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ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING BACTERIA ISOLATED FROM GARLIC *(allium sativum).*

Isolamento e caracterização de bactérias indutoras de crescimento vegetal de alho (Allium sativum).

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Abstract - Garlic has great economic and social importance in Santa Catarina. It is a demanding crop in nutrients, being necessary high amount of chemical fertilizers, increasing the cost of production and the environmental impact. An alternative to the use of inputs are Rizobacteria Promoting Plant Growth (RPCP). Among the RPCPs, the most studied genera are *Azospirillum, Bacillus* and *Pseudomonas* of the fluorescent group that have shown benefits for the growth of several plant species. In this context, isolates of RPCP obtained from the rhizosphere of garlic maintained in protected cultivation were phenotypically and genotypically characterized and a growth promotion test *in vivo* was carried out, in protected cultivation. The biochemical tests used for phenotypic characterization were glucose and sucrose fermentation, Methyl Red (MR), catalase production and urea hydrolysis. The potential of plant growth induction was also evaluated by analyzing the production capacity of IAA (Indol-Acetic Acid) and calcium phosphate solubilization *in vitro.* Twenty-seven isolates were obtained. For the biochemical tests, 21 isolates were able to ferment glucose and 26, sucrose. For the other tests, all isolates showed a positive reaction. As for the mechanisms of plant growth promotion, 81.5% and 66.6% of the isolates presented IAA production and phosphate solubilizing capacity, respectively. The *in vivo* growth promotion test using maize (*Zea mays*) as a model indicated that the tested isolates (EB02, EB16, EB14, EB26, EB23) showed similarity to the treatment with 100% recommended nitrogen fertilization. The isolates were identified by sequencing the 16S rRNA gene. Fourteen isolates (51, 85% of the collection) were identified as *Bacillus subtillis*, including those tested *in planta*. These microorganisms present potential for the promotion of plant growth.

Key words: growth promotion, rizobacteria, liliacea, corn.

Resumo – O alho possui grande importância econômica e social em Santa Catarina. É um cultivo exigente em nutrientes, sendo necessária elevada quantidade de fertilizantes químicos, incrementando o custo de produção e o impacto ambiental. Uma alternativa ao uso de insumos são as Rizobactérias Promotoras de Crescimento de Plantas (RPCP). Dentre as RPCPs, os gêneros mais estudados são *Azospirillum, Bacillus* e *Pseudomonas* do grupo fluorescente que têm demonstrado benefícios para o crescimento de várias espécies vegetais. Neste contexto, foram caracterizados, fenotípica e genotipicamente, isolados de RPCP obtidos da rizosfera de alho mantido em cultivo protegido e realizado teste de promoção de crescimento in vivo, também em cultivo protegido. Os testes bioquímicos utilizados para caracterização fenotípica foram fermentação de glicose e sacarose, Vermelho de Metila (VM), produção de catalase e hidrólise da ureia. Também foi avaliado o potencial de indução do crescimento vegetal, mediante análise da capacidade de produção de AIA (Ácido Indol-Acético) e de solubilização de fosfatos de cálcio in vitro. Foram obtidos 27 isolados. Para os testes bioquímicos, 21 isolados foram capazes de fermentar a glicose e 26, a sacarose. Para os demais testes, todos isolados apresentaram reação positiva. Quanto aos mecanismos de promoção de crescimento vegetal, verificou-se que 81,5% e 66,6% dos isolados apresentaram produção de AIA e capacidade de solubilizar fosfato, respectivamente. O teste de promoção de crescimento in vivo, utilizando milho (Zea mays) como modelo, indicou que os isolados (EB02, EB06, EB14, EB16, EB26) testados apresentaram similaridade com o tratamento com 100% de adubação nitrogenada recomendada. Os isolados foram identificados por sequenciamento do gene 16S rRNA. Quatorze isolados (51, 85% da coleção) foram identificados como Bacillus subtillis, incluindo os testados in planta. Estes microrganismos apresentaram potencial para a promoção do crescimento da planta.

