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Original

Endophytes bacterial growth promoters isolated to colosoana grass, Department of Sucre, Colombia

Bacterias endófitas promotoras de crecimiento aisladas de pasto colosoana, departamento de Sucre, Colombia

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Abstract:

Objective. Evaluate *in vitro* the efficiency of endophytic growth promoting bacteria isolated from different colosuana grass tissues in the municipality of Corozal, department of Sucre, Colombia. **Materials and methods**. Endophytic bacteria were isolated, population density was determined in CFU / g of tissue, then quantitative and qualitative tests of FBN activities, phosphate solubilization, siderophore production and AIA were carried out to finally identify by sequencing the bacteria that had positive growth promotion activity. **Results.** The largest populations were found in roots (5.0 X 10^{10} 3.8 X 10^{10} 2.8 X 10^{10} 2.4 X 1010 and 1.5 X10^{10} CFU / g of tissue, for the location of the Peñas, the Mamon, Canta gallo, Chapinero and Hato Nuevo, respectively) with respect to stem and leaf. A total of 53 isolated endophytes bacteria, 18 showed reducing capacity of N2 to ammonium; 15 morphotypes showed phosphate solubilizing capacity; 8 of indole acetic acid production and 12 of siderophore producers. **Conclusions.** This work isolated endophytes bacteria with the ability to promote plant growth. Two species of endophytic bacteria were identified as *Delftia tsuruhatensis* and *Pseudomonas hibiscicola* (*S. maltophilia*), which showed excellent solubilization results of phosphates, reduced N2 to ammonium, production of indol acetic acid and the production of siderophores.

Keywords: Microorganisms, plant tissue, nutrition.

Resumen:

Objetivo. Evaluar *in vitro* la eficiencia de las bacterias endófitas promotoras de crecimiento aisladas de diferentes tejidos de pasto colosuana en el municipio de Corozal, departamento de Sucre, Colombia. **Materiales y métodos.** Se aislaron bacterias endófitas, se determinó densidad poblacional en UFC/g de tejido, seguidamente se llevó a cabo pruebas cuantitativas y cualitativas de las actividades de FBN, solubilización de fosfatos, producción de sideróforos y de AIA para finalmente se realizó identificación por secuenciamiento de las aquellas bacterias que tuvieron actividad positiva de promoción de crecimiento. **Resultados.** Las mayores poblaciones fueron encontradas en raíces (5.0 X 10^{10} 3.8 X 10^{10} 2.8 X 10^{10} 2.4 X 10^{10} y 1.5 X10^{10} UFC/g de tejido, para los corregimientos de La Peñas, El Mamón, Cantagallo, Chapinero y Hato Nuevo, respectivamente) con respecto a tallo y hoja. Un total de 53 bacterias endófitas aisladas, 18 mostraron capacidad reductora de N_2 a amonio; 15 morfotipos mostraron capacidad solubilizadora de fosfatos; 8 de producción de ácido indol acético y 12 de productoras de sideróforo. **Conclusiones.** Este trabajo aisló bacterias endófitas con capacidad de promover el crecimiento vegetal. Dos especies de bacterias endófitas fueron identificadas como *Delftia tsuruhatensis* y *Pseudomonas hibiscicola* (*S. maltophilia*), las cuales mostraron excelentes resultados de solubilización de fosfatos, reducen N2 a amonio, producción de ácido índol acético y la producción de sideróforos.

Palabras clave: Microorganismos, tejido vegetal, nutrición.

INTRODUCTION

Cattle ranching in the department of Sucre, Colombia, occupies 768600 ha of pastures that represent 13.7% of the cattle area of the Caribbean region; in the sub-region Sabanas of Sucre there are 164.000 ha with Bothriochloa pertusa, which corresponds to 21.3% of the total pasture area of the department (1). In addition, the double purpose modality represents the main economic activity of the department of Sucre, where 94.9% of the total area devoted to livestock activity is dedicated exclusively to livestock grazing (2). Pasture and forage production in Colombia is mainly for use in livestock, as a source of feed for livestock (3).

B. pertusa grass, in the Colombian Caribbean region is widely distributed, covering extensive areas of the dry tropical forest (bs-T) and very dry tropical forest (bms-T) life zones in the departments of Cordoba, Sucre, Bolivar and Magdalena (4); but due to the physiographic factors the degenerative anthropogenic actions of the environment and the use of inadequate technologies, they have brought as a consequence the degradation of the physical, chemical and biological properties of the soil, which has limited the supply and quality of this pasture mainly in the dry season (5).

