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BIOTECHNOLOGICAL POTENTIAL OF SOYBEAN PLANT GROWTH-PROMOTING RHIZOBACTERIA[1]

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ABSTRACT – Technologies that use rhizobacteria to promote plant growth are increasing in agriculture, results have shown improvements in soil quality, increases in productivity, and decreases in the use of synthetic inputs, The objective of work was to characterize bacterial isolates regarding their biological activity and growth promotion of soybean plants grown in a controlled environment. Fifteen bacteria were isolated from soils with continuous use of biological fertilizer. They were evaluated for enzymes production (amylase and protease), nitrogen fixation, antagonistic activity to phytopathogenic fungi, and indoleacetic acid (IAA) production, Soybean seeds were inoculated with bacterial isolates in a greenhouse and evaluated for plant development and soil chemical attributes. The results showed that 8 of the 15 isolates presented production of amylase, protease, or both and 4 isolates presented nitrogen-fixing capacity. The percentage of isolates with high or moderate inhibitory action against the fungi *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, and *Fusarium solani* were 73.3%, 66.6%, and 73.3%, respectively. The IAA production varied from 8.56 to 31.33 μg mL^{-1} (5 isolates had low, 6 had moderate, and 4 had high production). The soybean development was significantly higher in 80% of the treatments with inoculation with bacterial isolates. Five bacterial isolates effectively present all characteristics for use as inoculant (biofertilizer) to promote the development of soybean plants.

Keywords: Bioprospecting. Indoleacetic acid. PGPR. Inoculant. *Glycine max*.

POTENCIAL BIOTECNOLÓGICO DE RIZOBACTÉRIAS PROMOTORAS DE CRESCIMENTO DE PLANTAS DE SOJA

RESUMO – O uso de tecnologias utilizando rizobactérias para promover o crescimento de plantas é crescente na agricultura, resultados demonstram melhoria na qualidade dos solos, aumentando a sua produtividade e diminuindo uso de insumos sintéticos. O objetivo do trabalho foi caracterizar os isolados bacterianos quanto a sua atividade biológica e promoção do crescimento de plantas de soja em ambiente controlado. Foram isoladas 15 bactérias de solo com uso contínuo de adubo biológico. Estes foram avaliados quanto à produção de enzimas (amilase e protease), fixação de nitrogênio, atividade antagônica a fungos fitopatogênicos e produção de Ácido Indol Acético (AIA). Em casa de vegetação, sementes de soja inoculadas com os isolados bacterianos foram avaliadas quanto o desenvolvimento da plantas de soja e os atributos químicos do solo. Os resultados demonstraram que dos 15 isolados 8 apresentam a produção de amilase ou protease, ou ambos e 4 isolados apresentam capacidade de fixar nitrogênio livre. A porcentagem dos isolados com ação inibitória de moderada a alta contra os fungos *Sclerotinia sclerotiorum*, *Macrophomina phaseolina* e *Fusarium solani* foram 73,3%, 66,6% e 73,3% respectivamente. E, quanto a produção AIA variou de 8,56 a 31,33 μg mL^{-1} (5 isolados com baixa produção, 6 moderada e 4 alta). O desenvolvimento da soja foi significativamente maior em 80% dos tratamentos inoculados com os isolados bacterianos. Conclui-se que 5 isolados bacterianos efetivamente apresentam todas características para utilização como inoculante (biofertilizante) no desenvolvimento de soja.

Palavras chave: Bioprospecção. Ácido indol acético. RPCP. Inoculante. *Glycine max.*

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INTRODUCTION

The rhizosphere is a changeable habitat; its composition and structure are affected throughout the vegetative cycle of plants (TERRA et al., 2019). The soil microbial community population varies according to the plant species; moreover, the release of exudates by the root can cause chemical and physical changes in the rhizosphere, which benefits or inhibits microbial growth in this region. Thus, the microbial community in the rhizosphere is higher than that of the rhizoplane (MCNEAR JR, 2013).

Plant growth promoting rhizobacteria (PGPR) promote plant growth by colonizing roots; they are essential in assisting the plant establishment and development under nutrient deficit conditions (BENEDUZI; AMBROSINI; PASSAGLIA, 2012). This developmental stimulus to plants can be caused by different direct and indirect forms of action, such as the increase in nutrient availability, mainly phosphorus, caused by the solubilization of P forms of low solubility; increase in nitrogen, by biological fixation of atmospheric nitrogen; production of phytohormones; and control of pathogens (MANRIQUE et al., 2019).

PGPR are important agents of biological control; they can suppress pathogenic microorganisms in the rhizosphere by producing β-1.3-glucanase, siderophores, hydrocyanic acid, and antibiotics (MAKSIMOV, ABIZGIL'DINA; PUSENKOVA, 2011).

