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ORIGINAL ARTICLE

Biotechnological potential of *Pectobacterium* sp. endophyte on the growth of soy and bean plants

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ABSTRACT: Fertilizers and chemical pesticides supply plants' nutritional requirements and protect them against pathogens. However, they may cause harm to the environment. *Pectobacterium* sp. has been reported as an endophyte with the capacity of a plant-growth promoter, especially in the control of phytopathogens. The current paper evaluates the capacity of the endophytic bacterium *Pectobacterium* sp. MG-60 isolated from *Mikania glomerata* Spreng. (Asteraceae) to antagonize phytopathogens *in vitro*, produce IAA, and fix nitrogen (*nifH*). Growth promotion assessments of bean and soybean plants under greenhouse conditions were also performed. The rate of antagonism against *Sclerotinia sclerotiorum* was 52.5%. The endophyte *Pectobacterium* sp. MG-60 produced 3.54 $\mu\text{g mL}^{-1}$ of IAA when 5 mM L-tryptophan in the culture medium was used. Under greenhouse conditions, significant results for *Pectobacterium* sp. MG-60 in the growth of soybean and common bean plants were observed, especially in the height of common bean (19%) and soybean (20%), fresh weight of shoot (18.5%) and fresh weight of roots (46.8 %) of soybean plants. This is the first report on *Pectobacterium* isolated as an endophyte from *M. glomerata* with plant growth-promoting agent abilities with biotechnological potential for the agricultural field.

Keywords: endophyte; IAA; *Mikania glomerata*; *nifH*; plant-growth promoter.

Potencial biotecnológico de *Pectobacterium* sp. endofítico no crescimento de plantas de soja e feijão

RESUMO: Fertilizantes e defensivos químicos atuam suprindo as necessidades nutricionais das plantas e protegendo contra patógenos; entretanto, podem acarretar danos ambientais. *Pectobacterium* sp. já foi reportada atuando como endófito e apresentando capacidade de agente promotor do crescimento de plantas, especialmente no controle de fitopatógenos. Assim, o objetivo desta pesquisa foi avaliar as habilidades de antagonizar fitopatógenos *in vitro*, produzir AIA e fixar o nitrogênio (*nifH*) da bactéria endofítica

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Pectobacterium sp. MG-60, isolada de *Mikania glomerata* Spreng. (Asteraceae). Avaliações da promoção do crescimento de plantas de feijão e soja em condições de casa de vegetação também foram realizadas. O índice de antagonismo contra *Sclerotinia sclerotiorum* foi de 52,5%. Empregando 5 mM de L-triptofano em meio de cultura, o endófito *Pectobacterium* sp. MG-60 produziu 3,54 µg mL⁻¹ de AIA. Em condições de casa de vegetação, uma atuação significativa de *Pectobacterium* sp. MG-60 no crescimento das plantas de soja e feijão foi observada, especialmente na altura do feijoeiro (19%) e da soja (20%), no peso fresco da parte aérea (18,5%) e no peso fresco das raízes (46,8%) das plantas de soja. Aqui, relatamos pela primeira vez uma *Pectobacterium* isolada como endófito de *M. glomerata* com habilidades de agente promotor do crescimento vegetal com potencial biotecnológico para o setor agrícola..

Palavras-chave: AIA; endófito; *Mikania glomerata*; nifH; promotor do crescimento vegetal.

1 Introduction

The interaction between plants and microorganisms may favor resistance to biotic/abiotic stress conditions, help promote growth through the production of plant hormones and even produce compounds from secondary metabolism that act as antimicrobial and anti-insecticide agents (CAVALCANTE *et al.*, 2007; SOUZA *et al.*, 2017).

Emphasis should be given to endophytic microorganisms, among those associated with plants, which are commonly defined as a polyphyletic group (fungi and bacteria) that live asymptotically inside plant tissues and that may or may not produce structures external to their hosts. In this way, these microorganisms can be isolated from tissues previously superficially disinfected (AZEVEDO; ARAÚJO, 2007; KUSARI; SINGH; JAYABASKARAN, 2014).

In agriculture, many producers use fertilizers and chemical pesticides during production to supply plants with nutritional requirements and protect them against pathogen attacks. However, environmental damage may be eventually caused (DAMIAN *et al.*, 2018).

