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RESEARCH ARTICLE

SOYBEAN GROWTH PROMOTION AND PHOSPHATE SOLUBILIZATION BY *Bacillus subtilis* STRAINS IN GREENHOUSE

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ABSTRACT

The aim of this work was to verify the *Bacillus subtilis* efficiency in soybean growth promotion, cultivated in soil with and without natural phosphate fertilization under greenhouse conditions. Seven *Bacillus subtilis* isolates and *B. subtilis* cocktail were used. The seeds were inoculated by *Bacillus subtilis* strains at a concentration of 2 x 10^8 CFU mL^{-1} using direct application technique of planting on graves. Biomass parameters, relative efficiency (RE), phosphorus content and phosphate utilization efficiency (P-UEF) were evaluated. The treatment with natural phosphate fertilization and *B. subtilis* UFTBs 06 and *B. subtilis* cocktail inoculation promoted a significant increase in biomass of 111% and 93% on relative efficiency (RE) in relation to the control, respectively. On the other hand, *B. subtilis* UFTBs 07 and *B. subtilis* cocktail were able to promote the biomass increase without natural phosphate fertilization of 85% and 95% on relative efficiency (RE) in relation to control, respectively. Therefore, *B. subtilis* cocktail without fertilization and *B. subtilis* UFTBs 07 with phosphate fertilization increased their phosphate utilization efficiency (P-UEF) in 256% and 150% in relation to the control, respectively. The most of isolates provided a higher phosphorus content available in soil and aerial part of soybean plants.

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INTRODUCTION

Microorganisms from soils can be classified according to their influence in plants, such as harmful, beneficial and neutral. The plant growth-promoting rhizobacteria (PGPR) belong to the beneficial microorganisms. PGPR are soil-dwelling bacteria, and they have the ability to promote plant growth and control phytopathogenic microorganisms. The mode of action of these bacteria in plants is linked to antibiotic production, siderophores production, systemic resistance induction, hormone production, asymbiotic nitrogen fixation, and phosphate solubilization (Chauhan*et al.,* 2015). Different species from several genera belong to the PGPR group, such as *Bacillus, Pseudomonas, Azotobacter, Arthrobacter, Klebsiella, Enterobacter, Serratia, Alcaligenes, Burkholderia, Rhizobium* and *Azosphillum*(Saharan and Nehra, 2011).Phosphorus (P) is a very important nutrient for the growth and development of plants, due to its important role in biomolecules (nucleic acids,phospholipids and nucleotides) (Barroso and Nahas, 2008).

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Phosphorus is also the most limiting macronutrient for plant growth in agricultural production under Brazilian conditions. However, soils may have large reserves of total phosphorus, but the amounts available for the plants are usually small. Highly weathered soils, such as tropical soils, are characterized by low availability of phosphorus. There are natural processes, which are able to make phosphorus unavailable in form available, such as the microbial solubilization of insoluble inorganic phosphates already existing or added to soil as phosphorite (Zaidi *et al.,* 2009).The soybean (*Glycine max* L.) is one of the most important crops in the world economy. Therefore, several studies have been carried out with *Bacillus subtilis* in order to obtain an increase in plant growth and yield, as well as a better knowledge of the ability of microorganisms to solubilize phosphates. Oliveira*et al.* (2016) evaluated the effect of different doses of *Bacillus subtilis* inoculated on bean seed and obtained significant benefits in the initial growth of culture in high vigor seeds. Raasch *et al.* (2013) studied the inoculation of *Bacillus subtilis* in eucalyptus mini-cuttings, where they observed an increase in the seedlings growth varying from 20.3 to 37.2%.Consequently, this study aimed to evaluate the ability of isolates of *Bacillus subtilis* in promoting growth and phosphate solubilization in soybean crops (*Glycine max* L.) under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Laboratory of Microbiology of the Federal University of Tocantins, Gurupi campus. Located at 11°43'45" S and 49°04'07" W, at 278 m height. Seven strains of *Bacillus subtilis* isolated from savanna soils of the crop areas in the State of Tocantins and stored in the strains bank of the Laboratory of Microbiology were used. Nine treatments were developed with *Bacillus subtilis* isolatesUFTBs 01, UFTBs 02, UFTBs 03, UFTBs 04, UFTBs 05, UFTBs 06, UFTBs 07, *B. subtilis* cocktail (UFTBs 01, UFTBs 02 and UFTBs 03) and a control without inoculation. The isolates were maintained in growth and replicated in LB medium (Luria-Bertani). Two independent tests with soil samples were conducted: natural phosphate fertilization (+NPF) and without natural phosphate fertilization (-NPF).

