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## Associative diazotrophic bacteria isolated from different biomes as potential plant growth promoters of common bean

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**Abstract.** Associative diazotrophic bacteria can promote plant growth; however, their efficiency depends on the compatibility among host, microorganism, and environment. This study evaluated bacterial strains with high *in vitro* potential for nitrogen (N) fixation and indole-3-acetic acid (IAA) synthesis, isolated from the Atlantic Forest (Sp7), Cerrado (Ab-V5), and Amazon (MS32 and MS52) biomes, regarding their ability to promote growth and nodulation of common bean (*Phaseolus vulgaris* L.). The experiment was conducted in a greenhouse, in a completely randomized design with eight replications. Emergence, chlorophyll index, morphological parameters, nodulation, and rhizospheric bacterial density were evaluated. Although the selected strains showed high *in vitro* potential for N fixation and IAA production, they did not promote common bean growth. These results indicate the need to investigate other growth-promotion mechanisms that may be more promising for selecting efficient bacteria for common bean growth promotion.

**Keywords:** Biological nitrogen fixation (BNF), *Azospirillum*, Plant growth-promoting bacteria (PGPB), *Phaseolus vulgaris*, Rhizosphere, Nodulation.

### Introduction

The common bean (*Phaseolus vulgaris* L.) plays a central role in food security across Latin America, Africa, and Asia, being cultivated under a wide range of edaphoclimatic conditions. Its shallow root system and high nitrogen (N) demand increase dependence on synthetic fertilizers, whose use is costly and environmentally harmful (Chojnacka et al., 2020; Silva et al., 2024). Biological nitrogen fixation (BNF) emerges as a sustainable alternative, mediated by nodulating bacteria (rhizobia) and non-nodulating bacteria such as plant growth-promoting bacteria (PGPB), which are capable of producing phytohormones and improving nutrient uptake (Kumar et al., 2024; Sarao et al., 2024). However, inoculation efficiency depends on the competitiveness of the strains, environmental adaptation, and compatibility with the host (Mendonza-Suárez et al., 2021).

Brazil, with its high biodiversity and edaphoclimatic heterogeneity, harbors six major biomes: Amazon, Savanna (Cerrado), Caatinga, Atlantic Forest, Pampa, and Pantanal. This wide

diversity represents a valuable source of microorganisms adapted to different environmental conditions, allowing the isolation of strains with high biotechnological potential (IBGE 2019; Lopes et al., 2025). This study aimed to evaluate associative diazotrophic bacterial strains isolated from the Atlantic Forest, Savanna, and Amazon biomes in Brazil, to investigate their potential to promote growth and nodulation of common bean plants (*Phaseolus vulgaris* L.).