According to data presented by the Food and Agriculture Organization of the United Nations (FAO, 2019), in 2019, approximately 333 million tons of soy were produced worldwide. Brazil was responsible for 114 million tons (34%). Further, world bean production was around 54 million tons, with Brazil contributing approximately 3,000 tons.

Since beans (*Phaseolus vulgaris* L.) and soybeans (*Glycine max* L.) are two crops of great importance in the Brazilian and world economic scenarios, the development of strategies that will guarantee such products, whether through enhancement of the growth of these plants or through the biological control of phytopathogens, is justified (OLIVEIRA *et al.*, 2020; RABHA *et al.*, 2014; RIBEIRO *et al.*, 2021; SILVA *et al.*, 2016). Several phytopathogenic fungi, such as *Colletotrichum* sp. and *Sclerotinia* sp., affect these crops and cause heavy losses during the production system.

The use of microbial agricultural inoculants, especially those with endophytic microbial consortia, may have positive impacts on the economy and the environment by playing a role that is similar to that of chemical pesticides/fertilizers, albeit without their adverse impacts on the environment (OSES *et al.*, 2018).

Pectobacterium sp. has already been reported to act as an endophyte and present the capacity of a plant growth promoter, especially in the control of

phytopathogens (SILVA *et al.*, 2012). As far as we know, there are no reports on *Pectobacterium* as a growth-promoting agent in beans and soybeans. Thus, the objective of current authors was to evaluate the abilities to antagonize *in vitro* *Colletotrichum* sp. and *S. sclerotiorum*, produce indoleacetic acid (IAA), and fix nitrogen (*nifH*) of the endophytic bacterium *Pectobacterium* sp. MG-60 isolated from *Mikania glomerata* Spreng. (Asteraceae). Growth promotion assessments of bean and soybean plants under greenhouse conditions were also performed.

The current work reports the study of an endophytic bacterium as a growth-promoting agent of two plants of agronomic interest, soybean and common bean, respectively. This type of study exemplifies an application for eco-friendly agriculture compared to harmful chemical strategies.

The methods employed to assess the antagonist activity of bacterium MG-60, the production of IAA, and the molecular identification of the presence of *nifH* genes are outlined in Section 2. Additionally, the process of conducting tests in a greenhouse environment also is discussed in Section 2. The findings, along with the relevant discussions, can be found in Section 3, while the final thoughts are presented in Section 4.

2 Material and methods

To evaluate the characteristics of the endophytic bacterium MG-60 as a plant growth-promoting agent, we performed *in vitro* and *in vivo* tests (greenhouse). First, we identified the microbial strain through DNA sequencing and phylogenetic analysis, and then evaluated its ability to antagonize two phytopathogens and produce indoleacetic acid. We also verified the presence of genes related to biological nitrogen fixation and, finally, we tested the inoculation of the endophytic bacterium in bean and soybean seeds under greenhouse conditions.

2.1 Microorganisms

MG-60 bacterium was isolated as an endophyte from *Mikania glomerata* Spreng. (Asteraceae) leaves in 2017. It belongs to the Collection of Endophytic and Environmental Microorganisms (Coleção de Microrganismos Endofíticos e do Ambiente – CMEA) of the Microbial Biotechnology Laboratory (Laboratório de Biotecnologia Microbiana – LBIOMIC) of the State University of Maringá (Maringá-PR/ Brazil).

The bacterial strain was chosen due to its biological nitrogen fixation in a semi-solid medium and to its antagonist activity against the phytopathogen *Fusarium oxysporum* (data not shown) (BULLA, 2017).

The bacterial strain preserved in 25% glycerol in TSB (Trypticase Soy Broth) medium, at -80 °C, was reactivated in TSB (pH 7.3) medium for 48 hours at 28 °C and later transferred to TSA (Trypticase Soy Agar, pH 7.3) medium and grown for 24 hours at 28 °C.

Phytopathogenic fungi *S. sclerotiorum* and *Colletotrichum* sp. CNPUV38 also belong to CMEA of LBIOMIC and were grown on PDA medium (Potato Dextrose Agar, pH 6.6) for seven days at 28 °C.

2.2 Extraction of bacterial DNA

After the cultivation of endophytic bacterium in TSB medium, 400 µL of saturated phenol solution were added, shaken (vortex®) and centrifuged at 16,000 xg for 5 minutes. The supernatant was collected and the phenolic step was repeated. After centrifugation, the supernatant was transferred to a new microtube for the addition of 400 µL of chloroform. The microtube was shaken (vortex®) and centrifuged for 5 min at 16,000 xg. The aqueous phase was transferred to another microtube, where 1 mL of ice-cold ethanol was added. To complete the DNA extraction process, the microtube was centrifuged for 3 min at 16,000 xg and a DNA pellet was formed. Ethanol was discarded and the tube was left at room temperature for 24 hours so that ethanol residues evaporated. The pellet was then eluted in 15 µL of autoclaved milli-Q water.

