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RESEARCH ARTICLE

Characterization and plant growth promoting potential of microbial groups associated with a *Coffea* sp. collection

María Esther González Vega[1], Annia Hernández Rodríguez[2], Yarelys Herrera Díaz[2], José Ángel Lacerra Espino[3], Merardo Ferrer Viva[3], Eduardo Fidel Héctor Ardisana[4], Sandra Pérez-Álvarez[5]*

[1]Instituto Nacional de Ciencias Agrícolas (INCA), Dpto. Genética y Mejoramiento de las Plantas, San José de las Lajas, Mayabeque, Cuba, CP: 32700, [2]Facultad de Biología. Calle 25 No. 455, entre J e I. Vedado. La Habana, Cuba, CP: 10400, [3]Estación Experimental Agroforestal de Jibacoa. INAF. Rincón Naranjo, Manicaragua, Villa Clara, Cuba, CP: 54590, [4]Instituto de Posgrado, Universidad Técnica de Manabí, Portoviejo, Ecuador, CP: EC13010, [5]Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Km 2.5, carretera Delicias-Rosales, Campus Delicias, CD. Delicias, Chihuahua, México, CP: 33000.

ABSTRACT

The genus *Coffea spp*. is the most important of the *Rubiaceae* family and both *Coffea arabica* L. and *Coffea canephora* P. constitute the most economically important species. This research aimed to characterize morphologically, physiologically and biochemically diazotrophic rhizobacteria associated with coffee plants from a living collection, and to study their plant growth promoting potential. Semi-solid nitrogen-free media NFb and JNFb were used to isolate the bacteria. Ten isolates were selected for micro-morphological, physiological-biochemical and plant growth potential characterization. The solubilization of calcium phosphate, the synthesis of indole compounds and the production of siderophores were analyzed. The results showed that high population levels of bacteria, fungi and actinomycetes are present in the rhizosphere of the coffee collection. 95 native isolates were selected, and 8 were classified as *Azospirillum* and *Herbaspirillum*. Phosphate solubilizing activity was detected in the ten isolates, highlighting the C8 isolate. Synthesis of indole compounds was also detected by the ten isolates, although in amounts significantly lower than the controls. Only isolates C2, C9 and C10 produced siderophores. The multivariate cluster analysis resulted in the formation of three groups, the C8 grouped with the positive control in one of them. The beneficial potential of this isolate makes it a source of interest to develop a biostimulant product that could be applied as a sustainable alternative for the cultivation of *Coffea* sp.

Keywords: Coffee; Diazotrophic rhizobacteria; Isolate; Microorganism collection

INTRODUCTION

Coffee (*Coffea spp.*) is one of the most extensively exported commodities in the international markets and it is an important agricultural crop for the economy of millions of people around the world (De Los Santos-Briones and Hernández-Sotomayor, 2006). Several countries depend on this item to obtain foreign exchange, which leads to the constant search for improved performance. This crop represents an important economic potential and has high value as a drinking beverage (Alemayehu, 2017).

The genus *Coffea* includes a hundred species; however, only *C. arabica*, and *C. canephora* are cited as the most commercially cultivated species (N'Diaye et al., 2005). It is known that biological diversity is currently affected at a rate never seen before. The influence of biotic and abiotic factors can change species interaction and how this vary with environmental condition, having negative impacts in agriculture (Lavergne et al., 2010; Agler et al., 2016); coffee is not exempt from it.

The rhizosphere has often been used as the preferential site for the isolation of plant growth promoting (PGP) microorganism with potential applications as biofertilizers (Bashan et al., 2014; de Souza et al., 2015). PGP microbes have a great influence not only in growth but also in yield of numerous crops and include microorganisms of different groups such as Actinobacteria, Bacteroidetes, Balneolaeota, Firmicutes, Proteobacteria and Spirochaetes. The growth promotion includes mechanisms like the facilitation of nutrient acquisition, increased resistance against stresses and the modulation of plant gene expression (Santoyo et al., 2016; Olanrewaju et al., 2017).

***Corresponding author:**
Sandra Pérez-Álvarez, Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Km 2.5, carretera Delicias-Rosales, campus Delicias, CD. Delicias, Chihuahua, México, CP: 33000. **E-mail:** spalvarez@uach.mx

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PGP bacteria are naturally occurring in soil and they are bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Yadav et al., 2017), that is why the aim of this research was to characterize morphologically, physiologically and biochemically diazotrophic rhizobacteria associated with coffee plants from a living collection, and to study their plant growth promoting potential.