Faced with this situation, the use of chemical fertilizers becomes an alternative to overcome this difficulty, which improves the productivity of the pastures, but causes an imbalance in the communities of microorganisms native to the soil, which fulfill important functions within ecosystems such as: the contribution of nutrients, moisture retention, better soil structure among others (6). In this sense, as an alternative to mitigate the effect of fertilizers in recent years, several studies conducted with endophytic bacteria associated with plant species, show an important potential for plant nutrition. Endophytes bacteria reside within the tissues of plants without causing damage to their hosts (7), They promote the growth of plants, remove pollutants, solubilize phosphates and fix nitrogen, and can be used as control of phytopathogens.

The reports presented Pérez et al (8), on the in vitro activity of nitrogen-fixing endophytic bacteria and phosphate solubilizers in colosoana grass in the Colombian Caribbean, they identified the presence of endophytes bacteria Aeromonas salmonicida and Pasteurella pneumotropica with simultaneous ability to solubilize phosphates and biologically fix nitrogen.

For all the above and to answer the question are endophytic bacteria a natural source that help provide nutrients to pastures?, the present study was raised with the objective of evaluating in vitro the growth promoting activity of endophytes bacteria isolated of different colosuana grass weavings of 5 livestock farms located in the municipality of Corozal, department of Sucre, Colombia.

MATERIALS AND METHODS

Sampling. The sampling was carried out in 5 livestock farms planted only with colossal grass located in the municipality of Corozal, during the second period of 2016. At each site, a random sampling was carried out in the form of a zigzag, collecting colosoana grass with roots, stems and leaves. Also, samples of soil at a depth of 20 cm were taken to know the physical-chemical conditions of the soils. The samples were labeled with the date of collection, farm and village. The samples of soil and plants of colosoana grass were stored and conserved for their transport to the Laboratory of Microbiological Investigations for the microbiological analyzes and the other part were sent to the laboratory of Soils and Waters of the University of Sucre for their physicochemical characterization.

Isolation of endophytic bacteria. The isolation of the endophytic bacteria was carried out in the following way:

Surface disinfection of tissues. To each plant collected, tissue was separated (root, stem and leaf). Each tissue separately was subjected to a superficial disinfection process by protocols described by Pérez et al (5).

Determination of population density. After the disinfection process, each tissue was deposited on porcelain plates, macerated with liquid nitrogen until homogeneous, and serial dilutions (10^{-1} to 10^{-8}) were prepared in triplicate from which aliquots were taken and deposited on the R2A agar surface, were incubated at 32°C for 72 hours. The population density of bacteria per tissue (CFU / g of tissue) was determined by direct counting of colonies on the surface of the plates and selected those colonies that differed in shape, texture, color and size (8).

In vitro evaluation of growth promotion activity of endophytic bacteria isolated from the colosoana grass tissues. The isolates of obtained endophytic bacteria were used for the in vitro evaluation of the growth promoting capacity (biological nitrogen fixation, phosphate solubilization, production of indole acetic acid and siderophore).

Biological nitrogen fixation. The qualitative evaluation of nitrogen fixation was carried out by direct seeding of each morphotype on the surface of the selective medium BURK agar (5 gr $MgSO_4$, 20 gr KH_2PO_4, 5 gr $K2HPO_4$, 3.25 gr $CaSO_4$, 1.45 gr $FeCl_3$, 0.253 gr $NaMoO_4$, 1000 ml of sterile distilled water), following the methodology proposed by (8). The amount of nitrogen fixed was determined by indirect evaluation of the reduced nitrogen amount in the form of ammonium ion, using the Berthelot colorimetric method, described by Barraza and Pérez (9).

Solubilization of phosphate. The qualitative evaluation of phosphate solubilization was carried out by direct seeding of each morphotype on the surface of the NBRID agar medium (10 gr Glucose, 5 gr $Ca_3(PO4)_2$, 5 gr $MgCl_2 * 6H_2O$, 0.25 gr $MgSO_4 * 7H_2O$, 0.2 g KCl, $(NH_4) SO_4$ in 1000 ml of distilled water), following the protocol proposed by Pérez et al (8). The isolates that showed positive activity were used to determine the efficiency of the capacity of the endophytes bacteria to solubilize phosphate in NBRID culture medium through the indirect measurement of dissolved phosphate according to the protocol proposed by Rodriguez et al (10). The data obtained were analyzed using the standardized standard curve in the biotechnology laboratory of the University of Córdoba (11).