According to Tilak et al. (2005), the demand of PGPR biofertilizers has increasing continuously due to the increasing importance of an organic agriculture with minimum use of chemical inputs. A sustainable agriculture can be reached by emphasizing the use of PGPR through inoculation of biofertilizers (SCHIPPERS et al., 1995), The production and preparation of biofertilizers can affect their chemical composition due to the decomposition time, material of origin, microbial community present, and abiotic factors in the soil, such as temperature and pH (MARROCOS et al., 2012). The soil microorganisms are responsible for organic matter decomposition, nutrient mineralization and solubilization, and release of organic compounds, such as metabolites, enzymes, acids, antibiotics, and vitamins (MEDEIROS; LOPES, 2006)

The effects of biofertilizer application on plant development and soil quality are cumulative and lasting; moreover, they are less harmful to the ecosystem than those of chemical fertilizers and pesticides (BHARDWAJ et al., 2014). The use of biological inoculants to improve sustainability of the agricultural production has becoming popular in several parts of the world (PAUL, 2014).

Therefore, the search for production methods that increase soil quality is increasingly necessary, as well as the understanding of the importance of preserving the soil. Soil quality is characterized by its capacity of functioning within the limits of the ecosystem and positively interact with the external environment (BÜNEMANN et al., 2018). In this context of environmental health, it is important to understand that the soil is under the effect of interactions between several biotic and abiotic factors that make it a complex and dynamic system, and any changes may result in beneficial or detrimental effects (PORTO et al.. 2009).

The objective of work was to characterize bacterial isolates regarding their biological activity and growth promotion of soybean plants (*Glycine max*) grown in a controlled environment.

MATERIAL AND METHODS

Isolation and determination of the functional group of the rhizobacteria

The isolation of rhizobacteria and bioassays were carried out at the Laboratory of Soil Microbiology of the State University of Northern Paraná, Luiz Meneghel campus, Bandeirantes, state of Paraná, Brazil, in 2018 and 2019. Roots samples were collected from soybean plants grown in areas with continuous application of commercial biological fertilizer and liquid compost; they were placed in plastic bags and sent to the laboratory for growth and isolation.

Ten grams of root soil of each sample was suspended in 90 mL of a saline solution (NaCl 0.85%), and subjected to serial dilution (1/10) up to 10^{-6} and incubated at 28 °C for 48 to 72 hours, the last three dilutions were plated in tryptone soy agar (TSA). The isolate bacteria were morphologically identified by gram color; the ones that were identified as gram-positive bacillus were subjected to a thermal treatment (water bath) at 80 °C for 20 minutes and plated in nutrient agar and incubated at 28 °C for 48 hours to determine the bacillus genus (BETTIOL, 1995), and then plated in agar nutrient plus 2% NaCl for confirmation (BUCHANAN; GIBBONS, 1975).

The functional activity of the isolates was evaluated by plating them in a culture media selective for proteolytic and amylolytic functional groups that determines the production of the protease and amylase enzymes, respectively. The atmospheric nitrogen fixation capacity of the isolates was also evaluated (Table 1).

Table 1. Selective culture media used for microbial analysis of the soil biological fertilizer.

Functional group	Culture media
Proteolytic	Proteolytic (PONTECORVO et al., 1953 – modified)
Amylolytic	Starch-casein (PONTECORVO et al., 1953)
Atmospheric Nitrogen Fixing	Nfb (DAY; DOBEREINER, 1976)

Antagonistic action of bacteria against phytopathogenic fungi

The *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, and *Fusarium solani* fungi were isolated from typical injuries on soybean, common bean, and cassava plants, respectively. The *S. sclerotiorum* and *M. phaseolina* fungi were identified morphologically according to Barnett and Hunter (1998) and based on the characteristic injuries on plants, *F. solani* was identified by the PCR technique, with amplification of the ITS region of the genome. The antagonistic effect of bacteria against phytopathogenic fungi was evaluated by the method of paired plating in Petri dishes containing TSA medium (ARAÚJO; HENNING; HUNGRIA, 2005). An 8 mm disc of the phytopathogen mycelium was collected from the center of each Petri dish (90 mm of diameter) and the bacterial isolates were inoculated in 4 equidistant points, totaling 4 replications per Petri dish. The fungi were grown in absence of bacteria as a control treatment. The Petri dishes were maintained in a BOD incubator at 25 °C, with photoperiod of 12 hours until the control had grown in all Petri dishes. The fungus mycelial growth was daily measured, as well as the control, to obtain the MGSI (mycelial growth speed index) (Equation 1) and inhibition percentage (%INB) (MENTEN et al., 1976).

Equation 1:

$$MGSI = \frac{\sum (D\text{-}Da)}{N}$$

where:
D = current colony diameter
Da = colony diameter at the previous day and
N = number of days after inoculation.