The soybean cultivar used in the experiment was M 9144 RR. The experiments were installed in 2 L black plastic pots, filled with soil collected from cultivation area with the following characteristics: 1.80 $cmol_c\ dm^{-3}$Ca, 0.75 $cmol_c dm^{-3}$ Mg, 0.00 $cmol_c\ dm^{-3}$ Al, 5.54 $cmol_c dm^{-3}$H+Al, 0.21 $cmol_c dm^{-3}$ K, 8.31 $cmol_c\ dm^{-3}$ CTC, 2.76 $cmol_c\ dm^{-3}$ SB, 83.54 mg dm^{-3} (ppm) K, 5.85 mg dm^{-3} (PP) P (Mel), 33.27% V, 0.00% M, 2.56% (25.59 g dm^{-3}) organic matter, pH 4.8 in $CaCl_2$, pH 5.38 in water (Embrapa, 2009).In the +NPF trial, the soil was supplemented with 0.3 g pot^{-1} insoluble natural phosphate (bound) at 100 mg kg^{-1} soil concentration (65 kg de P_2O_5 ha^{-1}). The phosphate concentrate used was Angico (32% total P_2O_5 content), obtained from Galvani (Fertilizer Industry in Luiz Eduardo Magalhães, western Bahia, Brazil). Then, six soybean seeds per pot were sown, initially inoculated with rhizobia (strains SEMIA 5079 and SEMIA 5080, *Bradyrhizibiumjaponicum*).The seeds were inoculated by *Bacillus subtilis* strains at planting with direct application in the grave on seeds in an amount of 1 mL pot^{-1} of a bacterial cells suspension obtained by scraping from Luria-Bertani (LB) medium plates with a minimum concentration of 2 x 10^8 CFU mL^{-1}. Irrigation was done manually, providing water to the plants until the soil field capacity. Seven days after planting was done thinning plants leaving only one plant per pot.Evaluations were made 45 days after planting.

The soil of the pots adhered to te roots was carefully removed and placed for drying to be analyzed for available phosphorus. Then, the root system was separated from the aerial part of the plants and the roots were washed in running water to remove the adhered soil. The nodules were removed from the roots and counted. After that, the material was placed for oven drying with forced aeration at 75 °C to constant mass. After drying, the material was weighed to obtain the dry mass of the aerial part (DMAP), root dry mass (RDM), total dry mass (TDM), number of nodules (NN) and dry mass of the nodules (DMN). The relative efficiency (RE) was determined using biomass data of each treatment in each experiment according to the equation: RE = (DMAP inoculated with isolates/ DMAP without inoculation) x 100. The DMAP was milled in a knife mill where a sample was taken to evaluate the content of phosphorus in the aerial part (Embrapa, 2009). The available phosphorus was determined with the dry soil samples by the Mehlich-1 method (Embrapa, 2009). The phosphate utilization efficiency (P-UEF) was determined with the phosphorus content in the aerial part in the soybean plants: P-UEF = [($DMAP^2$)/(Nutrient content)]. Data were submitted to analysis of variance with test F, and the treatment averages were grouped by the Scott-Knott test at 1 or 5% probability using the statistical program Assistat (Silva, 2008).