2.3 Amplification of the 16S rRNA region

Assay followed Procópio *et al.* (2009), with slight modifications, using oligonucleotide pairs R1378 (5'-GGTGTGTACAAGGCCCGGGAACG-3') and PO27F (5'-GAGAGTTTGATCCTGGCTCAG-3').

PCR reaction was performed in a buffer (200 mM Tris-HCl, pH 8.4 – 500 mM KCl, 1x concentrate), dNTP (2.5 mM), primers (10 pMol), Taq DNA polymerase (5 U mL⁻¹), MgCl₂ (50 mM), autoclaved milli-Q water, and DNA sample (20 ng µL⁻¹) in a final volume of 50 µL.

Conditions for amplification comprised initial denaturation at 94 °C for 4 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 1 min, extension at 72 °C for 1 minute and final extension at 72 °C for seven minutes. Amplification products were visualized in a 1.5% agarose gel stained with ethidium bromide, observed in photo documentation equipment.

2.4 Amplification of the *nifH* gene

The presence of the *nifH* gene was evaluated by universal degenerate oligonucleotide pairs *Z-nifHf* (5'-TGYGAYCCNAARGCNGA-3') and *Z-nifHr* (5'-ADNGCCATCATYTCNCC-3') (ZEHR; McREYNOLDS, 1989), *NifHf-2* (5'-AAAGGYGGWATCGGYAARTCCACCAC-3') and *NifHr-2* (5'-TTGTTSGCSGCRTACATSGCCATCAT-3') (SZILAGYI-ZECCHIN *et al.*, 2014).

PCR reactions were performed for a final volume of 25 µL containing buffer (200 mM Tris-HCL, pH 8.4 – 500 mM KCL, 1x concentrate), dNTPs (2.5 mM), primers (100 pMol), Taq DNA polymerase (5 U µL⁻¹), MgCl₂ (50 mM), milli-Q water and DNA sample (20 ng µL⁻¹).

Amplifications consisted of an initial denaturation at 94 °C for 2 minutes, followed by 30 cycles with denaturation at 94 °C for one min, annealing at 55 °C for 45 s, extension at 72 °C for three min, with a final extension at 72 °C for five min. The amplification products were visualized in a 2% agarose gel stained with ethidium bromide.

2.5 Sequencing and molecular identification

The purification of the amplification products of the 16S rRNA region and *nifH* were performed with the combination of the enzymes *Shrimp Alkaline Phosphate* (SAP) and *Exonuclease I* (EXO), incubation in a thermocycler for 1 hour at 37 °C, followed by another 15 min at 80 °C. The purified *amplicons* were sequenced with ABI-PRISM 3100 Genetic Analyzer equipment (Applied Biosystems) by Ludwig Biotecnologia LTDA. The sequences obtained were evaluated for their quality by BioEdit v.7.2.5. The 16S rRNA sequences were submitted for chimerism analysis by DECIPHER Find Chimeras web tool (WRIGHT; YILMAZ; NOGUERA, 2012).

So that the endophytic bacterium based on the 16S rRNA region could be identified, the nucleotide sequences were compared with those deposited at the National Center for Biotechnology Information Website (NCBI) database by BLASTn tool, applying *sequences from type materials* for greater data robustness. Identification was based on the best value obtained for identity.

GenBank sequences with high similarity rates with the obtained sequence were then retrieved and aligned by ClustalW in MEGA 6.05, together with the MG-60 isolate sequence. A phylogenetic tree was built with grouping by the neighbor-joining method (SAITOU; NEI, 1987), using p-distance for nucleotides with the pairwise gap deletion option and bootstrap with 10,000 repetitions. The sequence from *Erwinia aphidicola* (AB681773.1) was used as an outgroup.

2.6 *In vitro* antagonism

Further, *in vitro* antagonist of MG-60 strain against phytopathogens *S. sclerotiorum* and *Colletotrichum* sp. CNPUV38 was evaluated with five repetitions, following the methodology by Specian *et al.* (2016). The inhibition index was assessed by measuring the mycelial growth area of the phytopathogen using ImageJ v 1.46r software, according to Oliveira *et al.* (2020).