Palavras-chave: promoção de crescimento, rizobactérias, liliacea, milho

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INTRODUCTION

Garlic is the fourth most economically important vegetable in Brazil. In addition, there is a social importance, because mainly farmers of small properties (LEONÊZ, 2008) make its tillage. In the state of Santa Catarina, the cultivation was initially introduced into the Catarinense Plateau region. For many years, the area around the Curitibanos city (Santa Catarina state) had the highest garlic productivity in the state, because it had the favorable geographical and climatical characteristics to the crop (LUCINI, 2008). However, economic and phytosanitary factors led to a decrease in the production. Currently, many efforts are done to increase it, through alternatives methods that can reduce costs, for example, decreasing inputs, like nitrogen fertilizers. Garlic cultivation requires a high nitrogen amount, using approximately 200kg/ha of nitrogen fertilizer, after germination (LUCINI, 2010). Much of this can be lost by leaching process (AGOSTINHO; FERNANDO; CAMEIRA, 2007), causing economic and environmental losses.

The studies related to the decrease of agricultural inputs have been stimulated. Although, trying to maintain the productivity level of the farming areas (BULLA; BALBINOT JUNIOR, 2012). This can be achieved by using the bacteria called Plant Growth Promoting Rhizobacteria (PGPR). The benefits to plants through PGPR can be directly or indirectly performed (VOISARD et al., 1994). The direct effect is a consequence of disponibilization of soil nutrients by PGPR, as Biological Fixation of Nitrogen (MOREIRA et al., 2010), solubilization of mineral phosphates (SANCHEZ LÓPEZ et al., 2014; SOUCHIE; ABBOUD; CAPRONI, 2007). These bacteria can also produce plant hormones, such as Indole-Acetic Acid (IAA) (SANCHEZ LÓPEZ et al., 2014; ANGURO et al., 2014; CATTELAN, 1999). The conversion of tryptophan to IAA is a mechanism performed by several PGPR, since it contributes to the reduction of the excess of this amino acid in the rhizosphere, which can be deleterious to bacterial cells (BRUNETTA et al., 2010).

Several genera of rhizobacteria have already been described, interacting with different plants. The most studied genera are rhizobia, *Azospirillum*, *Bacillus* and fluorescent *Pseudomonas*, that stimulate diverse plants in their development, from the germination until the production, reinforcing sustainable agriculture (HUNGRIA; NOGUEIRA; ARAÚJO, 2016; HUNGRIA; NOGUEIRA; ARAÚJO, 2013; VOGEL et al, 2013; REPKE et al 2013; FIORINI et al 2012; FILHO, 2010; MOREIRA et al., 2010; SILVEIRA, 2007; HUNGRIA et al., 2001). In addition, some isolates of the genera can suppress phytopathogens (LUDWIG; MOURA; GOMES, 2013; LUDWIG et al., 2009) by producing secondary metabolites, such as siderophores and antibiotics (BOTELHO; MENDONÇA-HAGLER, 2006; VOYARD et al., 1994; LOPER, 1991). Several reports of the plant development improvement, such as corn, beans, cotton, soybeans and rice inoculated with isolates from genera *Bacillus* and fluorescent *Pseudomonas* are described (FERREIRA; KNUPP; MARTIN-DIDONET, 2014; CHAVES; ZUCARELI; JUNIOR, 2013; SILVA, 2013; ASSUNPÇÃO et al., 2009; ARAUJO, 2008; LAZZARETI MELO, 2005; ARAÚJO; HUNGRIA, 1999). These reports emphasize the potential use of these rhizobacteria to a larger spectrum of plants.

There is a few information of the rhizobacteria interaction to plants of the Liliaceae family. Marcuzzo, Cezar e Scolaro (2008) evaluated the colonization of 53 rhizobacteria isolated from different parts of garlic. They observed that five of them colonized efficiently (rates between 95,8% to 66,7%) the rhizosphere of two cultivars. It can suggest that PGPR influences the development and growth of the garlic. Some fluorescent *Pseudomonas* isolates from garlic´s rhizosphere solubilized phosphate, produced IAA, were able to fix Nitrogen and inhibited the eclosion of *Meloidogyne javanica in vitro* (TURATTO; BOTELHO, 2015).

There are few studies of beneficial bacteria association at the garlic culture. For this reason, the objective was to verify, isolate and characterize bacterial groups and evaluate their potential and efficiency in promoting in vitro plant growth.