Production of indole acetic acid (IAA). The qualitative evaluation of the production of indole acetic acid (AIA) was evaluated in liquid medium Burk Corea (0.41gr KH_2PO_4, 0.52gr K_2HPO_4, 0.05gr Na_2SO_4, 0.2gr $CaCl_2$, 0.1gr $MgSO_4 * 7H_2O$, 0.01gr Fe $SO_4 * 7H_2O$, 0.0025gr $NaMoO_4$, in 1000 ml of distilled water), and supplemented with 0.1 g tryptophan (precursor of indole acetic acid), by techniques proposed by Dawwam et al (12). For the quantitative determination, the standardized curve wasused through standard solutions of pure indole acetic acid according to the protocol carried out in the biotechnology laboratory of the University of Córdoba (11).

Siderophore production. The qualitative evaluation of siderophore production was carried out by direct seeding of each morphotype on the surface of the medium chromium azurol-S (CAS) described by Doncel et al (13).

Molecular identification of endophytic bacteria with positive growth promotion activity. The isolates of endophytic bacteria that showed *in vitro* positive activity for the reduction of nitrogen to ammonium ion, solubilization of phosphates, production of indol acetic acid and siderophores, were selected and proceeded to it is purification and identified by Gram stain for the use of the protocol for the extraction of genomic DNA from Gram-negative or Gram-positive bacteria. The extraction of genomic DNA from endophytic bacteria with the ability to promote growth was carried out using the protocol proposed by Oliveira et al (14).The conditions used in each amplification reaction were based on the protocol described by Oliveira et al (14), using a mastercycler nexus eppendorf thermal cycler. The products obtained from the amplification were purified and sequenced in Macrogen Korea.

The sequences obtained were compared with those stored in the Genbank. The alignment of the bases was performed in the Clustal W program, the phylogenetic inferences were obtained by the maximum similarity method based on the kimura-2-parameter model in the MEGA 7 program (15).

Statistic analysis. A block design with a factorial arrangement was applied for the differences between the population density (CFU / g of tissue) of endophytic bacteria according to location and type of tissue. The multiple range test (Tukey) was used to establish significant differences between communities of endophytic bacteria (CFU / g of tissues) in relation to location and type of colonized tissue. The data was analyzed in the InfoStat program.

RESULTS

The results of the means of the chemical parameters of the soil samples of the 5 livestock farms analyzed, indicate: values of strongly acidic to slightly acidic pH; contents of organic matter and calcium from very low to abundant; phosphorus and magnesium from low to abundant; potassium from very low to low; sodium from excessive to low and texture from clay loam, sandy loam to sandy loam (Table 1)

TABLE 1.
Means of chemical parameters of soils of livestock farms by location, municipality of Corozal, department of Sucre, Colombia.

Physicochemical parameters	Las Peñas	El mamón	Cantagallo	Chapinero	Hato Nuevo	Reference values
pH (water 1:1 P/V)	6.32 (slightly ácid)	6.10 (slightly ácido)	4.98 (strongly ácid)	5.2 (strongly ácid)	4.74 (strongly ácid)	5.80 – 7.20
Organic matter (%)	2.23 A	0.16 F	1.19 D	3.18 C	1.19 D	2.0 – 4.0
Phosphorus (ppm), Bray II	25.57 C	12.13 C	5.92 D	93.83 A	45.01 A	15 – 30
C.I.C. (meq/100 g of soil)	18.00 C	7.5 D	15.0 C	11.0 C	10 C	10 – 20
Calcium (Cmol.kg^{-1} soil)	11.0 A	1.6 F	2.0 F	3.0 D	2.8 F	5 – 7
Magnesium (Cmol.kg^{-1} soil)	5.0 A	3.73 B	3.67 B	3.67 B	2.2 C	2 – 3
Potassium (Cmol.kg^{-1} soil)	0.16 D	0.01 F	0.01 F	0.12 D	0.01 F	0.2-0.4
Sodium (Cmol.kg^{-1} soil)	1.92 E	0.6 C	1.0 A	1.00 A	0.9 B	<1.0
Exchangeable aluminium (Cmol.kg^{-1} soil)	-	-	0.77 B	0.98 B	0.58 B	<0.2
Texture (M. Bouyoucos)	F.AR. Sandy load	A.F. clay load	F.AR. Sandy load	A.F. Clay load	F.A. Clay load	
Sand (%)	72.50	78.21	69.58	81.25	77.92	20-50
Clay (%)	7.08	4.64	10,42	7.08	10.42	20-60
Load (%)	20.42	17.15	20	11.67	11.66	20-70
Saturation of calcium (%)	61.11 B	21.33 D	13.33 F	27.27 D	28.0 B	50 – 70
Saturation of magnesium (%)	7.78 B	49.73 A	24.47 B	33.36 A	22.0 A	20 – 30
Saturation of sodium (%)	10.67 B	8.0 C	24.47 D	9.09 C	9.0 B	<6.0
Saturation of potassium (%)	0.78 C	0.02 F	0.07 C	1.1 C	0.1 F	1.25 – 3.5
Saturation of aluminium (%)	-	-	5.13 C	8.91 C	5.8 C	<5.0
Relation calcium /magnesium	2.2 N	0.43 inverted	0.57 inverted	0.82 inverted	1.27 Close	2 – 4 (normal)
Electrical conductivity (MicroS/cms)	620 N	103.5 N	141 N	330 N	174 N	< 2.000
Salinity % in satured pasta	0.2 N	0	0	0	0.0 N	0.0 – 0.20

