/10.3389/fpls.2023.1270039>
- Lara-Capistrán, L., Hernández-Montiel, L. G., Reyes-Pérez, J. J., Preciado-Rangel, P., & Zulueta-Rodríguez, R. (2019). Respuesta agronómica de *Phaseolus vulgaris* a la biofertilización en campo. *Revista Mexicana de Ciencias Agrícolas*, 10(5), 1035-1046. <https://doi.org/10.29312/remexca.v10i5.936>
- Ley-López, N., Márquez-Zequera, I., Carrillo-Fasio, J. A., León-Félix, J., Cruz-Lachica, I., García-Estrada, R. S., & Allende-Molar, R. (2018). Efecto de biocontrol e inhibición germinativa de *Bacillus* spp. sobre zoosporas de *Phytophthora capsici*. *Revista Mexicana de Fitopatología*, 36(2). <https://doi.org/10.18781/R.MEX.FIT.1711-2>
- López, M., Martínez, R., Brossard, M., Bolívar, A., Alfonso, N., Alba, A., & Pereira, H. (2008). Efecto de biofertilizantes bacterianos sobre el crecimiento de un cultivar de maíz en dos suelos contrastantes venezolanos. *Agronomía Tropical*, 58(4), 391-401.
- López, M., Rodríguez, B., & España, M. (2010). Tecnologías generadas por el INIA para contribuir

- al manejo integral de la fertilidad del suelo. *Agronomía Tropical*, 60(4), 315-331.
- Magalhães, F. M., Baldani, J. I., Souto, S. M., Kuykendall, J. R., & Döbereiner, J. (1983). A new acid-tolerant *Azospirillum* species. *Anais da Academia Brasileira de Ciencias*. Rio de Janeiro, 55(4), 417-430.
- Márquez, R., Blanco, E. L., & Aranguren, Y. (2020). *Bacillus* strain selection with plant growth-promoting mechanisms as potential elicitors of systemic resistance to gray mold in pepper plants. *Saudi Journal of Biological Sciences*, 27(8), 1913-1922. <https://doi.org/10.1016/j.sjbs.2020.06.015>
- Marquina, M. E., González, N. E., & Castro, Y. (2011). Caracterización fenotípica y genotípica de doce rizobios aislados de diversas regiones geográficas de Venezuela. *Revista de Biología Tropical*, 59(3), 1017-1036.
- Marquina, M. E., Ramírez, Y., & Castro, Y. (2018). Efecto de bacterias rizosféricas en la germinación y crecimiento del pimentón (*Capsicum annuum* L. var. Cacique gigante). *Bioagro*, 30(1), 3-16.
- Marquina, M. E., Skwierinski, R. M., & Briceño, B. (2001-2002). Actividad reductora de acetileno de bacterias asociadas a las Glumifloras del páramo Loma Redonda, Mérida-Venezuela. *Pittieria*, 2(31), 57-69.
- Martínez-Álvarez, J. C., Castro-Martínez, C., Sánchez-Peña, P., Gutiérrez-Dorado, R., & Maldonado-Mendoza, I. E. (2016). Development of a powder formulation based on *Bacillus cereus* sensu lato strain B25 spores for biological control of *Fusarium verticillioides* in maize plants. *World Journal of Microbiology and Biotechnology*, 32(5), 75. <https://doi.org/10.1007/s11274-015-2000-5>
- Martínez-Martínez, T. O., Guerrero-Aguilar, B. Z., Pecina-Quintero, V., Rivas-Valencia, P., González-Pérez, E., & Ángeles-Núñez, J. G. (2020). Antagonismo de *Trichoderma harzianum* contra la fusariosis del garbanzo y su efecto biofertilizante. *Revista Mexicana de Ciencias Agrícolas*, 11(5), 1135–1147. <https://doi.org/10.29312/remexca.v11i5.2325>
- Martínez-Salgado, S. J., Andrade-Hoyos, P., Parraguirre-Lezama, C., Rivera-Tapia, A., Luna-Cruz, A., & Romero-Arenas, O. (2021). Biological control of charcoal rot in peanut crop through strains of *Trichoderma* spp. in Puebla, México. *Plants*, 10(12), 2630. <https://doi.org/10.3390/plants10122630>
- Martínez-Viera, R., López, M., Brossard, M., Tejeda, G., Pereira, H., Parra, C., Rodríguez, J., & Alba, A. (2006). Procedimientos para el estudio y fabricación de biofertilizantes bacterianos. Instituto Nacional de Investigaciones Agrícolas, 11, 8.
