EFFICIENCY OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) IN SUGARCANE

Eficiencia de las Bacterias Promotoras del Crecimiento Vegetal (BPCV) en Caña de Azúcar

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SUMMARY

Plant growth promoting rhizobacteria (PGPR) are an alternative for promoting sugarcane (*Saccharum* spp.) development. Growth promotion was evaluated in sugarcane vitroplants inoculated separately with twenty-four strains of seven different bacterial species. Total indole synthesis and phosphate solubilization activity were determined in each strain. The experimental unit was one 5 L pot filled with a sterile mixture of farm soil-agrolite and one plant. The experimental design was completely random. Inoculation consisted of 1.0 mL of bacterial suspension (1×10^7 CFU). Plant height, stem diameter, number of shoots, leaf area and dry matter of shoot and root were determined every two weeks. The *Ochrobactrum anthropi* strains N208 and IMP311 and *Pseudomonas luteola* IMPCA244 had the highest production of total indoles (116.69, 115.70 and 117.34 µg mL^{-1}, respectively). The *Stenotrophomonas maltophilia* strains CA158 and 79 exhibited the highest values of phosphate solubilization (222.43 and 216.38 µg mL^{-1}, respectively). In general, plant height increased 27.75%, stem diameter 30.75%, number of tillers 38.5%, leaf area 49%, aerial dry matter 59.75% and root dry matter 59.5%. *P. luteola*, *P. fluorescens*, *O. anthropi* and *S. maltophilia* exhibited the highest values of the leaf area index, net assimilation, and relative and absolute growth rates. *P. luteola* IMPCA244, *O. anthropi* IMP311, *Aeromonas salmonicida* N264, *Burkholderia cepacia* N172, *P. fluorescens* N50 and *S. maltophilia* 79 promoted the highest values in different response variables throughout the study. Before using these strains as sugarcane biofertilizer, additional studies are required.

***Index words*:** Saccharum; *PGPR; biofertilizers; plant growth; soluble phosphate; plant growth regulators.*

RESUMEN

Las Rizobacterias Promotoras del Crecimiento Vegetal (RPCV) son una alternativa para el desarrollo de la caña de azúcar (*Saccharum* spp.). Se evaluaron 24 cepas de siete diferentes especies bacterianas en el desarrollo de vitroplantas de caña de azúcar. Se determinó la producción de índoles totales y la solubilizar fosfatos de cada cepa. La unidad experimental fue una maceta de 0.5 L, con una mezcla estéril de suelo agrícola-agrolita y una plántula. El diseño experimental fue completamente al azar. Se inoculó 1.0 mL de suspensión bacteriana (1×10^7 UFC) por planta. Se determinaron quincenalmente la altura de la planta, diámetro del tallo, número de macollos, área foliar y materia seca de parte aérea y raíz. La producción más alta de índoles totales la presentaron *Ochrobactrum anthropi* N208 y IMP311 y *Pseudomonas luteola* IMPCA244 (116.69, 115.70 y 117.34 µg mL^{-1}, respectivamente). Los valores más altos de solubilización de fosfatos se obtuvieron con *Stenotrophomonas maltophilia* CA158 y 79 (222.43 y 216.38 µg mL^{-1}, respectivamente). En general, la altura de la planta se incrementó en 27.75%, el diámetro del tallo en 30.75%, el número de macollos en 38.5%, el área foliar en 49%, y el peso de materia seca aérea y de raíz en 50.75 y 59.5%, respectivamente. Los valores más altos de índice de área foliar, tasa de asimilación neta, tasa relativa de crecimiento y tasa absoluta de crecimiento, se obtuvieron con las especies *P. luteola*, *P. fluorescens*, *O. anthropi* y *S. maltophilia*. Las cepas *P. luteola* IMPCA244, *O. anthropi* IMP311, *Aeromonas salmonicida* N264, *Burkholderia cepacia* N172, *P. fluorescens* N50 y *S. maltophilia* 79 promovieron los valores más altos en diferentes variables respuesta. Antes de emplear estas cepas en la elaboración de biofertilizantes para caña de azúcar, son necesarios estudios adicionales.

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Palabras clave: Saccharum; *RPCV; biofertilizantes; desarrollo vegetal; fosfato soluble; reguladores del crecimiento vegetal.*

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are microorganisms capable of promoting plant growth through different mechanisms, such as improving nutrient absorption and mobility (Yang *et al*., 2009), antibiotic production, nitrogen fixing, plant hormone production, phosphate solubilization, siderophore production and biological control (Bashan and de-Bashan, 2005). The use of PGPR is an alternative for sugarcane (*Saccharum* spp.) nutrition since it is a crop that demands large quantities of nitrogen (N), varying from 120 to 320 kg ha^{-1} (Salgado *et al*., 2003). Brazil uses around 50 kg ha^{-1}, because normally PGPR's are applied in the fertilization (Oliveira *et al*., 2006). The PGPR associated mainly with gramineous plants are *Azospirillum lipoferum*, *A. brasilense* and *A. amazonense*, *Herbaspirillum seropedicae*, *Acetobacter diazotrophicus*, *Enterobacter agglomeran*, *E. cloacae*, *Bacillus azotofixans*, *B. polymyxa*, *Alcaligenes faecalis*, *Klebsiella* sp., *Azotobacter* and *Pseudomonas* (Loredo-Osti *et al*., 2004). Plant response to inoculation with several soil microorganisms has been attributed to the inoculum's ability to synthesize and excrete substances in the rhizosphere, including diverse plant growth regulators such as auxins, gibberellins, cytocinins, ethylene and absicic acid (ABA) (Tilak *et al*., 2006). Of the bacteria isolated in the rhizosphere, 86% produce auxins and other growth regulators, although this varies widely among species and strains of the same species. It is also affected by conditions of cultivation, growth stage and substrate availability (Vestergard *et al*., 2009). Moreover, microorganisms can participate in inorganic phosphate solubilization and in organic phosphate mineralization, as well as in its immobilization (Pérez *et al*., 2007). The microbial mechanisms for solubilizing phosphate consist of phosphatase enzyme activity and synthesis of organic acids, such as citric, butyric, malonic, lactic, succinic, malic and gluconic acids, among others, produced by diverse bacteria known as phosphate solubilizing bacteria (PSB), which make phosphate available for absorption by plants (Paredes-Mendoza y Espinosa-Victoria, 2010).

