Chapter 90

Agronomic Applications of *Azospirillum* and Other PGPR

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90.1 INTRODUCTION AND DISCUSSION

90.1.1 Plant Growth Promoting Rhizobacteria (PGPR)

Q2

The rhizosphere is the area of soil influenced by plant roots. It is composed of microbial populations that are somehow different than the rest of the soil populations, generally denominated as the "rhizosphere effect" (de Bruijn, 2013).

Among the microorganisms inhabiting the rhizosphere it is possible to isolate a wide variety of viruses, bacteria, protozoa, and fungi. Some microbial species are capable of promoting root and plant growth (de Bruijn, 2013). Other species are neutral and some are deleterious to plant growth (Helman et al., 2011). In this chapter, we deal with some well-characterized bacteria that are known to promote root and plant growth directly and have good potential to be commercially applied for increasing the yields of agricultural crops; they are generally denominated as PGPR (Plant Growth Promoting Rhizobacteria; de Bruijn, 2013). PGPR with potential to directly promote crop yield include species from the *Azospirillum, Herbaspirillum, Gluconacetobacter, Burkholderia, Pseudomonas,* and *Paenibacillus* genera, among others. The genus *Azospirillum* is the most widely commercially used in agriculture, especially in South America, and will be reviewed in more detail. We are not including in this chapter indirect biological control for plant growth promotion (see de Bruijn, 2013).

The genus *Herbaspirillum* (beta proteobacteria) comprises several diazotrophic species. Some colonize root surfaces but are also capable of endophytic and systemic colonization of several plant species (Reis et al., 2007). An additional nitrogen-fixing endophyte PGPR, which is capable of colonizing sugarcane and other plants, is *Gluconacetobacter diazotrophicus* (alpha proteobacteria) (Reis et al., 2007). Nitrogen-fixing, plant-associated *Burkholderia* (beta proteobacteria) represent great potential for agrobiotechnological applications (Caballero-Mellado et al., 2007). The bacterial genus *Pseudomonas* (gamma proteobacteria) also comprises many bacterial species with agricultural importance. Some such as *P. syingae* are plant pathogenic bacteria of important crops and have been extensively studied. Other *Pseudomonas* species are involved in disease suppression and/or direct plant growth promotion (Glick et al., 2007; de Bruijn, 2013). The genus *Paenibacillus* comprises over 30 species of facultative anaerobes and endospore-forming, periflagellated, heterotrophic, low G + C gram-positive bacilli. *Paenibacillus polymyxa* is

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of biotechnological potential, mostly due to its biological control properties (Lal and Tabacchioni, 2009). The *Azospirillum* genus (Baldani et al., 2005) belongs to the alpha proteobacteria and comprises free-living, nitrogen-fixing, vibrio- or spirillum-shaped rods that exert beneficial effects on plant growth and the yield of many agronomically important crops. Azospirilla are able to fix nitrogen in association with plants but most inoculation experiments and systems evaluated so far indicate that nitrogen fixation does not play a major role in plant growth promotion (Helman et al., 2011).

The PGPR species mentioned above have been subjected to extensive studies to elucidate their colonization capabilities of surfaces and of interior of plants, and their physiological and molecular mechanisms involved in promoting plant growth (see de Bruijn, 2013). Genomes of several species are now available and the extensive knowledge acquired will possibly enable a more comprehensible biotechnological use of the bacteria as commercial products in the near future.

The main issues that have been investigated on mechanisms of direct plant growth promotion and are commonly reviewed in the literature remain to some extent controversial and there are only few clear demonstrations of their involvement, especially under more natural field conditions (Helman et al., 2011, Cassán and García de Salamone, 2008). In this chapter, the PGPR effect will be evaluated with emphasis on the applied aspects of the *Azospirillum*–plant interactions. Q3

One of the most pronounced effects of inoculation with azospirilla and other diazotrophic PGPRs on root morphology is the proliferation of root hairs as observed in several grasses, cereals, and legumes. However, morphologically, the proliferation and morphology of root hairs is not similar when comparing inoculation with different PGPR species (Dobbelaere and Okon, 2007). Inoculation can also promote the elongation of primary roots and increase the number and length of lateral roots. Again, when observed in detail, the morphological effects differ with the different PGPR inoculum. The most clear and distinct effects on root morphology have been observed after inoculation with Azospirillum species and strains (Dobbelaere and Okon, 2007).

The morphological effects on roots are generally dependent on the inoculum concentration and are consistent with the exogenous indole-acetic acid (IAA) levels secreted by *Azospirillum,* indicating that they are mainly due to the production and secretion of IAA by the bacterium. There is evidence that secretion of cytokinins and gibberellins by the bacteria is also involved, but most important could be the IAA/cytokinin ratio (Spaepen et al., 2009). Another diffusible molecule involved, as in the case of *A. Brasilense,* is nitric oxide (NO), a key signaling molecule involved in a wide range of functions in plants (Molina-Favero et al., 2008; see also Chapter 78).

The described effects on root growth and activity mainly in the case of *Azospirillum* result in enhanced mineral and water uptake from the soil by the inoculated roots. This has been repeatedly demonstrated under greenhouse and field conditions in various important crops such as maize and wheat (Dobbelaere and Okon, 2007).

biological nitrogen fixation (BNF) in diazotrophic PGPR inoculated plants (maize, wheat and other grasses) as well as in sugarcane has been investigated in detail by various techniques including the acetylene reduction assay (ARA), the ^{15}N dilution technique, $^{15}N_2$ fixation, ^{15}N natural abundance, and Kjeldhal N-content measurements (Dobbelaere and Okon, 2007). In most grain and forage crops the contribution of nitrogen fixation by *Azospirillum* has been estimated to be no more than 10 kg N/ha/year. The quantities of fixed nitrogen supplied to some cultivars of sugarcane and rice by *G. diazotrophicus* and other PGPR have been estimated to be as high as 50% of the required plant nitrogen (Reis et al., 2007). Since there are many other diazotrophs colonizing the surface and internal tissues of sugarcane roots and rice, it has been difficult to assess the specific contribution of diazotrophic bacteria in the inoculum (Fibach-Paldi et al., 2012). Q4

Some of the unresolved questions for agronomic interactions of PGPR and plants deal with PGPR colonization dynamics, whether PGPR are continuously colonizing and promoting growth of developing roots or alternatively, the major effects are at early stages of root development. Comparable questions are whether nitrogen fixation by the association is continuously taking place or especially at certain stages of plant growth such as inflorescence and grain filling.

