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Review

Azospirillum spp. in grasses and forages. Review

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Abstract:

The *Azospirillum* genus includes plant growth-promoting bacteria found in different soil regions worldwide. When associated with plant roots these bacteria help augment productivity by increasing both the aerial and root portions. These benefits derive from excretion of growth phytonutrients, especially auxins. Use of *Azospirillum* spp. can help to bridge the gap between productivity and sustainability since inoculants based on this microorganism can reduce use of nitrogen fertilizers without affecting productivity, and generate savings and greater profitability. Inoculation of *Azospirillum* spp. strains with forage grasses can result in greater forage mass gains and less need for nitrogen fertilizer, improving pasture production system sustainability. Co-inoculation with other strains such as *Bradyrhizobium* sp. apparently potentiates growth promotion. Proper application methods must be followed for these growth-promoting bacteria to be effective. Growth promotion in response to *Azospirillum* has been described in grasses such as sugarcane, maize and forages, but further research is needed under different conditions to support adoption by producers. Application of bacterial inoculates can increase competitiveness vis-à-vis conventional agriculture methods. Inoculation of grasses in livestock systems can raise forage mass production, mitigate degradation risks and improve productive indices.

Key words: Bacteria, Agricultural microbiology, Rhizobacteria, *Azospirillum*.

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Introduction

Perhaps the greatest challenge intrinsic to agriculture is balancing productivity with sustainability. Apparently at cross-purposes both goals have become pressing concerns due to population growth, climate change, and consumer behavior. New technologies and techniques are needed to shift the paradigm away from traditional techniques for increasing production of grains, meat, milk, etc. through greater use of chemical fertilizers and the opening of new areas.

Nitrogen (N) can increase productivity in plants and is an essential fertilizer in commercial agriculture, especially in tropical regions. However, fertilization with N raises production costs by approximately 40 %(1), and can pollute soils and water, with serious environmental consequences.

Sustainable alternatives for plant nutrition are becoming increasingly important. For example, research into the potential of biological fixation of atmospheric nitrogen (BFN) is revealing it be essential for greater grassland productivity. This process is performed by diazotrophic bacteria, commonly known as plant growth-promoting bacteria (PGPB). It involves conversion of atmospheric nitrogen (N_2) into other nitrogen substances which are then incorporated by plants through synthesis of protein and nucleic acids(2).

However, the benefits provided by PGPB extend beyond BFN(3). These bacteria also stimulate production of growth hormones such as auxins, cytokinins and gibberellins, and improve absorption of other nutrients such as phosphorus. Inoculation with PGPB is a promising technology for augmenting agricultural production while reducing the environmental impacts of inadequate use of fertilizers.

Prominent among growth-promoting bacteria are those in the genus *Azospirillum*, the most studied of the PGPB[4]. This genus gained worldwide relevance in the 1970s in response to research showing it increased absorption of water and nutrients, resulting in greater drought tolerance and productivity. These responses derive from higher production of growth-promoting substances which alter root system morphology by increasing the number and diameter of secondary, lateral and adventitious roots[5].

Inoculation of *Azospirillum* in corn (*Zea mays*) resulted in a 17 % increase in average ear length and productivity versus a control[5]. Use of *Azospirillum* can also reduce the need for N fertilization[6], producing savings of 30 to 50 kg ha^{-1} N in corn at the beginning and end of the rainy season[7]. Considering this possibility, substitution of 50 % of the N fertilizer used in Brazil would result in reductions of 52 kg ha^{-1} N in corn and 35 kg ha^{-1} N in wheat, and a consequent savings of approximately $1.2 billion a year[8]. Sugarcane also requires heavy N fertilization, but interacts positively with PGPB[9]. Inoculation with *Azospirillum brasilense* produced a 10% increase in productivity compared to a treatment receiving no N fertilizer, which is equivalent to the use of 120 kg ha^{-1} N[10].

The benefits observed with *Azospirillum* inoculation in corn, a forage grass, can also be expected in tropical forage grasses, possibly reversing or minimizing soil degradation risks while improving forage mass production. This is supported by a report of N_2-fixing bacteria in the rhizosphere of forage grasses[9], suggesting a future of lower N fertilizer use in tropical forage grasses[9]. Indeed, inoculation of the grass *Brachiaria* spp. with *A. brasilense* strains raised average forage mass production by 13 %[1].

