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Isolation and selection of plant growth-promoting bacteria associated with sugarcane[1]

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ABSTRACT

Microorganisms play a vital role in maintaining soil fertility and plant health. They can act as biofertilizers and increase the resistance to biotic and abiotic stress. This study aimed at isolating and characterizing plant growth-promoting bacteria associated with sugarcane, as well as assessing their ability to promote plant growth. Endophytic bacteria from leaf, stem, root and rhizosphere were isolated from the RB 867515 commercial sugarcane variety and screened for indole acetic acid (IAA) production, ability to solubilize phosphate, fix nitrogen and produce hydrogen cyanide (HCN), ammonia and the enzymes pectinase, cellulase and chitinase. A total of 136 bacteria were isolated, with 83 of them presenting some plant growth mechanism: 47 % phosphate solubilizers, 26 % nitrogen fixers and 57 % producing IAA, 0.7 % HCN and chitinase, 45 % ammonia, 30 % cellulose and 8 % pectinase. The seven best isolates were tested for their ability to promote plant growth in maize. The isolates tested for plant growth promotion belong to the *Enterobacteriaceae* family and the *Klebsiella*, *Enterobacter* and *Pantoea* genera. Five isolates promoted plant growth in greenhouse experiments, showing potential as biofertilizers.

KEY-WORDS: *Zea mays* L.; *Enterobacteriaceae*; *Klebsiella*; *Enterobacter*; *Pantoea*.

RESUMO

Isolamento e seleção de bactérias promotoras de crescimento vegetal associadas a cana-de-açúcar

Os micro-organismos apresentam papel fundamental na manutenção da fertilidade do solo e da saúde vegetal, podendo atuar como biofertilizantes, aumentando a resistência ao estresse biótico e abiótico. Objetivou-se isolar e caracterizar bactérias promotoras de crescimento vegetal associadas a cana-de-açúcar e avaliar a capacidade dessas bactérias em promover o crescimento vegetal. Bactérias endofíticas da folha, colmo, raiz e rizosfera foram isoladas da variedade comercial RB 867515 e triadas quanto à produção de ácido indol acético (AIA), habilidade de solubilizar fosfato, fixar nitrogênio, produzir ácido cianídrico (HCN), amônia e as enzimas pectinase, celulase e quitinase. Foram isoladas 136 bactérias, 83 delas produzindo algum fator de crescimento vegetal, sendo 47 % solubilizadoras de fosfato, 26 % fixadoras de nitrogênio, 57 % produtoras de AIA, 0,7 % de HCN e quitinase, 45 % de amônia, 30 % de celulose e 8 % de pectinase. Os sete melhores isolados foram testados quanto à capacidade de promover o crescimento vegetal em milho. Os isolados testados quanto à promoção do crescimento vegetal pertencem à família *Enterobacteriaceae* e aos gêneros *Klebsiella*, *Enterobacter* e *Pantoea*. Cinco isolados promoveram o crescimento vegetal em experimentos em casa-de-vegetação, sendo potenciais candidatos a bioinoculantes.

PALAVRAS-CHAVE: *Zea mays* L.; *Enterobacteriaceae*; *Klebsiella*; *Enterobacter*; *Pantoea*.

INTRODUCTION

Sugarcane, one of Brazil's most important crops, is used primarily to produce sugar and ethanol. Sugarcane is a semi-perennial grass belonging to the *Poaceae* family and *Saccharum* genus, and is highly adapted to the tropical climate (Menezes et al. 2012). The major challenge in agriculture is increasing crop yield with less environmental impact.

Microorganisms associated with sugarcane play a vital role in maintaining soil fertility and plant health. Many of these mutualistic organisms can act as biofertilizers, increasing the efficiency of nutrient absorption by the plant and producing substances that promote growth. It is estimated that biofertilizers could reduce the use of common fertilizers by 50 % with no yield losses (Pereg & McMillan 2015, Suman et al. 2016), in addition to increasing tolerance to abiotic and biotic stresses by promoting biological control (Babalola 2010).

In general, microorganisms involved in optimizing plant growth are denominated plant

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growth-promoting microorganisms (PGPM) (Mishra & Sundari 2013). They stimulate plant growth and can be found in soil areas that are directly influenced by roots (rhizosphere), colonizing the roots surface (rhizoplane) or inside the plant tissue (endophytes) (Vessey 2003). Plant growth promotion by PGPM can occur directly, through the production of phytohormones and increased availability of key elements, such as nitrogen and phosphorus, or indirectly, in the form of protection against pathogens (Glick 2012).

