Australian Journal of Crop Science AJCS
AJCS 9(8):754-760 (2015) ISSN:1835-2707

Potential of the endophytic bacteria (*Herbaspirillum* spp. and *Bacillus* spp.) to promote sugarcane growth

João Manoel da Silva[1*], Tania Marta Carvalho dos Santos[2], Ludmilla Santos de Albuquerque[2], Yamina Coentro Montaldo[2], José Ubaldo Lima de Oliveira[2], Sybelle Geórgia Mesquita da Silva[2], Maisa Silva Nascimento[2], Raiza da Rocha Oliveira Teixeira[2]

[1]Federal University of Sergipe, São Cristóvão, Brazil
[2]Federal University of Alagoas, Rio Largo, Agricultural Microbiology Laboratory, Rio Largo, Alagoas, Brazil

***Corresponding author: jm.agro@hotmail.com**

Abstract

The necessity for increase in sugarcane production encouraged the development of some studies with the objective of optimizing production. Thus, the objective of this study was to evaluate the potential of the endophytic bacteria *Herbaspirillum* spp. and the *Bacillus* spp. in sugarcane growth promotion. The research was performed at the Agricultural Microbiology Laboratory of the Federal University of Alagoas. The isolates were obtained from sugarcane and subjected to qualitative production of Indole Acetic Acid (IAA), gibberellins, cytokinins, antagonism, phosphate solubilization and rout and budding of sugarcane gems. Four isolates (ISO1, ISO3, ISO4 and ISO5) were able to produce auxins *in vitro*. To detect the presence of gibberellins and cytokinins, isolate 4 (ISO4) showed the greatest proportional weight of cotyledons differing significantly than the other treatments and the control. However, there was less weight hypocotyl to the same strain. The isolates showed varying rates of solubilization, with a mean of 1.70 obtained by isolate 1, showing the higher solubilization. All isolates exerted an antagonistic effect against the pathogen. The increases in budding, as well as rooting and seedling growth varied according to the isolated and no deleterious effect was observed in any of them. The positive interaction between endophytic bacteria and sugarcane was noted.

Keywords: Phosphate solubilization, biological control, endophytic micro-organisms, phytohormones.
Abbreviations: NBRIP_National Botanical Research Institute's phosphate growth medium; ISO_Isolate; IAA_Indole Acetic Acid; TSA_Tripticase Soy Agar; SI_Solubilization Index.

Introduction

Some studies have explored the interaction between bacteria and sugarcane with a focus on developing varieties suitable for Southeast region of the country (Baldani et al., 2003). However, in the Northeast, there are few studies to meet and get practical applications of micro-organisms-plant interaction. This may be one of the factors contributing to the Northeast having submitted the lowest yield in the 2008 season, despite being the second in area and production (CONAB, 2008). Bacteria are the group of organisms most addressed in research studies applicable characteristics for the development of more sustainable inputs, whether for industry, agriculture or reclamation. Currently, there are several studies that point to the large existing of the bacterial community exist in both the soil and associated plants (Rathnayake et al., 2009; Sheng, et al., 2008; Kuiper et al., 2004; Penrose and Glick, 2003; Azevedo et al., 2000).

The endophytic micro-organisms are potentially useful to agriculture and industry, particularly in the food and pharmaceutical industries. Several selected species of endophytes have potential in crop protection industries, besides being used as genetic vectors (Souza et al., 2004). In recent years, the interests of using micro-organisms in agricultural practices has increased significantly, as both plant growth promotion and biological control of pests and plant diseases among the other applications. They are potential substitutes for chemicals that could favor the conservation of environment (Souza, 2001). Studies on sugarcane demonstrate that application of endophytic bacteria like *Acetobacter diazotrophic* can raise production without increasing costs such as nitrogen fertilizers (Döbereiner, 1992).

After nitrogen, phosphorus (P) is the most limiting nutrient for plant growth (Gyaneshwar et al., 2002). It participates as a structural component of nucleic acids and phospholipids, as well as adenosine triphosphate (ATP), being a key element of metabolic and biochemical pathways, such as various steps of the process of C3 and C4 plants of the Calvin cycle and glycolysis (Holford, 1997). The endophytic bacteria are shown to be effective biocontrol agents of various pathogens, such as bacterial and fungal (Hallmann et al. 1997). This usually happens due to a close affinity with plants. With the aim of obtaining agents that are potentially effective biological controls, a large number of antagonists have been pre-selected at *in vitro* tests, due to the difficulties presented by the selection methods in the field, such as cost, hand labor, time and space (Xiujun, et al. 1996).

