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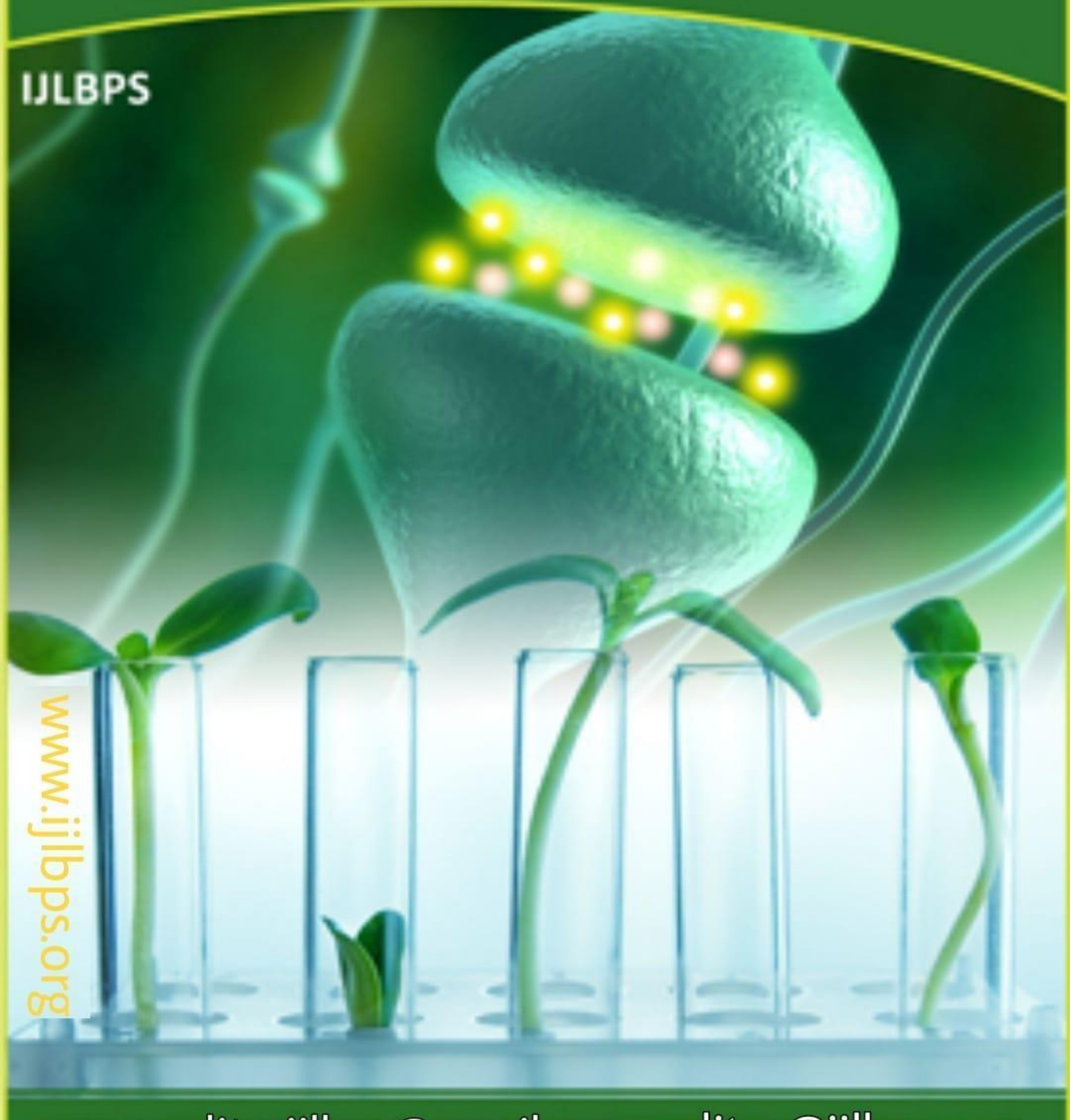

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Azospirillum: Diversity, Distribution, and Biotechnology Applications