Under field conditions, the success of inoculation depends on achieving a high number of bacteria on the root surface and/or the root tissue. There is a need for bacterial colonization in relatively high numbers (10^6–10^7 per g or cm of plant tissue) in order to obtain a significant impact on plant growth promotion derived from BNF, phosphorous solubilization, or disease control (Helman et al., 2011; Spaepen et al., 2009). P-solubilization and BNF are clearly observed and measured in pure cultures when there are $\sim 10^{10}$ cfu/ml; very seldom, high numbers (above 10^4–10^5 cfu) have been counted in plant tissues in the field.

The relevance of PGPR endophytic colonization and its possible advantages in comparison to colonization in the rhizosphere remain to be convincingly demonstrated mainly under field conditions. So far, researchers consider it somehow more logical that endophytic colonization would be more efficient for plant growth, but this needs more investigations (see de Bruijn, 2013). Recent detailed studies support the observations that dual inoculation of *Azospirillum* and some PGPR in legumes (alfalfa, beans, vetch, soybeans and chick peas among others) results in increased production of plant flavonoids and enhanced capacity to induce *Rhizobium nod*-genes expression

(Dardanelli et al., 2008). The presence of *Azospirillum* in the legume rhizosphere activates the hydrolysis of conjugated phytohormones and flavonoids in the legume root tissue, thus leading to release of compounds in their more active forms. Results from co-inoculation experiments suggest that IAA and NO produced by *A. brasilense* are key components of enhancement of secretion of *nod*-gene inducing flavonoids in the roots (Dardanelli et al., 2008; Star et al., 2012; Cassán et al., 2009; Hungria et al., 2013). Q6

90.1.2 Field Experiments

Extensive field inoculation experiments with *A. brasilense* carried out in Israel in the 1980s (Okon et al., 1988) clearly show an average significant increase in crop yield of maize, wheat, sorghum, and other forage grasses. In these cases, the seeds were inoculated with freshly prepared peat inoculants (Okon and Labandera-González, 1994; Helman et al., 2011; Cassán and García de Salamone, 2008). Q7 Q8

In earlier field experimentation, when the plant growing properties of *Azospirillum* were considered as derived from BNF and the number of bacteria in the inoculant was not carefully monitored, the commercial exploitation of *Azospirillum* was limited. At this stage, inoculation responses were considered as inconsistent. Further developments emphasize the importance of research and development for improved formulations, the existence of a regulatory frame guaranteeing inoculant quality, and also the need for a network of communication and diffusion systems, with a unique message for the farmers, in order to achieve good field results.

More recently (1998–2013), there have been hundreds of reports of field inoculation experiments utilizing mainly *A. brasilense* liquid or peat-based commercial inoculants, under varied climatic and soil conditions, mainly in Argentina, Uruguay, Brazil, and Mexico (Fuentes-Ramirez and Caballero-Mellado, 2005; Cassán and García de Salamone, 2008), and also in other parts of the world (Okon and Labandera-González, 1994; Helman et al., 2011).

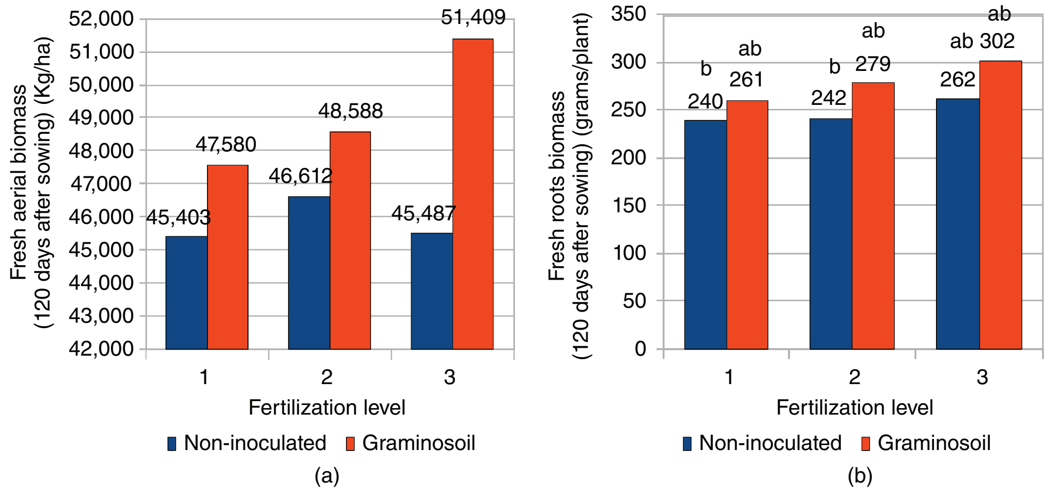
Statistically significant increases in crop yield have been obtained with intermediate and adequate levels of N, P, K, microelements and water, with high bacterial concentrations (10^9 cfu per g or ml) and inoculants of good physiological status (Spaepen et al., 2009). These inoculant products have been increasingly approved and registered by government agencies, inoculant companies, associations, and official agricultural research institutions (Helman et al., 2011). Thus, based on extensive field experimentation with proper agronomic design and accurate statistical evaluation of crop yield parameters, it is well accepted that the inoculation practice has the potential to promote crop yield in fields. Following the reported success of the PGPR inoculants, there has been an extension of research and inoculant quality controls for these microorganisms and at the same time different companies initiated development and commercialization of products. Commercial inoculants have been regularly applied in recent years; there has been a surge in the inoculation practice with *Azospirillum*, with estimations of about 2–2.5 million doses used in 2012 in Brazil, mainly in maize (Hungria et al., 2013). The MERCOSUR region was especially receptive to these developments. Q9

90.1.3 Field Experimentation in Uruguay: A Test Case for Development of PGPR Inoculants

In 1990, the Department of Soil Microbiology of Uruguay MGAP, [Department of Soil Microbiology, General Direction of Natural Renewable Resources, Ministry of Livestock, Agriculture and Fisheries (Departamento de Microbiología de Suelos – Dirección General de Recursos Naturales Renovables, Ministerio de Ganadería Agricultura y Pesca, Montevideo, Uruguay – DMS-DGRNR MGAP in the Spanish acronyms)] started a new research line on PGPR emphasizing on *Azospirillum* under controlled and field conditions in legumes and gramineous plants. Preliminary results proved to be very erratic, and because of this MGAP decided to invite Dr. Yaacov Okon in 1992–1993 to analyze available data on PGPR and develop suitable techniques for *Azospirillum* inoculant formulation and quality control. During the consultancy, the first regional workshop on *Azospirillum* took place in August 1993 (Okon and Labandera-González, 1994). The aim of the emerging PGPR project was to enlarge the use of inoculants to other crops following the success of using rhizobial inoculants for legumes.