Table 1. Qualitative Production of IAA by endophytic bacteria.

Isolate	IAA Production
ISO1	+
ISO2	-
ISO3	+
ISO4	+
ISO5	+

Fig 1. Selecting the capacity of IAA using membrane. The positive result is indicated by the color pink.

In plants, plant hormones (auxin, cytokinin, gibberellin, abscisic acid and ethylene) are organic substances, which play a role in growth regulation. However, some hormones have inhibitory effects, being more appropriate to consider them as chemical regulators. The production of chemical plant growth regulators is a mechanism of bacteria-plant interaction, being influenced by various factors such as the host's genotype and the actual micro-organism (Jain and Patroguin, 1985). The production of IAA which apparently does not function as a hormone in bacterial cells may have evolved due to their importance in plant-bacterium ratio (Patten and Glick, 2002).

Results

Auxins, gibberellins and cytokinins production

The observed results showed that four of the five isolates produced IAA in culture medium with the presence of L-tryptophan (Table 1). The production of IAA was more effective in isolates from leaf and root. The pinkish-red color characterizes the production of IAA by stimulation of L-tryptophan (Fig 1).

Detection of cytokinins and gibberellins was carried out using the Catellan (1999) method. The isolate ISO4 provided the greatest weight of cotyledons differing significantly ($P \leq 0.05$) from the other and the control (Fig 2). However, there was the lowest weight of hypocotyl to the same strain. Regarding the length of the isolated cotyledons, ISO4 and ISO2 showed the highest and lowest values of 10.78 and 7.50 mm, respectively. The supernatant of individual producers of cytokines enhances the weight of the cotyledons but not the growth of hypocotyls. Growers of gibberellins increased both the weight of the cotyledons and the hypocotyls length (Catellan, 1999). From our results, it appears that the ISO4 increased and the length of the cotyledon - with significant differences among the remaining strains but did not differ from control.

Phosphate Solubilization

The formation of halos on solid medium NBRIP solubilization was used as the parameter for evaluation of potential solubilization of calcium phosphate by endophytic bacteria isolated from sugarcane (Fig 3). Bacterial colonies that were able to form measurable halos were considered as solubilizing calcium phosphate. Halo solubilization was observed, except for the ISO2. The isolates showed varying rates of solubilization of low solubility, with an average of 1.70 obtained by ISO1. The high solubilization, with an average of 3.25 was found in ISO5. The highest SI was observed after 15 days of incubation; however, a lack of significance (Fig 4) for interaction indicates that there is no dependency between the two factors.

Antagonism

Table 2 shows the analysis of variance of the results obtained for inhibition index of plant pathogenic fungus without *C. inaequalis* percentage, the F-test did not detect significant differences ($p \geq 0.05$) among the isolates. All isolates exerted an antagonistic effect against the pathogen. The table 2 showed the efficiency of *in vitro* ability of inhibition of mycelial growth.

Induction of rooting and budding from sugarcane

The increments in budding, as well as rooting and seedling growth varied (Fig 5). The deleterious effect was not observed in any of them. The ISO2 was highlighted, with a gain of 100% over control to rooting and budding and greater seedling length (Table 3). All isolates grew around the roots, characterized by turbidity of the medium.

Discussion

Plant hormones naturally occur in plants and plant growth regulators are influencing physiological processes at low concentrations. The phytohormones can be classified as auxin (cell differentiation, root growth, promote and control the growth of fruit abscission), cytokines (growth regulation, differentiation and plant senescence), gibberellin (cell division and elongation interrupt dormancy and increases the fruit development). The action of growth regulators produced by endophytic bacteria has been studied in several plant species (Teixeira et al., 2007; Tsavkelova et al., 2007; Assumpção et al., 2009; Cerigioli, 2005; Phetcharat and Duangpaeng, 2012). The synthesis of phytohormones is characterized as a mechanism of direct action in promoting growth in plants (Lodewyckx et al., 2002).