Azospirillum Genus

The *Azospirillum* genus of bacteria was discovered by J. Dobereiner and became important in the 1970s for its ability to fix atmospheric nitrogen, hence the genus name *Azospirillum*[11]. The genus has a broad geographical distribution, and can be found in temperate and tropical regions[1].

Azospirillum-genus bacteria are Gram-negative and free-living. They are cane-shaped, have active movement, measure 0.8 to 2 µm in diameter and 2 to 4 µm in length, and have intracellular polyhydroxybutyrate granules[12]. Optimum development temperature range for these bacteria is 28 to 41 °C. These bacteria are strictly aerobic when in a N_2-free environment and supplied with nitrogen or microaerophilic sources, which requires them to fix nitrogen[11]. However, to promote a microaerophilic environment when in a semisolid medium these bacteria produce a thin veil-like film containing the oxygen concentration essential for nitrogen fixation and growth initiation.

Azospirillum sp. have a flexible carbon and nitrogen metabolism which augments their ability to compete for rhizosphere colonization(12). When present in the rhizosphere they colonize both the mucigel layer around the roots (external colonization) and root intercellular spaces (internal colonization)(8). Colonization occurs mainly in the areas of elongation and root hairs(11). They are highly competitive when colonizing the rhizosphere, and to maintain metabolism make use of different nitrogen sources such as ammonia, nitrite, nitrate, molecular nitrogen and amino acids, as well as carbon sources such as organic acids (malate, pyruvate, succinate and fructose)(10).

Fifteen *Azospirillum* species have been identified to date: *A. lipoferum*, *A. brasilense*, *A. amazonense*, *A. halopraeferens*, *A. irakense*, *A. largimobile*, *A. doebereinereae*, *A. oryzae*, *A. melinis*, *A. canadiana*, *A. zeae*, *A. rugosum*, *A. palatum*, *A. picis* and *A. thiophilum*. Of these the most widely researched are *A. lipoferum* and *A. brasilense*, both of which are common in tropical grasses and forages(1). Not all *Azospirillum* species can be found colonizing plants in various regions; *A. amazonense*, for instance, has only been isolated in Brazil(12). *Azospirillum brasilense*, however, is widely distributed in tropical and subtropical soils, a characteristic that contributed to its ample study(13). Experiments have shown that *A. brasilense* promotes plant growth and consequently increases productivity(14). It exhibits satisfactory results when associated with *Poaceae* family plants such as corn, oats, wheat and rice(15).

Inoculum for plants

Widely known as biofertilizer, inoculants intended to promote plant growth contain live microorganisms that implement various mechanisms. Commercial production of biofertilizers in Latin America began in 1898(16), and Brazil is one of the largest current markets in the region(17). Inoculants can improve competitiveness in the agricultural sector and is a promising and sustainable technology capable of partially replacing nitrogen fertilization(18).

Brazil’s Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento da Brasil - MAPAB)(19), requires that commercial inoculants contain at least 10^9 cells per gram or milliliter of product at their expiry date, that they must be prepared with a sterile vehicle and be free of unspecified microorganisms up to the $1x10^{-5}$ dilution factor. Traits decisive for inoculant efficacy in promoting plant growth include the strain, its competitiveness, and the number of necessary or viable cells for rapid colonization of the rhizosphere and plant tissue[20]. They need to be produced using non-toxic vehicles, and be water-soluble and associated with competitive lines[21].

Inoculation success depends on selection of efficient, stress-resistant strains, since diazotrophic bacteria are found in various soil types and their persistence is determined by soil salinity, pH and moisture[22]. *Azospirillum* genus bacteria promote plant growth through mechanisms such as greater tolerance to environmental stressors, phytohormone production and nitrogen fixation[23].