The mean values for population density of endophytic bacteria by location (Figure 1a), indicate that the location of the Peñas found the highest population densities ($4.48x10^{10}$ CFU / g of tissue), followed by Mamon ($3.31x10^{10}$ CFU / g of tissue), Canta gallo ($2.8x10^{10}$ CFU / g of tissue), Chapinero ($2.45 x10^{10}$ CFU / g of tissue), with respect to the Hato Nuevo location that obtained the lowest population density of endophytic bacteria 3.18 $x10^{8}$ CFU / g of tissue, respectively.

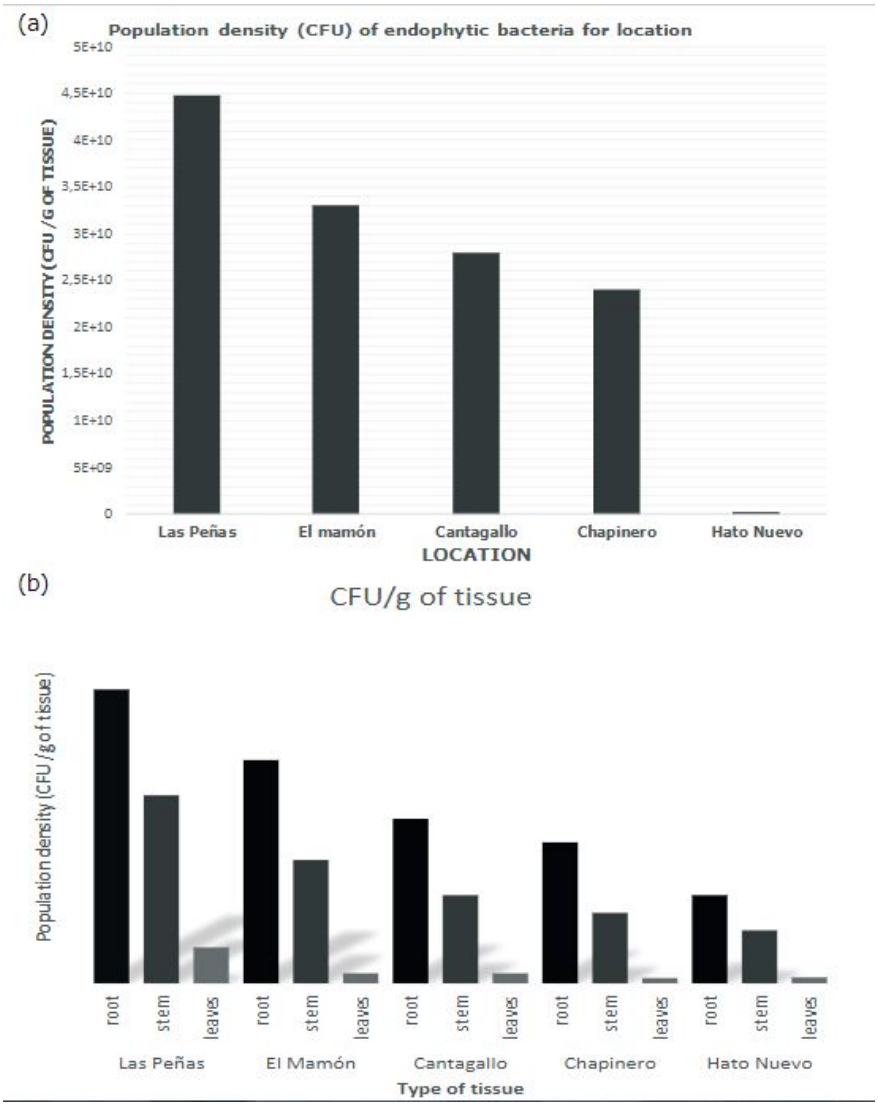

FIGURE 1
Population density of endophytic bacteria bylocation (a), and by tissue (b) isolated fromColosoana grass in the municipality of Corozal,Department of Sucre, Colombia, 2016.

A total of 53 endophytic bacteria were isolated, of which 18 showed reducing capacity of N2 to ammonium; 15 morphotypes showed phosphate solubilizing capacity; 8 production of indole acetic acid and 12 producers of siderophore. In the Figure 2 the qualitative in vitro activity of nitrogen fixation is observed, phosphate solubilization, production of indole acetic and siderophore production of the endophytic bacteria evaluated.