In 2004, a technical cooperation agreement was signed between the MGAP and Lage & Cia for the validation of a liquid formulation based on *Azospirillum* (Graminosoil brand). One of the relevant aspects in the working procedure was the methodology employed in the trials, where in addition to evaluating crop response to inoculation (dry matter production and grain yield), rhizospheric and endophyte *Azospirillum* determinations were always performed, comparing non-inoculated controls with inoculated treatments, to determine the plant–bacteria relationship and to have better elements for the interpretation of the results.

It is important to note that initial research work on *Azospirillum* in Uruguay focused on studying the bacteria's plant growth promoting and stimulant effects mainly under low levels of nitrogen fertilization. Nevertheless, mainly in the last decade, grain crop production systems in Uruguay were using relatively high levels of chemical fertilizers, with better adjustment to crop requirements. Consequently, it was understood that *Azospirillum* inoculants should aim to increase crop yields rather than intending a substantial decrease in the doses of chemical fertilizers. For this reason, recent field trials were conducted following actual



(a) (b)

Figure 90.1 Fresh above-ground maize biomass (a) and fresh root according (b) to seed treatment and level of fertilization. Fertilization levels: 1 – no fertilization (neither at planting nor 60 days after), 2 – 0-21/23-0 130 kg/ha at planting; no fertilization 60 days after planting, 3 – 0-21/23-0 130 kg/ha at planting; urea 120 kg/ha 60 days after planting (Casaretto and Labandera, 2008).

Q10

fertilization recommendations in terms of doses and ways of application. One of the main production factors that influence yield expression in dry crops in Uruguay is the water regime, since it varies extensively within and between seasons. For this reason, it is often difficult to optimize yield, since although there is a good adjustment of management factors (genetics, length of fallow, planting date, sowing technology and fertilization), rainfall ends up being crucial, either by lack or excess of rain at key moments. Knowing the mechanisms of action of *Azospirillum* and its ability to stimulate root growth, it was considered that *Azospirillum* inoculation could contribute to increase, but most importantly, stabilize yields.

Evaluations of Graminosoil application in maize and sorghum were performed in greenhouse and commercial fields during four consecutive seasons (2003/2004, 2004/2005, 2005/2006 and 2006/2007) (Figs. 90.1–90.4). The evaluated parameters were *Azospirillum* concentration in the inoculant and inoculated seed, *Azospirillum* concentrations in the rhizosphere and inside the root (endophytes), crop establishment, aerial and root dry matter at different physiological stages, and grain yield. Considering the expected responses, these determinations were performed frequently during the crop cycle.

In all experiments the liquid inoculant used had a high azospirilla concentration (10^9 cfu/ml), recovering 10^4 cfu/maize seed, both in non-treated seeds and those treated with chemical fungicides (Carbendazim 250 g/l + Thiram 250 g/l).

Five maize greenhouse trials were conducted, obtaining increases of 30–40% in root biomass and 14–27% in above ground biomass due to inoculation. Similar responses were also obtained in sorghum (C. Labandera, unpublished).

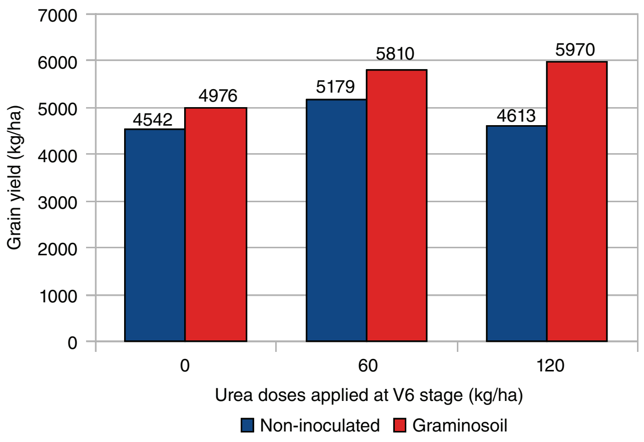


Figure 90.2 Production of maize grain yield in response to inoculation and urea doses at V6. Fertilization at planting: Urea 60 kg/ha + 18-46-0 60 kg/ha. Urea at V6 stage: 1 - none, 2 - 60 kg/ha urea. 3 - 120 kg/ha urea (Hoffman et al., 2008).

In maize field trials there was an increase in the number of bacteria due to inoculation, both in the rhizosphere and inside the roots, of about 2–5 times with respect to the uninoculated control. Following inoculation of maize roots and the aerial part, biomass was significantly increased at different growth stages. Increased production of roots has a direct effect on water and nutrient uptake in inoculated crops. Furthermore, there is an additional value of increased root and stubble input to the system, allowing higher organic matter levels and improving the physical conditions of the productive soil layers, especially in no-tillage systems (C. Labandera, unpublished; Helman et al., 2011).

Figure 90.1a and b shows the results obtained in the field trial performed in 2004/2005 season. In this trial, the greatest differences in aerial biomass production were obtained in

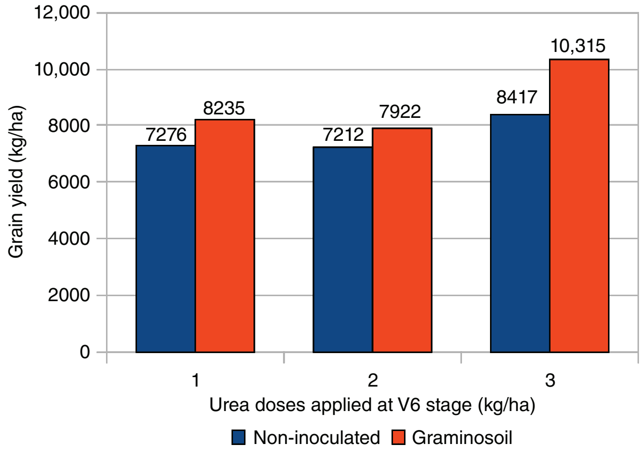


Figure 90.3 Production of maize grain yield in response to inoculation and urea doses at V6. Fertilization at planting: 0-21/23-0 200 kg/ha + 18-46-0 150 kg/ha + Kcl 80 kg/ha. Urea at V6 stage: 1 - none, 2 - 60 kg/ha, 3 - 120 kg/ha (Hoffman et al., 2008).