RESULTS AND DISCUSSION

In the treatments with natural phosphate fertilization (+NPF) for the evaluated characteristics of the dry mass of the aerial part (DMAP), root dry mass (RDM) and total dry mass (TDM), the treatments with isolates UFTBs 04, UFTBs 05, UFTBs 06, UFTBs 07 and *B. subtilis* cocktail were superior ($p<0.05$) to the other isolates and the control (Table 1). The treatments did not present significant difference in relation to the number of nodules (NN) and dry mass of nodules (DMN). In the treatments where the soil did not receive natural phosphate fertilization (-NPF), only the isolates UFTBs 07 and *B.* subtilis cocktail were superior ($p<0,05$) in relation to the other treatments and the control in the DMAP, RDM and TDM evaluations. For RDM, the treatments with UFTBs 01 UFTBs 02 and UFTBs 03 were also superior ($p<0.05$) to the control treatment. There was no significant difference between treatments in NN and DMN (Table 1).

Table 1. Analysis of biomass and nodulation of soybean (*Glycine max* L.) inoculated by *Bacillus subtilis* with (+) and without (-) natural phosphate fertilization (NPF)

Treatments	DMAP (g)	RDM (g)	TDM (g)	NN	DMN (mg)
+ NPF					
UFTBs 01	0.51 b	0.40 b	0.91 b	5.0 a	5.3 a
UFTBs 02	0.52 b	0.36 b	0.88 b	4.0 a	4.0 a
UFTBs 03	0.68 b	0.44 b	1.12 b	4.0 a	5.7 a
UFTBs 04	0.89 a	0.65 a	1.54 a	6.7 a	9.3 a
UFTBs 05	0.96 a	0.62 a	1.58 a	6.3 a	11.0 a
UFTBs 06	1.14 a	0.67 a	1.81 a	8.0 a	12.7 a
UFTBs 07	0.91 a	0.58 a	1.49 a	4.7 a	10.3 a
B. *subtilis* cocktail[1]	1.04 a	0.69 a	1.73 a	6.7 a	14.3 a
Control	0.54 b	0.42 b	0.96 b	3.0 a	5.3 a
CV (%)	31.9	15.5	23.5	34.1	61.5
- NPF					
UFTBs 01	0.60 b	0.43 b	1.03 b	4.3 a	3.7 a
UFTBs 02	0.55 b	0.44 b	0.99 b	3.3 a	3.7 a
UFTBs 03	0.53 b	0.41 b	0.94 b	3.3 a	3.0 a
UFTBs 04	0.57 b	0.36 c	0.93 b	3.7 a	3.0 a
UFTBs 05	0.62 b	0.34 c	0.96 b	5.3 a	4.7 a
UFTBs 06	0.57 b	0.26 c	0.83 b	4.0 a	3.0 a
UFTBs 07	0.76 a	0.52 a	1.28 a	6.3 a	5.7 a
B. *subtilis* cocktail[1]	0.80 a	0.58 a	1.38 a	6.7 a	6.7 a
Control	0.41 b	0.25 c	0.66 b	4.0 a	4.0 a
CV (%)	18.7	15.2	14.9	35.3	43.0

Averages followed by the same letter do not differ statistically by the Scott-Knott test at 5% significance. [1]*B. subtilis* cocktail (UFTBs 01, UFTBs 02 and UFTBs 03). *DMAP* dry mass of the aerial part. *RDM* root dry mass. *TDM* total dry mass. *NN* number of nodules. *DMN* dry mass of nodules. *CV* coefficient of variation.

The relative efficiency (RE), which evaluates the biomass of aerial part for the treatments with inoculation by *B. subtilis* isolates related to the control treatment without inoculation,in the treatments with natural phosphate fertilization (+NPF), the strains UFTBs 03, UFTBs 04, UFTBs 05 UFTBs 06, UFTBs 07 and *B. subtilis* cocktail presented averages above the control (Figure 1). Therefore, the highest averages were found with inoculation of the isolates UFTBs 06 and *B. subtilis* cocktail with RE increase of 111% and 93%, respectively, in relation to the control.