With the aim of implementing environment-friendly techniques that tend to reduce chemical fertilizer use in sugarcane production, the effect of 24 plant growth promoting bacterial strains on the commercial sugarcane variety MEX 69290 was assessed.

MATERIALS AND METHODS

Biological Material

The Molecular Plant-Microbe Interaction Laboratory of the Colegio de Postgraduados, Mexico, provided twenty-four bacterial strains isolated from sugarcane. The strains belong to the species *Aeromonas salmonicida*, *Burkholderia cepacia*, *Ochrobactrum anthropi*, *Pseudomonas* sp., *Shewanella putrefaciens*, *Sphingomonas paucimobilis* and *Stenotrophomonas maltophilia*.

For the bioassay, 25 day-old sugarcane seedlings of the variety MEX69290 produced *in vitro* were used. The seedlings were obtained from the Biotechnology Laboratory at the Motzorongo sugar factory in the state of Veracruz, México.

Determination of Total Indoles

A suspension (10% v/v) of each bacterial strain was inoculated in liquid Luria Bertani (LB) culture medium supplemented with tryptophan (0.1 g L^{-1}). The cultures were incubated at 28 ºC for 48 h and later centrifuged at 5000 rpm for 10 min (Ghevariya and Desai, 2014). The supernatants were mixed with Salkowski reagent (600 mL de H_2SO_4 18 M, 4.5 g $FeCl_3$ anhydrous gauged to 1 L with distilled water) in a 1:1 proportion. To estimate the concentration of total indoles, the supernatants were incubated at room temperature in darkness for 30 min, and absorbance at 539 nm was then read in a Genesys 20 spectrophotometer (Hartmann *et al*., 1983).

Determination of Phosphate Solubilization

The bacterial strains were cultivated in 50 mL Pikovskaya medium (Espinosa-Victoria *et al*., 2009) and incubated at 28 ºC with constant shaking (160 rpm) for 5 days. Then, the medium was centrifuged for 15 min at 5000 rpm. One milliliter of supernatant was taken and three mL distilled water and 1 mL of the reagent nitro vanadate-molybdate (yellow complex) were added. Absorbance was read at 470 nm after

20 min of incubation. Phosphate solubilization, in terms of the concentration of soluble phosphates in the medium, was determined using a standard KH_2PO_4 curve (Jeon *et al*., 2003).

Experimental Unit, Transplant and Inoculation

The experimental unit was one plastic pot with 500 g of a mixture of clay soil from sugarcane field and agrolite in a 1:1 proportion. The clay soil collected from farm plots of the Colegio de Postgraduados, Campus Córdoba, Veracruz, was dried at room temperature in the shade. Later, it was sieved through a 2 mm mesh and autoclaved two times at 120 ºC for 3 h. A sample of the farm soil was taken to make the following determinations: pH, water retention capacity, organic matter, total nitrogen, assimilable phosphorus, extractable potassium and texture (Benedicto-Valdés *et al*., 2005).

The 24 bacterial strains were propagated in nutrient broth. The culture media were incubated at 32 ºC under shaking (220 rpm) for 24 h (Ghevariya and Desai, 2014). One milliliter of the bacterial culture (1×10^7 UFC) was used to inoculate each plant at the base of its stem.

Variables Assessed and Sampling

The assessed response variables were height, stem diameter, number of tillers, leaf area, and aerial and root dry biomass. Leaf area was determined with an area integrator (LI-COR-Biosciences). Dry biomass was estimated at constant weight in dry samples, which were maintained for 72 h at 70 ºC. Sampling was destructive, and five plants of each treatment were used. Data of the different variables in sugarcane plants were taken 15, 30, 45, 60 and 75 days after inoculation (DAI).

Experimental Design and Data Analysis

The experiment was conducted in a greenhouse during 90 days. The experimental design was completely random distribution with 25 treatments (24 bacterial strains and one un-inoculated control) and 5 replications.

An analysis of variance and a test of comparison of means were performed on the data. The latter was done with the LCD test ($P \leq 0.05$) using the statistical software SAS (Statistical Analysis System Institute, 2009) for all of the variables.

For the case of estimations in growth analyses, logarithmic transformation was done for the respective test of means.