Bacterial IAA and a secondary metabolite are produced in the stationary phase of bacterial growth, but the length of the stationary phase depends on the species. Thus, it is necessary to know the behavior of each isolate for auxin synthesis at different times of the bacterial development. This enables the determination of the period in which the maximum synthesis of the hormone is reached (Cerigioli, 2005).

Pedraza et al. (2004) considered that the amounts of IAA excreted by isolates depend on the species or even the strain under study, as well as the conditions, under which the organisms are grown, such as presence or absence of the precursor of IAA in the culture medium (tryptophan), oxygenation, pH and growth phase.

Table 2. Efficiency of *in vitro* antagonistic ability indicated by inhibition of mycelial growth of *C. inaequalis* and inhibition index 100- (TM / MC) x100, after days of incubation. Means of five repetitions.

Isolates	Means of mycelial growth of *C. inaequalis* (cm)*	Im%**
Control	9.000	-
ISO1	2.492 a	72.31
ISO2	2.200 a	75.55
ISO3	2.200 a	75,55
ISO4	2.200 a	75.55
ISO5	2.785 a	69.05

*Means followed by the same letter do not differ by the Scott-Knott p≥0.05. ** Inhibition Index showed in percentage.

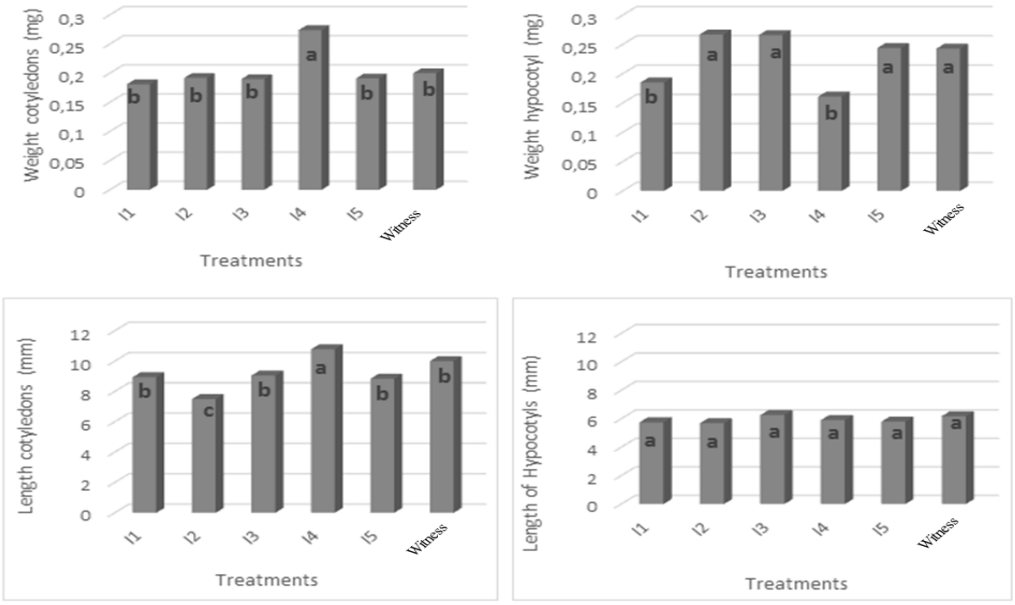

Fig 2. Production of gibberellins and cytokinins by endophytic isolates of cane sugar indicated by the weight of cotyledons (PC); weight of hypocotyls (PH); length of cotyledons (CC) and length hypocotyls (CH)) inoculated with endophytic bacteria isolated from cane sugar. Means of five repetitions. Means followed by different letters differ by the Scott-Knott test (P ≤0.05).

Table 3. Efficiency of endophytes isolated on rooting and budding from cane sugar *in vitro*, after fifteen days of incubation. Means of five repetitions*.

Isolate	Rooting (%)	Budding (%)	Seedling length (cm)
ISO1	50	25	6.3 a
ISO2	100	100	11.3 a
ISO3	75	50	4.5 a
ISO4	100	50	5.1 a
ISO5	75	75	5.3 a
Witness	0	0	
CV (%)		56.00	

*Means followed by same letter do not differ by the Scott-Knott p≥0.05.