MATERIALS AND METHODS

Soil sampling

The samples were taken from rhizospheric soil from a coffee collection of the *C. arabica* and *C. canephora* species established in the National Institute of Agricultural Sciences (INCA), belonging to the municipality of San José de las Lajas, Mayabeque province, Cuba. Sampling was done randomly according to the methodology described by Hernández et al. (2008).

The soil where the coffee work collection was established was classified as Ferralitic Red, leachate, typical eutrophic (Hernández et al., 2015), (Table 1).

Serial decimal dilutions of each rhizospheric soil sample were made in physiological saline solution (0.85% NaCl) (Bashan and Holguin, 1998). Subsequently, 100 µl of each dilution were inoculated in 100 mL Petri dishes in different culture media [Agar Oats (Merck); Nutrient Agar (Merck) and Malta Agar], this procedure was used for the characterization of the microbial groups present in the samples. Samples cultured in the media Nutrient Agar were incubated 24 h at 30°C and the ones cultured in Malta Agar and Agar Oats media were incubated 168 h at 30°C. The cell concentration was determined on the first and seventh day after incubation, depending on the growth rate of the microbial group under study.

Isolation

For the isolation of diazotrophic bacteria, 100 µL of each of the serial dilutions of the previously prepared rhizospheric soil samples were inoculated into flasks containing 5 mL of the semi-solid media free of nitrogen sources. Semi-solid selective culture media JNFb (Day and Dobereiner, 1976) and NFb (Baldani and Döbereiner, 1980) were used and each soil sample was studied in triplicate. The samples were incubated for 7 days at 28°C. The change in color and the appearance of a film about 4 mm from the surface of the culture media were taken as a positive result (Döbereiner, 1980). For the flask with positive results, a second sowing was carried out in a nitrogen-free environment. The cell concentration was determined by the most probable number method (PNM), (Pedraza et al., 2007).

Table 1: Agrochemical composition of red ferralitic soil

Agrochemical composition of the soil					
pH (H_2O)	Organic matter (%)	P (ppm)	K^+ (Cmolc. kg^{-1})	Ca^{+2} (Cmolc. kg^{-1})	Mg^{+2} (Cmolc. kg^{-1})
7.2	2.12	3.43	0.85	14.5	3.10

An aliquot was taken from the positive samples and sowed by depletion in 100 mL Petri dishes, containing the solid media NFb and JNFb, and incubated at 28°C for 7 days. Isolated colonies were sowed in 15 mL culture tubes containing 5 mL of Papa Agar solid medium (Döbereiner, 1988) and incubated 24 h at 28°C. The tubes were stored at 4°C for later use.

Characterization of diazotrophic bacterial populations

The micro-morphological characterization of the isolates in the solid media, Red Congo (Rodríguez-Cáceres, 1982) and Agar Potato were determined. The morphological studies of the cells were carried out according to Bergey and Holt (1994).

For the physiological-biochemical characterization, the API 20NE® system (Biomérieux, France) was used. The results were interpreted using the API analytical profile index (StandAlone® API 20NE®).

For the differentiation of the isolates, they were identified with the letter C of *Coffea spp*. and consecutive numbers were placed. The standard strains of *Azospirillum brasilense* Sp7 (ATCC 29145), *A. brasilense* 8I (ATCC 29709), *Pseudomonas putida* AI05 and *Herbaspirillum seropedicae* Z94, deposited in the strain collection of the CNPAB / EMBRAPA of Brazil were used.

Solubilization of tricalcium phosphate

The isolates were transferred to 15 ml Cornell tubes containing 5 mL of LB medium (Merck) and they were incubated in a shaker for 24 hours at 30°C. The phosphate solubilizing activity of each isolate was determined according to the protocol described by Mehta and Nautiyal (1999). Plates were incubated at 30°C for up to 14 days, and the solubilization halo was measured at 3, 7 and 14 days. The solubilization index of Kumar and Narula (1999) was used as a comparison criterion (IS = A / B, where: A = total diameter (diameter of the colony + diameter of the halo) and B = diameter of the colony). Three replicates were established per isolate and the experiment was repeated twice.

Synthesis of indole compounds

To quantify the production of indole compounds, the isolates were grown in liquid medium Triptone Soy Broth (Merck) supplemented with L-Tryptophan (0.1 g L^{-1}) (Tien

et al., 1979). The isolates were grown in a shaker at 150 rpm and 30°C for 24 hours.

The cell concentration was adjusted to 10^8 mL^{-1} cells, corresponding to tube 0.5 of the McFarland scale. The samples were then centrifuged at 5000 rpm (Eppendorf Centrifuge 5702, Germany) for 15 min and 0.5 mL of cell-free liquid was taken for auxin extraction according to Tien et al. (1979). For quantification, Salkowski's reagent (Sen and Leopold, 1954) was used in a 1:1 ratio (v/v). The mixture was incubated for 30 min in the dark and then the absorbance at 530 nm was measured. The concentration was calculated using a standard indoleacetic acid (IAA) standard curve.