MATERIAL AND METHODS

The soil was collected from Dias farm, in the locality of Horizolândia, around Curitibanos (SC). It was classified as Cambissolo associated to Bruno Nitossolo (Embrapa, 1998) and the withdrawal carried out according to the procedures described by the Comissão de Química e Fertilidade de Solo (2004). Garlic bulbs were planted in two pots of 5L containing the soil. Each pot was watered with 50 mL, four times a week.

Isolation of N-fixing rhizobacteria.

After four weeks, soil and root samples were taken for bacteria isolation. Ten grams of soil sample of each pot were homogenized and weighed. Each one was transferred to flasks containing 90 mL sterile 0.9% saline that was stirred for 30 min at 150 rpm. After carrying out the serial dilution, 0.1mL of 10^3, 10^4 and 10^5 dilutions were transferred to penicillin flasks containing 5mL of semi-solid NFB medium (DOBEREINER et al., 1995). For each dilution, there were three replicates, incubated at 28° C for seven days. After this period, the presence of the bacteria could be verified by a pellicle in

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the surface region. The Most Probable Number (MPN), using the McCrady's table for three replicates, evaluated the bacterial population. Then, the colonies were purified, as it will be described below.

For the isolation of the bacteria from the root, 10g of root of each pot were taken and disinfected by immersion in 10% bleach for 10 minutes, followed by three washes in sterile distilled water. Root samples were macerated and transferred to flasks containing and transferred to flasks containing 90 mL sterile 0.9% saline and similar procedures described above for soil sample were carried out.

The colonies purification consisted in transferring them to semi-solid NFB medium, four times. After this, the bacteria were grown onto solid NFB medium to obtain pure colonies. The pure cultures were stored at -20 ° C in cryotubes containing 40% glycerol.

Phenotypical characterization of isolates and potential for growth promotion of plants.

The phenotypic characterization observed morphological and biochemical features. Colonies morphological characteristics, such as diameter, border, shape, elevation and transparency of the isolates colonies were analyzed. Isolates were grown in Luria Bertani solid medium (LB) at 30ºC, for 24h.

The biochemical trials performed were Gram test, glucose and sucrose fermentation, catalase and urease production and methyl red (DÖBEREINER et al., 1999). Bacteria were grown in liquid LB medium for 24 hours at 30°C. For fermentation, urease and methyl red tests, an aliquot was transferred to the tubes containing specific media for each test (RIBEIRO; STELATO, 2011) in three replicates. These were incubated at 30°C for 48 hours. To determine the presence of the enzyme catalase, the samples were transferred to microscopic slides. Drops of H_2O_2 were added on them, and bubble formation were observed for positive result.

For the isolates plant growth induction potential, the phosphate solubilization capacity and IAA production were determined. To evaluate the solubilization capacity, a medium containing tribasic calcium phosphate (10g/L glucose; 5g/L NH4Cl; 1g/L $MgSO_4.7H_2O$; 4g/L $Ca_5(OH)(PO_4)_3$; 15g/L Agar – pH = 6.5) was used. The isolates were previously grown in liquid LB medium at 30°C for 24 hours. Then, 0.1 ml of the bacterial suspension was transferred to plates containing the phosphate medium, establishing four isolates per plate, arranged at equidistant points, with five replicates. After incubation at 30 ° C for seven days, the solubilizing capacity of phosphate was evaluated by the presence of colorless halo around the colonies.

The IAA production was evaluated using a colorimetric method described by Bric et al. (1991), with modifications. For this, each isolate was inoculated in tubes containing 2mL of liquid LB medium. The tubes were incubated for 24 h at 30°C. After this, they were shaken at 150 rpm for five minutes. Subsequently, Salkowski's solution (CATTELAN, 1999) was added to each tube and incubated at room temperature in the dark for 2 hours. It was observed color change to pink, as positive reaction.

Potential promotion of plant growth *in vivo*.

The corn was chosen as a model for growth promotion analysis, due to the large number of studies of the effect of PGPR on it (DARTORA et al., 2013; CARREIRA et al., 2012; CHAVES, 2013, QUADRADO et al., 2014) and because it is widely used in rotation with the garlic by the farmers of Curitibanos region, suggesting PGPR isolates effects to the two plants.