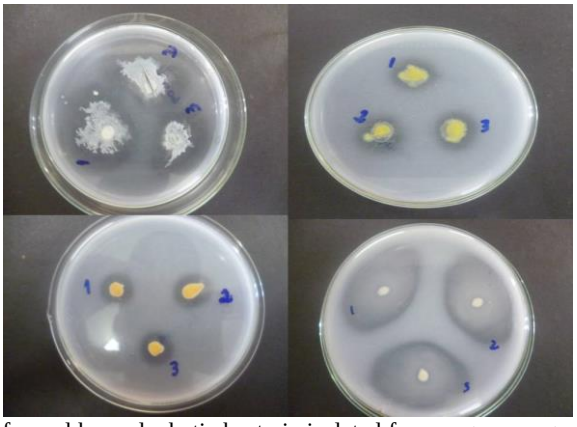

Fig 3. Phosphate solubilization halo formed by endophytic bacteria isolated from sugarcane grown on NBRIP medium pH 7.0 at 28 °C for fifteen days.

Table 4. Description of origin of endophytic bacterial isolates used in the experiment.

Isolate	Procedence	Genus
ISO1	Leaf	*Herbaspirillum*
ISO2	Culm	*Herbaspirillum*
ISO3	Root	*Bacillus*
ISO4	Leaf	*Bacillus*
ISO5	Culm	*Bacillus*

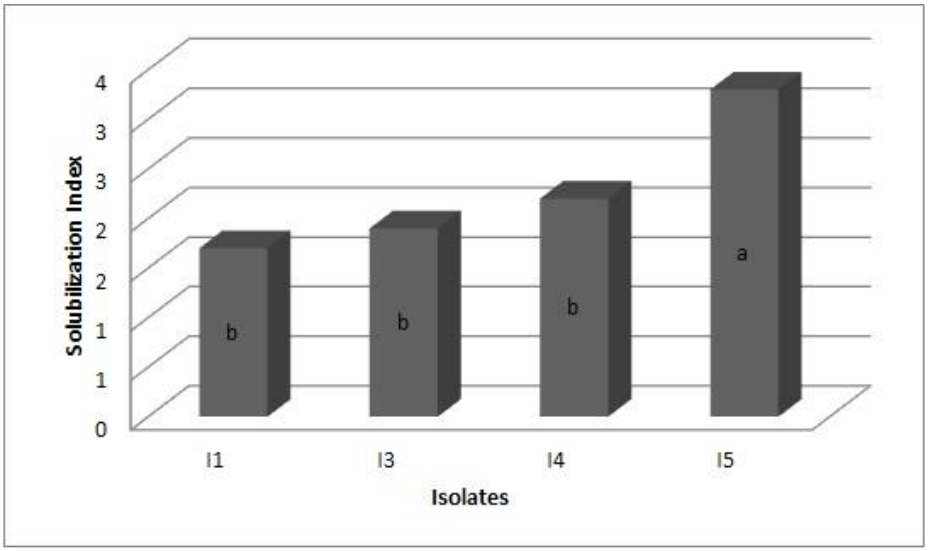

Fig 4. Efficiency of phosphate solubilization in NBRIP medium indicated by the solubilization index (SI) calculated as the ratio between the average diameter of the halos and the mean diameter of colonies of each isolate after 15 days of incubation. Means of three repetitions. Means followed by different letters differ by the Scott-Knott test ($P \leq 0.05$).

Fig 5. *In vitro* rooting and budding of gems of sugarcane inoculated with endophytic bacteria. From left to right, shows the rooting and budding of the isolated ISO1, ISO2, ISO3, ISO4, ISO5 and the witness (right side end).

While there has been production of IAA in the presence of the precursor L-tryptophan, there are also other routes by which the hormone can be produced by micro-organisms. Studies report evidence for the existence of more than one route for synthesis of IAA in a single bacterium and can select a particular route, depending on the ambient conditions (Patten and Glick, 1996; Dobbelaere et al., 1999).