Production of siderophores

The quantification of siderophores was performed according Schwyn and Neilands (1987). The appearance of an orange halo around bacterial growth was taken as an indicator of siderophores production and the diameter of the halo was measured in mm. A control was established without inoculation and three replications were made per treatment, the experiment was repeated three times.

Statistical analysis

All variables were tested for normality with Shapiro and Wilk test (1965), the simplified version of Shapiro and Francia (1972) was used, then parametric and non-parametric analyzes were performed as applicable. The $\chi 2$ test was applied to compare the percentages of microbial populations in the soil studied. The grouping of the isolates was carried out by means of the preparation of a dendrogram, in which the Full Ligation test was used and as a measure of distance the Euclidean distances were apply, and for its physiological characterization the Tukey honesty test was used, with the use of STATISTICA program version 8.0 on Windows.

RESULTS AND DISCUSSION

The concentration of microbial populations growing in the rhizosphere of *C. arabica* and *C. canephora*, which are part of a live collection was high (Table 2). In the samples analyzed, these values ranged from $3x10^{10}$ and $3x10^{11}$ CFU (Colony-forming units) g^{-1} for bacteria, $3.5x\ 10^7$ and $3.5x10^8$ CFU g^{-1} for fungi and $7x10^7$ and $5x10^8$ CFU g^{-1} in the case of actinomycetes. There were no significant differences in the population levels associated with the coffee species studied ($p < 0.05$), according to the statistical test of x^2 .

The high microbial concentrations found in this study are attributed to the characteristics of the soil from which they were isolated. The sampled rhizosphere soil was characterized by an average content of organic matter and calcium, low magnesium content, high phosphorus content and neutral pH, which favors the growth of microorganisms. In addition, the cation exchange capacity of this type of soil was high, and the clay texture in all its thickness, with a clay percentage greater than 40%. Several authors point out the importance of some of the properties of soil fertility such as pH and organic matter, in the survival and establishment of microorganisms in the rhizosphere (Hernández et al., 2008; Vázquez et al., 2000).

Another factor that can influence the frequency of occurrence of associative bacteria is the composition of radical exudates excreted by the plant. These constitute the main trigger for root colonization and the establishment of microbial associations (Walker et al., 2011). Regarding the microbial populations associated with the two species of the genus *Coffea* under study, *C. arabica* and *C. canephora*, no significant differences were found ($p < 0.05$); this indicates that there was no strict specificity between these coffee species and the associated microbial populations, for the conditions evaluated. These results differ from that observed by other authors for other crops of economic importance, such as canola and wheat, where significant differences were obtained between the endophytic and rhizosphere microbial communities associated with different cultivars (Siciliano et al., 2011).

In general, in the samples of rhizosphere soil studied, the presence of bacteria with concentrations in the order of 10^{10} and 10^{11} was favored. This response is related to the pH value of 7.2 in the soil studied, which favors the proliferation of bacterial populations, because the optimal growth of this microbial group is achieved with pH close to neutrality (Hernández et al., 2008).

Isolation

The values of concentrations from the diazotrophic bacterial populations associated with the *Coffea* species studied are shown in Table 3. The concentrations ranged

Table 2: Microbial populations present in the rhizosphere of a coffee collection (*Coffea* sp.)

Samples	Bacteria		Actinomycetes		Fungi	
	CFU g^{-1}	Log CFU g^{-1}	CFU g^{-1}	Log CFU g^{-1}	CFU g^{-1}	Log CFU g^{-1}
1	$6.2x10^{10}$	9.79	3.33×10^8	8.52	3.5×10^8	8.54
2	3×10^{10}	10.47	5×10^8	8.69	1.25×10^8	8.09
3	3×10^{11}	11.47	7×10^7	7.84	3.5×10^7	7.54

Samples 1 and 2: *Coffea arabica*, Sample 3: *Coffea canephora*

between $1.6x10^5$ and $1.4x10^8$ CFU g^{-1} of soil, without significant statistical differences between species.

A total of 95 isolates were selected from the rhizosphere of the coffee crop, given their ability to grow in nitrogen-free media NFb and JNFb; from these only 10 isolates (10.5%) were selected, considering the film formation at approximately 4 mm from the surface of the semi-solid culture media, typical of diazotrophic bacteria (Fig. 1).

The typical film formation, indicative of the presence of diazotrophic bacteria in the NFb medium, is considered a positive response, and suggests that these microorganisms have the ability to fix atmospheric nitrogen.