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Abstract

Plant growth-promoting bacteria (PGPB) are a well-studied group that can be found in the genus Azospirillum. Plants benefit from Azospirillum inoculation because of the bacterium's ability to fix atmospheric nitrogen and produce phytohormones like indole-3-acetic acid. Tolerance of abiotic and biotic stressors in plants may be mediated by phytohormones serving as signaling molecules, and recent research has assigned a significant role to Azospirillum in this process. In contrast to the systemic acquired resistance, which has been studied in relation to phytopathogens, the tolerance of biotic stresses is regulated by mechanisms of induced systemic resistance, mediated by elevated levels of phytohormones in the jasmonic acid/ethylene pathway. The NPR1 protein, which functions as a co-activator in the activation of defense genes, is involved in both of these processes. Induced systemic tolerance, which is mediated by antioxidants, osmotic adjustment, generation of phytohormones, and defensive tactics such the expression of pathogenesis-related genes, is another way by which Azospirillum may boost plant development. Insight into the Azospirillum-induced plant processes may lead to the discovery of PGPB's role as a significant method for reducing the negative impact of biotic and abiotic stressors on agricultural output. One of the primary initiatives for reducing fertilizers consumption is the creation of cultivars with increased nitrogen use efficiency (NUE), in combination with the application of plant growth-promoting bacteria. However, it has been reported that the production of phytohormones by Azospirillum strains used in commercial inoculants formulations plays a crucial role in plant growth promotion, so their use is generally recommended in conjunction with regular N-fertilizer doses. In addition, there is still reported high variability in the effectiveness of Azospirillum inoculants under field conditions, which means that the inoculation technology is being adopted more as an additional management practice than as a replacement for chemical inputs.

Azospirillum; Variety; Widespread; Practical Uses

Introduction :

The bacterial genus Azospirillum belongs to the Rhodospirillaceae family and is Gram-negative, microaerophilic, non-fermentative, and nitrogen-fixing.pathogenic bacteria [4]. As a microaerobic diazotroph, A. brasilense is able to fix nitrogen in environments with very little oxygen. First identified in 1925 in low-nitrogen soil in the Netherlands,Two] The bacterium Azospirillum may stimulate plantexpansion [3]. The Azospirillum brasilense strain of bacteria is athe ability to fix nitrogen (diazotroph), be investigated, and be genetically modifiable Negatively charged alpha-proteobacteria (Gn) Rhizospheres of grasses all throughout the globe contain this growth-promoting substance [5, 6].

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Hormone modulation vs. direct nitrogen transfer from bacteria to plant for growth promotion: which is more likely? [7, 8].Sp7 (ATCC 29145) and Sp245 are the two most investigated strains.A. brasilense Sp245 has had its genome sequenced, and it comes in at a manageable 7Mbp spread out throughoutSeven chromosomes. Due of its high GC concentration (70%) it is difficult to engineer. [9] Conjugation and elect roporation are two methods that may be used to introduce an OriV origin of replication plasmid into Sp245. The bacterium has inherent resistance to the medicines ampicillin and spectinomycin. As a selectable marker, kanamycin resistance stands out. Codon mutation and transposon hopping promote a rapid pace of evolutionary adaptation in A. brasilense.The Roseomonas fauriae strain was reclassified as A. brasilense. It was named after Yvonne Faur "for her contributions to public health bacteriology and, especially, for her contribution to the detection of pink-pigmented bacteria [10, 11]" after its discovery in 1971 from a woman's hand wound in Hawaii.Plant growth-promoting rhizobacteria (PGPR) belong to the genus Azospirillum and are free-living nitrogen-fixing bacteria that colonize the root surface or the intercellular spaces of the host plant by adhesion. Possible functions of the PGPR in commercially significant cereals and other grasses include inducing the development of nitrogen-fixing bacteria and phytohormones [12].Several species of Azospirillum are capable of producing plant growth-promoting phytohormones [13, 14]. These phytohormones include auxins, gibberellins, cytokinins, and nitric oxide. Previous research has revealed that different strains of Azospirillum have bigger genomes that are made up of several replicons, suggesting the possibility of genome plasticity [15]. When new megaplasmids are formed, replicons are often destroyed in the process [16, 17]. A large portion of the genome has also been lost in several Azospirillum species, according to genome sequencing.a horizontal purchase [17]. The genomes of just four Azospirillum species (A. brasilense, A. lipoferum, A. sp. B510, and a draft of A. amazonense) have been published thus far [18], despite the fact that 16 species have been defined.