The variation in the rate of solubilization of phosphate that we observed here is similar to others. For example, Santos et al. (2012) evaluated 15 endophytic bacteria and 15 isolates of rhizobacteria in sugarcane and found means ranging from 1.00 to 2.33 in the endophytic bacteria and 2.1 to 3.48 in rhizobacteria. Souza (2010) studed the efficiency of solubilization of the bacterial isolates obtained from solubilization ratios ranging from 1.00 to 4.06 on solid NBRIP and 0.67 to 2.76 Verma medium.

The ability to solubilize phosphate has been attributed to the ability of organism to change the pH of the environment through the release of organic acids such as citrate, lactate and succinate amongst others (Hariprasad and Niranjana, 2009). This ability is usually associated with root exudates and can act in solubilization of P in the depletion zone of the root (Nautiyal 1999; Rodriguez et al., 2000; Vasquez et al., 2000; Gyaneshwar et al., 2002; Vassilev and Vassileva, 2003).

The acids released can act directly on the mineral phosphate, making the exchange of phosphate anion by an anion in acidic substance contained or by complexing with Fe and Al ions present in phosphate rocks. The phosphorus solubilizing micro-organisms produce organic acids such as acetate, lactate, oxalate, tartrate, succinate, citrate, gluconate and glycolate (Gyaneshwar et al., 1998).

Amongst the various beneficial bacteria that exist in nature, endophytic may come to be used as biocontrol agents. Biological control seeks to maintain certain practices through agro-ecosystem balance. So, the hosts do not suffer significant damage in the presence of the pathogen due to the action of controlling non-pathogenic organisms (Grigoletti Jr et al., 2000). The growing interest in the use of endophytic micro-organisms in biological control is due to the ecological niche they occupy, which can be similar to that occupied by the pathogen. The ability of biocontrol can result from various mechanisms, such as the production of deleterious substances to pathogens or competing for space and nutrients, the production of growth promoting substances or inducing systemic resistance in the host.

Rooting parameter can be better considered as root colonization which has greater significance in the micro-organism-plant interaction. The symbiotic processes are most effective in roots, because the micro-organisms provide nutrients for the plants or protect them against attack by pathogens or pests. They also may start the process of internal colonization of the plant, which will begin the cycle of life endophyte, continuing the symbiotic processes.

The response observed in rooting can be result of the action of metabolites produced by bacterial isolates. The rooting can be divided into induction, initiation and elongation of roots. The first two steps are dependent on responsive auxin or may be due to similar mechanisms. Bastian et al. (1998) found that *H. seropedicae* is able to produce and release a significant amount of 3-indole acetic acid into the growth medium. In this sense, Radwan et al. (2004) inoculated rice and wheat with different species of *Herbaspirilum* and observed significant promotion of root growth, which directly related to the production of auxins. Olivares et al. (2002) found an increase in the activity of H + -ATPases of isolates from infected plants. The typical effect induced by auxin diazotrophic bacteria, evidenced by the increased number of sites of mitosis in roots emerged from the main root axis and consequently, by increasing the root area.

Canuto et al. (2003) found a positive effect on seedling growth of sugarcane by inoculation with diazotrophic bacteria. This leaded to observe the need for integration of alternative technologies aimed at optimizing crop productivity.

Materials and Methods

Collecting bacteria isolates

Five isolates of endophytic bacteria from sugarcane were isolated from the commercial variety RB8579, belonging to the collection of micro-organisms in Agricultural Microbiology Laboratory of Federal University of Alagoas (Table 4). The ISO1 and ISO2 belong to the genus *Herbaspirillum* and isolates ISO3, ISO4 and ISO5 belon to the genus *Bacillus*.

Production of indole acetic acid (IAA) in the presence of tryptophan

The analysis was performed according to the method of qualitative production of IAA in the presence of the precursor L-tryptophan, and the test was performed in triplicate.

Bacteria cultured in Nutrient Agar culture medium were inoculated with the aid of a platinum loop in triplicate petri plates containing TSA medium supplemented with 5 mM L-tryptophan, and covered with a nitrocellulose membrane previously sterilized, and incubated for 48 hours at room temperature. After this period, the membranes were removed from the plates and transferred to sterile plates clean forceps, which were saturated with Salkowski solution and incubated in dark at room temperature for a period of 30 min to 2h, where the presence of IAA has been detected from the presence of a pink-red halo paper after the treatment with the solution.