2.7 IAA production

The evaluation of IAA production using 10% TSB medium, added to 5 mM of L-tryptophan, and quantitative analysis with readings in a spectrophotometer (520 nm) was performed according to Emmer *et al.* (2021). Readings were normalized by standard curve $R^2 = 0.99$ obtained with different concentrations of the commercial standard of 3-indoleacetic acid (10, 25, 50, 100 and 150 $\mu\text{g mL}^{-1}$).

2.8 Greenhouse assay

The MG-60 endophytic bacterium was previously cultivated in TSB medium (pH 7.3) for 24 hours at 28 °C and later adjusted in 0.85% saline for a concentration of 10^8 CFU mL^{-1} , according to McFarland's scale 0.5. Commercially acquired soybean and bean seeds were superficially disinfected with a solution of sodium hypochlorite, 70% ethanol and distilled water in a laminar airflow chamber, according to the methodology by Oliveira *et al.* (2020).

Seeds were transferred to bacterial suspension, immersed for 30 minutes, transferred to pots with 90 g of autoclaved soil and incubated for 20 days in a greenhouse at the

Department of Agronomy of the State University of Maringá (Maringá, Paraná, Brazil). Controls consisted of seeds merely immersed in 0.85% saline without bacterial suspension.

The entire experiment was replicated ten times. Biometric parameters, such as the number of leaves, plant size, root size and fresh biomass, were analyzed after 20 days of incubation.

2.9 Statistical analysis

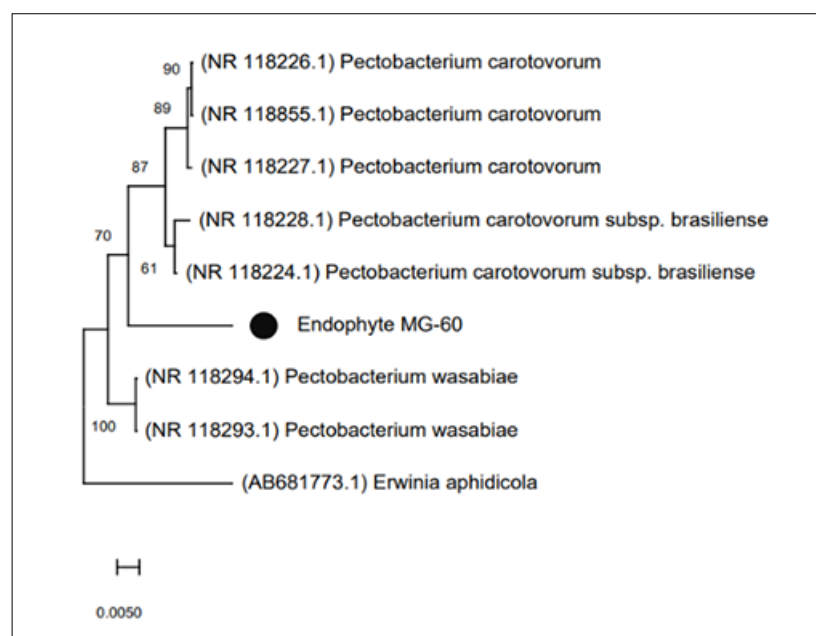
Data from the *in vitro* antagonism, the AI% values were analyzed using analysis of variance and means were compared by Scott-Knott test ($p < 0.05$) using SISVAR 5.6 (FERREIRA, 2011). In the case of greenhouse assay, means of biometric parameters were compared by the Scott-Knott test with statistical significance $p < 0.005$ (FERREIRA, 2011).

3 Results and discussion

According to BLASTn analyses, the endophytic bacterium MG-60 showed 96% identity with species of the genus *Pectobacterium* sp., such as *P. wasabiae* (access codes NR_118294.1 and NR_118293.1), *P. carotovorum* (NR_118226.1, NR_118855.1 and NR_118227.1) and *P. carotovorum* subsp. *brasiliense* (NR_118224.1 and NR_118228.1). Figure 1 shows molecular identification based on the 16S rRNA region. Foregrounded on the phylogenetic analysis using the neighbor-joining method and BLAST, the endophytic bacterium MG-60 was identified at the taxonomic level of the genus as *Pectobacterium* sp. (Figure 1). The sequence obtained was deposited in GenBank (accession number MZ491095).