Indole-3-acetic acid (IAA), the most common and best characterized phytohormone produced by PGPM, is one of the most physiologically active auxins. It is involved in plant cell division, differentiation and elongation, in addition to contributing to xylem and root growth (Bhardwaj et al. 2014).

Nitrogen is vital to plant growth, being required in the synthesis of chlorophyll, proteins, enzymes, DNA and RNA. It is largely distributed in the atmosphere as N_2, which is inaccessible to plants. Diazotrophs are microorganisms capable of converting N_2 into ammonia for use by plants, a phenomenon known as biological nitrogen fixation. The use of diatrophs is an alternative to reduce the dependency on synthetic nitrogen fertilizers (Santi et al. 2013).

The second most important element in plant growth is phosphorus, which participates in root branching and contributes to plant vitality and disease resistance. In the soil, it is typically found in insoluble forms, unavailable to plants. Some microorganisms are capable of solubilizing phosphate and making it available to plants (Ahemad & Kibret 2014).

Although auxin production, nitrogen fixation and phosphate solubilization are the most studied mechanisms *in vitro*, the search for microorganisms to use in agriculture should also evaluate indirect plant growth mechanisms. The production of siderophores, defined as Fe^{3+} binding agents, can prevent the harmful effects caused by phytopathogenic organisms. Other mechanisms of pathogen inhibition include the production of hydrogen cyanide (HCN), ammonia and chitinases. Ammonia is also involved in the supply of nitrogen to plants. Other important enzymes involved in the colonization of plants by microorganisms are pectinases and cellulases (Hayat et al. 2010).

Isolation, *in vitro* characterization of growth promotion factors, inoculation and plant response to bacteria associated with sugarcane have already been described (Mirza et al. 2001, Silva et al. 2012, Beneduzi et al. 2013, Schultz et al. 2014). However, little is known about the inoculation of PGPM isolated from sugarcane in other plant species. Although the use of native PGPM is more common, some inoculants show good results when tested in other plant species (Ma et al. 2011, Mengual et al. 2016).

The diversity of bacteria associated with a certain plant is closely linked to the host species and soil characteristics, although there are groups that are common to several species edaphic traits. This is the case of members of the *Enterobacteriaceae* family, which have been described as plant growth promoters in sugarcane and maize (Taulé et al. 2012, Silva et al. 2016).

This study aimed at isolating bacteria associated with sugarcane and evaluating their ability to produce plant growth-promoting factors *in vitro*. The best isolates were tested for their capacity to promote growth in maize (*Zea mays* L.).

MATERIAL AND METHODS

Plant samples were collected in May 2015, from six-months-old second growth of the RB 867515 sugarcane variety, in an experimental area at the Universidade Federal de Goiás (16º36'023''S; 49º16'956''W), in Goiânia, Goiás State, Brazil. Five plants were collected, approximately 100 m apart, to ensure a more heterogeneous sample.

The area is subjected to standard management techniques for major crops. At the time of collection, the soil was being used for an experiment with sugarcane, planted after soil harrowing and furrowing, with weeds controlled manually. Fertility was corrected after soil analysis. No irrigation was required, since planting was carried out at the beginning of the rainy season (September/October 2014).

After the plant samples were harvested, the different parts were washed with soap under running water and left to air dry on paper towel. Leaf surface sterilization was performed according to Araújo et al. (2002). The leaves were cut into 1 cm² squares and five fragments were inoculated per plate. All the plant parts and the rhizospheric soil were plated on the following media: nutrient agar (NA), tryptone soy agar (TSA), amino casein agar (AC) and King's B medium (K), in triplicate.

The methodology developed by Kuss et al. (2007) was used to sterilize roots and stem, which were fragmented, ground in sterilized phosphate buffer (20 mM, pH 7.4), serially diluted from 10^{-2} to 10^{-7} and plated in triplicate. Bacteria were isolated from the rhizosphere according to the methodology proposed by Mendes et al. (2007), whereby 1 g of soil adhered to the roots was incubated for one hour under agitation at 130 rpm, at room temperature, in 100 mL of phosphate buffer (20 mM, pH 7.4). After agitation, the material was serially diluted and concentrations of 10^{-8}, 10^{-9} and 10^{-10} were plated in triplicate.