To determine the leaf area index (LAI), the following equation was used: LAI= (LA/NP)*DP/10000 cm^2, where LA is leaf area (cm^2), NP is the number of sampled plants, and DP is the number of plants per m^2. Also, net assimilation rate (NAR), relative growth rate (RGR) and absolute growth rate (AGR) were calculated using the following equations: $NAR=[(PS_2-PS_1)/(LA_2-AF_1)]/[(lnLA_2-lnLA_1)/(T_2-T_1)]$, $RGR=(lnPS_2-lnPS_1)/(T_2-T_1)$ and $AGR=(PS_2-PS_1)/(T_2-T_1)$. PS_2 and PS_1 correspond to plant dry weight at times 2 and 1 (T_2 and T_1); and $lnLA_2$ and $lnLA_1$ are the natural logarithm of the leaf area at two times (Hunt, 2003).

RESULTS AND DISCUSSION

Table 1 shows the production of total indoles of the 24 bacterial strains used. The values range from 0.11 to 117.34 µg mL^{-1} total indoles, corresponding to the species *Stenotrophomonas maltophilia* 46 and *Pseudomonas luteola* IMPCA244, respectively. The highest values are important indicators that surpass the values of indole production (2 to 49.66 mg mL^{-1}) reported in other studies (Rana *et al*., 2011; Carcaño-Montiel *et al*., 2006). The species *Pseudomonas luteola* IMPCA244, *Ochrobactrum anthropi* IMP311 and *Ochrobactrum anthropi* N208 were statistically different ($P \leq 0.05$) from the other 21 strains.

Phosphate solubilization, expressed as soluble phosphates, of the 24 evaluated strains ranged from 0.13 to 222.43 µg mL^{-1} (Table 1); these two values corresponded to the bacteria *Pseudomonas fluorescens* N198 and *Stenotrophomonas maltophilia* CA158, respectively.

Sugarcane is predominantly cultivated in acid soils. In this study, pH was 5.8. For this reason, it is common to observe serious limitations in the supply of phosphorus available for the crop, underlining the importance of rhizobacteria that make available insoluble forms of P found in this type of soil.

Several studies have demonstrated that diverse microorganisms (Glickman and Dessaux, 1995) carry out *in vitro* synthesis of total indoles and other physiologically active growth regulators derived from tryptophan. The production of total indoles by a

Table 1. Total indoles production and phosphate solubilization by 24 bacterial species used to inoculated sugarcane seedlings.

Treatment	Bacterial species	Total indoles	Soluble phosphate
		- - - - - - - - - - µg mL^{-1} - - - - - - - - - -	
1	*Pseudomonas luteola* IMPCA244	117.34 a	0.37 d
2	*Shewanella putrefaciens* group 118	19.82 ef	1.35 d
3	*Stenotrophomonas maltophilia* IMP289	8.79 ghij	0.13 d
4	*Shewanella putrefaciens* group 174	7.44 hijk	8.21 cd
5	*Ochrobactrum anthropi* IMP311	115.70 a	24.75 cd
6	*Pseudomonas fluorescens* 285	6.34 hijk	5.67 cd
7	*Pseudomonas luteola* CA67	10.19 ghi	16.33 cd
8	*Stenotrophomonas maltophilia* IMPCA290	30.96 d	12.53 cd
9	*Stenotrophomonas maltophilia* 46	0.11 k	0.43 d
10	*Pseudomonas fluorescens* N198	5.19 ijk	0.13 d
11	*Stenotrophomonas maltophilia* CA158	15.13 fg	222.43 a
12	*Sphingomonas paucimobilis* 170	1.45 jk	30.68 bc
13	*Burkholderia cepacia* 7	13.53 fgh	1.57 d
14	*Shewanella putrefaciens* group CA88	111.15 ab	0.48 d
15	*Aeromonas salmonicida* N264	30.26 d	14.83 cd
16	*Burkholderia cepacia* N172	11.39 ghi	1.69 d
17	*Pseudomonas luteola* 136	9.09 ghij	5.38 cd
18	*Shewanella putrefaciens* group 20	74.95 c	1.75 d
19	*Ochrobactrum anthropi* N208	116.69 a	16.28 cd
20	*Stenotrophomonas maltophilia* 68C	12.63 fghi	1.69 d
21	*Pseudomonas fluorescens* 4	32.66 d	0.48 d
22	*Pseudomonas fluorescens* N50	106.11 b	56.79 b
23	*Sphingomonas paucimobilis* IMPCA190	27.36 de	1.18 d
24	*Stenotrophomonas maltophilia* 79	11.98 fghi	216.38 a

Different letters in the same column are statistically different (LCD; $P \leq 0.05$).

microorganism depends on several factors, such as the bacterial strain, the host plant, the culture conditions, crop development stage and the concentration of microorganisms in the substrate (Inui–Kishi *et al*., 2010).

Among the growth plant regulators produced by the bacteria, indolacetic acid (which forms part of total indoles) is one of the most important. It is widely distributed among cultivated plants, causing morphological changes in the root and directly regulating absorption of some minerals (Dobbelaere *et al*., 2002).

Table 2 shows that plants inoculated with *Pseudomonas luteola* 136, *Aeromonas salmonicida* N264, *Stenotrophomonas maltophilia* IMP289 and *Stenotrophomonas maltophilia* 79 were statistically different and superior in terms of plant height, relative to the other treatments 30, 45, 60 and 75 DAI. These species had a total indole production of 9.09, 30.26, 8.79 and 11.98 µg mL^{-1}, respectively, and for phosphate solubilization, values of 5.38, 14.83, 0.13 y 216.38 µg mL^{-1} of soluble phosphate, respectively. The results show that plant height is not directly related to the quantity of indoles produced or phosphate solubilized by the bacterial species. Nevertheless, it was observed that the bacteria that promoted greater plant height were those that synthesized less than 30 µg mL^{-1} total indoles.