Figure 1 ►

Dendrogram of MG-60 endophytic bacterium isolated from *Mikania glomerata* constructed with 16S rRNA gene cluster by neighbor-joining method. Probabilities (bootstrap) are shown on the nodes between each individual. Type-strain *Erwinia aphidicola* (AB681773.1) was used as an outgroup. Source: elaborated by the authors.



The *Pectobacterium* sp. MG-60 isolate showed amplification for the two genes of the *nif* region used (*Z-nifH* and *NifH-2*) and the sequences obtained were deposited in GenBank (accession numbers MZ501698-MZ501699).

Although the genus *Pectobacterium* has already been reported as pathogenic for several groups (NIEMI *et al.*, 2017; RAJAMANICKAM *et al.*, 2018), this genus of bacteria has also been reported as playing the role of endophytes (SILVA *et al.*, 2012).

The endophytic bacterium *Pectobacterium* sp. MG-60 showed inhibitory activity against the two fungal pathogens evaluated when compared statistically with controls featuring the phytopathogen only (Table 1). Compared to the phytopathogen control group that was inoculated at a single point on the petri dish (Control 1), the endophytic bacterium demonstrated the same level of efficacy against *S. sclerotiorum* as the commercial fungicide (Control 2) (Table 1, Figure 2). The antagonism index against *Colletotrichum* sp. was 18.14% (Table 1, Figure 2).

Table 1 ▶

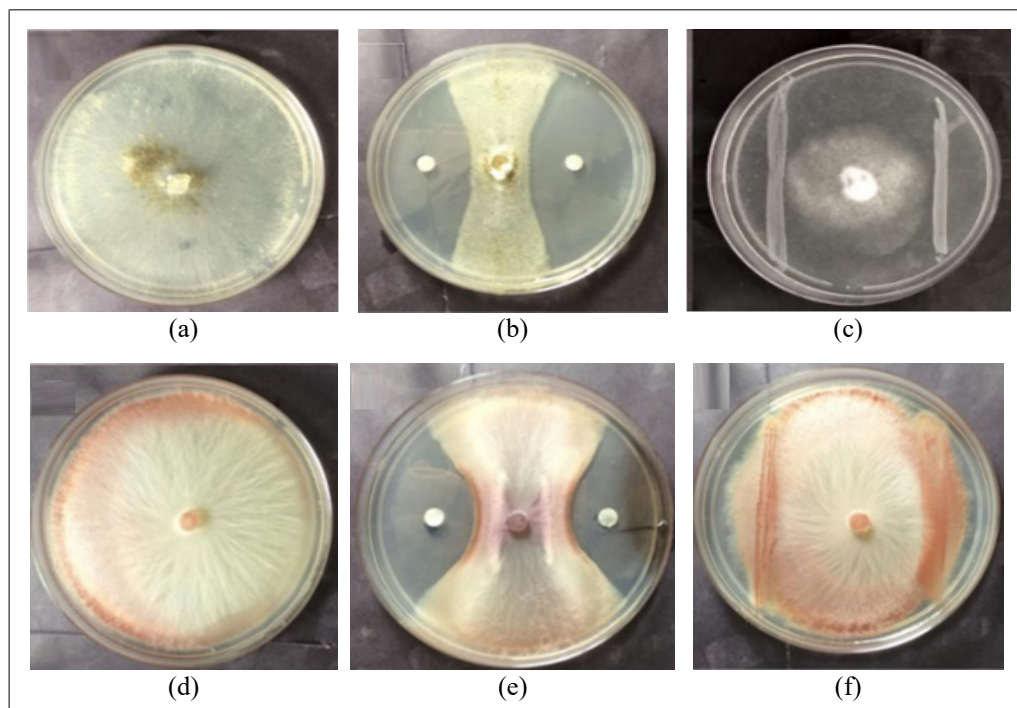
In vitro evaluation of the antagonistic activity of the endophytic bacterium MG-60 isolated from *Mikania glomerata* against pathogens *Colletotrichum* sp. CNPU378 and *Sclerotinia sclerotiorum*.
Source: survey data

<i>Colletotrichum</i> sp. CNPUV378		
Treatment	Mycelial growth (cm ²)	AI (%)
Control 1	53.1 c	---
Control 2	40.6 a	23.5
MG-60	43.4 b	18.1
<i>Sclerotinia sclerotiorum</i>		
Treatment	Mycelial growth (cm ²)	AI (%)
Control 1	54.3 b	---
Control 2	25.5 a	53
MG-60	25.8 a	52.5

Means of the five replicates. Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$). Control 1: Only the phytopathogen; Control 2: phytopathogen against commercial fungicide; AI (%): Antagonism index calculated following the methodology by Oliveira *et al.* (2020).