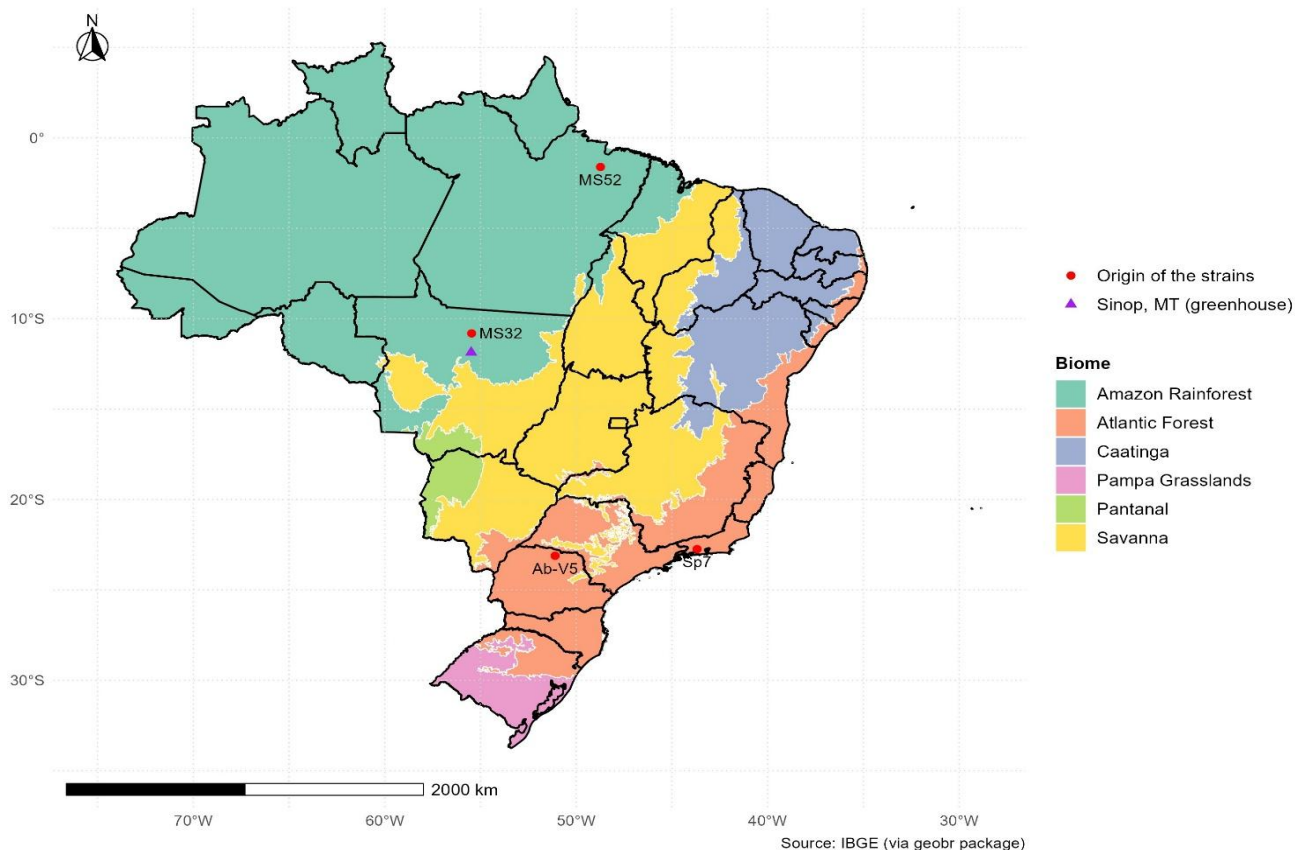
### Material and Methods

Four bacterial strains with high *in vitro* potential for N fixation and indole-3-acetic acid (IAA) production were tested: Sp7 (Atlantic Forest), Ab-V5 (Savanna), MS32 and MS52 (Amazon) (Figure 1). The strain Sp7 (*Azospirillum brasilense*) was isolated from the rhizosphere of pangola grass (*Digitaria decumbens*) (Somers et al., 2005), the strain Ab-V5 (*A. brasilense*) was obtained from maize (*Zea mays*) (Hungria et al., 2010), and isolates MS32 and MS52 were obtained from the rhizome and rhizosphere of arrowroot (*Maranta*

*arundinacea*) plants, respectively and characterized by Dias et al. (2020) and Rodrigues et al. (2020).

The bacterial strains were cultured in liquid medium, adjusted to  $1.5 \times 10^9$  CFU mL<sup>-1</sup>, and the seeds of common bean cv. Anfc5 were immersed in

the respective inoculant suspensions for 1 h. The following treatments were established: T2 – *Azospirillum brasilense* (Ab-V5), T3 – *A. brasilense* (Sp7), T4 – isolate MS32, and T5 – isolate MS52. Seeds from the control treatment (T1) were immersed only in sterilized liquid medium.



**Figure 1.** Distribution of associative diazotrophic bacterial strains (Ab-V5, Sp7, MS32 and MS52) isolated from different Brazilian biomes.