- Martiñón, A. S., Figueroa, R., Piña, J., Castro, C., Leana, J. L., & Aguilar, D. (2017). Evaluación de biofertilizantes y enraizador hormonal en jatropha (*Jatropha curcas* L.). *Revista mexicana de ciencias agrícolas*, 8(2), 463-469. <https://doi.org/10.29312/remexca.v8i2.66>
- Mendes, I. C., Hungria, M., & Vargas, M. A. T. (2004). Establishment of *Bradyrhizobium japonicum* and *B. elkanii* strains in a Brazilian Cerrado oxisol. *Biology and Fertility of Soils*, 40(1), 28-35. <https://doi.org/10.1007/s00374-004-0739-1>
- Mitra, D., Santos-Villalobos, S. D. L., Parra-Cota, F. I., Montelongo, A. M. G., Blanco, E. L., Lira, V. L., Olatunbosun, A. N., Khoshru, B., Mondal, R., Chidambaranathan, P., Panneerselvam, P., & Das Mohapatra, P. K. (2023). Rice (*Oryza sativa* L.) plant protection using dual biological control and plant growth-promoting agents: Current scenarios and future prospects. *Pedosphere*, 33(2), 268-286. <https://doi.org/10.1016/j.pedsph.2022.06.034>

- Montoya, C., Arias, K., Chacón, H., Sulbarán, J., & Ramírez, B. (2018). Evaluación de *Trichoderma asperellum* y mezclas de sustratos en la producción de semilla de papa (*Solanum tuberosum* L.) variedad Granola. *Revista Científica UNET*, 30(2), 444-451.
- Montoya-Martínez, A. C., Chávez-Luzanía, R. A., Olguín-Martínez, A. I., Ruíz-Castrejón, A., Moreno-Cárdenas, J. D., Esquivel-Chávez, F., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2024). Biological control of *Streptomyces* species causing common scabs in potato tubers in the Yaqui Valley, Mexico. *Horticulturae*, 10(8), 865. <https://doi.org/10.3390/horticulturae10080865>
- Montoya-Martínez, A. C., Parra-Cota, F. I., & de los Santos-Villalobos, S. (2022). Beneficial microorganisms in sustainable agriculture: Harnessing microbes' potential to help feed the world. *Plants*, 11(3), 372. <https://doi.org/10.3390/plants11030372>
- Mordor Intelligence. (2024). Market research company | Biofertilizer market size - Industry report on share, growth trends & forecasts analysis up to 2029. <https://www.mordorintelligence.com/es/industry-reports/global-biofertilizers-market-industry>
- Moretti, L. G., Lazarini, E., Bossolani, J. W., Parente, T. L., Caioni, S., Araujo, R. S., & Hungria, M. (2018). Can additional inoculations increase soybean nodulation and grain yield?. *Agronomy Journal*, 110, 715-721. <https://doi.org/10.2134/agronj2017.09.0540>
- Moronta-Barrios, F., Gionechetti, F., Pallavicini, A., Marys, E., & Venturi, V. (2018). Bacterial microbiota of rice roots: 16S-based taxonomic profiling of endophytic and rhizospheric diversity, endophytes isolation and simplified endophytic community. *Microorganisms*, 6(14). <https://doi.org/10.3390/microorganisms6010014>
- Nagrale, D. T., Chaurasia, A., Kumar, S., et al. (2023). PGPM: The treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol, and plant growth promotion in field crops. *World Journal of Microbiology and Biotechnology*, 39, 100. <https://doi.org/10.1007/s11274-023-03536-0>
- Nogueira, M. A., Prando, A. M., Oliveira, A. B., Lima, D., Conte, O., Harger, N., Oliveira, F. T., & Hungria, M. (2018). Ações de transferência de tecnologia em inoculação/coinoculação com *Bradyrhizobium* e *Azospirillum* na cultura da soja na safra 2017/18 no Estado do Paraná. Londrina: Embrapa Soja.
- Núñez, L., & Pavone, D. (2014). Tratamiento biológico del cultivo de arroz en condiciones de vivero empleando el hongo *Trichoderma* spp. *Interciencia*, 39(3), 185-190.
- O'Callaghan, M., Ballard, R. A., & Wright, D. (2022). Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. *Soil Use and Management*, 38(3), 1340-1369.