Figure 2 ▶

Dual culture assay of the MG-60 endophyte against *Colletotrichum* sp. CNPU378 and *Sclerotinia sclerotiorum*. (a) Control only with the phytopathogen *Sclerotinia sclerotiorum*. (b) *S. sclerotiorum* (center) against fungicide (on the sides). (c) Endophyte MG-60 against *S. sclerotiorum* (center). (d) Control only with the phytopathogen *Colletotrichum* sp. CNPUV378. (e) *Colletotrichum* sp. CNPUV378 (center) against fungicide (on the sides). (f) Endophyte MG-60 against *Colletotrichum* sp. CNPUV378 (center).
Source: authors' archive



Specian *et al.* (2016) isolated endophytic bacteria from *Malpighia emarginata* leaves and evaluated their abilities against different agronomically important phytopathogens. Considering isolates from the Enterobacteriales order, these authors found antagonism rates ranging between 0% and 34% against *S. sclerotiorum*. Silva *et al.* (2020), bioprospecting endophytic bacteria associated with *Aloe vera* roots, obtained a 45% inhibition rate. Results by authors above and those found in the current work with the endophyte *Pectobacterium* sp. MG-60 ($AI\% = 52.5\%$), corroborate the idea that endophytes of this order may act as antagonists of pathogens, especially against *S. sclerotiorum*.

Bacterial auxins may promote plant growth through several mechanisms, including adventitious root formation and stem and root elongation. The main auxin found in plants is IAA (OLANREWAJU; GLICK; BABALOLA, 2017). Endophyte *Pectobacterium* sp. MG-60 produced $3.54 \mu\text{g mL}^{-1}$ of IAA when 5 mM L-tryptophan in culture medium was used. This rate may favor plant growth since the phytohormone acts at low concentrations on the host's metabolism (BATISTA *et al.*, 2018; LACZESKI *et al.*, 2020; ZHAO; XU; LAI, 2018).

The current assay revealed that *Pectobacterium* sp. MG-60 showed an *in vitro* ability to produce IAA and the authors detected the presence of genes (*nifH*) that participate in the biological nitrogen fixation mechanism, in addition to antagonizing phytopathogens. The above suggests its potential as a plant growth promoter.

Results in Table 2 demonstrate the growth of soybean seedlings inoculated with the endophyte *Pectobacterium* sp. MG-60, with statistically significant differences for plant height and fresh biomass, when compared to those in control plants (Table 2, Figure 3).

Table 2 ►

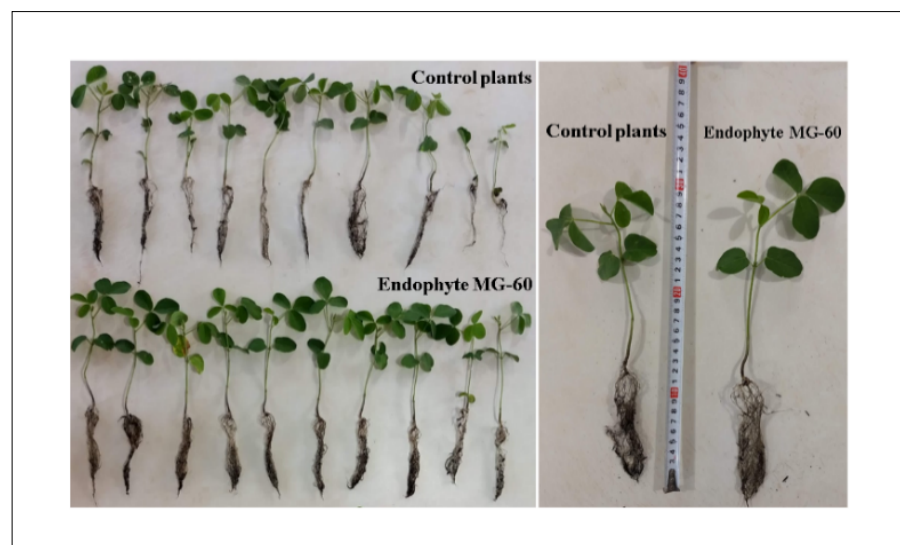
Greenhouse assay with endophytic bacterium MG-60 on *Glycine max* (soybean) growth. Biometric parameters were evaluated 20 days after inoculation.
Source: survey data

Trial	NF	Height	RL	FSW	FRW
Control	8.2 a	7.5 b	14.6 a	0.97 b	0.32 b
MG-60	8.3 a	9.0 a	14.9 a	1.15 a	0.47 a

This means of the ten replicates. Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$). NF: Number of leaves; RL: Root length (cm); FSW: Fresh shoot weight (g); FRW: Fresh root weight (g).