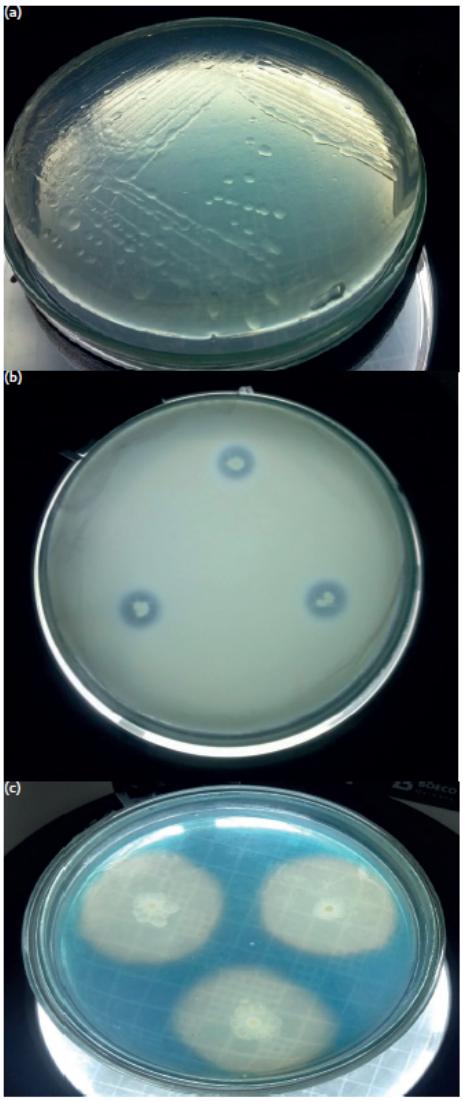

FIGURE 2
Shows the in vitro qualitative activityof nitrogen fixation (a), phosphatesolubilization (b) and siderophoreproduction (c) of the endophytic bacteriaevaluated.

With respect to nitrogen efficiency by endophytic bacteria, it was found that 18 isolates showed an ammonium ion production capacity in concentrations of 0.23 to 1.17 mg / L. The highest concentrations of ammonium ion production were found for M4HLP (1.17mg/L) isolated from colosoana grass leaves from cattle farms located in the location of the Peñas, followed by M2HEM (1.10 mg/L) morphotype isolated from leaf Colosoana grass of the location the Mamon. According to the results observed in figure 3a, it isindicated that the highest average concentrations in the production of the ammonium ion were found in the endophytic bacteria isolated from leaves, followed by the stem and the minor ones in roots.