A major initiative to develop *Azospirillum* inoculates in Brazil began in 1996 when the Department of Biochemistry and Molecular Biology of the Federal University of Paraná partnered with the Brazilian Agricultural Research Company (Empresa Brasileira de Pesquisa Agropecuária - Embrapa Soja) to do field research on *Azospirillum* efficiency. Years of research produced *Azospirillum* lines that survived longer in soils and produced more plant growth[16]. A total of eighteen trials were run which were divided into two sets of nine trials each. The first set evaluated peat inoculants containing a single *A. brasilense* or *A. lipoferum* strain in corn using five trials with three harvests and in wheat using four trials with two harvests. The second set evaluated a mixture of two *A. brasilense* strains (Ab-V5 and Ab-V6) in peat or liquid inoculants in four corn trials and four wheat trials. This research contributed to the release of four *A. brasilense* strains (Ab-V4, Ab-V5, Ab-V6 and Ab-V7) for commercial inoculant production. Only Ab-V5 and Ab-V6 are widely used as inoculants because they provide the most satisfactory results in corn and wheat[16]. Due to their ease of application liquid inoculants were chosen over peat inoculants for marketing. In 2010, a liquid inoculant containing *Azospirillum* strains and protective molecules for tropical conditions was developed in an association between Embrapa and the private sector.

Azospirillum strains are sold worldwide in countries including Argentina, Mexico, Italy, France, Australia, Pakistan, Germany, the United States, Africa, Belgium, India and Uruguay[24]. The success of *Azospirillum* inoculants is the result of their beneficial effects in agricultural crops; for instance, they have a 60 to 70 % success rate and provide 5 to 30 % productivity gains in various cereal crops[25].

Action mode of *Azospirillum* bacteria

Azospirillum spp. bacteria supply nitrogen to plants via different mechanisms (Table 1).

Table 1: Action modes of *Azospirillum* bacteria

Action modes	Source (Authors)
Plant growth regulator production (auxins, cytokinins and gibberellins)	Lambrecht *et al.*[27]
Assimilatory reduction of nitrate	Fages[23]
Biological fixation of N_2	Fernandes Júnior[24]
Resistence to hydric stress	Cohen *et al.*[34]
Growth of root system	Okon and Labandera-Gonzalez[25]
Greater water and nutrient absorption	Okon and Labandera-Gonzalez[25]
Biological control	Unno *et al.*[26]

Production of growth regulators is one of the main mechanisms by which these bacteria affect plant growth since they influence growth in plants, modify root morphology and maximize use of soil resources, which in turn augment BFN and reduction of the assimilable nitrate available in soils[24].

Azospirillum bacteria also reduce nitrate (NO_3^-) in the roots, promoting plant growth due to consequent lower energy expenditure required from the plant to reduce nitrate to ammonia; this energy is allocated to other vital processes. These bacteria can influence glutamine synthetase activity in the roots of corn plants[23]. Glutamine synthetase is extremely important in the nitrogen incorporation process and essential for plants to express their full productive potential[26]. Other indirect mechanisms through which *Azospirillum* bacteria can promote plant growth is by reducing the appearance of fungi or soil pathogens via production of siderophores, chitinases, glucanases and antibiosis.

Growth of the plant root system, which can occur in response to growth-promoting substances[25], can result in greater mineral and water absorption. Auxin, specifically indole-3-acetic acid (IAA), is the principal hormone promoting root growth[15]. *Azospirillum* lineages produce IAA in addition to other indole compounds such as cytokinins and gibberellins[8]. At least three biosynthetic pathways exist for IAA production in *Azospirillum*, two of which (indole-3-acetamide and indole-3-pyruvate) depend on tryptophan[27]. Indole-3-acetic acid is the main indole secreted by *Azospirillum*[24], although indole-3-butyric acid (IBA) has also been reported[23]. This indole is an important reserve source in the *Azospirillum* genus[28].

Production of IAA by *Azospirillum* very probably affects root growth. For example, inoculation of tomato and red pepper seeds with *A. brasilense,* which is known to produce IAA, increased average root length from 7.21 (control) to 9.68 cm in tomatoes and from 6.20 (control) to 6.76 cm in red pepper(29). The aerial portion of the plants in this study also grew more in the treatments than in the controls, highlighting the growth promoting ability of *A. brasilense*. Phytohormone synthesis in *A. brasilense* can differ between strains. In one study the 42 M line produced higher IAA levels than the sp7, Cd, Az39, 40 and 42 strains(6).

In *Azospirillum* strains IAA may be a signal needed to maintain a symbiotic plant-*Azospirillum* interaction(30), since this signaling function is shaped by coevolutionary processes between bacteria and their host plant(31). This phytohormone also affects photosynthesis, and biosynthesis of some metabolites and other phytohormones such as cytokinins and gibberellins(32). In plants cytokinins regulate cell division and new tissue formation both in the aerial part and roots(33). Gibberellins regulate plant growth, and promote division and elongation of primary roots(30).