Gibberellins and cytokinins production

For the production of gibberellins and cytokinins the Catellan (1999) method was adopted. First, the cucumber seeds were germinated in Petri dishes containing filter paper, moistened with sterilized distilled water and sterilized as well, and incubated in the dark at room temperature for a period of 72h. Then, the cotyledons and hypocotyls were separated and cotyledons and eight hypocotyls were placed in petri dishes containing moistened filter paper with the supernatant from each isolate, and distilled water for control. Smaller cotyledons placed with part of the ribs upside down and placed 5mm hypocotyl side of cotyledons. The plates were incubated at room temperature under continuous faint fluorescent light. After three days, cotyledons and hypocotyls were dried with absorbent paper and weighed on an analytical balance and measured where the weight was compared with that of control. The supernatant of isolates that produced cytokines promoted the weight in the cotyledons but not favor the growth of hypocotyls, while isolates that produce both gibberellins promoted the weight gain of the cotyledons and the hypocotyls by increasing the length.

Phosphate solubilization

The isolates were tested pealing them to Petri dishes containing NBRIP medium supplemented with 1.5% agar (Nautiyal, 1999). This method is based on adding to a medium-insoluble phosphate, causing turbidity. The analyses were done for a fortnight, where three readings were recorded, in which we observed the formation of a translucent halo around the colony, characterizing phosphate solubilization by bacteria. Readings were taken at 5, 10 and 15 days after inoculation (DAI). The solubilization index was calculated from the equation: IS = Ø halo / colony Ø (Berraqueiro et al., 1976). Then, the solubilization was classified according to the index (lower than SI = low solubility, SI = between 2 and 3 solubilization medium, SI 3 = greater than high solubilization) (Silva Filho and Viddor, 2000) Experimental design was completely randomized with five replications in a factorial design.

Antagonism

The direct comparison method was adopted, using 6 mm discs of a diameter of pathogen colonies grown at 28°C for 7 days. Pathogen and antagonist were inoculated at opposite poles of the Petri dish containing culture medium Sabouraud Agar. For the control, only the pathogen was inoculated on a pole of the plate. The visual observation was used to distinguish the period of time necessary to take the mycelium throughout the board surface as a parameter to indicate the time to assess inhibition. For the inhibition index calculation the following formula was used: 100-Im% = (MT / MC) x100, where, Im =% index in percentage inhibition of mycelial growth, MT = Average measured in cm to the treatment area, and MC = average of the control area in cm.

The experimental design was completely randomized with four replications, using the plant pathogenic fungus

Curvularia inaequalis, causing Curvularia Spot Disease newly detected in sugarcane in Brazil, causing damage to the cultivation of cane sugar.

Root colonization

Gems of sugarcane RB92579 commercial variety, obtained from Usina Santa Clotilde, the municipality of Rio Largo-AL were used for this test. The stems were washed in running water to remove environmental dirt and cut into pieces with size about 5cm to select the best gems (younger and vigorous). After selection, the gems were gone through a process of sterilization, which were immersed in 70% ethanol for 15 min, 2% sodium hypochlorite for 5 min and triple washing with sterile distilled water. The gems were then put into glass jars containing 100mL of semi-solid Agar-Water 2% in the absence of any nutrient. Then, 1mL of each isolate was grown in Nutrient Broth medium for 48 hours under constant agitation at 150 rpm. The inoculated jars were then incubated at room temperature for 15 days under 12h light and 12h dark. The experimental design was completely randomized with four replications, consisting of six treatments, where five of them corresponded to endophytic bacteria and a witness. After 15 days of incubation, the gems were removed from the jars and submitted to biometrics analysis, which evaluated the induction of rooting and budding of the gems. The data were evaluated from the rooting and budding of gems.

Statistical analysis

Data were subjected to analysis of variance ANOVA followed by a classification of means by agglomerative Scott-Knott test at 5% probability. This test aims to separation of treatment means in different groups, by minimizing the variation within and maximizing the variation between groups, with results showing greater objectivity and clarity (Borges and Ferreira, 2003). Statistical analyzes were performed using the program Assistat 7.6 beta (Silva and Azevedo, 2002).