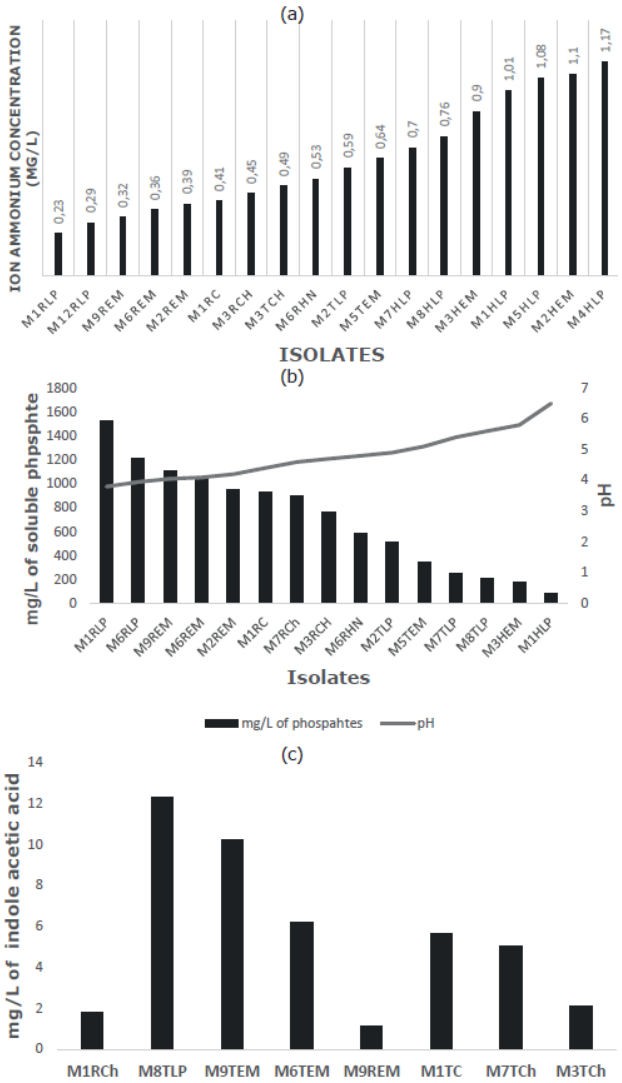

FIGURE 3
Determination of am monium ion concentration (a); soluble phosphate (b)and IAA (c) in mg/L of endophytic bacteriaisolated from colosoana grass, municipalityof Corozal, department of Sucre, Colombia.M: morphotype; R: root, T: stem, H: leaf;LP: Las Peñas, EM: El Mamon, C: Cantagallo, CH: Chapinero, AN: Hato Nuevo.

In Figure 3b, observe the results of phosphate solubilization activity of the 12 isolates of endophytic bacteria evaluated in NBRIP liquid medium, according to the results obtained the isolates with greater solubilizing activity corresponded to M1RLP (1530m/L), M6RLP (1210mg/L), M9REM (1112mg/L) and M6REM (1050mg/L), which came from different location, but all isolated from roots). The results indicate that the majority of isolates with phosphate solubilizing activity were isolated from the root, three of them from the stem and only two isolated from the leaf, showing this activity in a range of 85 (pH: 6.5) to 1530 mg/L (pH: 3.8).

The analysis of clustering sequences of 16 rDNA from endophytic bacteria with positive activity for growth promotion, was carried out from those isolates that had more than one activity. In Table 2, the selected isolates are related to the specific growth promotion activities observed in the in vitro test. The results of the clustering analysis of 16s rDNR sequences, of endophytic bacteria and their similarity with sequences of bacterial species stored in the GenBank database, can be seen in Figure 4. The isolates identified as M3RCh

and M2TLP showed high homology with bacteria from the Gamma-proteobacteria phylum (Figure 4a). The sequence of M2TLP presented greater homology with 99% identity and coverage with Pseudomonas hibiscicola (Stenotrophomonas maltophilia).

TABLE 2.
Isolates of endophytic bacteria with specific growth promotion activity, obtained from colosoana grass, municipality of Corozal, department of Sucre, Colombia.

Isolates	Activity promoter of growth			
	Ammonium	Phosphate	IAA	Siderophore
M1RLP	+	+	-	+
M2TLP	+	+	+	-
M9REM	-	+	+	+
M8TLP	+	-	+	+
M3RCh	+	+	+	-