Sugar-cane, maize, sorghum, and rice were all discovered to have Azospirillum amazonense associated with their roots and rhizospheres, indicating the bacterium has a wide ecological range in Brazil. In contrast to A. brasiliense, A. amazonense has been shown to be more closely related to Rhodospirillum centenum and Azospirillum irakense. When compared to other Azospirillum strains, A. amazonense is superior in its ability to colonize plant root tissuein acid conditions [19, 20] due to its ability to develop with sucrose as its primary carbon source. Genomic analysis of A. amazonense also uncovered a gene cluster (RubisCO) involved in carbon fixation and genes important for the usage of salicin as a carbon source (similar to A. irakense). However, there are still gapsin our knowledge about A. amazonense phytohormone synthesis.

Horizontal gene transfer may have played a role in the adaption and evolution of A. amazonense, which is likely connected to the flexible gene repertoire found in this bacterium's genome. Phylogenetic and gene tree analyses showed that the proteins involved in nitrogen fixation, carbon fixation (RubisCOs), and molecular hydrogen oxidation (hydrogenases) are most closely linked to members of the Rhizobiales genus.

A. amazonense's genetic diversity is very important, thus we used its complete coding sequences (CDS) to conduct an in silico comparative genomic study using subtractive hybridization against the genomes of closely related bacteria. Analysis of conserved and unique coding sequences from A. amazonense revealed characteristics that set it apart from other Azospirillum species. Furthermore, the specific interesting features related to phytohormone production may provide several cues to establish A. amazonense pathways for auxin biosynthesis.
Inoculation technology with plant growth-promoting bacteria (PGPB) has been presented worldwide as an important tool for reaching sustainability in agriculture due to its low environmental and production costs compared

with industrial inputs. However, different from symbiotic relationships, where plant-bacteria interactions have been widely exploited and are relatively well understood, the associative interactions driven by PGPB are discreet and elicited by factors that have only recently started to be clarified [21,22]. It is not difficult to realize that the broad adoption of PGPB inoculation as regular agricultural practice is somehow impaired by the lack of scientific knowledge regarding the ecology, physiology and biochemistry of associative plant-bacteria interactions. Although feasible, the replacement of chemical inputs in commercial agriculture with bioproducts developed from and based on the rational exploitation of plant-microbe natural relationships, such as nutrient-provider bacteria in substitution for soluble fertilizers, or biocontrol agents in substitution for pesticides, remains a major challenge [23]. In this sense, efforts to strengthen inoculation technology in non-leguminous crops with PGPB need to incorporate a broader understanding of the determinants of bacterial rhizocompetence and competitiveness necessary to successfully achieve a plant-PGPB interaction. In the same way, one must consider the physiological status of the inoculated microorganisms such that high viability ismaintained under adverse conditions found in soil and/or during storage. Such challenges are magnified if one considers the identification of PGPB with high biotechnological potential in distinct phylogenetic clusters, and the low probability of finding conditions to produce high-quality inoculants that can be universally applied for any PGPB [24].

Commercial inoculants carrying PGPB are generally available as dry or liquid formulations of different organic and/or inorganic materials, which may be prepared with cells grown in a liquid culture medium or via the direct use ofbacterial broth for producing liquid inoculants, or obtaining dehydrated cells that may be incorporated in a solid or liquid carrier [25, 26, and 27]. Liquid inoculants simplify both the industrial production and the field application, although compared with solid formulations, such as peat- or polymer-based inoculants, bacteria in liquid inoculants appear to be more sensitive to stressful conditions and can exhibit decreased viability when used on seeds or soil [28]. Effectiveness of PGPB inoculants has been improved by immobilization of inoculant cells in polymeric carriers, such as alginate and starch foam [29, 30]. Thus, the actual demand is for improved liquid inoculants formulations, which are replacing peat-based inoculants and currently comprise ~80% of doses sold for soybean crops in Brazil [31]. While the technical criteria required to produce high-performance peat-based inoculants are well defined, these same criteria aretreated as industrial secrets or proprietary information for liquid inoculants. Even considering the presence of high- performance Azospirillum-based liquid inoculants on the market, the information regarding the composition of these inoculants is mostly limitedto that presented on the product label, which increases the difficultly of conducting a thorough scientific analysis of the role of each ingredient in the formulation and its effects on the final quality of the product appliedin the field [24]. This may be best exemplified by inoculants formulations using the PGPB Azospirillum brasilense, which are prepared from different bacterial strains and are availablein a wide variety of commercial products worldwide; variations in theirperformance under field conditions are still reported.