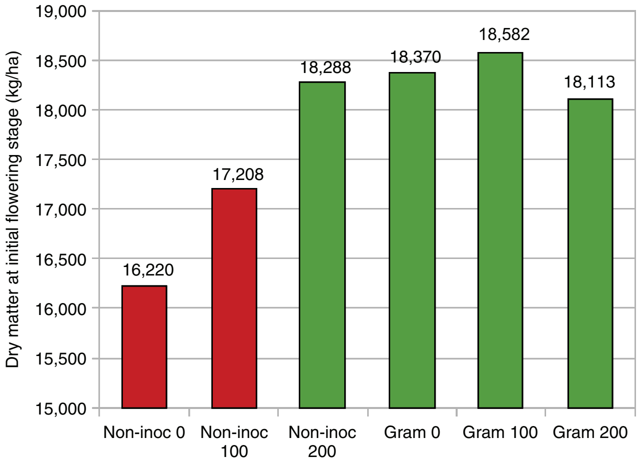


Figure 90.4 Aerial dry matter production of maize at the beginning flowering. Fertilization at planting: urea 50 kg/ha. Urea at V6 stage: 1 - none, 2 - 100 kg/ha, 3 - 200 kg/ha. gram-Inoculated with GRAMINOSOIL, Martino, M. personal communication 2008.

the inoculated treatments with higher doses of fertilizer; this would indicate a more efficient use. Moreover, inoculated plants without fertilization reached yields similar to those of the uninoculated control, with higher levels of fertilizer.

Other field trials to evaluate Graminosoil were conducted by private consulting firms in different crops (Figs. 90.2–90.6).

Although interaction in dry matter production and grain yield was not significant, inoculation with *Azospirillum* always maintained higher levels of biomass and grain yield, particularly with high doses of nitrogen (Fig. 90.2).

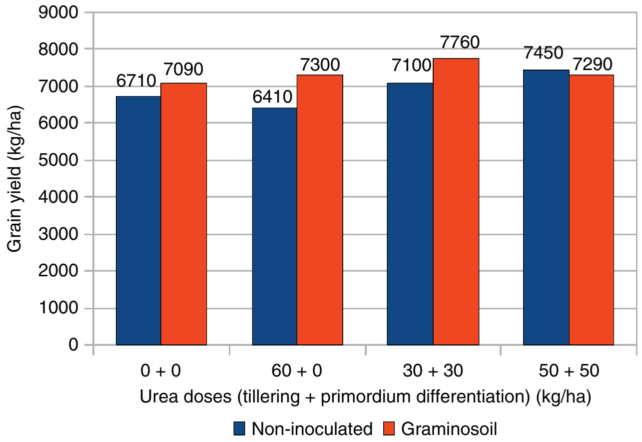


Figure 90.5 Rice yield response to inoculation and doses of urea. Fertilization at planting: 18-46-0 150 kg/ha. Urea at tillering and initial reproductive stage: 1 - none, 2 - 60 kg/ha at tillering, 3 - 30 kg/ha at tillering and 30 kg/ha at initial reproductive stage, 4 - 50 kg/ha at tillering and 50 kg/ha at initial reproductive stage. Chebataroff, N et al personal communication 2007.

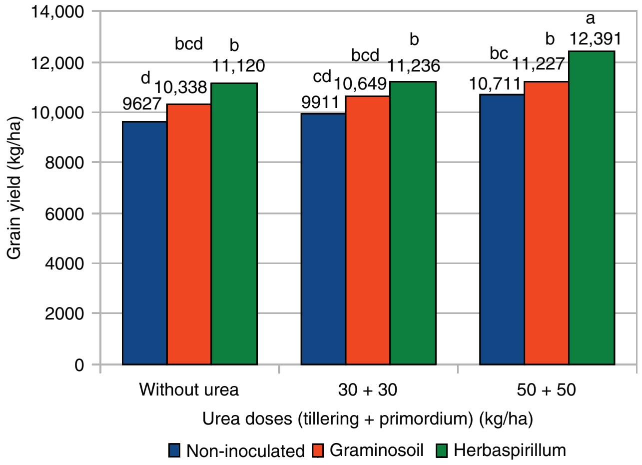


Figure 90.6 Rice yield response to inoculation and doses of urea. Fertilization at planting: 18-46-0 130 kg/ha. Urea at tillering and initial reproductive stage: 1 – none, 2 - 30 kg/ha at tillering and 30 kg/ha at initial reproductive stage, 3 - 50 kg/ha at tillering and 50 kg/ha at initial reproductive stage. Chebataroff, N et al., personal communication, 2009.

Increases in maize grain yield at higher levels of N fertilization (Fig. 90.3) and shoot dry matter yield (Fig. 90.4) were obtained in two more field experiments.

Yield increases in rice plots were obtained when seeds were inoculated with Graminosoil and showed to be important in productive and economic terms. Actually, this line of work includes agronomic evaluations of ENDO-RICE inoculant, formulated with a native strain of *Herbaspirillum* (Punschke, and Mayans, 2011).

In conclusion, there has been for several years a clear consistent plant growth promotion and yield increases of important crops in Uruguay, following inoculation with commercial formulations of PGPRs.

90.1.4 Inoculant Production

Following the extensive basic and applied research on PGPR, inoculants for more than 30 years (Helman et al., 2011) commercial inoculant products, field experimentation and international quality control procedures have been established.

The inoculant production process starts with the scaling up of recommended strain(s) for the target crop, according to the institution of reference in each country, when required. The pure cultures are normally maintained at −80 °C, delivered in agar slant tube cultures growing on the appropriate medium. Scaling up is carried out in a sterile liquid medium culture in an agitator and with controlled temperature for the appropriate period of time. When the growth period is completed, the purity of the culture is confirmed in agar plates by microscopic observation and Gram test; DNA fingerprinting procedures may also be performed (see de Bruijn, 2013). When that liquid culture is determined suitably, it is used to inoculate an industrial fermenter (Fig. 90.7).