- Oliveira, A. M., Costa, M. R., Graziotti, P. H., Abreu, C. M., Bispo, N. S., Roa, J. P. B., Silva, D. M., & Miranda, J. M. (2022). Brazilian scenario of inoculant production: A look at patents. *Revista Brasileira de Ciência do Solo*, 46, e0210081. <https://doi.org/10.36783/18069657rbcs20210081>
- Organización de las Naciones Unidas para la Alimentación y la Agricultura [FAO]. (2021). Marco estratégico para 2022-2031. <https://openknowledge.fao.org/server/api/core/bitstreams/45f12eb4-4625-4a59-af29-8305379fc710/content>
- Orozco-Mosqueda, M. del C., Fadji, A. E., Babalola, O. O., Glick, B. R., & Santoyo, G. (2022). Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. *Microbiology Research*, 263, 127137. <https://doi.org/10.1016/j.micres.2022.127137>

- Pavone, D., & Dorta, B. (2015). Diversidad del hongo *Trichoderma* spp. en plantaciones de maíz de Venezuela. *Interciencia*, 40(1), 23-31.
- Pavone-Maniscalco, D., Pinto, M., Pacheco-Calderón, R., Mora, D., Andara, C., Bello, A., Hernández, M., Correa, M. A., & Valbuena, O. (2020). A simple method for harvesting *Trichoderma asperellum* spores. *Journal of Microbiology, Biotechnology and Food Sciences*, 10(1), 65-67. <https://doi.org/10.15414/jmbfs.2020.10.1.65-67>
- Peña, H., & Reyes, I. (2007). Aislamiento y evaluación de bacterias fijadoras de nitrógeno y disolventes de fosfatos en la promoción del crecimiento de la lechuga (*Lactuca sativa* L.). *Interciencia*, 32(8), 560-565.
- Peres, J. R. R., Mendes, I. C., Suhett, A. R., & Vargas, M. A. T. (1993). Eficiência e competitividade de estirpes de rizóbios para a soja em solos do Cerrado. *Revista Brasileira de Ciência do Solo*, 17, 357-363.
- Ramírez-Guzmán, K., Torres-León, C., Martínez-Terrazas, E., De la Cruz-Quiroz, R., Flores-Gallegos, A. C., Rodríguez-Herrera, R., & Aguilar, C. N. (2018). Biocontrol as an efficient tool for food control and biosecurity. *Food Safety and Preservation*, 167-193. <https://doi.org/10.1016/b978-0-12-814956-0.00007-x>
- Reyes, I. (1986). Actividad biológica del suelo de un cafetal sometido a diferentes formas de manejo. Trabajo de Grado de Maestría, Universidad de Los Andes, Facultad de Ciencias. Mérida, Venezuela.
- Reyes, I. (1992). Dinámica del fósforo y aislamiento de algunos microorganismos en la mezcla pulpa de café - roca fosfórica. *Revista de la Facultad de Agronomía*, 17, 397-408.
- Reyes, I., Álvarez, L., El-Ayoubi, H., & Valery, A. (2008). Selección y evaluación de rizobacterias promotoras del crecimiento en pimentón y maíz. *Bioagro*, 20(1), 37-48.
- Reyes, I., Bernier, L., Simard, R., Tanguay, P., & Antoun, H. (1999b). Characteristics of phosphate solubilization by an isolate of a tropical *Penicillium rugulosum* and two UV-induced mutants. *FEMS Microbiology Ecology*, 28, 291-295.
- Reyes, I., Bernier, L., Simard, R., & Antoun, H. (1999a). Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. *FEMS Microbiology Ecology*, 28, 281-290.
- Reyes, I., & Valery, A. (2007). Efecto de la fertilidad del suelo sobre la microbiota y la promoción del crecimiento del maíz (*Zea mays* L.) con *Azotobacter* spp. *Bioagro*, 19(3), 117-126.
- Rezende, C. C., Silva, M. A., Frasca, L. L. M., Faria, D. R., Filippi, M. C. C., Lanna, A. C., & Nascente, A. S. (2021). Microorganismos multifuncionais: utilização na agricultura. *Research, Society and Development*, 10(2), e50810212725. <http://dx.doi.org/10.33448/rsd-v10i2.12725>
- Ribeiro, V. P., Marriel, I. E., de Sousa, S. M., Lana, U. G., Mattos, B. B., de Oliveira, C. A., & Gomes, E. A. (2018). Endophytic *Bacillus* strains enhance pearl millet growth and nutrient uptake under low-P. *Brazilian Journal of Microbiology*, 49, 40-46. <https://doi.org/10.1016/j.bjm.2018.06.005>
- Rivas, M., & Pavone, D. (2010). Diversidad de *Trichoderma* spp. en plantaciones de *Theobroma cacao* L. del estado Carabobo, Venezuela, y su capacidad biocontroladora sobre *Crinipellis pernicios* (Stahel) Singer. *Interciencia*, 35(10), 777-783.