The plates containing the fragmented plant parts and rhizosphere material were incubated at 30 ºC, for 15 days, and monitored daily, with isolation performed by the streak plate method. Once isolated, the bacteria were screened for their ability to produce IAA, HCN, ammonia, chitinase, pectinase and cellulase, and to fix nitrogen.

Quantitative production of IAA was determined using the colorimetric method described by Gordon & Weber (1951), with modifications. The isolates were initially cultivated in 10 % tryptic soy broth (TSB), added with 5 mM of L-tryptophan at 30 ºC. After 24, 48 and 72 h of growth, 1 mL aliquots were centrifuged at 10,000 *g* for 12 minutes and treated with Salkowski's reagent [50 mL of perchloric acid (35 %) and 1 mL of $FeCl_3$ solution (0.5 M)]. The resulting solution was analyzed in a spectrophotometer at 530 nm and the IAA concentration was calculated using the equation obtained from the standard curve for commercial IAA.

Phosphate solubilization was determined based on the cultivation of the isolates in NBRIP solid medium (Nautiyal 1999). Phosphate-solubilizing bacteria were detected by the formation of a clear halo around the colonies, with the solubilization index calculated according to the ratio between the halo and colony size (Lira et al. 2012).

Nitrogen fixing capacity was assessed as described by Döbereiner et al. (1995). The isolates were inoculated into nitrogen-free semi-solid media (NFb) incubated at 30 ºC, for 7 days, and successively subcultured five times. The isolates capable of growth that formed a visible film beneath the surface of the medium were considered nitrogen fixers.

Ammonia production was assessed in microplates (Cappuccino & Sherman 1992). The isolates were cultivated in 10 mL of peptone water for 96 hours, at 30 ºC. Next, 1 mL aliquots were centrifuged at 10,000 *g* for 10 minutes, adding 0.5 mL of Nessler's reagent to the supernatant. A brownish coloration develops in the event of ammonia production.

The ability of the isolate to produce HCN, chitinase and pectinase was determined according to Cattelan (1999), and cellulase production according to Stamford et al. (1998). For enzymatic activities, the enzymatic index was determined by dividing halo size by colony size (Alves et al. 2002).

The seven isolates that performed best in the *in vitro* assessment of plant growth promotion were identified and assessed for their ability to stimulate growth in maize. Molecular identification of the isolates was performed by amplifying and sequencing the 16S rRNA coding region. DNA extraction from the isolates followed the methodology described by Van Soolingen et al. (1994). Regions V1 to V9 of the 16S rRNA were amplified by polymerase chain reaction (PCR), using the universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1541R (5'-AAG GAG GTG ATC CAG CC-3'). The PCR products were purified and sequenced using the primers 27F, 1541R, 926F (5'-AAA CTY AAA KGA ATT GAC GG-3'), 530F (5'-TGA CTG ACT GAG TGC CAG CMG CCG CGG-3'), 519R (5'-GTN TTA CNG CGG CKG CTG-3') and 907R (5'-GTN TTA CNG CGG CKG CTG-3') (Kim et al. 2011). Sequence quality and the formation of a single *contig* for each isolate were assessed using the CodonCode Aligner software. The sequences obtained were compared to those deposited in the National Center for Biotechnology Information (NCBI) database.

Plant growth promotion was evaluated in maize, aiming at examining the response of bacteria isolated from sugarcane in other plant species. Maize was selected as a model organism because it also belongs to the *Poaceae* family, grows fast (Wang et al 2016), and because the same bacterial species have been described in both plants (Taulé et al. 2012, Silva et al. 2016).

Seeds from a landrace variety (purple maize or *Palha Roxa*, in Portuguese) of *Zea mays* L. not treated with fungicide or insecticide were initially sterilized (Kavamura et al. 2013). The seven previously selected microorganisms were cultured in 10 % TSB for 24 h and their concentration was adjusted in a spectrophotometer for 10^8 cells mL^{-1} (OD550nm = 0.1).

Microbiolization was performed by incubating seeds soaked in a culture medium containing microorganisms for 2 hours. The negative control consisted of seeds treated with 0.85 % saline solution. The seeds were air dried in a sterilized environment. The growth promotion experiment was conducted in a greenhouse, using soil collected from the experimental area.