Azospirillum strains can secrete abscisic acid (ABA) which forms part of the water stress defense mechanism in plants(34). This phytohormone induces plant responses to water, environmental and saline stress(35). For example, inoculation of *A. lipoferum* into corn plants resulted in high ABA levels and increased plant tolerance to drought probably related to ABA as well as prolines and polyamines(34). Polyamines (e.g. cadaverine, spermine and spermidine) are organic polymers associated with root growth and stress suppression in plants(33). In a study of rice seedlings inoculated with *A. brasilense* cadaverine production was associated with increased root growth and reduced osmotic stress(36). Corn plants inoculated with *A. brasilense* also are reported to exhibit greater drought resistance and higher biomass production(37), possibly due to the presence of cadaverine, among other phytohormones.

Growth-promoting bacteria such as *Azospirillum* can also act as biological control agents(35), which can occur through mechanisms such as parasitism, production of antibiotics, toxins and/or enzymes, interference in the plant-host recognition process and resistance induction(33). Facultative bacteria such as those in the genera *Pseudomonas*, *Burkholderia*, *Azospirillum* and *Bacillus*, produce and secrete secondary substances that can act as antibiotics, fungicides, antivirals and immunosuppressive agents(31). In plants endophytic bacteria of this type provide the best biological control of pathogens via natural colonization of the rhizosphere and invasion of internal tissues, both of which are essential means for successful treatment of diseases affecting subterranean plant tissues.

The *Azospirillum* genus is not strictly considered to exercise biocontrol but *Azospirillum* strains do produce chemical compounds that modify plant metabolism and defense activity. For instance, *A. brasilense* has been shown to effectively control *Agrobacterium tumefaciens* and phytopathogenic fungi(25). Although the inhibition mode was not well defined, phenylacetic acid, from the auxin group, was identified and found to have antimicrobial activity. Phenylacetic acid is used in the defense mechanism and bacterial competition in the host(25). Biological defense in plants follows two metabolic pathways involving jasmonic acid and salicylic acid(17), while bacteria-induced systemic resistance is activated by the jasmonic acid and ethylene signaling pathway(15).

Co-inoculation with rhizobia

Co-inoculation with bacteria that may or may not be symbiotic is common in legume crops(38). Different microorganisms are used that produce a synergistic effect that surpasses production results obtained when strains are used separately(39). *In vitro* assays indicate that some bacteria mixtures provide a synergistic interaction that can improve nutrient supply, remove inhibiting products and/or stimulate physical or biochemical mechanisms(35). For example, when associated with rhizobia in legumes *A. brasilense* produces a beneficial effect caused by production of phytohormones that promote root system development, thus allowing more effective exploitation of available soil volume(38).

In future co-inoculation between *Azospirillum* and other microorganisms may become a major research focus(35). Co-inoculation produces higher success rates in plants since it results in increased productivity due to balanced nutrition and improved absorption of nitrogen, phosphorus and other minerals(5). It also stimulates root nodule function and increases nodule numbers and weight. Co-inoculation of *Bradyrhizobium japonicum* and *A. brasilense* in soybeans provided better yields, even under water and nutrient deficit conditions(39). In a study of *B. japonicum*/*A. brasilense* co-inoculation with and without water deficit, co-inoculation increased nodule weight under water deficit conditions and produced better yields and a greater dry matter accumulation under both conditions(40). Studies of root growth and nodulation in soybean co-inoculated with *B. japonicum*/*A. brasilense* found that addition of *A. brasilense* helped to stimulate root growth and improved nodule onset and development through excretion of metabolic products, especially IAA(36). The co-inoculated plants also exhibited a greater number of nodules and a higher percentage of nodulated plants.

Azospirillum in forage grasses

Grassland degradation is a major problem in livestock systems. For example, an estimated 80 % of grasslands in Brazil exhibit some level of degradation(41). Of the factors that can lead to grassland degradation low nutrient supply for plants is the principal cause since it results in low grass quality and productivity(42). Among soil nutrients, N is the main limiting factor for grass growth and development in the tropics(43). Mineral fertilization is widely used to ensure proper grass growth, but this is expensive and environmentally damaging since around 50 % of applied N is lost due to volatilization or leaching(14).