The diazotroph A. brasilense is considered a model PGPB, and a great amount of information regarding the physiology of its growth and development has been published [32]. However, it is unclear when this available knowledge is in fact applied in the inoculants industry. Quality control assessments of agricultural inoculants are commonly defined by the presence of contaminants and the population density of viable cells in the final product throughout its shelf-life, and determining any information about the physiological state of the inoculants PGPB strain in a commercial product is not required. To consider inoculants production solely in terms of the yield of microbial biomass from a specific strain has the potential to produce poor-quality inoculants, which plays against the broad adoption of PGPB inoculation as an alternative to conventional agronomical practices. To implement this new paradigm in modern agriculture, commercial inoculants formulations should be produced with bacterial cells at a high population density and in high physiological state to enable them to face adverse conditions that occur both during storage (shelf life) and at the time of its use (on seeds and in soil) [24,28]. It is not difficult to realize that the broad adoption by the industrial and regulatory agencies of the available PGPB physiology knowledge, as well as the implementation of research studies aimed to better understand the physiological characteristics for which no such knowledge is yet available, should result in better inoculants with higher field performance.

At least in Brazil, the inoculation of cereals with diazotrophic PGPB, such as maize inoculation with A. brasilense, is considered an additional practice and its adoption occurs under the lowering the germination rate if the seeds are mechanically damaged during this process. Furthermore, inoculating seeds places the bacteria in contact with the pesticides and agrochemicals commonly found covering commercial seeds, which are then sown close to the fertilizers applied in the soil. Intending to determine whether conditioning the PGPB A. brasilense Ab-V5 to accumulate high amounts of polyhydroxybutyrate (PHB) and exopolysaccharides (EPS) during its growth leads to an improved inoculants performance in the field, a culture medium was developed, andgrowth conditions were defined to lead this bacterium to increase its biopolymer contents. The importance of the cellular content of PHB and EPS on improving the viability of A. brasilense was evaluated by a short-period assay carried out under greenhouse conditions. Bacterial biomass produced under the conditions defined so on was used to produce liquidinoculants, which were applied on seeds or topdressing in maize plants; the results of these treatments were compared with the performance of a peat inoculants applied on seeds.

Projections of population increases, especially in developing countries, as well as of life expectancy worldwide, imply greater needs for food and feed. To achieve higher productivity, agriculture isbeing intensified, mainly with monocultures highly dependent on increased chemical inputs, including pesticides and fertilizers [33, 34]. However, to ensure long-term food production, we must develop sustainable agricultural practices, based on conservationist practices, to achieve economic returns for farmers, but with stability in long-term production and

application of regular amounts of N fertilizer that can exceed 200 kg ha−1. Concerns leading to the distrust of the natural nitrogen inputs provided by diazotrophic PGPB substituting at least part of the nitrogen demand in commercial crops reflect the lack of scientific information available for the commercial inoculants formulations. In addition, there is a huge variability in inoculation efficiency as a result of the use of low-quality inoculants [25, 28]. Maize inoculants are mainly applied over seeds before sowing, which is an additional practice that presents risks of

minimal adverse impact on the environment [35]. In this context, the use of microbial inoculants plays akey role, and we may say that we are starting a “microgreen revolution.”