Five isolates (EB02, EB23, EB14, EB16 and EB26) were chosen by the ability to solubilize phosphate and to produce IAA, in addition to the biological fixation of N, for the *in vivo* experiment. There were eight treatments: five treatments containing the seeds inoculation with each selected isolate and three non-inoculated treatments, containing 0%, 50% and 100% of the amount of N recommended for the crop, respectively. The source of N was KNO_3 based on the solution of Hoagland and Arnon (1950). The experimental design was in randomized blocks with three replicates.

To the inoculum, flasks containing 250 mL of LB liquid medium were inoculated with each isolate that were grown at 30°C for 24 h. The seeds were previously disinfected by a wash in ethanol 95%, followed by immersion in 10% sodium hypochlorite solution for 3min, and then washed three times with sterile distilled water. Then, 20 seeds were dropped into each flask and kept for 1h, in the laminar flow chamber. For the non-inoculated treatments, the seeds were immersed in LB liquid medium without inoculation. Finally, the seeds were laid on paper towel for drying for 1 h, in the laminar flow chamber.

The pots used for sowing contained 5 kg of the mixture of 50% vermiculite, 25% sand and 25% crushed stones. Each pot received four seeds from each treatment. Every 48 hours, the pots received 50mL sterile distilled water for irrigation. After seven days of the sowing, started the application of the nutrient solution, based on HOAGLAND and ARNON (1950) that was applied once a week to each pot. When the corn plants reached the V3 stage, thinning was performed, leaving only two plants per pot.

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After 90 days of the sowing, the plants were collected and analyzed the following parameters: plant height in centimeters (which comprised the distance between the substrate surface region and the insertion of the last expanded leaf); wet weights of root and stem; Dry weights of root and stem (kept in the drying oven at 50°C for 72 hours). Data were submitted to analysis of variance and, if there was a significant difference, to the Tukey and Scott-Knott mean tests, with a significance level of 5%, using the statistical program R.

Isolates identification

All isolates were submitted to 16S rRNA gene sequencing, for determination of genera and/ or species. This procedure was carried out at Embrapa-Agrobiology (Seropédica - RJ).

RESULTS AND DISCUSSION

Phenotypical evaluations of the isolates

Twenty - seven diazotrophic isolates from the roots were obtained. There were no isolates from the soil. Through the semi-solid NFB medium, an environment with low oxygen level is created and there is no nitrogen present, like soil or plant conditions, where diazotrophic bacteria associated with plant roots are located. In the medium, the bacteria move to the center where the diffusion rate of O_2 is in equilibrium with the rate of respiration of the bacteria. Upon reaching a high concentration of cells, the O_2 flow occurs, activating the nitrogenase (DÖBEREINER et al, 1995) and forming a pellicle.

Despite the phenotypic analyzes developed were not sufficient for identification of the isolates. However, they were important to indicate that these characteristics are similar to those observed at bacterial genera, commonly found among rhizobacteria, especially *Azospirillum, Bacillus and Pseudomonas* of fluorescent group (GOMES, 2015; LIMA, 2010; SILVA; FELIPE; BACH, 2004; HOLT et al., 1994), capable of Biological Fixation of Nitrogen (BFN). Some tests, such as the Gram staining test carried out on very young culture of *Bacillus*, can lead to unreliable reactions, hindering the identification of bacteria and the choice of appropriate biochemical tests. Initially, several isolates showed negative reaction in Gram test and these were subsequently identified by sequencing.

The morphological structure of the colonies data are in table 1. Colony color of all the isolates was white. Fifteen of them (55.5%) were opaque white and 12 (44.5%), translucent white. The diameter of the colonies ranged from 0.5 to 4 mm. The predominant shape was circular form (25 from 27 isolates - 92.6%). Only two isolates presented rhizoid colony form. Majority of the isolates 81% presented smooth border colonies. Only five (19%) exhibited corrugated border. Regarding the elevation of the colonies, fifteen (55.5%) were convex and 12 (44.5%) flat.

Table 1 - Colonies characterization of bacteria isolated from garlic.