Determination of IAA (indoleacetic acid)

The rhizobacteria were grown in 20 mL of King-B culture medium, supplemented with 0.5 g L^{-1} of tryptophan, After inoculation, the microorganisms were maintained in an orbital shaker table for 72 hours, at a rotation of 220 rpm. The bacterial cultures were centrifuged at 5000 rpm for 15 minutes and 1 mL of the supernatant was mixed with 2 mL of Salkowsk's reagent (FeCl3 12 g L^{-1} in H_2SO_4 7.9 M) and left to rest in a dark environment for 30 min (GLICKMANN; DESSAUX, 1995). The indoleacetic acid of each sample was quantified in a spectrophotometer using a wavelength of 530 nm. The IAA production of each isolate was determined from a pure IAA curve (0, 5, 10, 15, 20, 25, 30, 35, 40 µg mL^{-1}), considering a King-B medium without inoculation as the control. The measurements were carried out with three replications (HERNÁNDEZ-MONTIEL et al., 2017).

Greenhouse assay

The experiment was conducted using 7-liter pots filled with soils from the 0-20 cm layer of a Typic Hapludox (Latossolo Vermelho eutroferrico), which presented the following chemical attributes: organic matter = 22.8 g Kg^{-1}; $pH_{(CaCl2)}$ = 5.1; P = 13.7 mg dm^{-3}; K = 0.7 cmolc dm^{-3}; Ca = 6.3 cmolc dm^{-3}; Mg = 0.90 cmolc dm^{-3}; H+Al = 5.61 cmolc dm^{-3}, sum of bases = 7.9 cmolc dm^{-3}; cation exchange capacity = 13.51 cmolc dm^{-3}, and base saturation = 58.5. A randomized block experimental design consisted of 16 treatments (15 with and a control without bacterial inoculation) was used. The treatments with bacterial inoculation were carried out with five replications and the control treatment with 10 replications, totaling 85 pots.

Four soybean (*Glycine max*) seeds of the cultivar TMG 7062 IPRO were seeded in each pot, and the bacterial inoculation was carried out using an automatic pipette at the emergence of the first trifoliate leaf. The minimum quantity was applied at the concentration of 10^9 colony forming units (CFU mL^{-1}). A thinning was carried out after the V3 soybean stage, leaving one plant per pot. The plant development and soil chemical attributes was evaluated at the end of the vegetative and beginning of the reproductive stage.

Agronomic analysis

The shoot and root dry weights and soil chemical attributes were evaluated at the stage R1. The dry weights were determined by drying the plants in a forced air-circulation oven for 7 days at 60 °C.

Preparation of soil samples for chemical analysis

The soil samples were collected, sieved in a 2 mm mesh, and cleaned for residues of animal or plant origin. Part of the sample was used to determine the moisture; these samples were taken to

an oven at 105 °C for 24 hours; the remainder samples were sent for chemical analyses (EMBRAPA, 2009).

Statistical analyses

The results obtained in laboratory (IAA production and antagonistic activity) were subjected to statistical quartile descriptive analysis. The results obtained in the greenhouse were subjected to analysis of variance and the means were compared by the Scott-Knott test at 5% probability, using the Sisvar program (FERREIRA, 2011). Plant growth was correlated with the bacterial isolates with antagonistic action against phytopathogenic fungi and the IAA production using Principal Component Analysis (PCA), in the RStudio 1.3.1056 program (RSTUDIO, 2016), with aid of the FactoMineR, factoextra, ggplot2, corrplot, and ggrepel packages.

RESULTS AND DISCUSSIONS

Fifteen rhizobacteria were isolated and named IS01, IS02, ISU03, IS04, IS05, IS06, IS07, IS08, IS09, IS10, IS11, IS12, IS13, IS14, and IS15. The isolates IS02 and IS10 presented no growth after thermal treatment at 80 °C and nutrient agar with 2% NaCl and, morphologically, they were gram positive coccus (tetrad); all other isolates were gram positive *Bacillus* spp.

Regarding the functional activity, the isolates IS01, IS02, IS03, IS04, and IS05 presented positive activity for amylase and protease, IS08 and IS15 were positive only for protease, and IS14 only for amylase, i.e., 33.3% of the isolates produced amylase and protease enzymes and 20% produced only amylase or protease; 26.6% of the isolates (IS02, IS06, IS08 and IS15) presented positive atmospheric nitrogen fixation (Table 2).

Table 2. Description of the functional activity of bacterial isolates for amylase, protease, and atmospheric nitrogen fixation.

Functional Groups	Bacterial Isolates														
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Proteolytic	+	+	+	+	+	–	–	+	–	–	–	–	–	–	+
Amylolytic	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–
N Fixation	–	+	–	–	–	+	–	+	–	–	–	–	–	–	+

Bahroun et al., (2018) found similar results, with bacterial isolates showing antifungal activity against *M. phaseolina*, *S. sclerotiorum*, and *F. solani*.

Amylases are a set of catalyst enzymes of starch hydrolysis to glucose units. The action of amylase in the soil make glycoside bounds of starch to hydrolyze, releasing maltose and glucose molecules at the end stage of hydrolysis, which are essential for the activity of microorganisms in nutrient cycling, and maintenance of the soil-plant system (BALOTA et al., 2013).