Characterization of diazotrophic bacterial populations

Morphological characterization of the isolates

The morphological characteristics of the selected isolates in the Congo Red and Agar Potato culture media, as well as the micro-morphological characteristics obtained by observation under an optical microscope are shown in Table 4. The isolates were grouped according to their characteristics, both cultural and micro-morphological, into three fundamental groups.

Isolates C1, C3, C4, C5, C6, C8 and C9, were obtained from a white film formed in the semi-solid JNFb medium, then they were sowed in Agar Potato medium, where they formed dark green colonies, and under the microscope they presented a short and medium bacillus shape, curvilinear, with spiral movement and a negative response to Gram staining. These isolates have characteristics very similar to the *Herbaspirillum seropedicae* Z94 strain, which could indicate that they belong to this genus (Marques et al., 2015).

The isolate C7 had characteristics very similar to the strain *Azospirillum brasilense* 8I, which suggests it belongs to the genus *Azospirillum*. A typical growth of this genus was observed in Congo Red Agar, that means scarlet red colored colonies, dry consistency, irregular edge, rough surface, with abundant growth; it was also negative for Gram staining and presented a bacillary form, with absence of spores and capsule.

The isolates C2 and C10 had different characteristics in culture compared to the rest of the isolates. They were characterized by forming white colonies in the Agar Potato medium, however their growth in Congo Red Agar was transparent, which differs from the expected characteristics. This response suggests that they could belong to other genera of Gram negative microaerophilic microorganisms with the ability to fix atmospheric nitrogen.

Table 3: Diazotrophic bacteria populations (CFU g^{-1}) in rhizospheric soil of a coffee collection

Samples	JNFb Medium		NFb Medium	
	CFU g^{-1}	Log CFU g^{-1}	CFU g^{-1}	Log CFU g^{-1}
1	$1.6 \cdot 10^5$	5.20	$2.0 \cdot 10^7$	7.03
2	$1.15 \cdot 10^6$	6.06	$1.4 \cdot 10^8$	8.14
3	$3.5 \cdot 10^6$	6.54	$1.4 \cdot 10^8$	8.14

Samples 1 and 2: *Coffea arabica*, Sample 3: *Coffea canephora*

Physiological – biochemical characterization of isolates

The analysis of the results of the physiological-biochemical tests (Table 5), obtained with the use of API 20NE, by calculating the Euclidean distance and complete ligation, as well as a clustering test, showed that the isolates are forming three groups (Fig. 2), which agree with the groupings obtained according to their micro-morphological and cultural characteristics.

Group I (Fig. 2), is formed by isolates C1, C3, C4, C5, C6, C8 and C9. The physiological-biochemical tests (Table 5), showed that this group of isolates belongs to the genus *Herbaspirillum*, because the isolates show physiological-biochemical characteristics similar to the control strain of *Herbaspirillum seropedicae* Z94. It is interesting to note that

Fig 1. Typical film of growth of diazotrophic bacteria, isolated from rhizospheric soil from a coffee collection. Semi-solid media: A- JNFb, B-NFb and C-Enlargement of B, the arrow indicates the whitish film.

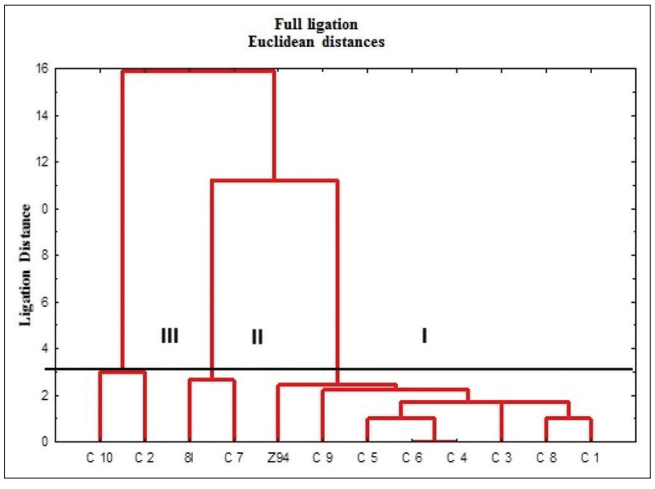

Fig 2. Dendrogram representing the distance of the rhizosphere isolates from a coffee collection, obtained from the analysis of the physiological-biochemical tests using API 20NE of the selected strains. *Azospirillum brasilense* 8I and *Herbaspirillum* seropedicae Z94 were used as a positive control. Roman numerals indicate the groups.