This situation highlights the potential benefits of BFN in tropical grasses as a means to restoring forage productivity and quality. Diazotrophic bacteria (a.k.a. PGPB) are the main force behind BFN. The process itself involves conversion of N_2 into other nitrogenous substances which are then assimilated by plants through synthesis of protein and nucleic acids(1). The nitrogen cycle in grassland ecosystems strongly depends on BFN and diazotrophic bacteria to supply N to plants. Inoculation of PGPB is therefore a promising technique for promoting growth and nutrition in forage plants. It is a viable alternative for totally or partially replacing N-fertilizer and thus contributing to natural resources conservation(44).

The benefits of PGPB in crops such as corn, wheat and rice are clear, but knowledge of the mechanisms involved requires extensive further research. Studies evaluating the effects of PGPB in establishing and maintaining pastures are fundamental to attaining cost-effective and sustainable grassland systems. Using BFN supports sustainability since it can totally or partially replace nitrogen fertilizers, which are produced from non-renewable fossil fuels(45). Any lowering of N fertilizer use will also mitigate environmental pollution derived from its production and use, with the added dividend of reducing greenhouse gas emissions(8).

A metanalysis of 22 yr of data on in-field PGPB inoculation concluded that *Azospirillum* spp. promotes growth in several grass species under different environmental conditions(5). These gains went beyond BFN to include modification of root production and a consequent broadening of absorption area and the volume of exploited soil. Most of the research supporting this conclusion has involved inoculation of *Azospirillum* in forage grasses.

Azospirillum brasilense was reported to increase dry matter production and foliar growth in pot-grown millet *Setaria italica*(46). In a study of forage grasses native to the Pantanal Matogrossense in South America, inoculation with diazotrophic bacteria had noticeable effects at 60 and 90 d of culture(47). Combined inoculation of an *A. brasilense*/*A. lipoferum* association in the same study resulted in greater dry matter production in the aerial portion and roots, as well as N accumulation. Higher dry matter production than in a control was also observed with inoculation of *A. brasilense* in the grasses in a natural pasture(48).

Inoculation is also reported to favor the simultaneous production of dry matter in the aerial portion and roots, and high levels of phosphorus (P) and N accumulation due to more efficient use of these nutrients(14). This higher dry matter accumulation rate may be related to an increase in photosynthetic enzyme activity and N assimilation(8). Similar increases in dry matter production have been observed with inoculation of Marandú grass, indicating this technique is a sustainable alternative for increasing forage production(49). *Azospirillum* inoculation has also increased productivity in other grasses(50), which may be due to excretion of plant hormones that enhance macro- and micronutrient absorption(35).

Use of PGPB is reported to raise nitrogen accumulation and overall yields. In a study using *Pennisetum* and *Panicum* species grasses inoculation with *A. brasilense* increased N content by approximately 40 kg ha^{-1} year^{-1} via BFN(25). In another study BFN was found to raise N accumulation 7 to 10 kg ha^{-1} mo^{-1} during the summer, which varied by grass genotype; this represents 39% of the N necessary for plant development and productivity(51). Dry matter accumulation rate in the same study was 15 kg ha^{-1} d^{-1} in the inoculated treatments, almost four-fold higher than the 4 kg ha^{-1} d^{-1} in a control without inoculation and fertilization.

Inoculation of *A. brasilense* in *Brachiaria brizantha* Staf cv. Marandu, produced better yield, greater tiller generation and a longer grazing period than uninoculated and unfertilized plants(49). In this same system root dry matter production increased with *A. brasilense* inoculation(33). Using the same grass cultivar, another study found that *A. brasilense* inoculation produced 9 % more leaves and 12 % more tillers than a control, although no differences in plant height were observed between inoculation and N fertilization treatments(52).

Application of *A. brasilense* in forage grass production contributes to increasing dry matter content, N content and plant height, making PGPB inoculation a viable alternative for partial replacement of N fertilizer. It can be a vital element in efforts to combine animal production with environmental conservation, which will become more feasible as soil biotechnology is broadly employed to promote soil conservation and fertility and improve plant nutrition. However, more research is needed on the mechanisms and effects of PGPB inoculation on dry matter content, N content, plant height, leaf area and crude protein content so recommendations can be made regarding its combination with N fertilizer(1). In addition, there is still no conclusive data indicating whether the effects of inoculation are due to BFN or hormonal action.