Proteases are enzymes responsible for protein catalysis by releasing several amino acids that can undergo deamination, releasing ammoniacal nitrogen. Thus, it is the first process in nitrogen protein mineralization from crop residues, incorporated organic fertilizers, and dead organisms (MELO et al., 2010). The biological capacity of the enzymatic conversion of substrates is dependent on the microbial activity (BALOTA et al., 2013).

Understanding possible functions and the importance of soil enzymes is essential for the maintenance of the soil quality, ecosystem fertility management, and microorganism functional diversity (SOUZA et al., 2015).

Nitrogen fixation activity by groups of microorganisms that present the nitrogenase enzyme is the main form of atmospheric nitrogen incorporation to soil systems and plant nutrition. The contribution of microorganisms is estimated in approximately 258 million Mg of nitrogen per year in the world, with 60 million Mg in agriculture (FUKAMI; CEREZINI; HUNGRIA, 2018).

The enzymatic activity of bacterial isolates with high plant growth promotion potential is due to the high positive proteolytic, amylolytic, and nitrogen fixation activities (RANA et al., 2017).

The antagonistic activity of bacterial isolates against phytopathogenic fungi (*S. sclreotiorum*, *M. phaseolina*, *F. solani*) were classified in a semi-quantitative form, dividing in quartiles. This division enables to group a large group into smaller groups, and determine individual positions inside each group (BISQUERRA; SARRIERA; MARTÍNEZ. 2009), with the quartile of high inhibition belonging to the first group, the one of low inhibition to the second, and the third formed by two quartiles of moderate inhibition.

The isolates IS02, IS03, IS10, and IS13 presented low inhibition (<54.65%) and the others had moderate to high (>71.20%) inhibitory activity

against *S. sclerotiorum*, i.e., totaling in 73.3% of isolates. The isolate IS02, IS05, IS06, IS08, IS12, and IS14 presented moderate inhibition against M. *phaseolina* (>0, <37.03%), and IS01, IS03, IS04, and IS15 presented high (>37.03%) inhibition, totaling 66.7% of isolates with moderate to high inhibitory action. The isolate IS02, IS07, IS08, IS11, IS12, IS14, and IS15 presented moderate inhibition (>19.15%, <46.94%) against *F. solani*, and IS01, IS03, IS04, and IS05 presented high inhibition. totaling 73.3% of isolates with moderate to high inhibitory action (Table 3). The bacteria that presented protease and amylase productions (Table 2) presented moderate to high inhibitory activity against the phytopathogenic fungi.

Biocontrol agents are organisms that decrease the intensity or incidence of diseases in plants, whereas organisms that present antagonistic activity to a pathogen are defined as antagonists. This activity in the rhizosphere is mainly noticed by the production of hydrolytic enzymes, such as β-glucanases, lipases, chitinase, protease, and amylases, that can cause injuries to the fungus cells (NEERAJA et al., 2010).

Bacteria may present other antagonism forms, such as competition for nutrients or ecological niches (KAMILOVA et al., 2005), production of ACC-deaminase enzyme that affect the regulation of ethylene levels in plants under stress caused by infections (VAN LOON, 2007), and production of siderophores and antibiotics (MAKSIMOV; ABIZGIL'DINA; PUSENKOVA, 2011).

Table 3. Mycelial growth speed index (MGSI) and inhibition percentage (%INB) of bacterial isolates against phytopathogenic fungi (*S. sclerotiorum*, *M. phaseolina*, *F. solani*).