Like the results for plant height, *Pseudomonas luteola* 136 and *Stenotrophomonas maltophilia* 79 promoted significantly superior plantlet stem diameter in comparison to the rest of the treatments at 30 and 45 DAI. On days 60 and 75 after inoculation, the species *Sphingomonas paucimobilis* IMPCA190

Table 2. Height and diameter of sugarcane seedlings inoculated with 24 bacterial species.

Treatment	Height				Diameter			
	Days after inoculation (DAI)							
	30	45	60	75	30	45	60	75
	- - - - - - - - - - - - - cm - - - - - - - - - - - - -				- - - - - - - - - - - - - mm - - - - - - - - - - - - -			
T1	33.2b	34.5defg	44.7abcd	52.0bcde	3.3bcde	4.0def	4.0h	4.9lm
T2	31.5bcde	31.7g	42.9bcdefgh	51.6bcde	3.0hij	3.6ghijk	4.3gh	4.7m
T3	30.4cdef	34.8def	46.3a	54.7ab	3.2bcdefg	3.5ijkl	5.1abcd	5.8defg
T4	24.3kl	38.8abc	40.8fgh	50.8cde	2.9ij	3.9efg	4.7def	5.8defgh
T5	29.3efghi	39.1ab	43.4abcdefg	50.5cde	2.9ijk	4.0bcde	4.5efg	5.4ghijk
T6	25.9jk	33.7fg	40.7gh	49.9cde	2.9ijk	3.5hijkl	4.5efg	5.3ijkl
T7	28.9fghi	34.6defg	44.3abcde	53.7abc	2.8jkl	3.4kl	5.1abcd	6.1abcde
T8	28.2fghij	37.4abcd	41.5efgh	49.5e	3.2defgh	4.4ab	4.5fg	5.3ijkl
T9	30.6cdef	37.2bcde	42.3cdefgh	51.9bcde	2.8jkl	4.1bcde	4.3gh	5.2ijkl
T10	31.4bcde	37.4abcd	42.6cdefgh	52.1bcde	3.2bcdefg	4.0cde	5.3abc	6.3abc
T11	29.9cdefg	33.5fg	44.1abcde	51.4bcde	3.0ghi	3.6hijkl	5.3abc	6.2abcd
T12	32.1bc	33.8fg	43.7abcdefg	50.7cde	3.0hij	3.8efghi	5.2abc	6.1abcde
T13	33.6b	38.6abc	42.0defgh	51.2bcde	3.4bcd	4.0cdef	5.3abc	6.2abcd
T14	28.3fghij	35.9cdef	42.9bcdefgh	53.0abcde	3.5ab	3.8efgh	4.8def	5.9bcdef
T15	23.3l	40.3a	42.2defgh	49.8de	2.7kl	3.8efghi	4.8def	5.6fghij
T16	33.2b	33.7fg	41.7defgh	51.5bcde	3.3bcdef	3.5jkl	4.9cde	5.9cdef
T17	36.9a	39.1ab	44.3abcde	53.3abcd	3.7a	4.3abc	5.0bcd	6.2abcd
T18	29.4defgh	33.7fg	42.2defgh	52.5abcde	3.2cdefg	3.6hijkl	4.8def	5.7efghi
T19	31.8bcd	33.0fg	40.2h	49.5e	2.9hij	3.8efghij	4.2gh	5.2jkl
T20	28.7fghi	34.8def	41.3efgh	52.6abcde	2.6l	3.9efg	5.3ab	6.5a
T21	27.7ghij	37.0bcde	45.3abc	51.3bcde	2.6l	4.3bcd	4.4fg	5.3ijkl
T22	30.2cdef	38.4abc	44.0abcde	54.9ab	3.4abc	3.8efgh	4.4fg	5.4hijk
T23	26.9ij	34.3efg	43.8abcdef	52.8abcde	3.0ghj	3.7fghijk	5.4a	6.4ab
T24	27.1hij	39.7ab	45.8ab	56.0a	3.1fghi	4.6a	5.1abcd	6.1bcdef
T25	28.9fghi	33.4fg	35.4i	41.8f	3.1efghi	3.3l	4.2gh	5.1klm

Different letters in the same column are statistically different (LCD; $P \leq 0.05$). T1-T24: Bacterial species inoculated (See Table 1). T25: Control (not inoculated).

and *Stenotrophomonas maltophilia* 68C had higher values for stem diameter than the other species; they synthesized 27.36 y 12.63 µg mL^{-1} total indoles, respectively. Regarding soluble phosphate, the same species had values of 1.18 and 1.69 µg mL^{-1}, respectively. Again, it is observed that it is not necessarily the bacteria with the highest values of total indole synthesis and phosphate solubilization that induce larger stem diameter in seedlings.