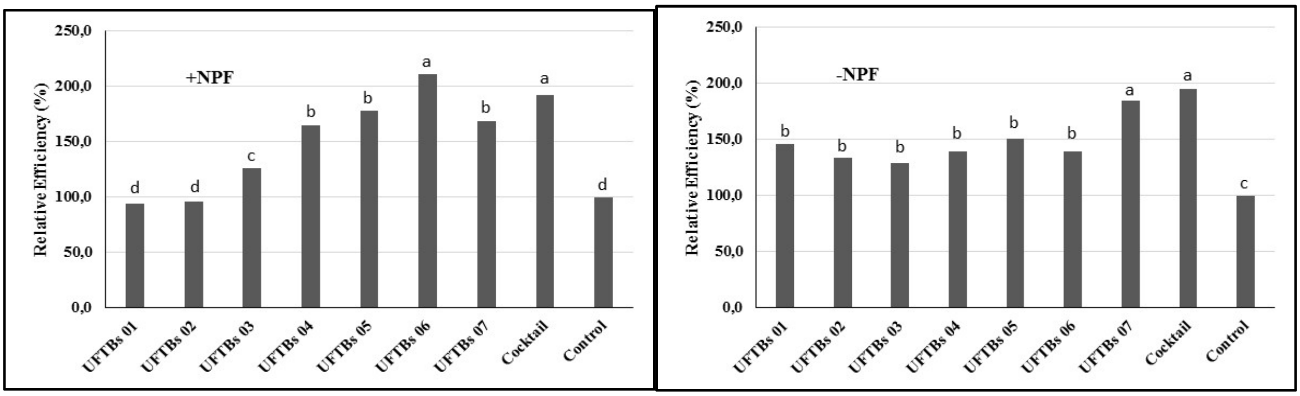

Figure 1. Soybean relative efficiency inoculated by *Bacillus subtilis* isolates with (+) and without (-) natural phosphate fertilization (NPF) in relation to control without inoculation. Different letters on bars indicate significant differences between treatments according to Scott-Knott test (p<0.05)

In the soybean without natural phosphate fertilization (-NPF), all treatments had an efficiency relative (RE) higher than the control, especially to the treatments with UFTBs 07 isolate and *B. subtilis* cocktail showing an increase of 85% and 95%%, respectively, in relation to the control (Figure 1).In the analysis of the soil phosphorus content, the UFTBs 05, UFTBs 06 and UFTBs 07 isolates without natural phosphate fertilization (-NPF) showed values higher than 6.1 g kg^{-1} phosphorus (P) compared to the others ($p<0.05$) (Table 2). Consequently, these isolates increased the soil phosphorus content at 30, 43 and 38%, respectively. On the other hand, UFTBs 04, UFTBs 06 and *B. subtilis* cocktail with natural phosphate fertilization (+NPF) provided higher soil phosphorus content ($p<0.05$), followed by isolates UFTBs 01, UFTBs 02, UFTBs 03, UFTBs 05 and UFTBs 07 with values higher than the control without inoculation by *B. subtilis* . Thus, the inoculation by *B. subtilis* strains (+NPF) increased the soil phosphorus content from 57 to 155% relative to the control (Table 2).

Table 2. Mean values of soil phosphorus content cultivated with soybean and inoculated by *B. subtilis* strains with and without natural phosphate fertilization

	-NPF		+NPF	
Treatments	P (g kg^{-1})	% P increase[1]	P (g kg^{-1})	% P increase[1]
UFTBs 01	4.9 c	104	11.9 b	157
UFTBs 02	4.6 c	98	14.3 b	188
UFTBs 03	4.7 c	100	14.3 b	188
UFTBs 04	5.1 b	109	17.0 a	224
UFTBs 05	6.1 a	130	15.1 b	199
UFTBs 06	6.7 a	143	19.4 a	255
UFTBs 07	6.5 a	138	14.1 b	186
B. subtilis cocktail[2]	4.9 c	104	16.5 a	217
Control	4.7 c	100	7.6 c	100
CV (%)	12.2	-	15.3	-