The nomenclature “plant-growth-promotingbacteria (PGPBs)” has been increasingly used for bacteria able to promote plant growth by a variety of individual or combined mechanisms. By this definition, rhizobia—studied and used in commercial inoculants for more than a century— are also PGPBs. Undoubtedly, besides rhizobia, the most studied and used PGPB is *Azospirillum*, encompassing bacteria with a remarkable capacity to benefit a range of plant species [36,37,38, 39, and 40]

The genus *Spirillum* was first reported by Beijerinck (1925), [41] and decades later reclassified as *Azospirillum*, because of its ability to fix atmospheric nitrogen (N2), discovered and reported by the group of Dr. Johanna Döbereinerin Brazil, in the 1970s [4]. After the discovery that *Azospirillum* was diazotrophic, several studies evaluated its capacity to fix N2 and to replace N- fertilizers when associated with grasses [42], including sugarcane (*Saccharum* spp.), grain crops such as maize (*Zea mays* L.), wheat(*Triticum aestivum* L.), and rice (*Oryza sativa* L.), pastures such as *Brachiaria* (= *Uruchloa*), among others[32, 39, 40, 43, 44, 45]. Twenty speciesof *Azospirillum* (DSMZ 2018) have been described so far, but *A. brasilense* and *A. lipoferum* have been the subjects of the highest numbers of physiological and genetic studies [46, 47].

Beneficial results have been obtained consistently with *Azospirillum* applied to a variety of crops [40, 48, 49] in dozens of commercial inoculants worldwide [50]. Intriguingly, although the Brazilian research group headed by Dr. Döbereiner contributed to dozens of studies with *Azospirillum* [46, 51 and 52], it was only in 2009 that the first commercial inoculant containing *A. brasilense* started to be commercialized in the country [37, 38]; however, more than 3 million doses of inoculants are now applied annually by farmers, for inoculation both of non-legumes and for co-inoculation of legumes.

Although the most prevalent reported benefit of *Azospirillum* has been its capacity of fixing N2, an increasing number of studies describe other properties that imply growth-promotion. One main property of *Azospirillum* relies on the synthesis of phytohormones and othercompounds, including auxins [53], cytokinins [6], gibberellins [54], abscisic acid [55], ethylene [56], and salicylic acid [57]. Phytohormones greatly affect root growth, resulting in improvements in uptake of moisture and nutrients [58]. Some *Azospirillum* strains can solubilize inorganic phosphorus, making it more readily available to the plants and resulting in higher yields [59]. There are also reports of *Azospirillum* helping in the mitigation of abiotic stresses, such as salinity and drought [60, 61, 62], by triggering induced systemic tolerance (IST). *Azospirillum* has also been reported to helpin the mitigation of excessive compost and heavy metals [36, 63]. Another important feature of *Azospirillum* is related to biological control of plant pathogens [64,65, 66 and 67], enabled by the synthesis of siderophores, and limiting the availability of iron (Fe) tophytopathogens [67], or causing alterations in themetabolism of the host plant, including the synthesis of a variety of secondary metabolitesthat increase plantresistance to infection by pathogens, a mechanism known as induction of systemic resistance (ISR) [68]. Due to the several mechanisms reported to promote plant growth, Bashan and De-Bashan (2010) [36] proposed the "theory of multiple mechanisms" in which the bacterium acts in a cumulative or sequential pattern of effects, resulting from mechanisms occurring simultaneously or consecutively. In this review we will give emphasis to the mechanisms of *Azospirillum* that can improve plant tolerance of biotic and abiotic stresses.

Conclusion

Azospirillum is currently one of the most broadly studied and commercially employed PGPB.Previous studies with *Azospirillum* emphasize its capacity of fixing atmospheric N2, followed by benefits in promoting plant growth via synthesis of phytohormones. More recently, it has been shown that the benefits should be extended to the capacity of some *Azospirillum* strains to protect plants from biotic stresses, triggering ISR defense mechanisms, and from abiotic stresses, through IST. The mechanisms discussed in this review of tolerance of abiotic and biotic stresses promoted by inoculation of *Azospirillum* in plants, encompassing detoxification of oxidative stress, ISR and IST. The mechanisms that PGPB use to cope with biotic and abiotic stresses vary with the plant species and cultivar and with the bacterial species and strains, and also depend on the phytopathogen and the intensity of the abiotic stress. Further studies to elucidate the mechanisms of action of PGPB—as well as of the response of plants to stresses—are of fundamental importance for understanding the potential and increasing the use of PGPB as an important and sustainable strategy to mitigate the effects of biotic and abiotic stresses in agriculture.

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