Figure 3 ►

Growth-promoting evaluation of the *Pectobacterium* sp. MG-60 endophytic bacterium on soybean plants.
Source: authors' archive



In the case of bean plants (Table 3, Figure 4), there was a statistical difference for root length (8.03 cm) and height (6.1 cm) of plants inoculated with the endophyte when compared to those in control plants (root length = 7.14 cm; height = 5.1 cm).

Table 3 ▶

Greenhouse assay with endophytic bacterium MG-60 on the growth of *Phaseolus vulgaris* (common beans). Biometric parameters were evaluated 20 days after inoculation.

Source: survey data

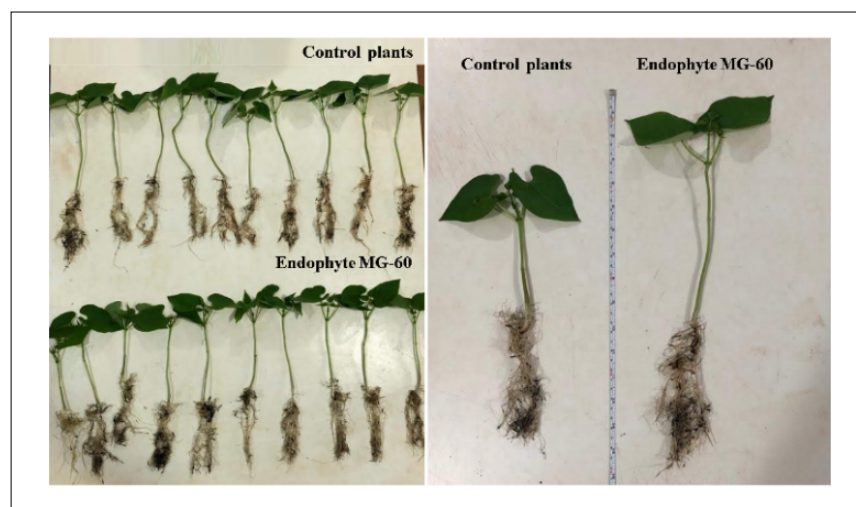
Trial	NF	Height	RL	FSW	FRW
Control	3.8 a	5.1 b	7.14 b	2.28 a	1.40 a
MG-60	4.4 a	6.1 a	8.03 a	2.42 a	1.25 a

This means of the ten replicates. Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$). NF: Number of leaves; RL: Root length (cm); FSW: Fresh shoot weight (g); FRW: Fresh root weight (g).

Figure 4 ▶

Growth-promoting evaluation of the *Pectobacterium* sp. MG-60 endophytic bacterium on bean plants.

Source: authors' archive



Emmer *et al.* (2021) evaluated three endophytic bacteria belonging to the order Enterobacteriales (*Erwinia* sp. and *Pantoea* sp.) isolated from the ornamental plant *Echeveria laui*. The growth-promoting capacities of these bacterial isolates on the growth of the common bean were investigated. The authors also detected positive results regarding the height/shoot and biomass of plants inoculated with these endophytes when compared to their non-inoculated controls.

The results in Tables 2 and 3 suggest a significant role of *Pectobacterium* sp. MG-60 on the growth of soybean and common bean plants evaluated, especially height for beans (19%) and soybean (20%), shoot fresh weight (18.5%) and root fresh weight (46.8 %) of soybean plants. Zhao, Xu and Lai (2018) evaluated the response of endophytic bacterial isolates on soybean growth and also obtained significant indices ranging between 13% and 38% for the analyzed biometric parameters of treated plants in relation to untreated soy plants.