Sampling of industrial fermenters is done daily and not only at the end of fermentation. In addition to the already mentioned controls, an estimation of microbial growth is carried out by spectrophotometry. For peat-based inoculants, the peat has to be previously neutralized, ground to 200 mesh, raked in polyethylene bags, and sterilized by Gamma radiation. Impregnation is made aseptically, puncturing the bag with a needle and then sealing the hole. For liquid inoculants, the broth is packaged aseptically (Fig. 90.8). The final inoculant product is subjected to quality control. However, it is highly recommended to have an additional quality control performed by the reference institution (RI).

90.1.5 MERCOSUR: Regulatory Framework and Quality Control

The achievements of the use of PGPR inoculants, mainly following field experimentation in the world has been summarized (Okon and Labandera-González, 1994; Helman et al., 2011).

PGPR research and inoculant development in MERCOSUR was initiated during the workshop on *Azospirillum* held at MGAP, Montevideo, August 1993. Its conclusions were published (Okon and Labandera-González, 1994). In 1998, all MERCOSUR countries signed “Recommendation 9/97, SGT 8 Agriculture, MERCOSUR/GMC/RES 28/98,” which includes the obligation to register inoculants and to establish a “RI” for each country, as well as defining its responsibilities. Actually, each country has adapted its own regulations according to it.

Argentina has IMYZA INTA Castelar as its RI and the Secretary of Agriculture SENASA is in charge of registering inoculants. Az39 is the recommended *Azospirillum* strain to inoculate maize and wheat. In 2005, the network “Red de Control de Calidad de Inoculantes (REDCAI) (Inoculants

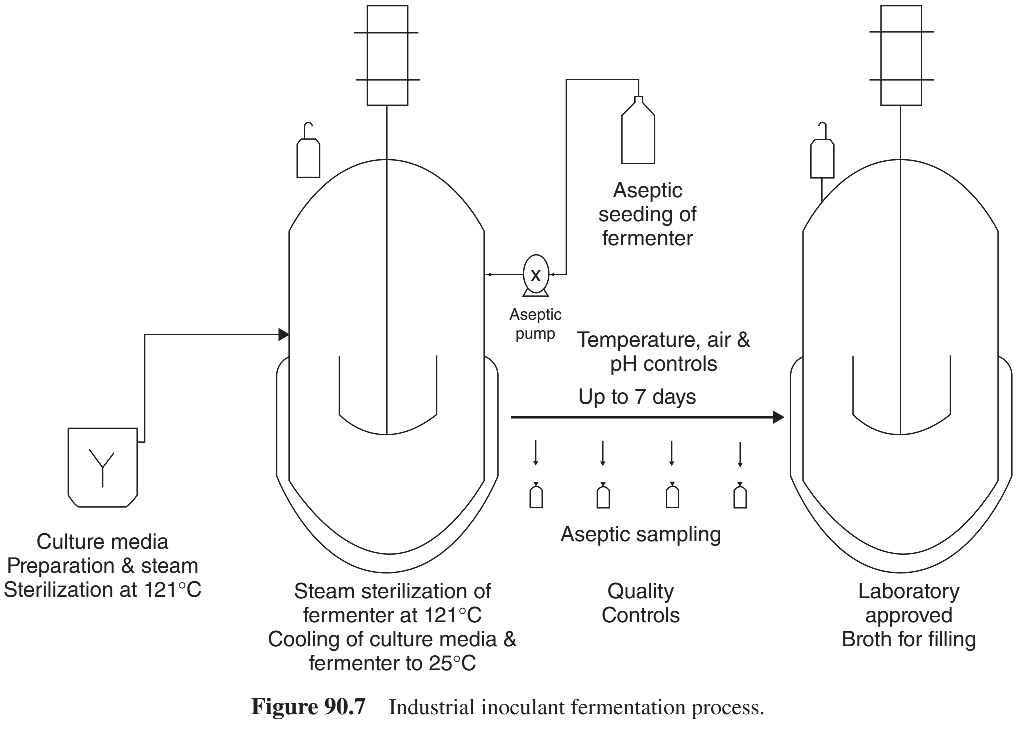


Figure 90.7 Industrial inoculant fermentation process.

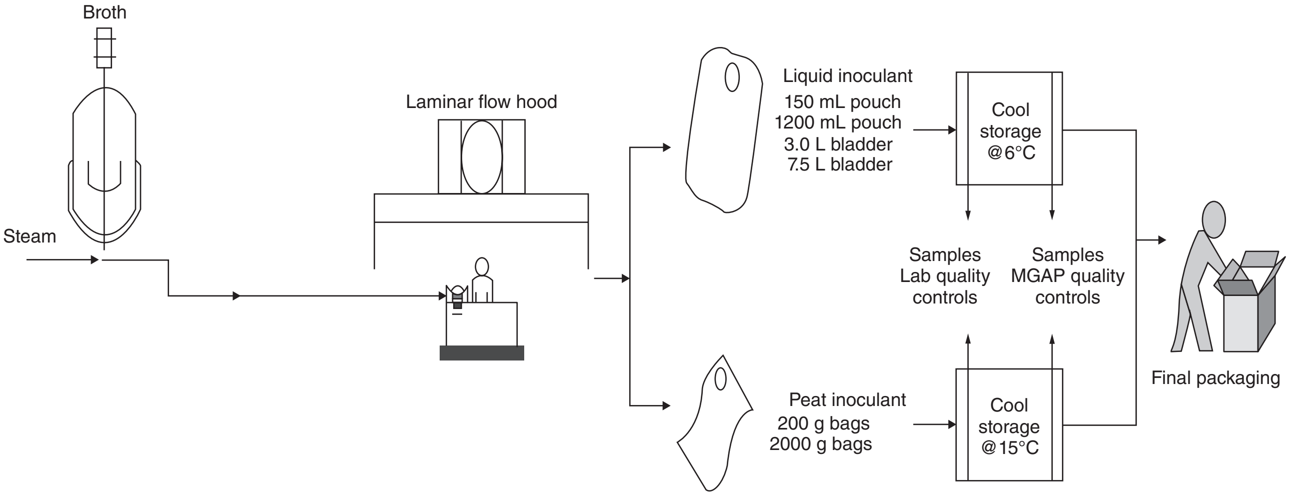


Figure 90.8 Inoculant aseptic filling and packing.

Quality Control Network)" was established as a member of the Division for Agricultural and Environmental Microbiology of the Argentinian Association of Microbiology, with the aim of creating and validating a set of methodological tools for the evaluation of inoculants (I Taller Iberoamericano REDCAI – RED BIOFAG, Asociación Argentina de Microbiología, Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo, October 2010) (I Ibero-American Workshop REDCAI-REDBIOFAG, Argentinean Association of Microbiology, Ibero-American Science and TECHNOLOGY Program for Development). Procedure document 2 of Q11 REDCAI (Cassán et al., 2010) established the protocol for the quality control of *Azospirillum* inoculants.