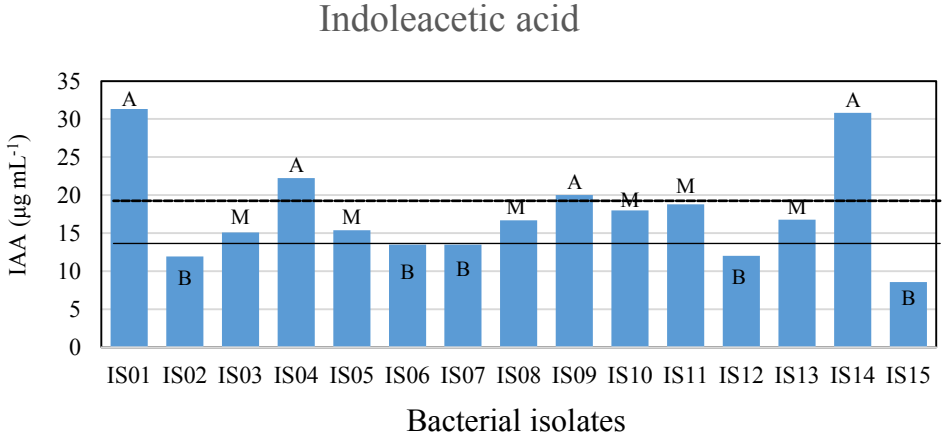
Treatments	*Sclerotinia sclerotiorum*		*Macrophomina phaseolina*		*Fusarium Solani*	
	MGSI	%INB	MGSI	%INB	MGSI	%INB
Control	5.92	0	15.88	0	11.57	0
IS01	1.38	76.69 [a]	9.56	39.80 [a]	5.36	53.67 [a]
IS02	2.75	53.55 [c]	10.25	35.45 [b]	7.43	35.78 [b]
IS03	3.36	43.24 [c]	9.62	39.42 [a]	6.07	47.54 [a]
IS04	1.50	74.66 [a]	9.88	37.78 [a]	5.29	54.28 [a]
IS05	1.79	69.76 [b]	10.25	35.45 [b]	5.64	51.25 [a]
IS06	2.50	57.77 [b]	11.50	27.58 [b]	10.00	13.57 [c]
IS07	2.08	64.86 [b]	16.38	-3.15 [c]	8.71	24.72 [b]
IS08	2.58	56.42 [b]	10.50	33.88 [b]	7.21	37.68 [b]
IS09	1.92	67.57 [b]	18.75	-18.07 [c]	10.64	8.04 [c]
IS10	2.75	53.55 [c]	18.44	-16.12 [c]	11.00	4.93 [c]
IS11	2.62	55.74 [b]	17.75	-11.78 [c]	8.54	26.19 [b]
IS12	1.62	72.64 [a]	11.50	27.58 [b]	6.79	41.31 [b]
IS13	5.08	14.19 [c]	17.81	-12.15 [c]	10.86	6.14 [c]
IS14	2.17	63.34 [b]	10.12	36.27 [b]	7.71	33.36 [b]
IS15	1.20	79.73 [a]	8.12	48.87 [a]	6.21	46.33 [b]

The inhibitory activity of the isolates was grouped by quartile analysis into high [a], moderate [b], and low [c] inhibition percentage for each phytopathogenic fungus.

The IAA production was analyzed in a semi-quantitative form by dividing the results in quartiles (BISQUERRA; SARRIERA; MARTÍNEZ, 2009); this method was already used for antagonistic activity analysis. The lowest IAA showed productions ≤13.5 µg mL^{-1} in the first quartile, the highest showed productions ≥19.40 µg mL^{-1}, and the moderate production showed productions between these values. The determination of quartiles showed the IAA production of all isolates: 5 isolates had low production (IS02, IS06, IS07, IS12, and IS15), 6 isolates had moderate production (IS03, IS05, IS08, IS10, IS11, and IS13), and 4 isolates had high production (IS01, IS04, IS009, and IS14) (Figure 1).

The application of rhizobacteria that produce IAA to soybean plants increases the plant growth rate, leaf sizes, chlorophyll contents, root and shoot dry matter weight, and number of pods per plant (KHALIMI et al., 2017).

Rhizobacteria, such as *Pseudomonas putidas*, produce IAA in the soil, but this production may be dependent on tryptophan availability in the medium. Rhizobacteria can obtain tryptophan from exudates of plants, which can control the IAA production by the bacterium according their needs (DUCA et al., 2014).



Continuous line (−) = first quartile; dotted line (---) = third quartile, [A] = high IAA production; [M] = moderate IAA production; [B] = low IAA production.

Figure 1. Quantity of IAA (indoleacetic acid) produced by bacterial isolates in soils with continuous use of biological fertilizer.

The results of the soil chemical attribute analysis are shown in Table 4, which indicate that the isolates tested presented similar dynamics, i.e., with significant decreases in pH and, consequently, increases in H+Al contents. The results found for the isolates IS14 and IS15 were similar to those of the control soil. These results are confirmed by those of sum of bases, which were lower in all treatments when compared to the control, and directly affected the bases saturation, which followed the same decreasing dynamic of the sum of bases, except for the isolates IS14 and IS15, whose results were similar to those of the control soil.

The soil acidity in treatments with bacterial inoculation is related to the production of organic acids from the organic matter continuously decomposed by microorganisms, which potentiates the mineralization and solubilization. Moreover, the reaction of calcium and magnesium carbonates in the soil form complex organic acids with multipurpose cations that leach and makes the soil more acid (PAVINATO; ROSALEM, 2008).

In addition, acidification can occur due the factors of natural origin, such as reactions that generate protons and absorption of cations by plants. When a cation is absorbed, hydrogen is released in the same proportion, contributing to the soil acidification. Soils with high acidity tend to present low base saturation due to fixation to non-soluble forms and increases in exchangeable Al^{3+} contents (RHODEN et al., 2017).

The results found are consistent with those presented by Anjos et al., (2017), who found high positive correlation between the microbial activity and soil chemical properties, especially for Ca^{+} and Mg^{2+} (r=0.990), sum of bases (r=0.985), and K^{+} (0.999).