Conclusion

The bacteria tested were able to produce IAA, gibberellins and cytokinins through the tests. The ISO1, ISO3, ISO4 and ISO5 isolates showed to be efficient *in vitro* phosphate solubilization in NBRIP medium. All strains were able to inhibit the growth of the pathogen. Of the five isolates tested, only the ISO1 showed low efficiency in inducing rooting and budding from sugarcane.

References

Assumpção LC, Lacava PT, Dias ACF, Azevedo, JL, Menten JOM (2009) Diversidade e potencial biotecnológico da comunidade bacteriana endofítica de sementes de soja. Pesq Agropec Bras. 44(5):503-510.

Azevedo JL, Sarabia S, Maccheroni Jr. W, Pereira JO, Araújo WL (2000) Endophytic microrganisms: Aareview on insect control and recent advantages on tropical plants. Environ Biotechnol. 3(1):31.

Baldani JI; Reis, VM; Baldani VLD.; Döbereiner J. (2002) A brief story of nitrogen fixation in sugarcane – reasons for sucess in Brazil. Func Plant Biol. 29:417-423.

Bastian F, Cohern A, Piccoli P, Luna V, Baraldi R, Bottini R (1998) Production of indole-3- acetic acid and gibberellins A(1) and A(3) by Acetobacter diazotrophicus and Herbaspirillum seropedicae in chemically-defined culture media. Plant Growth Regul, Dordrecht, 24:7-11.

Berraqueiro FR, Baya AM, Cormenzana AR (1976) Establecimiento de índices para el estudio de la solubilizacion de fosfatos por bactérias del suelo. ARS Pharmac. 17:399-406.

Borges LC, Ferreira DF (2003) Poder e taxas de erro tipo I dos testes Scott-Knott, Tukey e Student-Newman-Keulssob distribuições normais e não normais dos resíduos. Rev de Mat e Est. 21:67-83.

Canuto EL, Salles JF, Oliveira ALM, Perin L, Reis VM, Baldani JI (2003) Resposta de plantas micropropagadas de cana-de-açúcar à inoculação de bactérias diazotróficas endofíticas. Agr. 37, 67-72.

Cattelan JA (1999) Métodos qualitativos para determinação de características bioquímicas e fisiológicas associadas com bactérias promotoras de crescimento vegetal. Londrina, Embrapa Soja.

Cerigioli MM (2005) Diversidade de bactérias endofíticas de raízes de milho (Zea mays L.) e potencial para promoção de crescimento. (Tese de Doutorado) – Universidade Federal de São Carlos, São Carlos. 2005.

CONAB - Companhia Nacional de Abastecimento. Available in: <http://www.conab.gov.br/conabweb/download/safra/3_levantamento2008_dez2008.pdf>. Acessed in: January 2014.

Dobbelaere S, Croonenborghs A, Thys A, Van de Broek A, Vanderleyden J (1999) Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. Plant Soil. 212:155–164.

Döbereiner J (1992) History and new perspectives of diazotrophs in association with nonleguminous plants. Symbiosis. 13:1-13.

Grigoletti Júnior A, Santos AF, Auer CG (2000) Perspectivas do uso do controle biológico contra doenças florestais. Rev Flor. 30:155-165.

Gyaneshwar P, Kumar GN. Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil. 245:83-93.

Gyaneshwar P, Naresh Kumar G, Parekh LJ (1998) Effect of buffering on the phosphate solubilizing ability of microorganisms. World J Microbiol Biotechnol. 14:669–673.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J of Microbiol. 43:895-914.

Hariprasad P, Niranjana SR (2009) Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil. 316(1-2):13-24.

Holford ICR (1997) Soil phophorus: its measurement and, its uptake by plants. Aust J of Soil Res. 35:227-239.

Jain DK, Petroguin DG (1985) Characterization of a substance produced by Azospirillum which causes branching of wheat root hairs. Can J of Microbiol. 31:206-210.

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg JJ (2004) Rhizoremediation-A beneficial plant-microbe interaction. Amer Phytopathol Soc. 17:6- 15.

Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, Van Der Lelie D (2002) Endophytic bacteria and their potential applications. Crit Rev in Plant Sci. 21:583-606.

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Let. 170:265-270.