The number of tillers of sugarcane plantlets (Table 3) is an important variable since in the field this datum is essential for crop yield projections. The species *Aeromonas salmonicida* N264 promoted the largest number of tillers 30 and 45 DAI, with 30.26 total indoles and 14.83 µg mL^{-1} phosphate solubilization. At 60 and 75 DAI the species *Burkholderia cepacia* N172 and *Ochrobactrum anthropi* IMP311 induced a larger number of tillers than the other species, with values of 11.39 and 115.701 µg mL^{-1} total indoles and 1.69 and 24.75 µg mL^{-1} soluble phosphate, respectively.

Leaf area is another important parameter (Table 3) since the size of the photosynthetic apparatus determines crop yield. The species *Ochrobactrum anthropi* N208 promoted the largest leaf area 15 DAI, with total indoles and phosphate solubilization values of 116.69 and 16.28 µg mL^{-1}, respectively. At 45, 60 and 75 DAI, the strain *Pseudomonas fluorescens* N50 induced the largest leaf area with values of 106.11 µg mL^{-1} total

Table 3. Number of tillers and leaf area of sugarcane seedlings inoculated with 24 bacterial species.

Treatment	Number of tillers				Leaf area			
	Days after inoculation (DAI)							
	30	45	60	75	30	45	60	75
					- - - - - - - - - - - - - dm^2 - - - - - - - - - - - - -			
T1	7.2abc	7.6ab	7.6bcd	7.6cde	42.5fgh	76.5ij	114.4hij	137.4hi
T2	5.2fgh	7.0bcde	7.4cd	7.6cde	45.9def	88.0ef	111.7hij	140.3gh
T3	5.4efg	5.6gh	5.6g	6.8fgh	56.4c	90.9de	108.8ijk	137.6hi
T4	5.4efg	6.6def	6.6ef	6.8fgh	37.2ij	97.0bcd	97.9lm	115.4jk
T5	4.6hi	6.4ef	7.6bcd	8.6a	39.0ij	80.7ghi	88.2m	107.9k
T6	4.2i	6.8cdef	7.4cd	7.6cde	39.9hi	76.4ij	98.8klm	125.2ij
T7	4.6hi	6.6def	7.4cd	8.4ab	36.2ijk	78.6hij	129.6def	156.0ef
T8	5.6ef	5.6gh	6.4ef	6.6gh	32.3kl	91.5cde	99.6kl	125.0ij
T9	4.6hi	6.4ef	6.4ef	6.6gh	31.3l	85.3efgh	98.0lm	131.5hi
T10	6.0de	6.4ef	6.4ef	6.4h	31.5l	72.1jk	126.9efg	158.2de
T11	4.6hi	4.6i	6.4ef	6.4h	35.3jkl	77.4ij	126.5efg	172.8bc
T12	6.2de	6.4ef	6.6ef	6.8fgh	43.1fgh	67.8kl	141.7bc	177.3ab
T13	6.0de	6.4ef	6.4ef	7.8bcde	43.2fgh	64.8lm	121.2fgh	141.7fgh
T14	7.0bc	7.2abcd	7.4cd	7.6cde	41.5gh	59.0m	142.9ab	166.8bcde
T15	7.8a	7.8a	8.0abc	8.0abcd	42.4fgh	66.0kl	125.7efg	159.0cde
T16	7.6ab	7.6ab	8.4a	8.4ab	66.5b	91.3de	131.6cdef	158.1de
T17	7.4ab	7.6ab	7.6bcd	8.2abc	49.9d	86.1efg	135.1bcde	189.2a
T18	5.2fgh	6.4ef	6.4ef	7.2efg	63.6b	98.2bc	110.4ij	138.9ghi
T19	5.4efg	7.0bcde	8.2ab	8.2abc	76.4a	101.4b	122.0fgh	152.6efg
T20	6.0de	6.4ef	7.4cd	7.4def	48.4de	103.2b	121.0fgh	138.1ghi
T21	6.0de	6.2fg	6.4ef	7.8bcde	34.8jkl	101.9b	115.0hij	136.3hi
T22	4.8ghi	5.6gh	7.0de	7.6cde	32.5kl	115.8a	152.7a	188.1a
T23	6.6cd	6.6def	7.0de	7.6cde	46.3def	78.9hi	117.2ghi	157.0de
T24	5.4efg	7.4abc	8.2ab	8.2abc	44.3efg	118.2a	138.7bcd	170.7bcd
T25	5.4efg	5.4h	5.6g	7.4def	45.8def	82.2fghi	104.8jkl	133.2hi

Different letters in the same column are statistically different (LCD; $P \leq 0.05$). T1-T24: Bacterial species inoculated (See Table 1). T25: Control (not inoculated).

indoles and 56.79 µg mL^{-1} soluble phosphate. This species was the most consistent in inducing the largest leaf area during the bioassay, relative to the other bacterial species and had high values of total indole synthesis and phosphate solubilization.

Aerial dry matter weight (sum of leaf area weight plus main stem and tiller weight) is highly relevant since it reflects the effect of PGPR on development of sugarcane plants.

Table 4 shows that *Shewanella putrefaciens* group 20 promoted more aerial dry matter 30 DAI with total indole values of 74.955 µg mL^{-1} and 1.75 µg mL^{-1} of soluble phosphate. At 45 DAI, *Stenotrophomonas maltophilia* 79 was statistically superior in aerial dry matter induction with values of total indoles and soluble phosphates of 11.989 and 216.38 µg mL^{-1}, respectively.