The agronomic evaluation of plants showed no significant difference between the treatments and the control for stem diameter. The shoot dry weight was significantly higher in all treatments when compared to the control; however, treatments IS07, IS08, IS14 and IS15 had lower increases than the other treatments. Regarding the root volume attribute, the isolates that presented the highest shoot dry weight had significantly higher root volume, which was also found for the root dry weight (Table 5).

Table 4. Chemical properties of soils with continuous use of biological fertilizer, inoculated with soil bacterial isolates, and without inoculation.

T	OM^{ns}	pH	P$^{(ns)}$	K	Ca	Mg	H+Al	SB	CEC^{ns}	BS
	g Kg^{-1}	$CaCl_2$	mg dm^{-3}	cmol$_c$ dm^{-3}						%
C	22.79	4.87 a	7.73	0.30 a	5.43 a	2.67 a	4.86 b	8.40 a	13.26	63.27 a
IS01	20.14	4.43 b	8.29	0.11 b	4.23 c	2.67 a	6.70 a	7.01 c	13.71	51.09 b
IS02	19.69	4.40 b	6.82	0.11 b	4.17 c	2.40 a	6.77 a	6.68 c	13.45	49.66 b
IS03	19.69	4.63 b	9.29	0.11 b	4.77 b	2.50 a	6.21 a	7.51 b	13.72	54.77 b
IS04	20.89	4.50 b	8.23	0.11 b	4.07 c	2.57 a	6.81 a	6.75 c	13.56	49.76 b
IS05	21.48	4.43 b	7.94	0.11 b	3.80 c	2.93 a	6.43 a	6.84 c	13.28	51.54 b
IS06	20.58	4.43 b	7.85	0.12 b	3.63 c	2.97 a	6.61 a	6.72 c	13.33	50.40 b
IS07	21.03	4.47 b	10.85	0.14 b	4.00 c	2.70 a	6.50 a	6.84 c	13.35	51.26 b
IS08	19.69	4.53 b	7.09	0.11 b	4.67 b	2.03 b	6.11 a	6.81 c	12.92	52.71 b
IS09	18.79	4.53 b	6.96	0.13 b	4.63 b	1.90 b	6.23 a	6.66 c	12.90	51.67 b
IS10	19.69	4.47 b	8.03	0.11 b	5.07 b	2.30 b	6.68 a	7.48 b	14.16	52.80 b
IS11	21.03	4.50 b	7.79	0.11 b	4.57 b	1.93 b	6.57 a	6.61 c	13.18	50.18 b
IS12	22.79	4.47 b	9.50	0.14 b	4.80 b	1.93 b	6.42 a	6.88 c	13.30	51.74 b
IS13	21.52	4.53 b	9.08	0.16 b	5.03 b	1.93 b	6.35 a	6.83 c	13.18	51.69 b
IS14	21.03	4.80 a	8.62	0.17 a	6.17 a	2.10 b	5.45 b	8.53 a	13.98	61.00 a
IS15	22.79	4.80 a	7.64	0.31 a	5.17 a	2.17 b	5.12 b	7.58 b	12.70	59.79 a
CV	8.20	2.31	16.52	14.15	9.46	12.34	6.82	7.16	3.93	5.43

Means followed by the same letter in the columns are not different by the Scott-Knott test at 5% significance level; ns = not significantly different when comparing treatments. T = treatment OM = organic matter; H+Al = acidity potential; SB = sum of bases; CEC = cation exchange capacity; BS = base saturation; CV = coefficient of variation (%).

Table 5. Agronomic evaluation of soybean plants inoculated with bacterial isolates.

Treatment	SD^{ns}	SDW	RV	RDW	IAA
	cm	g	mL	g	µg mL^{-1}
Control	8.08	25.59 c	33.60 b	3.66 f	-
IS01	10.85	36.15 a	50.80 a	8.78 b	31.33 a
IS02	10.33	38.42 a	54.00 a	7.71 c	11.93 e
IS03	10.55	36.99 a	52.20 a	9.68 a	15.10 d
IS04	10.08	37.53 a	49.60 a	7.72 c	22.25 b
IS05	10.38	37.34 a	48.00 a	7.26 c	15.39 d
IS06	9.88	35.84 a	49.80 a	5.12 e	13.49 e
IS07	9.79	33.20 b	26.20 b	3.47 f	13.50 e
IS08	9.71	35.17 b	26.00 b	2.75 f	16.70 c
IS09	10.09	37.40 a	54.20 a	10.28 a	20.02 b
IS10	10.49	35.96 a	48.80 a	6.39 d	17.97 c
IS11	10.82	37.07 a	46.00 a	5.42 e	18.77 c
IS12	11.32	36.51 a	45.20 a	7.71 c	12.02 e
IS13	10.81	37.71 a	63.00 a	10.05 a	16.75 c
IS14	8.95	34.25 b	38.00 b	7.71 c	30.83 a
IS15	9.91	32.28 b	38.00 b	6.07 d	8.56 f
CV (%)	12.08	6.36	22.31	10.71	7.73

Means followed by the same letter in the columns are not different by the Scott-Knott test at 5% significance level; ns = not significantly different when comparing treatments. SD = stem diameter; SDW = shoot dry weight; RV = root volume; RDW = root dry weight; IAA = indoleacetic acid.