The soil chemical analysis showed the following results: pH ($CaCl_2$) = 4.8; Al ($mmol_c\ dm^{-3}$) = 0; organic matter ($g\ dm^{-3}$) = 14; P ($mg\ dm^{-3}$) = 12.8; K ($mg\ dm^{-3}$) = 150.0; Ca ($cmol_c\ dm^{-3}$) = 0.5; Mg ($cmol_c\ dm^{-3}$) = 0.3; V% = 29; CEC ($cmol_c\ dm^{-3}$) = 3.1. The soil collected was sieved, autoclaved (two 12-hour cycles at 121 ºC) and distributed into 3 L pots. Ten seeds previously treated with microorganisms were planted in each pot. Thinning was performed after germination, leaving 5 plants per pot.

The experimental design consisted of 8 treatments: 7 bacteria and one negative control. The mean of the growth parameters of the five plants was calculated for each pot, constituting the data from each experimental unit. Each treatment consisted of 40 repetitions (pots). Leaf area and stem length were assessed at 40 days after planting. The root system was separated from the shoots of the plant and washed under running water to remove any soil. All the plant material was dried in an oven at 65 ºC, during 72 h, to evaluate the root and shoot dry weight.

The individual growth data obtained in the greenhouse experiments were submitted to analyses of variance, and means were anlayzed by the Dunnett's test, using the Statistica 7 software (Statsoft) at 5 %.

RESULTS AND DISCUSSION

A total of 136 bacterial isolates were obtained from leaves, stem, roots and rhizosphere of sugarcane and 83 presented some plant growth substance and/or trait (Table 1). Concerning the isolated bacteria, 30.88 % were from roots, 26.48 % from the rhizosphere, 32.35 % from leaves and 10.29 % from stem. These data corroborate those reported by Silva et al. (2012) and Leite et al. (2014), who isolated bacteria in nonselective media and also found a higher number of microorganisms from root, when compared to the rhizosphere.

Our data indicate that the number of rhizobacteria was lower than the endophytes (Table 1). Literature shows that the number of rhizobacteria is more vulnerable to variations in soil components, so it may vary. Endophytes, especially those that live in leaves and roots, exhibit a higher number of individuals (Bodenhausen et al. 2013). The microorganisms found in each part of plants are highly specific. However, some species are capable of colonizing different regions simultaneously (Glick 2012).

The potential of the isolated microorganisms to promote plant growth was assessed *in vitro*. The performance of endophytes isolated from different sites was superior than that of the rhizobacteria (Table 1). The screening for growth-promoting factors *in vitro* is considered an effective tool in the investigation of microorganisms that can be used as biofertilizers. These tests are extremely important because they allow the selection of microorganisms with better agronomic potential before testing them in plants (Szilagyi-Zecchin et al. 2016).

IAA is a plant hormone that can be produced by a large number of microbial species, particularly endophytes. In addition to its role in plant growth, microbial IAA also serves as a signaling molecule in several plant-microorganism interactions (Lin et al. 2012). IAA production in the presence of tryptophan was observed in 57 % of the isolates, varying 5.09-83.24 $\mu g\ ml^{-1}$ in 24 h, 17.88-124.12 $\mu g\ ml^{-1}$ in

Table 1. Number of bacteria obtained by isolation from leaves, stem, roots and rhizosphere of the RB 867515 sugarcane variety and production of growth-promoting factors.

Source	Total	Number of isolates producing plant-growth promoting factors							
		IAA	Phosphate	Nitrogen fixation	HCN	Ammonia	Cellulase	Pectinase	Chitinase
Leaf	44	29	22	10	-	20	13	2	-
Stem	14	8	3	5	-	5	5	1	-
Root	42	25	24	17	-	24	18	5	1
Rhizosphere	36	15	15	3	1	12	4	3	-
Total	136	77	64	35	1	61	40	11	1

48 h and 21.05-139.21 µg ml^{-1} in 72 h. Table 2 shows the number of IAA-producing isolates according to the isolation site and production ranges. The root isolates produced higher amounts of IAA at 72 h, when compared to other groups. The rhizosphere isolates were the worst IAA producers at all times. The classification suggested by Farina et al. (2012) was used in order to present, in a simplified way, the large number of values obtained in the IAA production test.