- Roche, L., Vera, R., Galvis, J., Moreno, B. (2018). Evaluación de la aplicación líquida o granulada de *Trichoderma* spp. para promover el crecimiento de *Allium cepa* y *Lactuca sativa*. *Revista Científica UNET*, 30(1), 139-149.

- Rojas-Padilla, J., Chaparro-Encinas, L. A., Robles-Montoya, R. I., & de-los Santos Villalobos, S. (2020). Promoción de crecimiento en trigo (*Triticum turgidum* L. subsp. durum) por la co-inoculación de cepas nativas de *Bacillus* aisladas del Valle del Yaqui, México. *Nova Scientia*, 12(24). <https://doi.org/10.21640/ns.v12i24.2136>
- Rondina, A. B. L., dos Santos Sanzovo, A. W., Guimarães, G. S., et al. (2020). Changes in root morphological traits in soybean co-inoculated with *Bradyrhizobium* spp. and *Azospirillum brasilense* or treated with *A. brasilense* exudates. *Biology and Fertility of Soils*, 56, 537–549. <https://doi.org/10.1007/s00374-020-01453-0>
- Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación [SAGARPA]. (2017, 4 de julio). Acuerdo por el que se dan a conocer las Reglas de Operación del Programa de Fomento a la Agricultura de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación para el ejercicio 2018. https://dof.gob.mx/nota_detalle.php?codigo=5509548&fecha=29/12/2017#gsc.tab=0
- Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación [SAGARPA]. (2019). Agricultura. <https://www.inegi.org.mx/temas/agricultura/#Tabulados>
- Sahu, P. K., Gupta, A., Singh, M., Mehrotra, P., & Brahmaprakash, G. P. (2018). Bioformulation and fluid bed drying: A new approach towards an improved biofertilizer formulation. En Sengar, R., & Singh, A. (Eds.), *Eco-friendly agro-biological techniques for enhancing crop productivity* (pp. 47-62). https://doi.org/10.1007/978-981-10-6934-5_3
- Sánchez, L. A., Dávila, B., Briceño, J., & Valery, A. (2018). Los biofertilizantes como una herramienta de la agricultura sostenible en los cultivos del pimentón y del café. *Revista Científica UNET*, 30(2), 435-443.
- Sánchez, L. A., & Reyes, I. (2018). Incidencia de la inoculación con microorganismos rizosféricos benéficos y roca fosfórica sobre el crecimiento del pimentón (*Capsicum annuum* L.). *Revista Científica UNET*, 30(1), 122-128.
- Sansinenea, E. (2021). Application of biofertilizers: current worldwide status. En *Biofertilizers* (pp. 183–190). Woodhead Publishing. <https://doi.org/10.1016/B978-0-12-821667-5.00004-X>
- Santillano-Cázares, J., Turmel, M. S., Cárdenas-Castañeda, M. E., Mendoza-Pérez, S., Limón-Ortega, A., Paredes-Melesio, R., ... & Ortiz-Monasterio, I. (2021). Can biofertilizers reduce synthetic fertilizer application rates in cereal production in Mexico?. *Agronomy*, 12(1), 80.
- Santos, F., Melkani, S., Oliveira-Paiva, C., Bini, D., Pavuluri, K., Gatiboni, L., Mahmud, A., Torres, M., McLamore, E., & Bhadha, J. H. (2024). Biofertilizer use in the United States: definition, regulation, and prospects. *Applied Microbiology and Biotechnology*, 108(1), 1-16. <https://doi.org/10.1007/s00253-024-13347-4>
- Santos, M. S., Nogueira, M. A., & Hungria, M. (2019). Microbial inoculants: Reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express*, 9, 205. <https://doi.org/10.1186/s13568-019-0932-0>
- Santos, M. S., Nogueira, M. A., & Hungria, M. (2021). Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: Lessons that farmers are receptive to adopt new microbial inoculants. *Revista Brasileira de Ciência do Solo*, 45.
- Savostin, P. (1950). La eficiencia de la inoculación artificial de las plantas leguminosas en Venezuela. Ministerio de Agricultura y Cría. Caracas.