The experiment was carried out in a greenhouse in Sinop, Mato Grosso, Brazil, within the Amazon biome (Figure 1), from January to March 2020, using non-sterilized soil. The local climate is tropical, with a rainy season (October–April) and a dry season (May–September). A randomized complete block design was adopted with five treatments and eight replications, with two plants per replication. The soil used was a Dystrophic Red Latosol, corrected according to recommendations for common bean (Sousa & Lobato 2004). Half of the recommended N dose ( $40 \text{ kg ha}^{-1}$ ),  $90 \text{ kg ha}^{-1}$  of  $\text{P}_2\text{O}_5$ , and  $60 \text{ kg ha}^{-1}$  of  $\text{K}_2\text{O}$  were applied, following standard crop management practices. Because the experiment was conducted in irrigated pots, N, P, and K doses were quadrupled. Foliar fertilizer was applied at phenological stages V4 and R5.

In all experimental plots, the percentage of emerged seedlings was recorded at the V2 phenological stage, while at stage R6, plant height, stem diameter, leaf area, shoot and root dry mass,

and number of nodules were evaluated. Leaf chlorophyll content was measured using a ClorofiLOG® chlorophyll meter (model CFL-1030, Falker®) at the following phenological stages: R5, early R6, and late R6. Measurements were taken on two young trifoliates per plant.

At stage R6, rhizospheric soil was collected to estimate the density of associative diazotrophic bacteria. Ten grams of soil were suspended in 0.85% NaCl, serially diluted ( $10^{-1}$  to  $10^{-5}$ ), and inoculated in triplicate into semi-solid NFB medium, which is semiselective for associative diazotrophic bacteria, particularly those of the genus *Azospirillum*. After seven days at approximately  $30^\circ\text{C}$ , pellicle formation was assessed, and the results were expressed as the most probable number (MPN) of bacteria per gram of soil (Baldani et al., 2014).

Data were subjected to analysis of variance (ANOVA,  $p < 0.05$ ), and means were compared using the Scott–Knott test, performed in SISVAR.

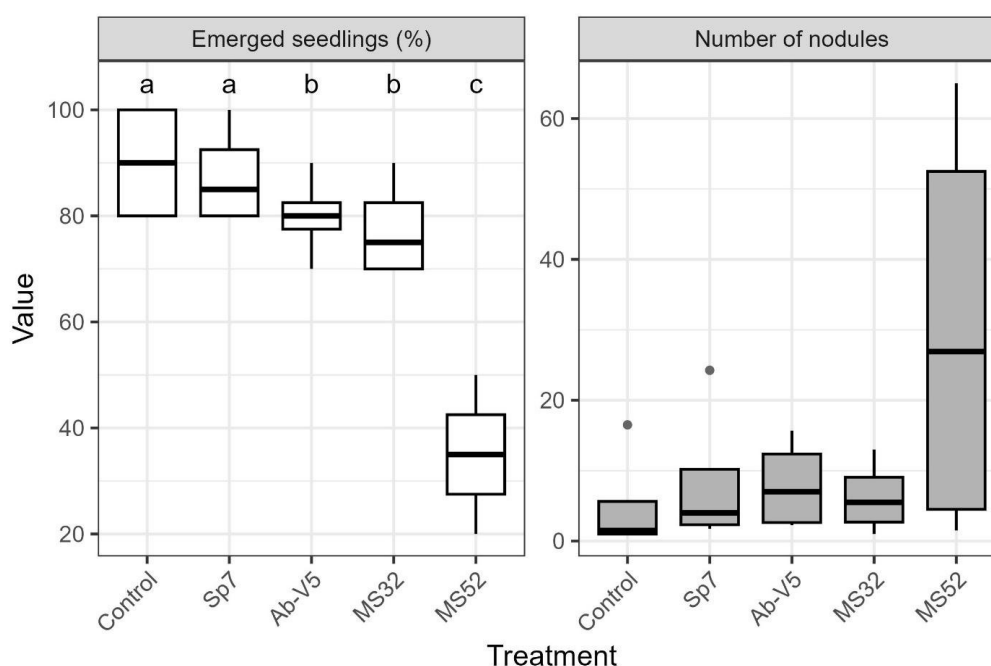
Bacterial counts were  $\log_{10}(x)$ -transformed prior to analysis.