Means followed by the same lowercase letter in the column do not differ from each other by the Scott-Knott test at 5% probability. [1] Phosphorus content increase in relation to control. [2]*B. subtilis* cocktail (UFTBs 01, UFTBs 02 and UFTBs 03). *P*, phosphorus content. *–NPF*, without natural phosphate fertilization. *+NPF*, with natural phosphate fertilization. *CV*,coefficient of variation.

For the phosphorus content in the aerial part of the soybean plants, the isolate UFTBs 07 was superior ($p<0,05$) to the other treatments, being 58% superior to the control in the treatments –NPF (Table 3). In the case of phosphorus utilization efficiency (P-UEF), the highest values ($p<0.05$) were found in the treatments inoculated by *B. subtilis*, being higher ($p<0.05$) for the *B. subtilis* cocktail treatment.

The increase in the P-UEF percentage ranged from 67 to 256% for the isolates evaluated relative to the control. The phosphorus content in the aerial part for soybean, where the soil was fertilized with natural phosphate (+NPF), was higher in the isolates UFTBs 04 and UFTBs 06, followed by the isolates UFTBs 03, UFTBs 05 and cocktail, being superior to the other treatments and the control. The highest values of P-UEF were evidenced by *B. subtilis* UFTBs 07 and *B. subtilis* cocktail. The increase in the P-UEF percentage ranged from 8 to 150% for the *B. subtilis* isolates in +NPF conditions relative to the control (Table 3).

Table 3. Mean values of phosphorus content in aerial part (P) and phosphorus utilization efficiency (P-UEF) in soybean by *B. subtilis* under -NPF and +NPF conditions

Treatments	P (g kg^{-1})	% P increase[1]	P-UEF	% P-UEF increase[1]
-NPF				
UFTBs 01	2.0 b	105	0.18 b	200
UFTBs 02	1.9 b	100	0.16 b	178
UFTBs 03	1.9 b	100	0.15 b	167
UFTBs 04	1.7 b	90	0.19 b	211
UFTBs 05	2.0 b	105	0.19 b	211
UFTBs 06	1.9 b	100	0.17 b	189
UFTBs 07	3.0 a	158	0.19 b	211
B. subtilis cocktail[2]	2.0 b	105	0.32 a	356
Control	1.9 b	100	0.09 c	100
CV (%)	8.9	-	11.2	-
+NPF				
UFTBs 01	1.6 c	64	0.16 c	133
UFTBs 02	2.9 c	116	0.09 d	75
UFTBs 03	3.7 b	148	0.13 d	108
UFTBs 04	5.0 a	200	0.16 c	133
UFTBs 05	4.3 b	172	0.21 b	175
UFTBs 06	5.6 a	224	0.23 b	192
UFTBs 07	2.8 c	112	0.30 a	250
B. subtilis cocktail[2]	3.9 b	156	0.28 a	233
Control	2.5 c	100	0.12 d	100
CV (%)	9.8	-	10.3	-

Means followed by the same lowercase letter in the column do not differ from each other by the Skott-Knott test at 5% probability. [1] Increase in relation to control. [2]*B. subtilis* cocktail (UFTBs 01, UFTBs 02 and UFTBs 03). *P*, phosphorus content. *P-UEF*, phosphorus utilization efficiency. *–NPF*, without natural phosphate fertilization. *+NPF*, with natural phosphate fertilization. *CV*,coefficient of variation.