Isolated	Color (Whith)	Diameter	Shape	Border	Elevation
5E22	Opaque	0,5	Circular	Smooth	Convex
5E31	Opaque	0,5	Circular	Smooth	Flat
3E32	Translucent	1	Circular	Corrugated	Flat
4E12	Translucent	4	Circular	Smooth	Flat
4E22	Opaque	1	Circular	Smooth	Convex
3E13	Translucent	0,5	Circular	Smooth	Flat
4E21	Opaque	1	Circular	Smooth	Convex
3E14	Opaque	1	Circular	Smooth	Convex
4E23	Opaque	3	Rizhoidal	Corrugated	Flat
4E13	Translucent	1	Circular	Smooth	Flat
4E212	Opaque	1	Circular	Smooth	Convex
4E32	Opaque	0,5	Circular	Smooth	Flat
5E32	Translucent	3	Rizhoidal	Corrugated	Convex
3E31	Translucent	0,5	Circular	Smooth	Convex
5E21	Opaque	0,5	Circular	Smooth	Flat
5E34	Opaque	1	Circular	Smooth	Convex
3E21	Opaque	1	Circular	Smooth	Convex
3E11	Translucent	1	Circular	Smooth	Convex
5E331	Translucent	3	Circular	Corrugated	Flat
4E33	Translucent	0,5	Circular	Smooth	Flat
5E23	Opaque	1	Circular	Smooth	Convex
4E211	Translucent	0,5	Circular	Smooth	Flat
3E13	Opaque	1	Circular	Smooth	Convex
5E332	Opaque	0,5	Circular	Smooth	Flat
4E11	Translucent	1	Circular	Corrugated	Convex
4E332	Opaque	2	Circular	Smooth	Convex
4E333	Translucent	1	Circular	Smooth	Convex

The morphological characteristics observed at the isolates resemble the features of the genera *Bacillus*, *Pseudomonas* and *Azospirillum* (CARDOSO, 2008; GOMES, 2013; BENTO, 2013), rhizobacteria commonly found in several plant species ((HUNGRIA; NOGUEIRA; ARAÚJO, 2016; GOMES, 2013;

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MOREIRA et al, 2010; BOTELHO; MENDONÇA-HAGLER, 2006).

Biochemical evaluations demonstrated that for the glucose and sucrose fermentation test, 21 (78%) and 26 (97%) of the isolates showed positive reaction, respectively (table 2). The results are in agreement with several reports that some rhizobacteria are able to ferment a number of sources of carbon. Bueno (2010) described that *Bacillus* isolates had the ability to use various sources of carbon, including glucose and sucrose. *Bacillus* uses glucose to produce acids and hydrolyzes starch (LIMA, 2010). According to Holt et al. (1994), *Azospirillum amazonense* is the only species of the genus capable of metabolizing different carbon sources, such as glucose and sucrose. *Pseudomonas* also present the potential of using different C sources (GOMES, 2015) (Table 2).

For the methyl red test, which verified the capacity of producing stable organic acids from the fermentation of glucose, all the isolates obtained positive reaction (Table 2). This test is used to aid in the identification of gram-negative bacterial species (OLIVEIRA, 2000) of Enterobacteriaceae family, such as *Pseudomonas*.

The catalase presence test indicated that 85,2% in the isolates (24) were capable of releasing oxygen from hydrogen peroxide (Table 2). All they changed the color of the medium, from yellow to reddish, because of the pH increase caused by the final products of urea breaks generated by the action of urease (Table 2). These characteristics are similar to those observed at the genera *Azospirillum*, *Pseudomonas* and *Bacillus* (FERREIRA; KNUPP; MARTIN; DIDONET, 2014; GOMES, 2013; SILVA, 2013; MANDELBAUM; ALLAN; WACKETT, 1995).

Table 2- Biochemical profile and mechanisms of growth promotion in plants.

Isolate	Fermentation						
	Glucose	Sucrose	MR	Catalase	Urease	IAA production	P solubilization
EB01	+	+	+	+	+	+	+
EB02	+	+	+	+	+	-	+
EB03	+	+	+	+	+	+	+
EB04	+	+	+	+	+	+	+
EB05	+	+	+	-	+	+	+
EB06	+	+	+	+	+	-	+
EB07	+	+	+	+	+	+	-
EB08	+	+	+	+	+	+	+
EB09	-	-	+	-	+	+	-
EB10	+	+	+	+	+	+	+
EB11	-	+	+	-	+	-	-
EB12	+	+	+	+	+	+	-
EB13	+	+	+	+	+	+	+
EB14	+	+	+	+	+	+	+
EB15	+	+	+	+	+	-	-
EB16	+	+	+	+	+	+	-
EB17	+	+	+	+	+	+	+
EB18	+	+	+	+	+	+	-
EB19	-	+	+	-	+	+	+
EB20	-	+	+	+	+	+	-
EB21	+	+	+	+	+	+	-
EB22	-	+	+	+	+	+	+
EB23	+	+	+	+	+	+	+
EB24	-	+	+	+	+	+	+
EB25	+	+	+	+	+	+	+
EB26	+	+	+	+	+	+	+
EB27	+	+	+	+	+	+	+

* MR - Methyl Red test; (-) Negative reaction.