The dry weight of the root is an important indicator, reflecting the quantity of effective root for plant nutrient and water absorption. For this reason, indoles and soluble phosphates are of vital importance for plant development. Table 4 shows that *Burkholderia cepacia* 7 promoted a greater effect on root growth of the sugarcane seedlings at 30 DAI, with values of total indoles and soluble phosphates of 13.53 and 1.57 µg mL^{-1}, respectively. *Ochrobactrum anthropi* IMP311, induced better results in root dry weight

Table 4. Aerial and root dry matter of sugarcane inoculated with 24 bacterial species.

Treatment	Aerial dry matter				Root dry matter			
	Days after inoculation (DAI)							
	30	45	60	75	30	45	60	75
	- g -							
T1	0.50jkl	1.29mno	2.30a	2.94a	0.46n	1.19klm	2.59a	3.39a
T2	0.89c	1.55bcdefg	1.82ij	2.22ghijk	0.68def	1.30ijk	2.09bcd	2.87bc
T3	0.90c	1.23no	1.80ij	2.50cdef	0.62fghij	1.27jkl	1.98defgh	2.73cd
T4	0.46kl	1.56bcdef	1.62kl	1.92lm	0.56jkl	1.45fgh	1.48m	1.82l
T5	0.82cd	1.58bcd	2.01efgh	2.21hijk	0.53klm	1.71a	1.83ghij	2.21hijk
T6	0.74def	1.38ijklm	1.60kl	2.39efghi	0.58hijkl	1.49defg	1.99defg	2.48efg
T7	0.99b	1.49defghij	1.91ghi	2.31fghij	0.60ghijk	1.56cdef	1.99defg	2.02k
T8	0.77d	1.64abc	1.89hij	2.39efghi	0.66efg	1.37ghij	1.66kl	2.10jk
T9	0.68efg	1.52cdefgh	1.91ghij	2.34fghij	0.52lmn	1.29ijk	1.87efghi	2.16ijk
T10	0.89c	1.55bcdefg	1.93ghi	2.71bc	0.66efg	1.35hij	1.85ghij	2.20ijk
T11	0.53ijk	1.36jklmn	2.11bcde	2.47def	0.64fgh	1.48defg	2.03cde	2.56def
T12	0.67fg	1.07p	2.10bcdef	2.48def	0.75c	1.48defg	1.80ijk	2.35ghi
T13	0.76de	1.33klmno	1.88hij	2.36fghi	1.26a	1.58bcde	2.02cdef	2.67cde
T14	0.63gh	1.49defghi	1.93fghi	2.46def	0.57ijkl	1.40ghi	1.96defghi	2.55def
T15	0.45lm	1.44fghijk	2.01efgh	2.64bcd	0.46n	1.56cdef	2.16bc	2.67cde
T16	0.65g	1.40hijklm	2.07cdefg	2.63bcd	0.74cd	1.46efgh	2.46a	3.06b
T17	0.79d	1.57bcde	2.04defgh	2.59bcde	0.63fghi	1.64abc	1.90efghi	2.40fgh
T18	1.10a	1.30lmno	1.74jk	2.21hijk	0.68def	1.25jkl	2.03cde	2.60de
T19	0.89c	1.45efghijk	1.63kl	2.12jkl	0.92b	1.60abcd	1.70jk	2.27hij
T20	0.37mn	1.38ijklm	1.95efghi	2.43defgh	0.48mn	1.45fgh	1.82hijk	2.15ijk
T21	0.37n	1.43ghijkl	1.55l	1.77m	0.39o	1.49defg	1.50lm	1.79l
T22	0.80d	1.65ab	2.26ab	2.80ab	0.38o	1.71ab	2.23b	2.62de
T23	0.45kl	1.05p	1.89hij	2.50cdef	0.64fgh	1.11m	2.04cde	2.72cd
T24	0.55hij	1.76a	2.21abcd	2.44defg	0.56jkl	1.56cdef	1.86fghij	2.29ghij
T25	0.61ghi	1.21o	1.61kl	2.18ijk	0.71cde	1.15lm	1.66kl	2.17ijk

Different letters in the same column are statistically different (LCD; $P \leq 0.05$). T1-T24: Bacterial species inoculated (See Table 1). T25: Control (not inoculated).

46 DAI, with synthesis of total indoles and soluble phosphates at values of 115.70 and 24.75 μg mL^{-1}, respectively. Finally, *Pseudomonas luteola* IMPCA244 promoted higher values of root dry weight in comparison with the rest of bacterial strains at 60 and 74 DAI. It is important to emphasize that some *Burkholderia cepacia* strains have been recognized as a human opportunistic pathogen in hospitals and associated with cystic fibrosis (Ibarguren *et al*., 2011).

The leaf area index (LAI) revealed significant permanent increases on the sampling dates (Table 5). However, the PGPR inoculated treatments had variable increases on different dates, while the control showed constant increases with no large variations.

In the case of net assimilation rate (NAR) and relative growth rate (RGR), the effect of inoculation with *Pseudomonas luteola* IMPCA244 was the highest and most constant, although on the early dates the effect of *Stenotrophomonas maltophilia* 79 is outstanding, but it declines later on. Absolute growth rate (AGR) had behavior similar to NAR and RGR, although the effect of inoculation with *Pseudomonas fluorescens* N50 showed a constant trend, as observed in Figure 1.

Table 5. Leaf Area Index (LAI) of sugarcane seedlings inoculated with five strains of bacterial species.