Studies on plant growth promoting rhizobacteria (PGPR) conducted by El Habil-Addas et al. (2017) also showed increases in fresh weight, growth, and root length of plants.

The increases in SDW, RDW, and RV were attributed mainly to the IAA production by the bacterial isolates (Table 5). Moreover, the good development of plants inoculated with the isolate IS02 and IS12, which did not produce IAA, can be related to other factors, such as antagonistic activity (Table 3) and soil microbial activity. Contrastingly, IS14 presented large IAA production, but low increases in plant development, which may be related to the rate used, since the availability of a high IAA concentration can be toxic or not used by plants.

The efficiency of IAA in promoting plant growth depends on the concentration; low concentrations are inefficient and high concentrations can be toxic. This is found for all phytohormones, which present a specific ideal concentration range to promote growth of plant species (SCHLINDWEIN et al., 2008).

Plant growth is affected by PGPR by direct and indirect forms; directly by providing nutrients, and synthesized compounds, such as phytohormones, to the plant; and indirectly by controlling pathogens and producing enzymes or secondary metabolites that prevent or decrease deleterious effects (GLICKMANN; DESSAUX, 1995). PGPR use one or combinations of processes to induce plant development, which increase crop yields (BENEDUZI; AMBROSINI; PASSAGLIA, 2012).

The computational analysis by PCA combined the treatments into groups based on the variables that promoted growth. The first group was formed by the bacterial isolates IS01, IS02, IS03, IS04, and IS05, which promoted plant growth (SDW and SD), directly, by producing IAA, and indirect by control phytopathogenic fungi, IS01 is highlighted by its significant effect due to its high IAA production. The second group was formed by the bacterial isolates IS09 and IS13, which promoted a slight plant growth, but is highlighted by acting in root growth (RDW and RV), which makes them interesting organisms for rooting promotion studies. The third group was formed by the bacterial isolates IS06, IS07, IS08, IS10, IS11, IS12, IS14, and IS15, which had little effect on plant growth. The control treatment was isolated distant from the other treatments evaluated (Figure 3)

In this analysis, the two first principal components explained more than 60% of the variation (Figure 2).

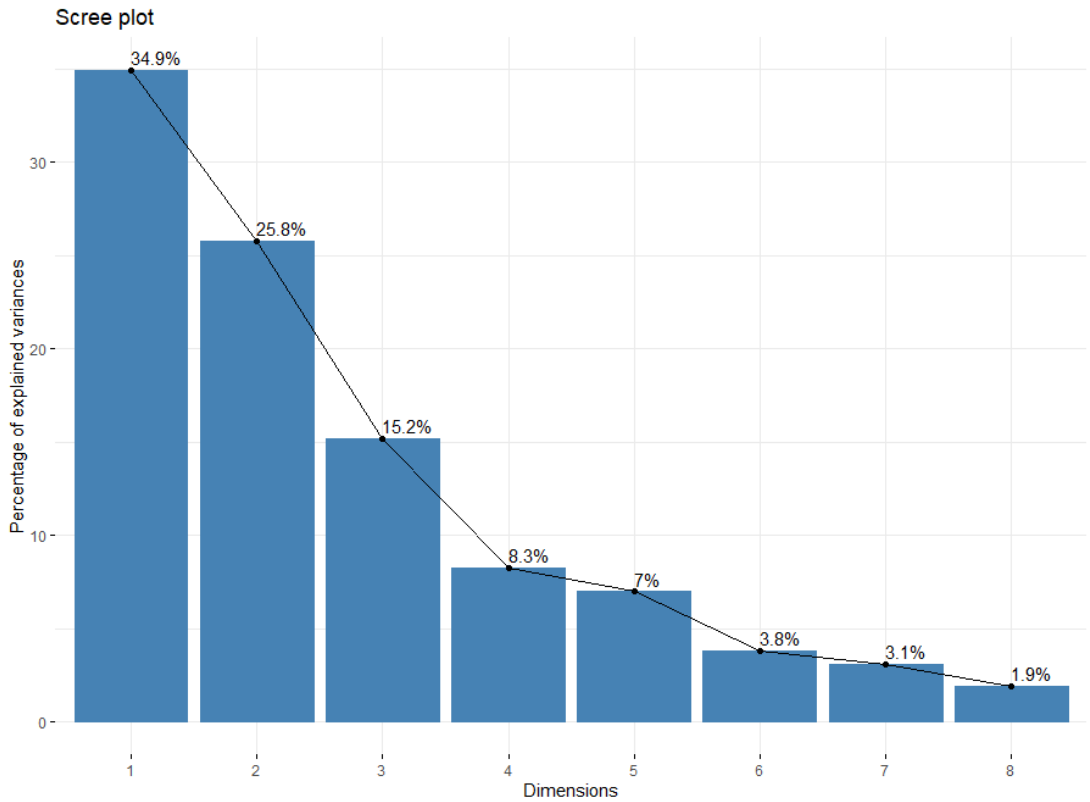


Figure 2. Eigenvalues of dimensions (scree plot), showing the percentage of variations explained by each principal component.