Table 4: Cultural and micro-morphological characteristics of the rhizospheric soil isolates of the coffee collection, *Azospirillum brasilense* 8I and *Herbaspirillum seropedicae* Z94 strains

Characteristics		Isolates											
		8I	Z94	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Characteristics in Agar Potato culture	Shape	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
	Elevation	Flat	Concave	Concave	Flat	Concave	Concave	Flat	Concave	Flat	Flat	Flat	Flat
	Surface	Smooth	Smooth	Smooth	Rough	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Rough
	Edge	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole
	Color	White	Green	White	White	Green	Green	Green	Green	White	Green	Green	White
	Optical details	Opaque	Sparkly	Sparkly	Opaque	Sparkly	Sparkly	Sparkly	Sparkly	Opaque	Sparkly	Sparkly	Opaque
Features in Congo Red Agar culture	Shape	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
	Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat
	Surface	Dry	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Dry	Dry	Dry	Dry
	Edge	Whole	Regular	Irregular	Whole	Whole	Whole	Whole	Whole	Whole	Irregular	Irregular	Whole
	Color	Scarlet red	Red/ white	Red	Transparent	Red	Red	Red	Red	Scarlet red	Red	White	Transparent
	Optical details	Opaque	Sparkly	Sparkly	Sparkly	Sparkly	Sparkly	Sparkly	Sparkly	Opaque	Sparkly	Sparkly	Sparkly
Micro-morphological characteristics	Gram	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	Shape	Short bacilli	Short bacilli / medium	Short bacilli	Short bacilli	Medium bacilli	Short bacilli	Medium bacilli	Short bacilli	Short bacilli	Medium bacilli	Short bacilli	Medium bacilli
	Motility	Very motile	Very motile	Motile	Motile	Motile	Very motile	Motile	Motile	Very motile	Very motile	Very motile	Slow

Table 5: Physiological-biochemical characteristics of bacterial isolates from rhizospheric soil from a coffee collection

Test	Isolates											
	8I	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10	Z94
Enzymatic activity												
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-	+	-	-	-	-
Glucose Fermentation	-	+	-	+	+	-	+	+	+	+	+	+
Arginine Dihydrolase	+	+	-	+	+	+	+	+	+	+	+	+
Urease	+	-	-	-	-	-	-	-	-	-	+	+
Hydrolysis of Esculin	+	+	-	+	+	+	+	+	+	-	+	+
Gelatin Hydrolysis	-	+	+	+	+	+	+	+	+	-	+	+
β-Galactosidase	+	+	-	+	+	+	+	+	+	+	+	+
Assimilation Tests												
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	-	+	-	+	+	+	+	+	+	+	+	+
Mannose	+	+	-	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
N- acetyl glucosamine	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	-	+	+	+	+	+	+	+	+	+
Potassium gluconate	+	+	+	+	+	+	+	+	+	+	+	+
Capric acid	+	+	+	+	+	+	+	+	+	+	+	+
Adipic acid	-	+	+	+	+	+	+	+	+	+	+	-
Malate	+	+	+	-	-	-	-	-	+	+	+	+
Citrate	+	+	+	-	+	+	+	+	+	+	+	+
Phenylacetic acid	+	+	-	+	+	+	+	+	+	+	+	+

within subgroup III different subgroups were obtained, which could indicate the presence of different species of this genus.

Group II (Fig. 2) is formed by isolates 8I and C7, which indicates that C7 belongs to the genus *Azospirillum*, because it manifests physiological-biochemical characteristics similar to the control strain *Azospirillum brasilense* 8I (Table 5).

Group III (Fig. 2), is formed by isolates C2 and C10. The physiological-biochemical tests (Table 5), indicate that the isolates of this group do not belong to the *Azospirillum* and *Herbaspirillum* genera, so they could be found within other genera of microaerophilic diazotrophic bacteria, Gram negative, with bacillary morphology and that have been frequently isolated in the rhizosphere of economically important crops.

Among the microaerophilic diazotrophic microbial genera described in the literature the following are listed: *Azospirillum* (Baldani and Döbereiner, 1980); *Herbaspirillum* (Brasil et al., 2005); *Burkholderia* (Guimarães et al., 2007) and *Sphingomonas* (Videira et al., 2009). Research carried out in recent years shows that *Azospirillum* and *Herbaspirillum* genera are more frequently associated with the rhizosphere of different economically important crops (Baldani et al., 1997); however, other genera of diazotrophic microorganisms could be associated with coffee cultivation.

These results also indicate that although the semi-solid media NFb and JNFb, are considered specific for *Azospirillum spp*. and *Herbaspirillum spp*., respectively, they also allow the isolation of other nitrogen-fixing microbial genera. The diversity observed could be favored by the characteristics of the soil studied, such as the percentage of clay and organic matter content, which corresponds to that proposed by other authors (Caballero-Mellado et al., 2007).