Biological nitrogen fixation (BNF) may positively contribute to plant growth (PURI; PADDA; CHANWAY, 2015). Studies on agents that promote plant growth, in particular soy and beans, normally focus on those bacteria that are nodules or on those present in the rhizosphere. As a result, the leaf endophytic community remains understudied. As mentioned above, the endophytic bacterium *Pectobacterium* sp. MG-60 presented two coding regions of the *nifH* gene (accession numbers MZ501698-MZ501699) that participate in the expression of the nitrogenase enzyme complex (KNEIP *et al.*, 2007).

The nitrogenase complex is made up of two main functional subunits, dinitrogenase reductase and dinitrogenase. The structural components of these subunits are the *nif* proteins – *nifH* and *nifD*. Three types of nitrogenases are known based on the composition of their metal centers: iron and molybdenum (Fe/Mo), iron and vanadium (Fe/V) or simply iron (Fe) (KNEIP *et al.*, 2007; REES; HOWARD, 2000).

Studies developed by Moyes *et al.* (2016) demonstrate that leaf endophytes may effectively contribute towards nitrogen fixation. To support this hypothesis, the authors did not find significant differences in the reduction of acetylene in the leaf interior. The above-indicated nitrogenase activity since acetylene may inhibit nitrogenase activity (SCHÖLLHORN; BURRIS, 1966).

Aryantha and Hidiyah (2018) performed the inoculation of an endophytic diazotrophic bacteria in palm leaves. The authors found that there was an increase of up to 39% in NH_4^+ present in the leaves, indicating the conversion of N_2 into ammonia.

Findings by these authors suggest that the biological fixation of N_2 by endophytic bacteria present in leaves may occur through ammonification. Atmospheric N_2 enters the cell through the stomatal openings and, since the endophyte has the genetic machinery to express the nitrogenase complex, it converts the N_2 into NH_4^+ . Consequently, BNF would occur through ammonification. An increase in ammonium ions within the cell may cause a cellular imbalance to become toxic. This fact would be resolved by the action of enzymes glutamate synthase and glutamine synthase that converts NH_4^+ into amino acids and are incorporated into the plant's metabolism (BREDEMEIER; MUNDSTOCK, 2000; MIYAZAWA *et al.*, 2018).

The high sensitivity of nitrogenase to oxygen would be an impasse since the leaf is a tissue specialized in carrying out photosynthesis. However, some authors suggest that a protection mechanism used by several species to protect nitrogenase from O_2 comprises fixation at night when the photosynthetic rate is lower (DOTY, 2017; MARCHAL; VANDERLEYDEN, 2000).

These hypotheses, coupled with the ability of the bacterium to produce IAA, the presence of the *nifH* gene and results in Tables 1, 2, and 3 suggest its potential as a plant growth-promoter, especially in beans and soybeans, since fertilizers are based on microorganisms (biofertilizers) which act in the production of phytohormones, nitrogen fixation and pathogen control (BAKHTIYARIFAR; ENAYATIZAMIR; KHANLOU, 2021; COCKING, 2003; KUKLINSKY-SOBRALE *et al.*, 2004).

Microorganism-based inoculants have shown prominence not only in agricultural systems for being more sustainable, but also in the industrial production scenario. For the production of these biological products to be successful, it is important that the culture medium used for their production is low-cost and allows the large-scale production of the desired microbial cells (ELNAHAL *et al.*, 2022; SANTOS; NOGUEIRA; HUNGRIA, 2019). As an example, we can mention the use of agro-industrial by-products (ROMANO *et al.*, 2020) or even the use of methods such as *on Farm*, which may contribute to reducing the production costs associated with this process (CZERNIAK; STURMER, 2014).

4 Conclusion

The current paper reports for the first time a *Pectobacterium* isolated as an endophyte from *Mikania glomerata* with abilities as a plant growth-promoter, in particular from two plants of great importance for the world and national agriculture, such as beans and

soybeans. While producing IAA and fixing nitrogen, *Pectobacterium* sp. MG-60 may also antagonize phytopathogens, especially *S. sclerotiorum*. The chemical elucidation of bioactive compounds produced by this endophyte with antifungal capacity is necessary for the future. Further, evaluations at other stages of development and application in cropping systems employing this endophyte are also required since promising results have been found, which will enable the formulation of an eco-friendly endophytic biofertilizer.

Acknowledgments

We would like to dedicate this work to Prof. Dr. João Alencar Pamphile, who painfully passed away as a result of COVID-19. We are extremely grateful for his contributions. Our affection, love and admiration. Rest in peace!

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Conflict of interest

The authors declare no conflict of interest.

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