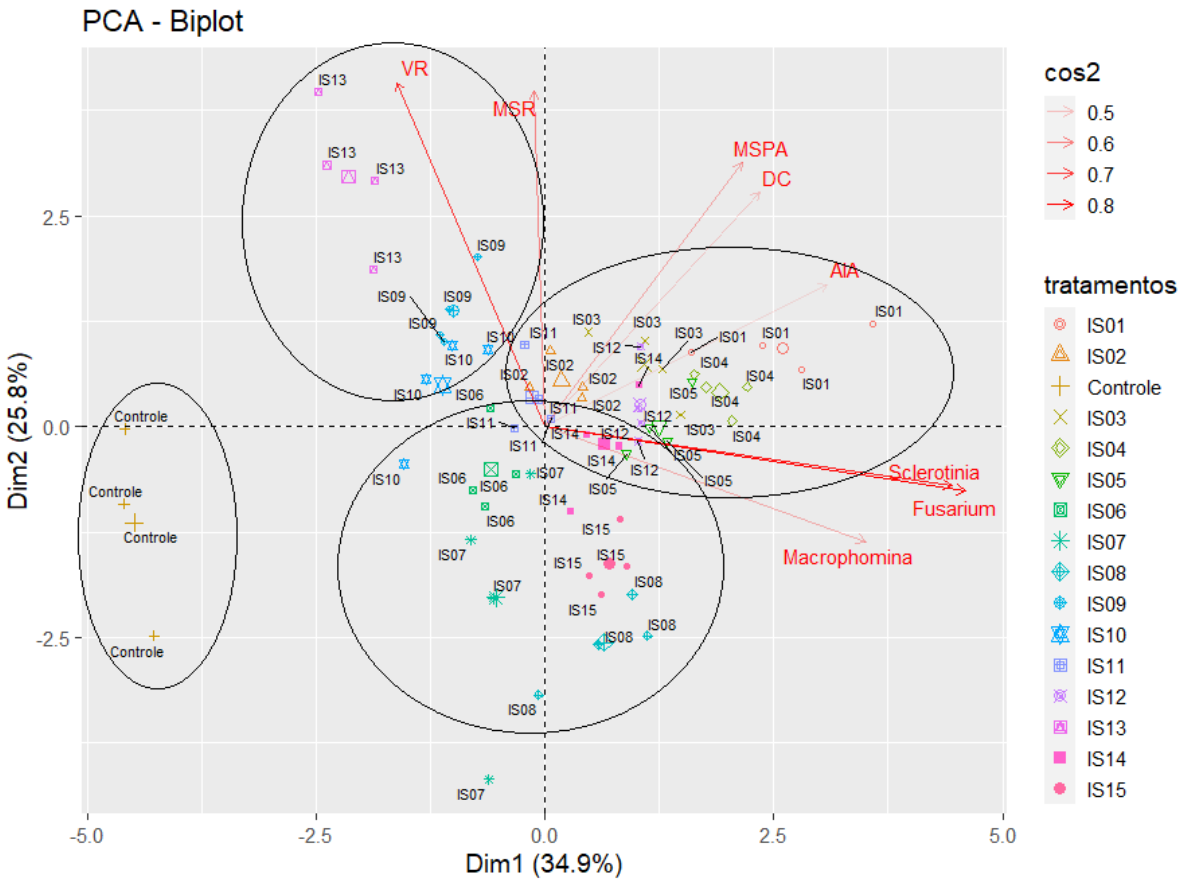


Figure 3. Graph of variables generated by PCA. Variables positively correlated are indicate by their location in same side of the graph. Variables negatively correlated are indicate by their location in opposite sides of the graph.

CONCLUSION

The use of inoculation with rhizobacteria to promote increases in plant growth is viable, since they efficiently mineralize and make nutrients available. In addition, many of them present antagonistic action against pathogens and production of growth phytohormones. The results showed the importance of the contribution of microorganisms associated to soybean crops. The isolates IS01, IS02, IS03, IS04, and IS05 were highlighted for all variables evaluated, presenting a biotechnological potential. The isolates IS09 and IS13 presented large potential for the development of rooting products. However, the efficiency and biotechnological potential of these bacterial isolates should be confirmed in the field to evaluate the effects of these microorganisms associated to biotic and abiotic factors.

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