In this study, a greater increase of DMAP, RDM and TDM was observed. These increases were provided by the most of *B. subtilis* isolates in soybean plants. This result can be connected to various mechanisms by which these bacteria acts, such as hydrocyanic acid production, phytohormones, enzymes, in the nutrients availability, such as phosphorus and nitrogen, acting in the biological control of phytopathogens, among others. The increment provided may also be related to the ability of rhizobacteria to produce plant growth regulators (PGPR), which are organic compounds that influence the physiological processes of plants at low concentrations, such as indole-3-acetic acid (IAA) and the ability to solubilize organic soil phosphates (Ashrafuzzaman *et al.,* 2009). Cerqueiraet al. (2015) in their work using four isolates of *Bacillus* spp. performed *in vitro* tests where it confirmed the production of indole acetic acid (IAA) and ACC-deaminase by these isolates. Saharan and Nehra (2011) observed the use of *Bacillus* species to improve different root parameters, such as rooting, root length and dry matter content, where inoculation with IAA-producing isolates increased the absorption of some nutrients, promoting the growth of sweet potatoes and greater rooting of eucalyptus seedlings. Lima*et al.* (2011) concluded that seed inoculation by *Bacillus subtilis* improved the development and increased the corn grains yield.Araújo *et al.* (2005),using isolates of *B. subtilis* found that the AP-3 isolate increased root yield, whereas the PRBS-1 isolate increased the lateral roots in soybean, similarly may have occurred in the present study, where be observed for the MSR of some treatments with *B. subtilis* inoculation (Table 1).This increase in root dry mass (RDM)wasalsoobserved byOliveira*et al.* (2016)with increase in the primary root length of common bean seedlings, showing that there is contribution of the association of this rhizobacteria to the plant. In relation to the number of nodules (NN) and dry mass of nodules (DMN) in soybean crop, no treatment was statistically different in both soils with natural phosphate fertilization (+NPF) as without (-NPF), however the best values were found by *B. subtilis* isolates inoculation (Table 1).The availability and solubilization of nutrients such as phosphorus and nitrogen is a factor by which the isolates tested may have acted in biomass increment. In addition, other plant growth stimulating mechanisms are also related to soil microbial metabolism, such as the production of nitrogenase, chitinases and glucanases enzymes. Most of *B. subtilis* isolates tested in this study influenced the phosphorus content in aerial part and on nutrient availability in the soil with and without natural phosphate fertilization (Table 2). This demonstrates that in these treatments some soil event could have occurred as an increase of enzymatic activity (phosphatases) or greater availability of the natural phosphate that provided an increase in the nutrient availability in the soil. Phosphorus solubilizing bacteria act on the insoluble phosphate through phosphatases, mainly acid phosphatases, with the production of organic and inorganic acids and/or pH reduction, thus obtaining the phosphate available to the plant (Vassilev and Vassileva, 2003).

Even though the natural phosphate Angico (32% de P2O5), a slightly soluble source, resulted in a greater availability of phosphorus in the soil compared to the experiment where there was no fertilization of natural phosphate. A few isolates of *B. subtilis* promoted a higher P content in the aerial part where it was fertilized, as observed in Table 3.Araújo (2008), in his work using *B. subtilis* (strain PRBS-1) formulated with oyster flour under greenhouse conditions, there was an increase in the emergence of the culture of cotton and soybeans, also found a greater increase in the corn dry matter at 40 days after emergence, higher phosphorus concentration in cotton and corn leaves, and higher nitrogen content in corn leaves. Andrade (2012) verified the *in vitro* solubilization capacity of insoluble calcium phosphate by different species of *Bacillus*, among them *B. subtilis* .Therefore, it is possible to justify the biomass gain and higher phosphorus content in soybean when inoculated with *B. subtilis* isolates, due to the probable synthesis or stimulation of phytohormones production, as well as the phosphate solubilization. Therefore, acting on the available phosphorus in the soil as well as on the natural phosphate supplement, as seen in the study, providing a greater amount of phosphorus available in the soil for the plant. Consequently, the most of *B. subtilis* isolates tested in this work demonstrated potential for plant growth promotion as well as phosphate solubilization. Future studies should be done to check the real effectiveness of these isolated in the field as well as *in vitro* tests.