Treatment	Days after inoculation (DAI)				
	15	30	45	60	75
T1	0.011b	0.027c	0.048d	0.071bc	0.086d
T17	0.023a	0.031b	0.054c	0.084a	0.118a
T19	0.017ab	0.048a	0.063b	0.076b	0.095c
T22	0.019a	0.020d	0.072a	0.073bc	0.118a
T24	0.020a	0.028c	0.074a	0.087a	0.107b
T25	0.022a	0.029bc	0.051cd	0.066c	0.083d

T1, T17, T19, T22 y T24 = strains of four bacterial species (See Table 1). T25 = non-inoculated plants.

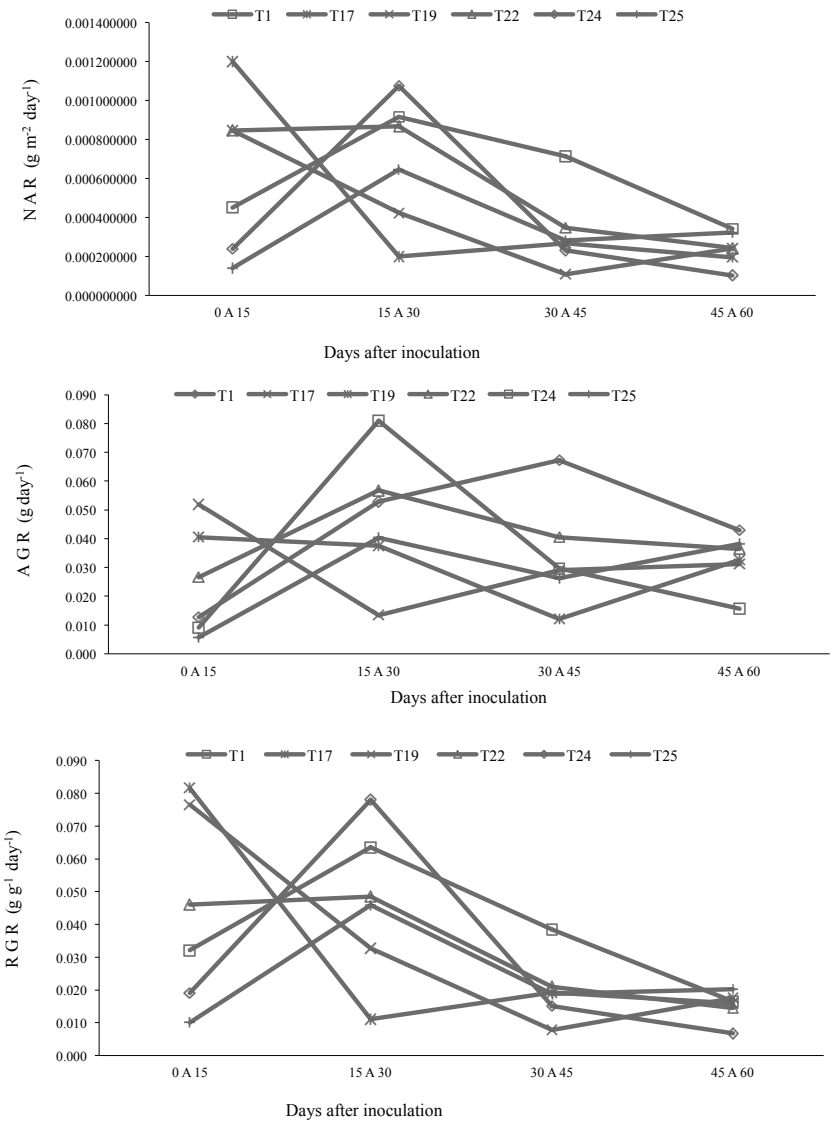

Figure 1. Dynamics of the Net Assimilation Rate (NAR), Absolute Growth Rate (AGR) and Relative Growth Rate (RGR) in sugarcane plants inoculated with five bacterial strains: T1, T17, T19, T22 and 24 = strains of four bacterial species (See Table 1). T25 = control, non-inoculated plants.

CONCLUSIONS

- The species *Ochrobactrum anthropi* (strains IMP311 and N208) and *Pseudomonas luteola* IMPCA244 were statistically superior in total indole production. The species *Stenotrophomonas maltophilia* 79 was that with the highest values of phosphate solubilization.
- The species *Pseudomonas luteola* IMPCA244, *Ochrobactrum anthropi* IMP311, *Aeromonas salmonicida* N264, *Burkholderia cepacia* N172, *Pseudomonas fluorescens* N50 and *Stenotrophomonas maltophilia* 79 outstandingly promoted different response variables in sugarcane throughout the bioassay.
- The sugarcane plants inoculated with the species *Pseudomonas luteola* (strains IMPCA244 and 136), *P. fluorescens* N50, *Ochrobactrum anthropi* N208 and *Stenotrophomonas maltophilia* 79 had the highest leaf area indexes and growth rates.
- The results of this study indicate that the gains in sugarcane plant height are not necessarily related to the amount of indoles synthesized or to phosphates solubilized by the bacterial species used for inoculation. Nevertheless, it is observed that the bacteria that promoted greater plant height synthesized less than 30 μg mL^{-1} total indoles.
- Before using some of the species evaluated in this research in the production of biofertilizers for sugarcane, the absence of human pathogenic genes in their genomes must be corroborated. In the same way, antibiosis studies are required to determine the feasibility of using them individually or in consortiums. Particularly, the use of *Burkholderia cepacia* N172 is not recommended unless it can be proved that it does not carry in its genome genes that are pathogenic for humans.