Argentina is a pioneer in the development of PGPR inoculants and their field application. In the early 1980s, Silder Barrios and Enrique Rodríguez Cáceres began their work in obtaining several isolations of *Azospirillum* from diverse areas of their country. They formulated peat-charcoal based inoculants, which reached 84 days of shelf life in concentrations Q12 higher than 1×10^8 cfu/g (Puente et al., 2008). These researchers followed the guidelines of J. Döbereiner and Y. Okon to obtain several strains (Rodriguez Cáceres Q13 et al., 2008). The solid culture media "RC" was developed for isolation and identification of *Azospirillum* (Rodríguez Q14 Cáceres, 1982). Those strains were the initial material of the collection of the current Institute of Agricultural Microbiology and Zoology, IMYZA INTA Castelar. At the urging of Edgardo Muñoz Ratto, SENASA was the first institution of MERCOSUR to open registration for *Azospirillum*-based inoculants.

Proyecto Inocular emerged at the beginning of the last decade, led by Alejandro Perticari in a partnership between INTA and 25 inoculant companies, with the aim of promoting the technology of soybean inoculation, later extending it to other PGPRs, with more emphasis on *Azospirillum*. The local companies were the ones that started to develop *Azospirillum* inoculants (Table 90.1), with some of them that formulate mixtures of *Azospirillum* and other PGPR (Table 90.1). Laboratorios Alquimia was the first to launch a commercial product, Graminante. Its carrier is a wettable powder consisting of calcium carbonate and magnesium carbonate, registered as 20259 for maize and 20260 for wheat in 1992.

A few years later, work began simultaneously in Uruguay and Argentina with Graminosoil, a sterile peat-based inoculant, reaching high initial concentrations of *Azospirillum* per gram but with rapid death of the bacteria; authorization for open sale in Uruguay was obtained in 1992; and the registration number was 20284 for maize and 20285 for wheat in Argentina in 1994. Owing to difficulties in the use of peat-based inoculants in both countries, it was decided to change the carrier of Graminosoil to an aqueous media with at least 1×10^8 cfu/ml with 6 months shelf life.

Brazil has FEPAGRO as it RI. Strains Ab-V4, Ab-V5, Ab-V6, and Ab-V7 are recommended to inoculate maize and Ab-V1, Ab-V5, Ab-V6, and Ab-V8 for wheat (Hungria, 2011). The current recommendation for *Azospirillum* strains arises in part from the work carried out by Fabio Pedrosa at the Molecular Biochemistry and Biology Department of the Federal University of Paraná (Araujo, Solón, personal communication, March 2013). *Azospirillum*-based inoculants need to be registered at the Ministry of Agriculture, Livestock, and Food Supply (MAPA, in its Portuguese acronym) before being sold on the market and they must contain at least one of the recommended strains for each crop. Normative Instruction 30 sets the official methods for inoculant analysis. The Network of Laboratories for Recommendation, Standardization, and Dissemination of Microbial Inoculants of Agricultural Interest (Red de Laboratorios para Recomendación, Padronización y Difusión de Tecnología de Inoculantes Microbianos de Interés Agrícola - RELARE)

Table 90.1 List of Inoculant Products in the MERCOSUR

Company	Brand	*Azospirillum* Strains	Concentration at Expiry Date (cfu/ml-Liquid, cfu/g-Solid or WP)
Argentina			
AGRO FRANQUICIAS	AXION PLUS TRIGO	*Azospirillum* Az39	
AGRO INVEST	FULL BACTER	*Azospirillum* Az39	
ALQUIMIA	GRAMINANTE MAIZ	AZM3	5×10^4 WP
ALQUIMIA	GRAMINANTE TRIGO	AZT5	5×10^4 WP
ALTERBIO	ALTER PROMAZ TLS	*Azospirillum* spp.	1×10^7
ARBO	ENE 2 MAIZ	*Azospirillum* Az39	1×10^8
ARBO	ENE 2 TRIGO	*Azospirillum* Az39	1×10^8
AYUI	FACYT AZ	*Azospirillum* Az39	
BECKER UNDERWOOD	NITRAFIX	*Azospirillum* BR 1100	Origin of the inoculant-Brazil
BENEFICIAL GERMS	AZOGERMS	*Azospirillum* Az39	
BERANEK	NITROPLUS TRIGO		
BILAB	NITRO-FIX AZ ARROZ	*Azospirillum* Az39	2×10^8
BILAB	NITRO-FIX AZ MAIZ	*Azospirillum* Az39	2×10^8
BILAB	HOBER AZOS TRIGO	*Azospirillum* Az39	2×10^8
BILAB	HOBER AZOS MAIZ	*Azospirillum* Az39	2×10^8
BILAB	NITRO FIX AZ GIRASOL	*Azospirillum* Az39	2×10^8
BILAB	NITRO FIX AZ ALGODON	*Azospirillum* Az39	2×10^8
BIOTECH	N AZOSPIRRILLUM MAIZ	*Azospirillum* AbV5 + AbV6	1×10^7
BIOTECH	BIO NITROSEM MAIZ	*Azospirillum* spp.	1×10^7
CAMPOMAX	AZOMAX		
CERGEN	AZOSNITRO	*Azospirillum* Az39	
CHEMICAL – BIO	GRAMIBAC	*Azospirillum* spp.	1×10^8
CHEMICAL – BIO	RADIXIUS ONION	*Azospirillum* spp.	1×10^8
CKC	RHIZOFLO TRIGO	92078 ACTA 1390	1×10^6
CKC	RHIZOFLO GIRASOL	92079 ACTA 1390	1×10^6
CKC	RHIZOFLO MAIZ	*Azospirillum* Az39	1×10^6
ECOFERTIL	RAY GREEN TRIGO	*Azospirillum* ATCC 1003	1×10^8
ECOFERTIL	RAY GREEN MAIZ	*Azospirillum* ATCC 1003	1×10^8
ECOFERTIL	RAY GREEN GIRASOL	*Azospirillum* ATCC 1003	1×10^8
EMFAG	G3 AZUBAC	*Azospirillum* spp.	1×10^6
F.B.N.	F.B.N. TRIGO		
FARMCHEM	SOWER TRIGO		
FITOGENIA	AZOS-B		
FITOQUIMICA	FORTE	*Azospirillum* Az39	1×10^7
FPC ARGENTINA	AZP 2000	*Azospirillum* Az39	1×10^7
FPC ARGENTINA	AZP 2000	*Azospirillum* Az39	1×10^7
FRAGARIA	TRIGALAZO	*Azospirillum* Az39	1×10^8
FRAGARIA	GRAMINAZO PLUS	*Azospirillum* Az39	1×10^8
FRAGARIA	MAIZAZO	*Azospirillum* Az39	1×10^8
GREEN QUALITY	FOSTRIGON		
LANTHER QUIMICA	AZO LQ		
LOPEZ	NODUMAX	*Azospirillum* spp.	
MARKETING AGRICOLA	MARKETING AGRICOLA TRIGO	*Azospirillum* Az39	
MENAGRO	BIOCAMPO MAIZ		
MENAGRO	BIOCAMPO TRIGO		
NITRAP	AZOTRAP	*Azospirillum* Az39	
NITRAP	AZOTRAP PLUS – LETTUCE	*Azospirillum* Az39	
NITRASOIL	BIO-ENHACE	*Azospirillum* Az39	1×10^8
NITRASOIL	GRAMINASOIL MAIZ	*Azospirillum* Az39	1×10^8