Conclusions

The *Bacillus subtilis* isolatesUFTBs 04, UFTBs 05, UFTBs 06, UFTBs 07 and cocktail promoted biomass increase in the soybean crop with natural phosphate fertilization (+NPF). On the other hand, *B. subtilis* UFTBs 07 and cocktail were able to promote he largest increase in plant biomass without natural phosphate fertilization (-NPF). The most of isolates tested provided a higher phosphorus content in soil and aerial part of plants, under both conditions of natural phosphate fertilization and without fertilizer use. Then, *B. subtilis* isolates showed potential to promote plant growth.

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REFERENCES

Andrade, L.F. 2012. Bactérias endofíticas em bananeira Prata Anã: fixação de nitrogênio, solubilização de fosfato e produção de ácido indol-3-acético. 76 f. Dissertação (Mestrado em Produção Vegetal no Semiárido). Universidade Estadual de Montes Claros, Montes Claros, Brasil.

Araújo, F. F. 2008. Inoculação de sementes com *Bacillussubtilis,* formulado com farinha de ostras e desenvolvimento de milho, soja e algodão. *Ciência e Agrotecnologia*, 32: 456-462.

Araújo, F. F.; Henning, A., Hungria, M. 2005. Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development. *World Journal of Microbiology & Biotechnology*, 21: 1639-1645.

Ashrafuzzaman, M., Hossen, F. A; Ismail, M. R., Hoque, M. A., Islam, M. Z., Shahidullan, S. M., Meon, S. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *AfricanJournalofBiotechnoogy*, 8: 1247–1252.

Barroso, C. B.; Nahas, E. 2008. Solubilização do fosfato de ferro em meio de cultura. *Pesquisa Agropecuária Brasileira*, 43: 529-535.

Cerqueira, W. F., Morais, J. S., Miranda, J. S., Melo, I. K. S., Santos, A. F. J. 2015. Influência de bactérias do gênero *Bacillus*sobre o crescimento de feijão comum (*Phaseolusvulgaris*L.).*Enciclopédia Biosfera*, 11: 20-31.

Chauhan, H., Bagyaraj, D. J., Selvakumar, G., Sundaram, S. P. 2015. Novel plant growth promoting rhizobacteria - Prospects and potential. *Applied Soil Ecology*, 95: 38–53.

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). 2009. Manual de análises químicas de solos, plantas e fertilizantes. 2 ed., Brasília-DF, Brasil.

Lima, F.F., Nunes, L. A. P. L., Figueiredo, M. V. B., Araújo, F. F., Lima, L. M., Araújo, A. S. F. 2011. *Bacillussubtilis* e adubação nitrogenada na produtividade do milho. *Revista Brasileira de Ciências Agrárias*, 6:657-661.

Oliveira, G.R. F., Silva, M. S., Marciano, T. Y. F., Proença, S. L., Sá, M. E. 2016. Crescimento inicial do feijoeiro em função do vigor de sementes e inoculação com *Bacillussubtilis*. *Brazilian Journal of Biosystems Engineering*, 10:439-448.

Raasch, L. D., Bonaldo, S. M., Oliveira, A. A. F. 2013. *Bacillussubtilis:* enraizamento e crescimento de miniestacas de eucalipto em Sinop, norte de Mato Grosso. *Bioscience Journal*, 29:125-140.

Saharan, B.S., Nehra, V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. *Life Sciences and Medicine Research*,21:1-30.

Silva, F. A. S. 2008.ASSISTAT.Versão 7.6 beta. Disponível em: <http://www.assistat.com/indexp.html>. Access in: 10 dez. 2016.

Vassilev, N.,Vassileva, M. 2003. Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. *Applied Microbiology and Biotechnology*, 61: 435-440

Zaidi, A.,Khan,M. S., Ahemad, M., Oves, M.2009. Plant growth promotion by phosphate solubilizing bacteria. *ActaMicrobiologicaetImmunologicaHungarica*,56: 263-284.

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