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REFERENCES

Bashan, Y. and L. E. de-Bashan. 2005. Bacteria/plant growth-promotion. pp. 103-115. *In*: D. Hillel (ed.). Encyclopedia of soils in the environment. Elsevier. Oxford. UK.

Benedicto-Valdés, G. S., C. Hidalgo-Moreno, V. Ordaz-Chaparro, R. Sánchez-Hernández y D. J. Palma López. 2005. Cambios en las propiedades físicas de un suelo arcilloso por aportes de lombricompuesto de cachaza y estiércol. Interciencia 30: 775-779.

Carcaño-Montiel, M. G., R. Ferrera-Cerrato, J. Pérez-Moreno, J. D. Molina-Galán y Y. Bashan. 2006. Actividad nitrogenasa, producción de fitohormonas, sideróforos y antibiosis en cepas de *Azospirillum* y *Klebsiella* aisladas de maíz y teocintle. Terra Latinoamericana 24: 493-502.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, Y. Okon, and J. Vanderleyden. 2002. Effect of inoculation with wild type *Azospirillum brasilense* y *A. irakense* strains on development y nitrogen uptake of spring wheat y grain maize. Biol. Fertil. Soils 36: 284-297.

Espinosa-Victoria, D., L. López-Reyes, and A. de la Cruz-Benítez. 2009. Use of 16S rRNA gene for characterization of phosphate-solubilizing bacteria associated with corn. Rev. Fitotec. Mex. 32: 31-37.

Ghevariya, K. K. and P. B. Desai. 2014. Rhizobacteria of sugarcane: *In vitro* screening for their plant growth promoting potentials. Res. J. Recent. Sci. 3: 52-58.

Glickman, E. and Y. Dessaux. 1995. A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl. Environ. Microbiol. 61: 793-796.

Hartmann, A., M. Singh, and W. Klingmuller. 1983. Isolation and characterization of *Azospirillum* mutants, excreting high amounts of indoleacetic acid. Can. J. Microbiol. 29: 916-923.

Hunt, R. 2003. Growth analysis, individual plants. pp. 588-596. *In*: B. Thomas, D. J. Murphy, and D. Murray. (eds.). Encyclopaedia of applied plant sciences. Academic Press. London, UK.

Ibarguren P., M., N. Cobos-Trigueros, A. Soriano, J. A. Martínez, Y. Zboromyrska, M. Almela y J. Mensa. 2011. Bacteriemias por *Burkholderia cepacia*: Análisis prospectivo de 33 episodios. Rev. Esp. Quimioter. 24: 209-212.

Inui–Kishi, R. N., L. Takeshi, S. C. Picchi, J. C. Barbosa, M. T. Olivério, J. Marcondes, and E. G. de Macedo. 2010. Phosphorus solubilizing and IAA production activities in plant growth promoting rhizobacteria from brazilian soils under sugarcane cultivation. ARPN J. Eng. Appl. Sci. 7: 1446-1454.

Jeon, J. S., S. S. Lee, H. Y. Kim, T. S. Ahn, and H. G. Song. 2003. Plant growth promotion in soil by some inoculated microorganisms. J. Microbiol. 41: 271-276.

Loredo-Osti, C., L. López-Reyes, and D. Espinosa-Victoria. 2004. Bacterias promotoras del crecimiento vegetal asociadas con gramíneas: Una revisión crítica. Terra Latinoamericana 22: 225-239.

Oliveira, A. L. M., E. L. Canuto, S. Urquiaga, V. M. Reis, and J. I. Baldani. 2006. Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. Plant Soil 284: 23-32.

Paredes-Mendoza, M. y D. Espinosa-Victoria. 2010. Ácidos orgánicos producidos por rizobacterias que solubilizan fosfato: Una revisión crítica. Terra Latinoamericana 28: 61-70.

Pérez, E., M. Sulbarán, M. M. Ball, and L. A. Yarzábal. 2007. Isolation y characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol. Biochem. 39: 2905-2914.

Rana, A., B. Saharan, M. Joshi, R. Prasanna, K. Kumar, and L. Nain. 2011. Identification of multi-trait PGPR isolates y evaluating their potential as inoculants for wheat. Ann. Microbiol. 61: 893-900.

Salgado G., S., R. Nuñez y L. Bucio. 2003. Determinación de la dosis optima económica de fertilización en caña de azúcar. Terra Latinoamericana 21: 267-272.

SAS Institute. 2009. The SAS system for windows, ver. 9.0. SAS Institute. Cary, NC, USA.

Tilak, K., R. Anganayakin, and M. Anoharacharic. 2006. Synergistic effects of plant-growth promoting rhizobacteria and *Rhizobium* on nodulation y nitrogen fixation by pigeonpea (*Cajanus cajan*). Eur. J. Soil Sci. 57: 67-71.

Vestergard, M., L. Bjornlund, F. Henry, and R. Roon. 2009. Decreasing prevalence of rhizosphere IAA producing y seedling root growth promoting bacteria with barley development irrespective of protozoan grazing regime. Plant Soil 295: 115-125.

Yang, J., J. W. Kloepper, and C. M. Ryu. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14: 1-4.