IAA production is a common characteristic. Beneduzi et al. (2013) recorded 71 % of positive sugarcane bacteria using the IAA test, with production ranging 0.1-264 µg ml^{-1} in 72 h. Dagnaw et al. (2015) obtained low IAA production values for rhizosphere microorganisms. Even at small amounts, IAA production by rhizosphere microorganisms is important, since some plant management strategies are based on L-tryptophan soil application. Most microorganisms need tryptophan to produce IAA, given that four of the five routes of synthesis for auxin are tryptophan dependent (Ul Hasan & Bano 2015).

Phosphate solubilization was observed in 47.05 % of the isolates, with the solubilization index varying from 1.11 to 6.32. According to Silva Filho & Vidor (2000), solubilization indices below 2 are considered low, between 2 and 3 medium and above 3 high. Most of the isolates are classified as low solubilizers (Table 3). Phosphate solubilization is a highly variable characteristic among bacteria. Lira et al. (2012) found that 75 % of isolates were phosphate solubilizers, while Silva et al. (2012) reported a positive rate of 90 %.

Concerning the phosphate solubilizing bacteria, 61 % were from the root and rhizosphere. Moreover, rhizobacteria exhibited the highest solubilization indices. This is very desirable for their application as biofertilizers, since phosphate solubilization occurs mainly through the secretion of organic acids. They are responsible for solubilizing insoluble phosphates into soluble orthophosphate ($H_2PO_4^-$ or HPO_4^{-2}). For phosphate solubilization, the bacteria must be close to the phosphorus source (Taurian et al. 2010). Although rhizobacteria are the most promising as biofertilizers, phosphate solubilizing endophytic bacteria are also described. Oteino et al. (2015) demonstrated that the inoculation of endophytes into the rhizosphere increased growth in plants suffering from limited phosphate supply.

Nitrogen fixing ability was found in 26 % of the isolates, predominantly those from roots and leaves (Table 1). Nitrogen fixation is the most desirable trait in the study of plant growth promotion by bacteria, since the use of nitrogen inputs increases agricultural costs (Mirza et al. 2001, Lin et al. 2012). Although a variety of organisms are capable of nitrogen fixation, endophytes have an advantage over rhizobacteria. Their location inside the plant means that their habitat is protected, more uniform and with lower oxygen levels, favoring nitrogen fixation (Sala et al. 2005).

HCN production was only observed for the KRZ5 isolate, while ammonia was produced in 45 % of the samples (Table 1). Similar values were reported by Szilagyi-Zecchini et al. (2014). HCN and ammonia production are considered indirect mechanisms of growth promotion. HCN is a volatile product that exhibits antifungal action. Ammonia production can help satisfy the nitrogen demand of the host plant and in excess reduces the colonization of plants by pathogens. Microorganisms produce

Table 3. Number of phosphate solubilizers according to their degree of solubilization and origin.

Isolation origin	Low SI*	Medium SI	High SI
Leaf	16	5	1
Stem	3	0	0
Root	21	3	0
Rhizosphere	2	8	5

* SI: solubilization index = halo size/colony size.

Table 2. Number of IAA-producing isolates associated with sugarcane after 24, 48 and 72 h of growth on 10 % TSB media, in the presence of tryptophan.

Source	24 h			48 h			72 h		
	0.1-50	51-100	> 100	0.1-50	51-100	> 100	0.1-50	51-100	> 100
	µg ml^{-1}								
Leaf	24	5	0	17	9	3	10	12	7
Stem	8	0	0	2	6	0	0	4	4
Root	24	1	0	6	18	1	1	9	15
Rhizosphere	14	1	0	13	2	0	11	4	0

ammonia by hydrolyzing urea in ammonia and carbon dioxide (Schippers et al. 1990, Babalola 2010, Mbai et al. 2013).

Cellulase was produced by 30 % of the isolates, with enzymatic indices varying from 1.255 to 10. The KRC 1.1 isolate was particularly noteworthy, exhibiting cellulase indices of 10. Chitinase was produced only by the ACRA 2.2 isolate, with an enzymatic index of 3.5, while pectinase production was observed for 8.08 % of the bacteria, with enzymatic indices ranging from 1.33 to 5. Most producers of cellulase and pectinase are endophytic organisms from leaf and root. These enzymes are hydrolases involved in the penetration of plant tissue by endophytes. Chitinases are associated with protecting the plant against pathogenic fungi by acting on the fungal cell wall (Castillo et al. 2016, Suman et al. 2016). Although direct plant growth mechanisms are more important when selecting a microorganism for use as an inoculant, the presence of indirect mechanisms helps the microorganisms to establish in the plant and contribute to plant protection (Hayat et al. 2010).