Solubilization of tricalcium phosphate

Table 6 shows the solubilization values for phosphate obtained from the isolates collected in the rhizospheric soil of *Coffea* sp.

Solubilization index (SI) <2 (low), 2≤ SI ≤4 (medium), SI >4 (high). Early solubilization (solubilization in the first three days); late solubilization (solubilization after the third day). Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

All the isolates solubilized phosphate after 3 days of bacterial growth (data not shown). The analysis of the solubilization results showed no significant differences seven days after inoculation; however, after 14 days, differences were observed, highlighting firstly the isolate C8, and also C1, C4, C6 and C9 because of high solubilization rates, with values between 4.25 and 6.37.

Table 6: Phosphate solubilization of isolates from rhizospheric soil of *Coffea* sp.

Strains	Solubilization index (SI)		Solubilization level
	7 days	14 days	
8I	2.15	2.27 b	Medium / late
Z94	1.94	2.71 b	Medium
C 1	3.75	4.25 ab	High / Early
C 2	4.00	3.50 b	Medium / Early
C 3	2.95	3.54 b	Medium / Early
C 4	3.79	4.70 ab	High / Early
C 5	3.25	3.31 b	Medium / Early
C 6	4.00	5.00 ab	High / Early
C 7	3.50	3.04 b	Medium / Early
C 8	5.37	6.37 a	High / Early
C 9	4.54	4.64 ab	High / Early
C 10	3.83	2.67 b	Medium / Early

The solubilization of phosphate in the rhizosphere is the mechanism of action most commonly developed by plant growth promoting rhizobacteria, which increases the availability of nutrients for plants (Bergey and Holt, 1994). In this study, 100% of the isolates solubilized tricalcium phosphate, which was confirmed by the formation of a transparent halo around the microbial growth, coinciding with that indicated by Nico et al. (2012) which is shown in Fig. 3.

The solubilization index (SI) depends on different factors, among which the type of microorganism isolated, the production of organic acids, the substrate used as a source of phosphorus, the culture medium and the carbon source used are the most important (Lemke et al., 1995). It is known that the consumption of carbon sources such as sucrose or glucose generates the production of organic acids that acidify the pH of the medium. It has been demonstrated that the solubilization of phosphorus present in soils is due, in some cases, to the production of organic acids such as oxalic, citric, lactic, gluconic acid, among others, as a result of glucose metabolism (Bashan et al., 2013).

The SI observed in this research are superior to those reported by previous workers (Ambrosini et al., 2012) when evaluating phosphate solubilization in strains of the genus *Bacillus* in rice cultivation (*Oryza sativa* L.). Sasaki et al. (2010), obtained results similar to those of the present investigation, with respect to phosphate solubilization, for the *Azospirillum* B510 strain, which is a very good promoter of plant growth in rice plants (*O. sativa*). The efficiency of the isolates under study, in terms of the ability to solubilize inorganic phosphate, could indicate their potential as plant growth promoting agents, once the mechanisms of their interaction with the plant are characterized.

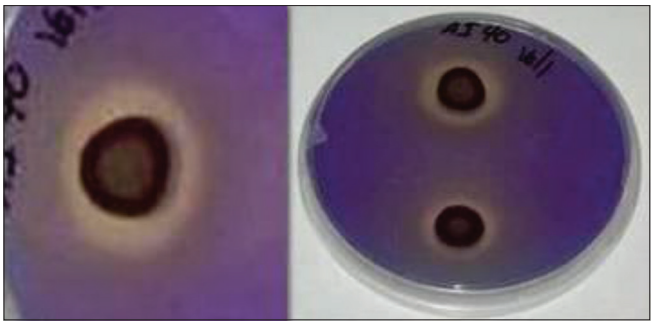

Fig 3. Tricalcium phosphate solubilization haloes formed around the microbial colonies of the rhizospheric soil isolates of a coffee tree collection.

Production of indole compounds

In Fig. 4, it is observed that 100% of the selected isolates produced indole compounds, which are of great importance in plant phytostimulation. No statistically significant differences were observed between the isolates, keeping the values in a very narrow numerical range between 0.93 and 2.72 $\mu g\ mL^{-1}$.

The concentrations of indole compounds obtained in this study are considered low compared to the values obtained for the two control strains, 8I and Z94, and values reported for other genera of nitrogen fixing bacteria, producing auxins (Dobbelaere et al., 2003; Islam et al., 2009). However, with very low amounts of this compound it is possible to achieve phytostimulation (Gravel et al., 2007).

Siderophores production

Some of the selected isolates have the ability to produce siderophores under iron-limiting conditions (Acid Casamino Agar culture medium, to which the CAS indicator solution is added), which is denoted by the formation of a halo orange around the bacterial colony (Fig. 5).