Azospirillum in corn

Yield in corn responds to a combination of genetic potential, environmental conditions and soil fertility. However, attaining maximum yields requires application of large amounts of fertilizers, particularly nitrogen. One option for reducing use of N fertilizers with corn is application of *Azospirillum* spp. strains, since it can reduce their use by 30 to 50 kg ha^{-1}(7).

More specifically, inoculation with *A. brasilense* increases photosynthesis and dry matter accumulation rates(8). When associated with unimproved and cultured genotypes with low N availability, *Azospirillum* inoculation leads to greater dry matter production, grain yield and N accumulation in corn(5). It can also increase overall grain production by 9 %(53), and productivity by 30 %(8). Some authors report better results when inoculating *Azospirillum* in corn seeds, with average increases of 70 % in dry root mass and 43.5 % in aerial dry mass(54).

Inoculation process

Inoculation is a simple procedure in which seeds are mixed with an inoculant. It is preferably done in the shade in the morning to prevent direct sunlight and excessive heat from killing the bacteria. Mixing is done in a rotating drum, cement mixer, seed treatment machine or other machine. It is important to uniformly distribute the inoculant on the seeds and verify that each seed is covered by inoculant before sowing. For this technique to be effective seeds must be of good quality and the proper dose of inoculant/kg seed must be applied. After inoculant application the seeds are dried for about 30 min before sowing, all the while protected from sunlight and heat. For peat mixture inoculants a sugar solution or other adhesive substance (gum arabic, arrowroot flour, cassava flour, wheat flour and/or various types of cells and polymers) is recommended to moisten the seeds. In this case, a 10 % sugar solution (100 g sugar/L water) is prepared, the seeds mixed into it at a proportion of 250 to 300 ml sugar solution per 50 kg seeds, and finally the peat mixed in at the proportion indicated by the manufacturer[8]. In either process, the seeds have a thin layer of inoculant on their surface when it is complete. It is extremely important to verify that all seeds have been covered with inoculant since this will ensure nitrogen fixation and expression of their full genetic potential.

Inoculated seeds should be sown the same day they are inoculated to ensure the highest number of viable microorganisms. If this is not feasible, the seeds can be stored in ventilated sheds by spreading them out on a smooth surface in layers less than 30 cm thick[12]. The inoculant contains live microorganisms which are sensitive to hot environments[16], and seeds should therefore be sown a maximum of 24 h post-inoculation. If this period is exceeded, the seeds must be re-inoculated to improve efficacy. During sowing, the seed container on sowing machines can become quite hot (35+ °C), which can kill the bacteria; should this occur sowing should be stopped and the container cooled. Direct inoculation in the seed container is not recommended because inoculant distribution is not uniform, which reduces inoculation efficiency[16].

Inoculation of small lots of seeds can be done with clean plastic bags, basins or buckets previously sterilized with 70% alcohol, as long as all seeds are covered with a thin layer of inoculant. Other aspects of the process follow the same criteria as when using a rotating drum or concrete mixer, namely that it be done in the shade, that inoculated seeds be allowed to dry for 30 min before sowing, and that doses be calculated following manufacturer instructions to ensure maximum efficiency[12].

For beans planted in small areas direct inoculation in the furrow is an alternative technique. Application can be done with a manual or motorized sprayer directly in the furrow. If using this technique, the inoculant must be mixed with water following manufacturer instructions and the sprayer to be used cannot have residues of agrochemicals such as pesticides or herbicides as these can decrease bacteria viability and lower efficiency. Even though the inoculant consists of soil bacteria, the operator must wear personal protection gear.

Final considerations

Use of *Azospirillum* spp. in grasses can span the gap between productivity and sustainability. Inoculants based on these microorganisms can potentially reduce nitrogen fertilizer use without affecting productivity, resulting in savings and greater profitability. Grasses such as sugarcane, corn and forages are known to respond well to *Azospirillum* sp. inoculation, but further study is needed under different conditions to support its broader adoption among producers. This technique can increase competitiveness for some agricultural products vis-à-vis conventionally-grown crops. Grassland inoculation in livestock production can increase forage mass production, mitigate degradation risks and improve production rates.

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