Evaluation of mechanisms of plant growth promotion *in vitro*.

Most of the isolates were able to produce IAA and solubilize phosphate (Table 2b). The results agree with several reports describing these mechanisms as common among PGPR.

The analysis results indicated that 22 of the isolates (81.5%) were positive to produce Indol-Acetic Acid (IAA). Kuss (2007) disclosed that *Azospirillum* helps the plants, not only for N_2 fixation, but also by other mechanisms, including the production of phytohormones, such as IAA that benefits root growth. Consequently, there is an increasing of water and nutrients absorption (BONILLA, 2011). Many fluorescent *Pseudomonas* have the capacity of indole- acid production (VACHERON et al., 2016; COELHO, 2009).

Andrade (2012) reports that *Bacillus* can synthesize IAA in banana crop, both by the tryptophan-dependent pathway and by alternative routes. Sicuia, Dinu e Constantinescu (2016) reported that an IAA-producing *Bacillus subtilis* isolate increased the germination percent and seedlings vigor of tomato.

The solubilizing capacity trials of calcium phosphate showed that 66.7% of isolates (18) had positive reaction, i.e., produced transparent halo around the colonies. This capacity can help the plant in several soil conditions. When the insoluble P-Ca form is in the soil, due to the excess of liming, for instance, phosphate-

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solubilizing bacteria can help plant absorption of this element (TIRLONI, 2006). Rodríguez and Fraga (1999) described that *Bacillus* and *Pseudomonas* have the ability to solubilize inorganic phosphate compounds. Kang et al (2014) related that a phosphate solubilizing *Bacillus megaterium* isolate enhanced the growth of mustard plant and Oteino et al. (2015) reported that phosphate solubilizing *Pseudomonas fluorescens* L321 strain increased *Pisum sativum* L. growth. Ayyaz et al (2016) also observed improvement of wheat growth inoculated with *Azospirillum* strains.

Potential promotion of plant growth *in vivo*.

Concerning the plant height, there is a significant difference between without nitrogen fertilization treatments, those with nitrogen fertilization (100% and 50%) and inoculated treatments (Figures 1a and 1b). However, inoculated and nitrogen fertilizations treatments did not differ between them, indicating that the isolates in the greenhouse were able to maintain the development of the plant (Figure 1).

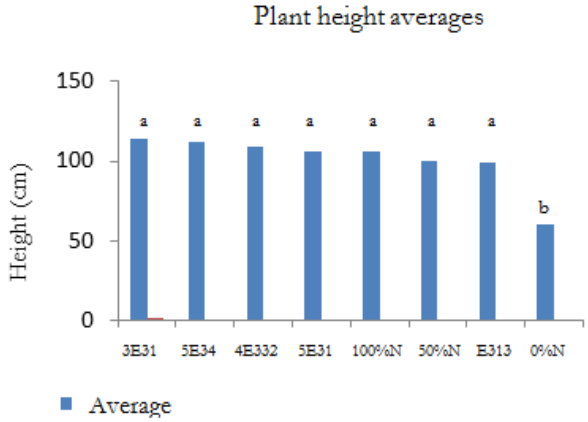

Figure 1 - Effect of bacteria isolates inoculation at plant height.

These results coincide with others that used PGPR for corn inoculation. The inoculation of *B. subtilis* OG strain on bean (LAZZARETI; MELO, 2005) provided larger and more vigorous plants. The inoculation significantly stimulated the growth of the crop. The same effect was observed when *A. brasilense* strains (BR11005 - Sp 245, AbV5 and AbV6) (PEDRINHO, 2009; BASI, 2013) were inoculated on corn. De Oliveira et al. (2015) also describes that *Pseudomonas fluorescens* provided higher corn plant height and ear size.