Olivares FL, Reis VM, Façanha AR, Baldani JI, Baldani VL, Ferreira FP, Batista QR, Barreto BR, Silva LG, Azevedo IG, Brasil FC, Rossielo ROP, Miguens FC, James EK (2001) The role of endophyticdiazotrophs in sugarcane root morphogenesis and development. In: International N_2 Fixation Congress, Nitrogen Fixation-Global Perspectives, 13. Hamilton. Proceedings.Oxford, Cabi Publishing, p.471-471, 2001.

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J of Microbiol. 42:207-220.

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* Indol Acetic Acid Development of the Host Plant Root System. Appl Envir Microbiol. 68(8):3795-3801.

Pedraza RO, Ramires-Malta A, Xiqui MA, Baca BE (2004) Aromatic amino acid aminotransferase activity and indole-3-acetic production by associative nitrogen-fixing bacteria. FEMS Microbiol Let. 233:15-21.

Penrose DM, Glick BR (2003) Methods for isolating and characterizing accdeaminase-containing plant growth-promoting rhizobacteria. Physiol. 118:10–15.

Phetcharat P, Duangpaeng A, (2012) Screening of Endophytic Bacteria from Organic Rice Tissue for Indole Acetic Acid Production. Proc Engin. 32:177-183.

Radwan T, El-Sayed El-Desouk, Mohamed ZK, Reis VM (2004) Efeito da inoculação de *Azospirillum* e *Herbaspirillum* na produção de compostos indólicos em plântulas de milho e arroz. Pesq Agropec Bras. 39:987-994.

Rathnayake VN, Mallavarapu M, Bolan N, Naidu N (2009) Tolerance of heavy metals by Gram-positive soil bacteria. World Acad Sci Engg Technol. 53:1185-1189.

Rodriguez H, Gonzalez T, Selman G (2000) Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. J Biotecnol. 84:155-161.

Santos IB, Lima DRM, Barbosa JG, Oliveira JTC, Freire FJ, Kuklinsky-Sobral J (2012) Bactérias diazotróficas associadas a raízes de cana-de-açúcar: solubilização de fosfato inorgânico e tolerância à salinidade. Biosci J. 28:142-149.

Sheng XHL, Wang Q, Ye, H, Jiang J (2008) Effects of inoculation of biosurfactant producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium amended soil. J Haz Mat. 155(1):17–22.

Silva FAS, Azevedo CAV (2009) Principal Components Analysis in the Software Assistat-Statistica Attendance. In: World Congress on Computers in Agriculture, 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers, 2009.

Silva Filho GN, Vidor C (2000) Solubilização de Fosfato por Microrganismos na presença de fontes de carbono. Rev Bras de Ciênc do Sol. 24:311-319.

Souza AQL, Souza ADL, Astolfi Filho S, Belém Pinheiro ML, Sarquis MIM, Pereira JO (2004) Atividade antimicrobiana de fungos endofíticos isolados de plantas tóxicas da amazônia: Palicourea longiflora (aubl.) rich e Strychnos cogens bentham. Acta Amazôn. 34(2):185-195.

Souza CA (2010) Solubilização de fósforo por bactérias endofíticas. 2010. 38 p. Dissertação (Mestrado em Ciência do Solo) – Universidade Federal Rural de Pernambuco, Recife, 2010.

Souza ML (2001) Utilização de microrganismos na agricultura. Biotecnol. 21:28-31.

Teixeira MA, Melo IS, Vieira RF, Costa FEC, Harakava R (2007) Microrganismos endofiticos de mandioca de áreas comerciais e etnovariedades em três estados brasileiros. Pesq Agropec Bras. 42:43-49.

Tsavkelova EA, Cherdyntseva TA, Klimova SY, Shestakov AI, Botina SG, Netrusov AI (2007) Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxin. Arch Microbiol. 188:655–664.

Vassilev N, Vassileva M (2003) Biotechnologycal solubilization of rock phosphate on media containing agroindustrial wastes. Appl Microbiol Biotechnol. 61:435-440.

Xiujun Z, Shixiang W, Yi Z, Qi Z (1996) Screening biocontrol agents for *Pythium*-induced disease by germinating carrot seeds in Petri dish – a rapid and effective method. In: WENHUA, T.; COOK, J.R.; ROVIRA, A. Advances in Biological Control of Plant Diseases. Beijing: China Agricultural University Pressp. 140-144.