### Results and discussion

Seedling emergence was significantly affected by inoculation ( $p < 0.05$ ). While Sp7 maintained emergence rates comparable to the control, MS52 markedly reduced germination (Figure 2). Despite this early effect, vegetative growth parameters, including plant height, stem diameter, leaf area, and biomass, did not differ significantly among treatments (Table 1). Although Ab-V5 numerically increased leaf area and shoot dry

mass, these increments were not statistically supported.

At the R6 stage, only Ab-V5 showed a reduction in chlorophyll content, whereas MS32 and MS52 remained stable between R5 and R6. MS52 stimulated intense but highly variable nodulation (Table 2). However, even under high nodule numbers, no corresponding increases in chlorophyll content or biomass were observed. Rhizospheric diazotrophic density was similar across treatments, including the non-inoculated control (Table 1 and 2). Rhizospheric bacterial density did not differ among treatments ( $p > 0.05$ ), ranging from  $0.81 \times 10^5$  (MS32) to  $1.72 \times 10^5$  most probable number of bacteria  $g^{-1}$  soil (Ab-V5).



**Figure 2.** Boxplots of common bean seedling emergence (%) and number of nodules under different treatments with associative diazotrophic bacterial seed inoculation. Boxes represent the interquartile range (25th–75th percentile), the horizontal line indicates the median, and dots represent outliers. Letters denote differences according to the Scott-Knott test ( $p < 0.05$ ) only for seedling emergence; no significant differences were observed for nodule number.

**Table 1.** Plant height (PH), stem diameter (SD), leaf area (LA), shoot dry mass (SDM), and root dry mass (RDM) of common bean, cv. Anfc5, inoculated via seeds with associative diazotrophic bacterial strains. Experiment conducted in a greenhouse in a municipality located within the Amazon biome, Sinop, Mato Grosso, Brazil.

Treatments	PH (cm)	SD (cm)	LA (cm <sup>2</sup> )	SDM (g)	RDM (g)
Control	38,87	3,97	290,00	3,29	2,55
Sp7	35,54	4,52	337,94	3,52	2,85
Ab-V5	51,96	4,94	483,34	4,67	2,84
MS32	41,17	4,44	341,54	3,64	2,80
MS52	39,48	4,45	469,40	3,45	2,68
CV (%)	27,31	12,36	43,41	23,67	30,79

Not significant by the F test ( $p < 0.05$ ) for all variables; CV (%): coefficient of variation. Values correspond to the arithmetic means of evaluations conducted in the experiment.

**Table 2.** Mean leaf chlorophyll content (Chlorophyll) at different phenological stages of common bean, cv. Anfc5, inoculated via seeds with associative diazotrophic bacterial strains. Experiment conducted in a greenhouse in a municipality located within the Amazon biome, Sinop, Mato Grosso, Brazil.

Treatments	Chlorophyll (R5)	Chlorophyll (Early R6)	Chlorophyll (Late R6)	Mean
Control	37,51 a A	29,56 a B	27,94 a B	31,67 a
Sp7	35,52 a A	29,22 a B	28,35 a B	31,03 a
Ab-V5	29,98 b A	29,98 a A	27,03 a B	29,00 b
MS32	29,74 b A	29,74 a A	28,91 a A	29,46 b
MS52	29,62 b A	29,62 a A	29,11 a A	29,45 b
CV (%)	32,47 A	29,62 B	28,27 C	

Means followed by the same lowercase letter within columns or uppercase letter within rows did not differ from each other according to the Scott-Knott test at 5% probability. CV (%): coefficient of variation. Chlorophyll - estimated using a ClorofiLOG® chlorophyll meter (model CFL-1030, Falker®). R5 - plants were at pre-flowering. Early R6 - flowering, with approximately 50% of the flowers open. Late R6 - flowering, with all flowers fully open. Values correspond to the arithmetic means of evaluations conducted in the experiment.