Table 90.1 (*Continued*)

Company	Brand	*Azospirillum* Strains	Concentration at Expiry Date (cfu/ml-Liquid, cfu/g-Solid or WP)
NITRASOIL	GRAMINOSOIL TRIGO	*Azospirillum* Az39	1×10^8
NIVELAGRO	NIVEL AZO		
NOVA	PROMOZION	*Azospirillum* Az39	
NOVA	INOCULANTE AGH		
NOVOZYMES BIOAG	NITRAGIN MAIZ	*Azospirillum* Az39	
NOVOZYMES BIOAG	NITRAGIN WAVE	*Azospirillum* Az39	
NOVOZYMES BIOAG	NITRAGIN SEMILLERO	*Azospirillum* Az39	
NOVOZYMES BIOAG	NITRAGIN NEW WAVE	*Azospirillum* Az39	
RAPARO, ANGEL	BUSCADOR N TRIGO		WP
RAPARO, ANGEL	BUSCADOR N MAIZ		WP
RAYBAC	RAYBAC		
RED SURCOS	DOMOX		
RIZOBACTER	RIZOSPIRILLUM	*Azospirillum*	
SAN PABLO	AZOLLUM H	*Azospirillum* TUC 27/85	
SAN PABLO	AZOLLUM TRIGO	*Azospirillum* TUC 27/85	
SAN PABLO	AZOLLUM MAIZ	*Azospirillum* TUC 27/85	
SAN PABLO	MACROMIX GIRASOL	Azopirillum TUC10/1	
SEMILLERA GUASCH	ZADPIRILLOM MAIZ	*Azospirillum* Az39	1×10^8
SEMILLERA GUASCH	ZADEN GRAMINEAS	*Azospirillum* Az39	1×10^8
SERV – QUIM	NITROFULL - G	*Azospirillum* spp.	
SINTESIS BIOLOGICA	BETERSEED	*Azospirillum* Az39	1×10^6
SINTESIS QUIMICA	NOCTIN AZO	*Azospirillum* Az39	1×10^8
SINTESIS QUIMICA	NOCTIN TURBA AZO	*Azospirillum* Az39	1×10^8(peat)
TRES E PRODUCTOS	AZZEA UNO		
WEIZUR	RIZOGROWTH AZP	*Azospirillum* Az39	
ZALAZAR, RODOLFO	LABZA LIQ AZO	*Azospirillum* Az39	1×10^7

Argentina-Continuation, Combined Inoculum

Company	Brand	PGPR #1	PGPR #2	Concentration
AGRO ADVANCE	PHOEBUS	*Azospirillum brasilense*	*Pseudomonas fluorescens*	
AGRO ADVANCE	PHOEBUS	*Azospirillum brasilense*	*Pseudomonas fluorescens*	
ALTERBIO	ALTER PSE		*Pseudomonas fluorescens*	1×10^8 cfu/ml
BILAB	NITRO FIX PF GIRASOL		*Pseudomonas fluorescens* BNM 233	1×10^7 cfu/ml
BILAB	NITRO FIX PF MAIZ		*Pseudomonas fluorescens* BNM 233	2×10^7 cfu/ml
BILAB	NITRO FIX PF TRIGO		*Pseudomonas fluorescens* BNM 233	1×10^7 cfu/ml
GREEN QUALITY	ECOFOS		*Pseudomonas* PS3	
BIAGRO	BIAGRO PSA LIQUID		*Pseudomonas aurantica*	1×10^7 cfu/ml
BIAGRO	BIAGRO PRO SOL		*Pseudomonas fluorescens*	1×10^7 cfu/ml
DEGSER	DEGFERTIL GIRASOL	*Azospirillum brasilense*	*Pseudomonas fluorescens*	
DEGSER	DEGFERTIL MAIZ	*Azospirillum brasilense*	*Pseudomonas fluorescens*	
DEGSER	DEGFERTIL TRIGO	*Azospirillum brasilense*	*Pseudomonas fluorescens*	
LIPHATECH LA	DESANGOSSE TRIGO		*Pseudomonas*	
LIPHATECH LA	DESANGOSSE MAIZ		*Pseudomonas*	
PALAVERSICH	BIOPOWER	*Azospirillum brasilense*	*Pseudomonas fluorescens*	
RIZOBACTER	RIZOFOS		*Pseudomonas sp.*	
RIZOBACTER	RIZOFOS-LIQ		*Pseudomonas fluorescens*	1×10^9 cfu/ml
RIZOBACTER	RIZOFOS-LIQ		*Pseudomonas*	1×10^9 cfu/ml

(*continued*)

Table 90.1 (*Continued*)

Brazil

Company	Brand	*Azospirillum strains*	Concentration at Expiry Date
GRUPO BIOSOJA	BIOMAX PREMIUM L	*Ab-V5*	2×10^8 cfu/ml
LABORATORIOS FARROUPILHA	AZOS		
NOVOZYMES BIOAG PRODUTOS	AZOMAX	*Ab-V5 + Ab-V6*	2×10^8 cfu/ml
SPRAYTEC	NODOFIX AZP MILHO/TRIGO		2×10^8 cfu/ml
STOLLER DO BRASIL	MASTERFIX GRAMINEAS	*Ab-V5 + Ab-V6*	2×10^8 cfu/ml
TOTAL BIOTECNOLOGIA	AZOTOTAL	*Ab-V5 + Ab-V6*	1×10^8 cfu/ml