Regarding the dry and wet weight of the stem, results similar to those obtained with plant height was observed. There was a significant difference between the treatment without Nitrogen fertilization, inoculated treatments and those with N fertilization. However, there was no significant difference between the latter (Figure 2). The results emphasize again that the isolates can induce plant growth. Possibly, by helping in the nutrients availability, especially in processes such as biological N fixation, requiring more detailed analysis.

Several studies describe the action of PGPR in mass accumulation by plants. Authors (KUAN et al, 2016; LIMA, 2010; LAZZARETI; MELO, 2005) reported that *Bacillus* showed a positive effect on the development of beans and corn plants. In corn, *B subtilis* (PRBS-1 and OG lineage) associated to N fertilization caused effective responses in growth and grain yield (LIMA, 2010). Inoculation of PGPR strains, such as UPMB10 (*Bacillus subtilis*) increased in plant-N uptake, dry biomass and ear yield of maize (Kuan et al, 2016). Ramos et al (2010) mentioned that the interaction of *A. lipoferum* BR 11084 strain on corn showed a significant development as far as nitrogen fertilization. Costa (2015) also observed the positive effect of *A. brasiliense* Ab-V5 and Ab-V6 strains and nitrogen fertilization association at vegetative growth stage of maize and at dry mass of stalk and leaves. De Oliveira et al. (2015) reported that *Pseudomonas* supported corn plants in their growth and increased the length and diameter of ears.

Regarding the root wet weight data (Figure 2), the mean test of Scott-Knott was used because there was great variation within treatments. This test aims to homogenize the data within the treatments, minimizing the existing variations (Da SILVA, 2007). Possibly, this great variation was due to the intense root growth during the experiment, which hampered its development inside the pots. This made it impossible to obtain the dry weight of the roots.

However, for the root wet mass it was observed a significant difference between the group formed by the treatments inoculated with the isolates EB02 (T8), EB16 (T7), EB14 (T6) and the total nitrogen fertilization and that formed by the other (isolated EB26 -T5; EB23 - T4 and with half of the nitrogen fertilization).

The isolates EB02 (T8), EB16 (T7), EB14 (T6) did not differ from the total nitrogen fertilization, indicating that they showed similar performance in relation to the root mass accumulation. This suggests that they can induce the plant to produce more roots, increasing the uptake of water and nutrients. Araujo (2008) described the *Bacillus subtilis* potential for inducing root production in corn, cotton and soybeans. The author reported the possibility that bacteria to enhance the plant nutrition. Hartmann et al. (1983), studying the inoculation with *Azospirillum*, reported a morphological modification of the corn root system, increasing not only the number of radicels, but also the diameter of the lateral and adventitious roots.