The reduced emergence observed for MS52 may reflect host incompatibility or the production of secondary metabolites, as similar effects have been reported for diazotrophic isolates derived from arrowroot sharing the same origin as MS52 (Dias et al., 2020). The absence of significant vegetative responses indicates that associative diazotroph performance in common bean is strongly context-dependent and may be constrained by host genotype and environmental conditions.

The changes in chlorophyll content at R6 suggest a limited contribution to foliar nitrogen accumulation during early reproductive growth, a stage typically associated with peak biological nitrogen fixation (BNF) (Hungria & Neves, 1986). Moreover, the wide variation in nodulation observed for MS52 reinforces that nodule number alone is not a reliable indicator of BNF efficiency, particularly given the low effectiveness of native rhizobia in nitrogen fixation (Moura et al., 2022).

Similar lack of growth responses to diazotrophic inoculation has been reported in bean (Peres et al., 2018; De Souza; Simonetti, 2019; Mellini et al., 2020). In the present study, the short growth cycle of the genotype (80 days) may have restricted the temporal window necessary for effective associative establishment. The comparable rhizospheric bacterial densities across treatments further indicate the presence of native diazotrophs capable of competing with inoculated strains.

Plant growth promotion by Amazonian PGPB strains is not consistently associated with in vitro IAA production or BNF capacity (Canche-luit et al., 2025), suggesting that additional mechanisms may be involved. Future research should further characterize isolates MS32 and MS52 by exploring alternative growth-promotion pathways and identifying common bean genotypes with greater responsiveness to BNF. Investigating the role of other phytohormones may also improve the selection of effective plant growth-promoting bacteria for this crop.

## Conclusion

Seed inoculation with associative diazotrophic bacteria did not significantly improve

common bean growth. Nodulation and the presence of diazotrophs in all treatments, including controls, indicate that a native soil community likely limited the establishment of the introduced isolates. Despite their strong in vitro capacity for nitrogen fixation and auxin production, these traits did not translate into measurable plant growth promotion under the evaluated conditions.

## References

- Baldani, J.I.; Reis, V.M.; Videira, S.S.; Boddey, L.H.; Baldani, V.L.D. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. *Plant and Soil*, v. 384, p. 413-431. 2014.
- Canche-luit, C.E.; Uchôa, S.C.P.; Matos, K.da S.; Schurt, D.A.; Silva, G.F. da.; Maffei, M. et al. Selection of chilli-pepper seedling's growth promoting rhizobacteria from an Amazonian savanna. *Acta Amazonica*, v. 55, p. 55ag23260. 2025.
- Chojnacka, K.; Moustakas, K.; Witek-krowiak, A. Bio-based fertilizers: a practical approach towards circular economy. *Bioresource Technology*, v. 295, p. 122223. 2020.
- De Souza, S. L. S.; Simonetti, A. P. M. Inoculação e coinoculação de *Rhizobium* e *Azospirillum* na cultivar de feijão BRS FC 104. *Revista Cultivando o Saber*, p. 14-23. 2019.
- Dias, I.G.; Versari, L.R.; Rodrigues, L.C.; Felipe, R.T.A.; Couto, M.F.; Sabino, D.C.C. Capacidade de produção de auxina e promoção do crescimento de plântulas de arroz por isolados de *Azospirillum*. In: Silva, R.J. Iniciação Científica e Suas Múltiplas Aplicabilidades 1. [S. I.]. *Stricto Sensu*, Rio Branco, p.80-92. 2020.
- Hungria, M.; Neves, M.C.P. Ontogenia da fixação biológica do nitrogênio em *Phaseolus vulgaris*. *Pesquisa Agropecuária Brasileira*, v. 21, p. 715-730. 1986.