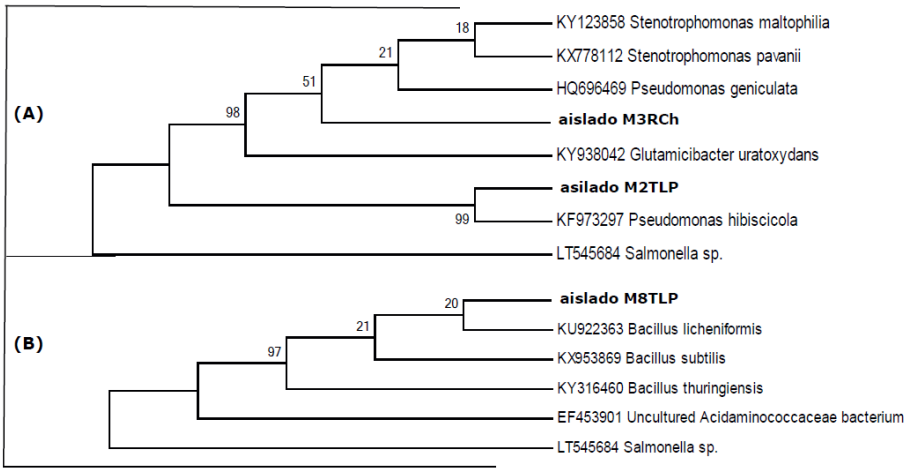

FIGURE 4
Phylogenetic tree of the M3RCh and M2TLP isolates and their relationships with species of bacteria ofthe genus Pseudomonas (A) and of the isolate M8TLP and its relationship with bacterial species of thegenus Bacillus (B). M: morphotype; R: root; T: Tallo, Ch: Chapinero and LP: Las Peñas.

On the other hand, the sample of genomic DNA of the isolated M1RLP amplified with the ologonucleotides F948 and R1492 it is sequences showed high homology with the sequence of the bacterium Delftia tsuruhatensis, a Betaproteobacteria of the order Burkholderiales, with 100% identity and coverage by analysis in Blast, at the moment of doing the phylogenetic reconstruction, the sequence obtained presented few information sites which prevented the construction of a tree.

DISCUSSION

The population values of endophytic bacteria obtained in the present study ranged from 3.10 x 108 ± 4.48 x 1010 CFU/g of tissue, and show higher values of means than those reported by Pérez et al (8), In a study conducted with the same bacteria associated with colossal grass in three municipalities of the department of Sucre, the population densities found by the authors correspond to 3.24 x 109± 2.2 x 1010 CFU/g of tissue.

Figure 1b shows the densities values of endophyte bacteria isolated from roots (5.0 X 1010, 3.8 X 1010, 2.8 X 1010, 2.4 X 1010 and 1.5 X10 CFU/g of tissue), indicating that the highest values of endophyte bacteria isolated from roots were for the location of The Peñas, The Mamon, Canta gallo, Chapinero and Hato Nuevo, respectively, while the lowest values were found in stems and leaves respectively. On the other hand, the soils of The Peñas location presented pH (6.32) slightly acidic and values of high organic matter, with respect to the location of Hato Nuevo, soils with pH values of 4.74, strongly acid reaction and values of organic matter predominated of deficient. Possibly the difference found with respect to the population density of endophytic bacteria and locality, can be supported to what is posed by Axelrood et al (16), who state that bacteria do not work efficiently in acid soils. As the pH of the soil decreases, the activity of the microorganisms that decompose the organic matter and provide nutrients to the plants also decreases. Although most bacteria work best at soil pH levels of 7.0, their effectiveness rapidly decreases until pH levels are below 6.0.

With respect to the results found in the in vitro test of qualitative activity of ammonium ion production by endophytic bacteria isolated from colossal grass, values of 0.23 to 1.17 mg/L were found. At the date of this study, no information is reported in national databases, which allow the results obtained to be compared. However, the results of the study carried out by Lara et al (17) with asymbiotic nitrogen-fixing bacteria in the agricultural area of San Carlos, Córdoba, Colombia, reported the presence of 14 ammonium-producing isolates at a concentration range of 0.9 to 5.2 mg/L. The genus of bacteria Azotobacter sp, A16PG and A26M1P, produced values of 5.1545 and 5.1743 mg/L of ammonium, respectively, while the values obtained for the genus Azospirrillum spp, A5M1G was 4.6741 mg/L of ammonium. These authors concluded that the biological fixation of nitrogen (FBN) by diazotrophic bacteria has contributed to increase yields in crops, reducing the need for nitrogen fertilizers and the emission of toxic gases such as N2O, obtaining economic and environmental benefits in the farms.