Uruguay

Company	Brand	Origin	*Azospirillum* Strains	Concentration at Expiry date
CALISTER	BIOPROM AZ39	URUGUAY	*Azospirillum brasilense Az39*	1×10^9 cfu/ml
LAGE & CIA	GRAMINOSOIL	URUGUAY	*Azospirillum sp.*	1×10^8 cfu/ml
RUSPER	NITRAGIN MAIZ/WAVE	ARGENTINA *Az39*	*Azospirillum brasilense*	1×10^7 cfu/ml

Paraguay

Company	Brand	Origin	PGPR	Concentration at Expiry Date (cfu/ml-Liquid, cfu/g-Solid or WP)
AGROGANADERA PIRAPEY	AZP 2.000	ARGENTINA	*Azospirillum brasilense*	1×10^9 cfu/ml
AGROCONSULT	GRAMINANTE TRIGO	ARGENTINA	*Azospirillum brasilense*	5×10^4 cfu/g WP
AGROCONSULT	GRAMINANTE MAIZ	ARGENTINA	*Azospirillum brasilense*	5×10^4 cfu/g WP
BASF PARAGUAYA S.A.	NITRAGIN MAIZ	ARGENTINA	*Azospirillum brasilense*	1×10^9 cfu/ml
AGROLAND	RHIZOFLO LIQUID MAIZ	ARGENTINA	*Azospirillum brasilense*	1×10^7 cfu/ml
FRAGARIA DEL PARAGUAY	INOCULANTE GRAMINAZO	ARGENTINA	*Azospirillum brasilense*	2×10^9 cfu/ml
CHEMTEC	NUTRICHEM	PARAGUAY	*Azospirillum + Pseudomonas*	$5 \times 10^8 + 5 \times 10^8$ cfu/ml
BASF PARAGUAYA	NITRAGIN SEMILLERO	ARGENTINA	*Azospirillum brasilense*	1×10^8 cfu/ml WP
MATRISOJA	AZO TOTAL	BRAZIL	*Azospirillum brasilense*	2×10^8 cfu/ml
AGROFERTIL	GELFIX GRAMINEAS	BRAZIL	*Azospirillum BR 11005*	1×10^9 cfu/ml
ALQUIMICA	NOCTIN AZO	ARGENTINA	*Azospirillum brasilense*	1×10^9 cfu/ml
AGROLAND	RHIZOFLO PREMIUM	ARGENTINA	*Azospirillum + Pseudomonas*	$1 \times 10^9 + 1 \times 10^8$ cfu/ml
CAMPO FERTIL	BIOMAX PREMIUM MILHO	BRAZIL	*Azospirillum brasilense Ab-V5*	2×10^8 cfu/ml
DIAGRO	GRAMMY CROP	BRAZIL	*Azospirillum BR 11005*	1×10^9 cfu/ml
PLANAGRO	RADIMAX INOCULANTE	ARGENTINA	*Azospirillum brasilense*	1×10^9 cfu/ml

meets on a biannual basis, bringing together people from MAPA, EMBRAPA, Universities, ANPII (the inoculants chamber), and representatives from companies that are not associated to ANPII. Each RELARE may suggest that MAPA incorporate or discharge strains from FEPAGRO's recommendations. Table 90.1 summarizes the strains actually used by Brazilian companies.

Paraguay is a country that has received a large number of Argentinian and Brazilian farmers, who brought along with them their agricultural practices, among them, grain inoculation, and have created demand for the products they used in their own countries. NUTRICHEM, of CHEMTEC Company, is the first co-inoculant producer in Paraguay that contains *Azospirillum* and *Pseudomonas* (Table 90.1). Resolution 564 and its Annex regulate the registration of inoculants with Servicio Nacional de Calidad y Sanidad Vegetal y de Semillas (SENAVE; National Service for Plant and Seed Quality and Health) (Galeano Samaniego, M and Marecos, C, personal communication, 2013).

Uruguay appointed MGAP as its reference institution. More than 95% farmers inoculate their legume seeds. Adoption of this practice comes from a successful strategic country plan that started in the early 1950s, developed by MGAP with the objective to increase animal productivity through the improvement of natural pastures by the use of phosphorous fertilizers and inoculated legumes focusing in the diffusion of the technology to reach farmers. *Rhizobium* legume inoculant production and its use are regulated in the country by Decrees MGAP 546/81 and MGAP 7/99 while other PGPR inoculants are by Resolution MGAP 03/2013. There are three *Azospirillum* inoculants on the market (Table 90.1) and one product, ENDO RICE, that contains a strain of *Herbaspirillum* isolated from local flood irrigated rice systems (Punschke and Mayans, 2011).

MERCOSUR may allow registration of an inoculant with strains that are not recommended, but the registrant company shall submit reports of efficacy under laboratory and greenhouse conditions, together with data from at least 3 years of field trials in three different agro-ecological zones.

90.1.6 Summary and Concluding Remarks

The experience achieved with *Rhizobium* inoculants in relation to storage, use, and management of inoculants and treated seeds has been transferred to PGPR products. Good inoculation procedures are important to achieve uniform distribution and high average load of bacteria on every seed. It is imperative for the efficiency of the process to use specifically designed inoculation machinery for each kind of seed. It is always recommended to follow the guidelines stated on the label. Every inoculant company should have information about compatibility of their product with chemical treatments used on the seeds, such as fungicides, insecticides, and bird repellents, as they may affect viability of bacteria on inoculated seeds. The timing between inoculant applications and sowing, as well as crop management (previous crop, type of sowing, seed density, fertilization, and irrigation) need to be planned for an optimal inoculant response.

It is important to point out that the degree of adoption and relative agronomic success of the use of PGPR inoculant technologies will depend on the quality of the products and their characteristics (powdered, peat based or liquid) and the way of application. Therefore, in order to ensure permanent adoption of the technology, it is necessary to

- have a coordinated multidisciplinary research institution to provide technical bases for inoculant formulation and application, such as carriers, strains, concentration, transportation and storage conditions, shelf period, recommendations, and precautions for use;
- have a regulatory framework setting high standards to assure inoculant quality in the market;
- develop a unique communication program to be transferred to agricultural engineers, extension agents, and farmers, based on local agronomic expected responses.

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