- Hungria, M.; Campo, R.J.; Souza, E.M.; Pedrosa, F.O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil*, v. 331, p. 413-425. 2010.
- IBGE. Instituto Brasileiro de Geografia e Estatística. Biomas e sistema costeiro-marinho do Brasil: compatível com a escala 1:250 000. 164 p. 2019. Available in: <https://biblioteca.ibge.gov.br/index.php/biblioteca-catalogo?view=detalhes&id=2101676>. Accessed on 01 dez 2025.
- Kumar, A.; Naroju, S.P.; Kumari, N.; Arsey, S.; Kumar, D.; Gubre, D.F. et al. The Role of Drought Response Genes and Plant Growth Promoting Bacteria on Plant Growth Promotion under Sustainable Agriculture: A Review. *Microbiological Research*, v. 286, p. 127827. 2024.
- Lopes, M.J.S.; Cardoso, A. F.; Dias-Filho, M. B.; Gurgel, E.S.C.; Silva, G.B. da. Brazilian Amazonian microorganisms: A sustainable alternative for plant development. *AIMS Microbiology*, v. 11, p. 150-166. 2025.
- Mellini, B. S et al. Inoculação e co-inoculação no desenvolvimento agrônomo do feijoeiro. *Cadernos de Agroecologia*, v. 15, n. 1. 2020.
- Mendoza-Suárez, M.; Andersen, S.U.; Poole, P.S.; Sánchez-Cañizares, C. Competition, nodule occupancy, and persistence of inoculant strains: key factors in the rhizobium-legume symbioses. *Frontiers in Plant Science*, v. 12, p. 690567. 2021.
- Moura, F.T.; Ribeiro, R.A.; Helene, L.C.F.; Nogueira, M.A.; Hungria, M. So many rhizobial partners, so little nitrogen fixed: The intriguing symbiotic promiscuity of common bean (*Phaseolus vulgaris* L.). *Symbiosis*, v. 86, p. 169-185. 2022.
- Peres, A.R et al. Efeito do cultivo de feijão com co-inoculação (*Rhizobium tropici* e *Azospirillum brasilense*) e lâminas de irrigação sobre a qualidade fisiológica das sementes produzidas. *Investigación Agraria*, v. 20, n. 1, p. 11-21. 2018.
- Rodrigues, L.C.; Pereira, K.G.S.; Versari, L.R.; Dias, I.G.; Couto, M.F.; Sabino, D.C.C. Influência do meio de cultura na caracterização fenotípica de isolados de *Azospirillum*. In: Silva, R.J. Iniciação Científica e Suas Múltiplas Aplicabilidades 2. [S. l.]. Stricto Sensu, Rio Branco, p.82-96. 2020.
- Sarao, S.K.; Boothe, V.B.; Das, B.K.; Gonzalez-Hernandez, J.L.; Brozel, V.S. *Bradyrhizobium* and the soybean rhizosphere: Species level bacterial population dynamics in established soybean fields, rhizosphere and nodules. *Plant and Soil*, v. 508, p. 515-530. 2024.
- Silva, C.G.M.; Moreira, S.F.; Moraes, L.C.; Gaudencio, J.R.F.; Pimentel, G.V. Nutrient extraction and export by determinate and indeterminate common bean cultivars1. *Pesquisa Agropecuária Tropical*, v. 54, p. e78404. 2024.
- Somers, E.; Ptacek, D.; Gysegom, P.; Srinivasan, M.; Vanderleyden, J. *Azospirillum brasilense* produces the auxin-like phenylacetic acid by using the key enzyme for indole-3-acetic acid biosynthesis. *Applied and environmental microbiology*, v. 71, p. 1803-1810. 2005.
- Sousa, D.M.G.; Lobato, E. Cerrado: correção do solo e adubação. 2nd ed. Embrapa Cerrados, Brasília. 416p. 2004.