In vitro tests on the production of soluble phosphate by endophytic bacteria show a decrease in pH as the concentration of soluble phosphate increases. Study carried out by Barraza and Pérez (9), on the evaluation of the efficiency in the production of phosphate by isolated endophytic bacteria of different rice varieties in the department of Córdoba, observed a decrease in the pH value as the soluble phosphate concentrations increase. This decrease in pH is possibly due to the direct relationship between the production of acids and the release of phosphate-type compounds. However, as other studies point out, pH decrease does not always occur, because not all bacterial species use the same mechanism to release phosphate and make it available to plants (18).

With respect to the results indicated in Figure 3c, it is observed that the production values of IAA ranged from 1.15 to 12.3 mg / L for 8 isolates of endophytic bacteria evaluated. The values found in the present studies were not purchased with other studies because there are no similar works in national and national databases. However, although the research on evaluation of the production of AIA show high or low values when compared to each other, it is demonstrated that low concentrations of phytohormone are able to stimulate plant development and high concentrations inhibit and reduce the area of elongation; taking into account that native microorganisms are adapted to their own conditions and environments, only performing bioassays in vivo can find the appropriate dose and check the effect exerted on the crops to be applied (19).

On the other hand, 12 isolates of endophytic bacteria showed qualitative capacity to produce siderophores. It has been shown that endophytic bacteria have various indirect mechanisms for promoting the growth of plants, which include: the production of antimicrobial metabolites and lytic enzymes, the induction of

systemic resistance, competition for nutrients (production of siderophores) and the saturation of ecological niches (20).

The species of related endophytic bacteria such as Delftia tsuruhatensis was isolated from colossal grass roots belonging to the Peñas location, which according to in vitro growth promotion tests, showed phosphate solubilization capacity, siderophore production and N2 reducing capacity to ammonium. Meanwhile, the species of endophytic bacteria identified as Pseudomonas hibiscicola isolated from colosoana grass stalk in the same location of The Peñas, showed reducing capacity of N2 to ammonium phosphate solubilization, production of siderophore.

Several studies have found that Pseudomonas hibiscicola (S. maltophilia) has been reported as an endophytic bacteria of black pepper plant (Piper nigrum), isolating 20 strains of bacteria related to the species of Ps. hibiscicola and the tests carried out proved that this species has the capacity to produce siderophores and in a lesser amount of IAA (21). Another study indicates that this species of bacteria is associated with medicinal plants from China in which the production capacity of IAA (8.51 μg/mL) was demonstrated, and biological control activity exerted against the phytopathogens Dothiorella gregaria and Botryosphaeria dothidea (22). Likewise, the nitrogen fixation capacity of this bacterium in legume (Parkia roxburghii) was reported by Singh and Mazumder (23). Other works relate to this bacterium as an endophyte of corn seeds (Zea mays) (24). And in Aloe Vera (Aloe vera), where it was found producing metabolites with antioxidant activity (25).

Recent studies show Stenotrophomonas maltophilia CR71 (Ps Hibiscicola) as an endophytic bacterium that according to the results of in vitro tests presents excellent antagonistic action against the fungal mycelia of Fitopatógeno Botrytis cinerea, through the emission of volatile organic compounds (VOCs). acronym in English). In greenhouse trials in this, same species of bacteria inoculated in tomato plants) showed efficient activity as a growth promoter in these plants (26).

With respect to the species of bacteria known as Delftia tsuruhatensis, it has been isolated from roots of rice cultivation (27), and its potential promoter growth promoting activity has been described by Han et al (28) when found in rice rhizoplane and in wheat cultivation (29). Likewise, this bacterium was reported by Wurdig et al (30), in a study on the diversity of diazotrophic endophytic bacteria associated with millet plants of the Rio Grande do Sul region of Brazil, the results showed that there is a high diversity of endophytic bacteria among which was identified D. tsuruhatensis with nitrogen fixation capacity in millet culture.

In conclusion, these two new species of endophytic bacteria were identified as Delftia tsuruhatensis M1RLP and Pseudomonas hibiscicola (S. maltophilia) M2TLP, isolated from colossal grass, with excellent results due to their direct capacity to promote growth through the solubilization of soluble phosphates, biological fixation of nitrogen (production of ammonium), production of indole acetic acid and the production of siderophores. Finally, these species of endophytic bacteria isolated from colossal grass in the present study can be used as microbial consortiums to effectively act as growth promoters and for biological control simultaneously in said pasture of livestock farms in the municipality of Corozal, department of Sucre, Colombia.

Conflict of interests

The author (s) did not declare potential conflicts of interest with respect to the investigation, authorship and/or publication of this article.

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