- Secretaría de Agricultura, Ganadería y Desarrollo Rural. (1997, 8 de enero). NOM-032-FITO-1995: Requisitos y especificaciones fitosanitarias para la realización de estudios de

- efectividad biológica de plaguicidas agrícolas y su dictamen técnico. Diario Oficial de la Federación. https://dof.gob.mx/nota_detalle.php?codigo=4864052&fecha=08/01/1997
- Secretaría de Agricultura y Desarrollo Rural. (2020, 24 de abril). NOM-077-FITO-2000: Estudios de efectividad biológica en insumos de nutrición vegetal de uso agrícola y su Dictamen Técnico. Diario Oficial de la Federación. https://www.dof.gob.mx/nota_detalle.php?codigo=5592222&fecha=24/04/2020
- Solano-Báez, A. R., Leyva-Mir, S. G., Núñez-Pastrana, R., Quezada-Salinas, A., & Márquez-Licon, G. (2021). Biocontrol of damping-off of zucchini squash seedlings with *Bacillus subtilis* QST 713. *Revista mexicana de fitopatología*, 39(2), 302-313. <http://dx.doi.org/10.18781/R.MEX.FIT.2101-2>
- Soumare, A., Boubekri, K., Lyamlouli, K., Hafidi, M., Ouhdouch, Y., & Kouisni, L. (2020). From isolation of phosphate solubilizing microbes to their formulation and use as biofertilizers: Status and needs. *Frontiers in Bioengineering and Biotechnology*, 7, 425. <https://doi.org/10.3389/fbioe.2019.00425>
- Sulbarán, J., Barrios, R., López, M., & Ferrer, J. (2011). Beneficios de los biofertilizantes en cebolla. Bioestimulación del crecimiento (Parte I). INIA Divulga.
- Sulbarán, J., Barrios, R., López, M., & Ferrer, J. (2012). Beneficios de los biofertilizantes en cebolla. Rendimientos, costos de producción y socialización de resultados (Parte II). INIA Divulga.
- Suyal, D. C., Soni, R., Sai, S., & Goel, R. (2016). Microbial inoculants as biofertilizer. Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1. *Research Perspectives*, 311-318. https://doi.org/10.1007/978-81-322-2647-5_18
- Tarrand, J. J., Krieg, N. R., & Döbereiner, J. (1978). A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. *Canadian Journal of Microbiology*, 24(8), 967-980. <https://doi.org/10.1139/m78-160>
- Tortolero, J., & Pavone, D. (2012). Efecto de *Trichoderma* spp. sobre *Rhizoctonia solani* y algunos parámetros fisiológicos en *Zea mays* L. bajo condiciones de vivero. *Fitopatología Venezolana*, 25, 10-15.
- Vázquez-Martínez, J. A., González-Cárdenas, J. C., Chiquito-Contreras, R., Sangabriel-Conde, W., & Alvarado-Castillo, G. (2019). Evaluación del potencial biofertilizante de cinco especies de *Trichoderma* en la producción de maíz elotero nativo e híbrido bajo condiciones de campo. *Información Técnica Económica Agraria*. <https://doi.org/10.12706/itea.2019.006>
- Vessey, J. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255, 571-586.
- Yadav, A., & Yadav, K. (2024). Challenges and opportunities in biofertilizer commercialization. *SVOA Microbiology*, 5(1), 1–14. <https://doi.org/10.58624/SVOAMB.2024.05.037>
- Yahya, M., Rasul, M., Sarwar, Y., Suleman, M., Tariq, M., Hussain, S. Z., ... & Yasmin, S. (2022). Designing synergistic biostimulants formulation containing autochthonous phosphate-solubilizing bacteria for sustainable wheat production. *Frontiers in Microbiology*, 13, 889073. <https://doi.org/10.3389/fmicb.2022.889073>
- Zaheer, M. S., Raza, M. A. S., Saleem, M. F., Khan, I. H., Ahmad, S., Iqbal, R., & Manevski, K. (2019). Investigating the effect of *Azospirillum brasilense* and *Rhizobium pisi* on agronomic traits of wheat (*Triticum aestivum* L.). *Archives of Agronomy and Soil Science*, 65(11), 1554-

1564. <https://doi.org/10.1080/03650340.2019.1566954>

Zeffer, D. M., Perini, L. J., Silva, M. B., ... & Azeredo, L. S. (2019). Azospirillum brasilense promotes increases in growth and nitrogen use efficiency of maize genotypes. *PLoS One*, e0215332. <https://doi.org/10.1371/journal.pone.0215332>