After 24 hours of incubation, only three of the 10 selected isolates showed siderophores production, the C2, C9, and C10 isolates, with siderophores production halos ranging from 12.25 to 16.75 mm. The largest production halos were those of the C2 isolate, comparable to the *Pseudomonas sp.* AJ13. Secondly, the C10 isolate was highlighted, which presented values comparable to that of the *Pseudomonas sp.* AI05, although it differed significantly, from the statistical point of view, from strain AJ13 and isolate C2. The lowest halo was observed for the C9 isolate, which presented inferior and statistically significant results with respect to C2 and C10 (Fig. 6).

Selection of promising isolates

The analysis of Hierarchical Conglomerate and Complete Ligation, based on the Euclidean distance, allowed to group the isolates obtained from the rhizosphere of coffee, by the

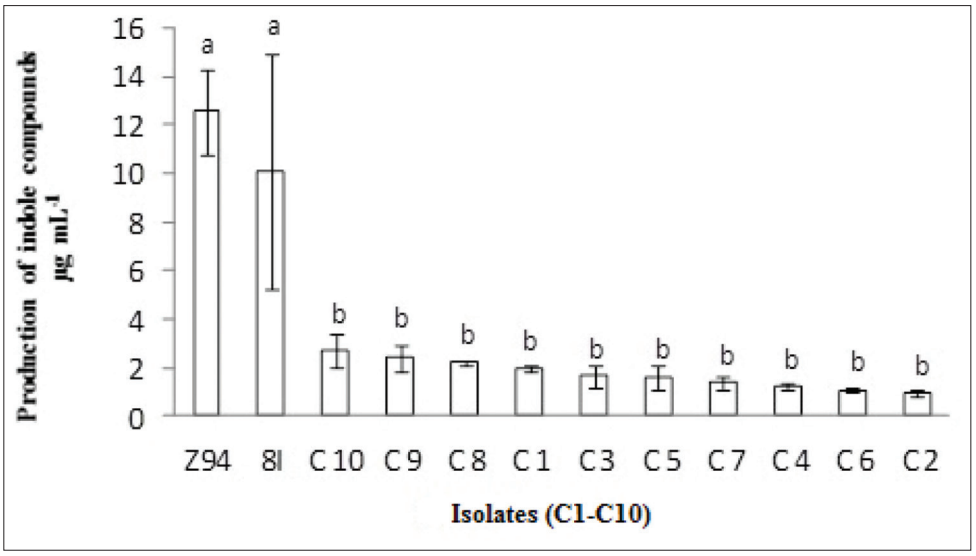

Fig 4. Characterization of the selected isolates, from rhizospheric soil of the coffee collection, in terms of the production of indole compounds. *Azospirillum brasilense* 8I and *Herbaspirillum* seropedicae Z94 were used as a positive control. Uncommon letters indicate significant differences between the means according to Tukey's test ($p \leq 0.05$).

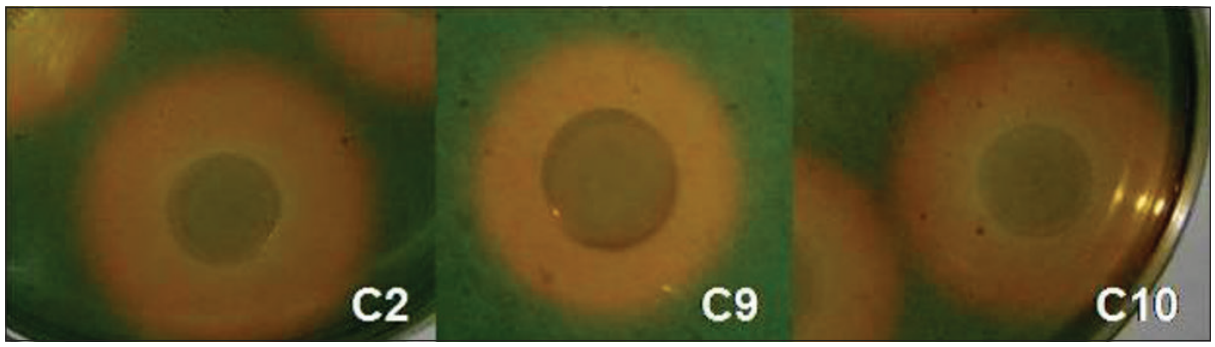

Fig 5. Production of siderophores by the rhizospheric soil isolates of the coffee collection, C2, C9, and C10, in agar acid Casamino medium to which the CAS indicator solution was added. Siderophores production is denoted by the appearance of an orange halo around the bacterial colony.