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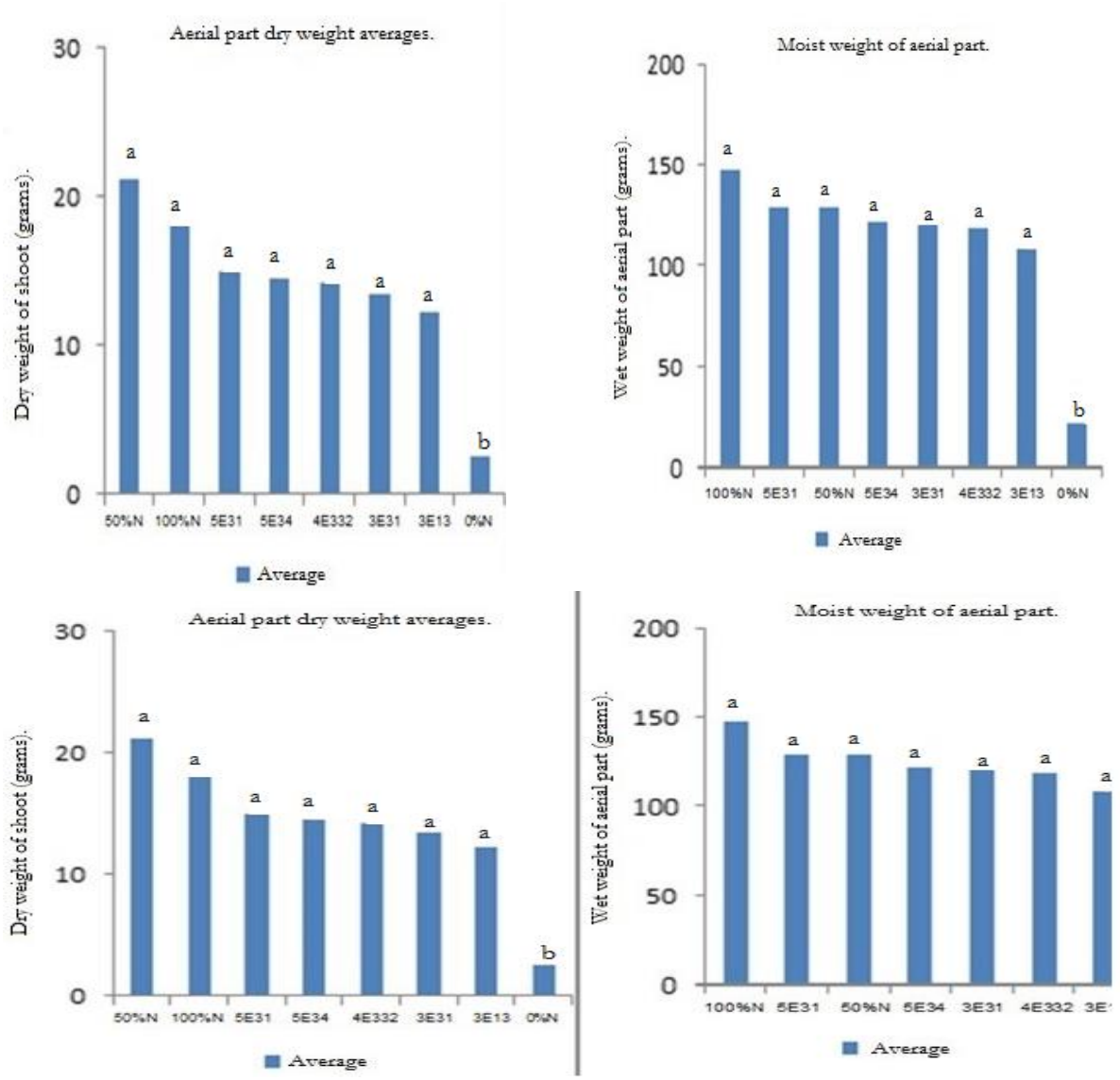

Figure 2 - Effect of inoculation of bacterial isolates on the shoot weight.

Identification of the isolates.

Sequencing of the 16S rRNA gene of bacteria revealed that 14 isolates belong to *Bacillus subtilis* species, including the five used for evaluations *in planta*. The remaining isolates could not yet be identified. The results are in agreement with the phenotypic characterization of the isolates, since many of the characteristics match to those observed in genus *Bacillus*. *Bacillus* can fix N_2 and this characteristic can be observed initially in bacteria that form pellicule in semi solid NFB medium, as well as the shape of the colony in solid medium (ARAUJO, 1999; FILHO, FERRO, PINHO, 2010), generally is circular, with irregular borders, cream or white. Andrade (2012) and Lazzaretti and Melo (2005) described the potential *B. subtilis* for production of IAA and to phosphate solubilization, resembling the results found in the tests *in vitro* with the isolates. The authors also reported that the bacteria assisted bean and banana plant growth. Several isolates from cowpea nodules grown in the Cerrado were identified as belonging to the genus *Bacillus*. Those were able to solubilize phosphate and produce IAA *in vitro* and some

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of them were able to nodulate cowpea roots (Da COSTA et al, 2013).

CONCLUSION

Phenotypic evaluations are important for the characterization of rhizobacteria. However, some analyzes, such as Gram-test must be done at different stages of the bacterial growth in order to avoid dubious reactions, such as to *Bacillus*.

The sequencing showed that 14 of the isolates belong to the *Bacillus subtilis* species. These presented productions of AIA and phosphate solubilization *in vitro*. Five of these isolates (EB02, EB16, EB14, EB23 and EB26) were tested *in vivo* and demonstrated potential to promote corn growth, including biological fixation of nitrogen. The evaluated parameters showed similar efficiency to the treatments with 50% and 100% of nitrogen fertilization recommended.

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