Based on the values obtained in *in vitro* testing, four endophytic microorganisms (KFA 1.3, KFA 1.2, KRC 2.2 and KRB 1.2) and three rhizosphere isolates (KRZ 5, KRZ 6 and KRZ 23) were selected for growth promotion tests in maize (Table 4). Among the chosen microorganisms, endophytes displayed the highest IAA values and accumulated the largest number of indirect plant growth promotion factors. Rhizobacteria show good results for phosphate solubilization, which, in conjunction with nitrogen fixation, are considered the most important plant growth promotion factors (Gaiero et al. 2013). The potential of endophytic and rhizospheric microorganisms as plant growth promoters has been debated. Endophytes are efficient at making nitrogen available to the host plant by fixing nitrogen at lower oxygen pressures, when compared to those found in the soil (Döbereiner 1992). Rhizosphere microorganisms are responsible for supplying a large amount of nutrients to plants (Mendes et al. 2013).

All the isolates identified by partial sequencing of the 16S rRNA gene code belong to the *Enterobacteriaceae* family (Table 5). Loiret et al. (2004) and Taulé et al. (2012) reported the prevalence of isolates from this family, commonly described in association with roots and the rhizosphere, showing potential as phosphate solubilizers and producers of a large amount of indoles.

Considering the seven isolates analyzed, four of them increased the leaf area and shoot dry weight of *Zea mays* L., when compared to the control ($p < 0.01$) (Table 6). Five bacteria enhanced stem length ($p < 0.01$), KRZ5 (38 %), KRC 2.2 (35.7 %), KRZ23 (25 %), KRZ6 (22 %) and KFA 1.3 (12.9 %). Two isolates increased root dry weight ($p < 0.01$): KFA 1.3 - *Klebsiella* sp. promoted a 57.83 % rise, in relation to the control, and KRZ5 - *Pantoea* sp.

Table 5. Identification of the 7 bacterial isolates from sugarcane by comparing the partial sequence of the 16S rRNA coding region to sequences available in the GenBank database.

Isolate	Fragment size (pb)	Closest species	Similarity %	e-value
KFA 1.3	1,294	*Klebsiella* sp.	100	0.0
KFA 1.2	1,040	*Klebsiella* sp.	100	0.0
KRC 2.2	1,346	*Klebsiella* sp.	99	0.0
KRB 1.2	1,496	*Enterobacter* sp.	99	0.0
KRZ5	868	*Pantoea* sp.	99	0.0
KRZ6	1,027	*Enterobacter* sp.	99	0.0
KRZ23	1,200	*Enterobacter* sp.	99	0.0

Table 4. Summary of plant growth promotion factors *in vitro* of the bacterial isolates selected for growth promotion tests in maize.

Isolate	Origin	IAA	PHOS	N_2	HCN	AP	CEL	PEC	CHI
		µg ml^{-1}	SI[1]				EI[2]		
KFA 1.3	Leaf	126.49	1.5	+	-	+	1.25	-	-
KFA 1.2	Leaf	127.84	1.4	+	-	+	1.75	1.33	-
KRC 2.2	Root	116.75	2.0	+	-	-	2.0	-	-
KRB 1.2	Root	109.22	2.75	+	-	+	-	-	-
KRZ5	Rhizosphere	69.39	3.55	+	+	+	-	-	-
KRZ6	Rhizosphere	64.30	3.88	+	-	+	-	-	-
KRZ23	Rhizosphere	74.55	6.32	+	-	+	-	-	-

[1] SI: solubilization index (halo size/colony size). [2] EI: enzymatic index (halo size/colony size); IAA: indole acetic acid production in 72 h; PHOS: phosphate solubilization; N_2: nitrogen fixation; HCN: hydrogen cyanide; AP: ammonia production; CEL: cellulase; PEC: pectinase; CHI: chitinase.