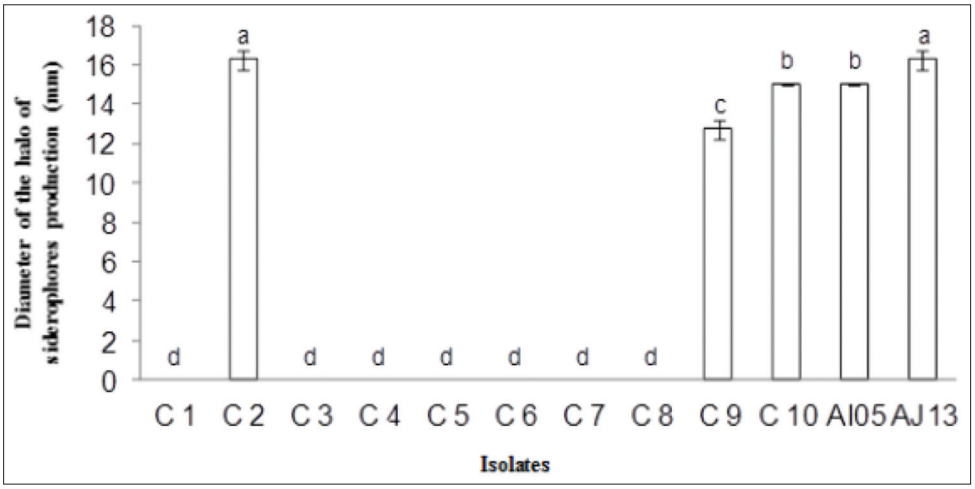

Fig 6. Production of siderophores by the selected rhizospheric soil isolates from the coffee collection. Pseudomonas sp. AI05 and AJ13 as a positive control. Different letters indicate significant differences between the means according to Tukey's ($p \leq 0.05$).

ability to produce the metabolites under study, in three different groups (Fig. 7); within groups, patterns were observed.

In Group I, only the isolate C8 and the positive control (CP) were clustered. C8 is an isolate with outstanding potential

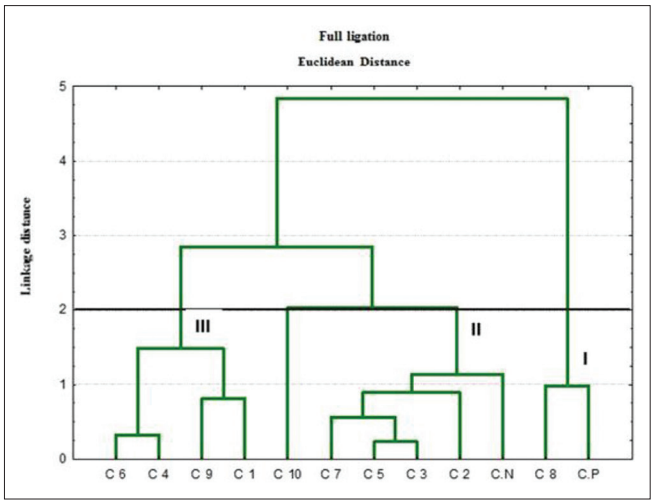

Fig 7. Dendrogram calculated from the analysis of the concentrations / solubilization index of the metabolites under study. The positive control (CP) is formed by the highest concentrations / solubilization index of indole compounds and siderophores produced by the rhizospheric soil isolates of the coffee collection, and the negative control (CN) by the lowest. Roman numerals indicate the groups.

for phosphate solubilization, a discrete synthesis of indole compounds and non-producing siderophores.

Group II consisted of isolates C2, C3, C5, C7, C10 and the negative control (CN). These isolates have lower performance in terms of the tests implemented, so it is considered that they do not meet the characteristics required to be recommended for the production of future biopreparations of microbial origin.

Group III included isolates C1, C4, C6 and C9, which were grouped independently and showed that their performance was medium. However, other tests could be carried out to support its potential as promoters of plant growth.

CONCLUSIONS

High population levels of bacteria, fungi and actinomycetes were found in the rhizosphere of the collection of *C. arabica* and *C. canephora*, not depending on the species. From 95 native isolates, 8 were classified as *Azospirillum* or *Herbaspirillum*. All the selected isolates have the capacity to produce indole compounds and solubilize phosphates; the isolates C2, C9 and C10 also produce siderophores, and the C8 isolate stands out for the greatest potential as a plant growth promoting agent.

Authors' contributions

María Esther González Vega, Annia Hernández Rodríguez, Yarelys Herrera Díaz, José Ángel Lacerra Espino, Merardo Ferrer Viva, designed study, wrote the document and analyzed data; Eduardo Fidel Héctor Ardisana and Sandra Pérez-Álvarez wrote the document, analyzed data and interpreted the results.

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