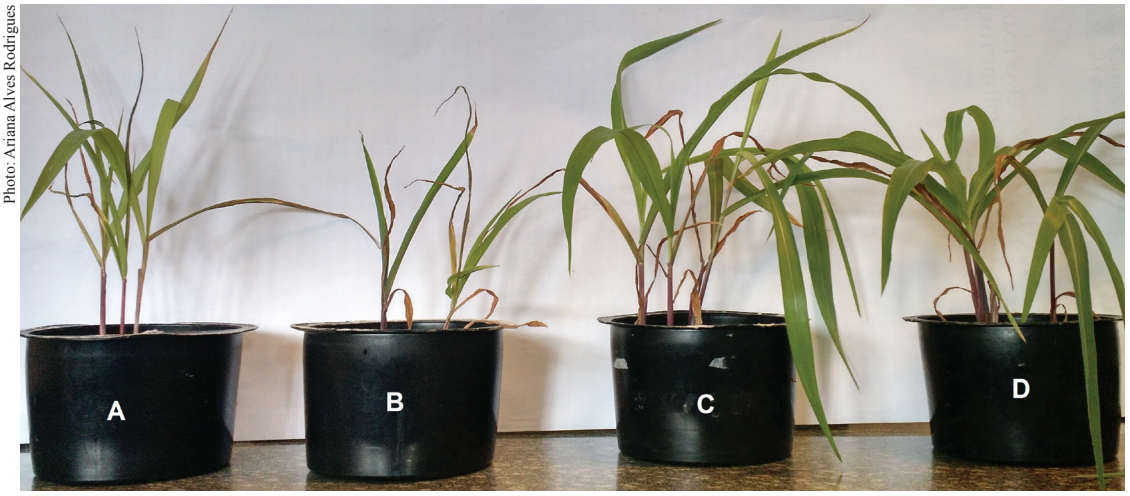

Figure 1. Results of the plant growth promotion test in *Zea mays* L. A - KRB 1.2; B - negative control; C - KRC 2.2; D - KRZ5.

Table 6. Growth promotion of *Zea mays* L. by bacterial isolates associated with sugarcane.

Treatment	Leaf area (cm^2)	Stem length (cm)	Shoot dry weight (g)	Root dry weight (g)
Negative control	31.58	8.24	0.2650	0.3014
KFA 1.3 - *Klebsiella* sp.	41.49*	9.98*	0.3972*	0.4757*
KFA 1.2 - *Klebsiella* sp.	37.17	8.50	0.3099	0.3399
KRC 2.2 - *Klebsiella* sp.	46.96*	11.18*	0.4435*	0.3901
KRB 1.2 - *Klebsiella* sp.	36.33	9.44	0.3446	0.3945
KRZ5 - *Pantoea* sp.	45.64*	11.37*	0.4071*	0.4264*
KRZ6 - *Enterobacter* sp.	35.54	10.05*	0.2614	0.3444
KRZ23 - *Enterobacter* sp.	39.70*	10.30*	0.3805*	0.3811

Mean values were obtained from 40 repetitions for assessment of leaf area, stem length, shoot and root dry weight. * Statistically different from the negative control, according to the Dunnett's test at 5 %.

41.47 %. These were the only two isolates capable of increasing all the plant growth variables assessed, while the KRB 1.2 and KFA 1.2 bacteria showed no effect on plant growth (Figure 1). None of the isolates exhibited a harmful, inhibitory growth effect on maize.

The treatment that promoted the highest growth (KRC 2.2) did not display the highest number of plant growth-promoting characteristics *in vitro*. Unlike the results reported by Grobelak et al. (2015), not all the microorganisms with the highest IAA values were capable of increasing plant growth. Phosphate solubilization also varied among microorganisms that increased maize growth. It is suggested that the maize growth was caused by a sum of factors, and not by individual values obtained *in vitro*. Araújo & Guerreiro (2010) emphasized that high *in vitro* IAA values do not always reflect plant growth. Microorganisms used in plant tests should ideally exhibit different growth promotion factors.

The predominant plant growth-promoting bacteria were from the *Klebsiella* genus. Babalola & Odhiambo (2008) also found that this genus promoted growth in maize. *Enterobacter* and *Pantoea* have also been described as promoting growth in this crop (Morales-García et al. 2011, Kavamura et al. 2013).

CONCLUSIONS

1. The *Enterobacteriaceae* family predominates among plant growth-promoting isolates associated with sugarcane.
2. Both endophytic and rhizobacteria promote growth in maize plants, indicating that plant growth-promoting microorganisms isolated from sugarcane have potential for growth promotion in other grasses.
3. The high indole acetic acid values obtained do not reflect an overall predictable increase in plant growth.

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