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Comparative plant growth promoting traits and distribution of rhizobacteria associated with heavy metals in contaminated soils

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ABSTRACT: The heavy metals at high concentration are generally toxic to the plants for their metabolism and growth; therefore, interactions among metals, rhizosphere microbes and plants have attracted attention because of the biotechnological potential of microorganisms for metal removal directly from contaminated soils or the possible transference of them to the plants. The aim of this study was to compare the relationships between the physiological *in vitro* characteristics of rhizobacteria isolated from plant metal accumulators and their distribution relating with the heavy metals content in contaminated soils. The results of this study showed that the heavy metals present in the rhizosphere of the plant species analyzed, decrease the microbial biomass and content of heavy metals caused a different distribution of rhizobacteria found. Gram negative rhizobacteria (90 %) and gram positive rhizobacteria (10 %) were isolated; all of them are metal-resistant rhizobacteria and 50 % of the isolated rhizobacteria possess both traits: higher indol acetic acid and siderophore producers. The inoculation with these rhizosphere microorganisms that possess metal-tolerating ability and plant growth promoting activities, can be recommended with a practical importance for both metal-contaminated environment and plant growth promotion.

Keywords: *Phytohormones; Phytoremediation; Plant growth-promoting rhizobacteria; Siderophores*

INTRODUCTION

The interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals, making the microorganisms an important component of phytoremediation technology (Wenzel *et al.*,1999; Glick, 2003; Nouri *et al.*, 2011). The heavy metals are generally toxic to most of the plants for their metabolism and growth, if the heavy metals concentrations exceed the maximum permissible limit. Thus, the development of phytoremediation strategies like the interactions in the rhizosphere of plants growing in contaminated soils are important because of the biotechnological potential of microorganisms for metal removal directly from soil or the possible transference of them to the plants (Guo *et al.*, 1996). The rhizosphere provides a complex and dynamic microenvironment where microorganisms, in association with roots, form unique communities that have considerable potential for plant growth promotion (Belimov *et al.*, 2005) and detoxification of hazardous compounds (Black *et al.*, 1993; De Souza *et al.*, 1999). The addition of certain metal resistant microorganisms like *Psychrobacter* sp. SRA1, *Bacillus cereus* SRA10, *Bacillus weihenstephanensis* SRP12, *Sphingomonas macrogoltabidus and Microbacterium liquefaciens,*

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can affect the trace metal mobility and availability to the plants through release of chelating agents, acidification, phosphate solubilization and redox changes (Abou-Shanab *et al.*, 2003; Idris *et al.*, 2004; Ma *et al.*, 2009).

In mining sites, toxic heavy metals are known to adversely affect the number, activity and diversity of soil organisms, as well as to restrict the growth of the most tolerant plants (Wong, 2003). The endemic biodiversity present in many metalliferous sites offers huge potential for the development of environmental technologies. Unfortunately, the management of metalliferous ecosystems lacks the ecological understanding of how contaminants interact with ecosystem functions and biological communities (Ramsey *et al.*, 2005); because aboveground plant and belowground microbial communities of terrestrial ecosystems are are closely related (Epelde *et al.*, 2010).

Although many soil bacteria are tolerant to heavy metals and play important roles in mobilization or immobilization of heavy metals (Gadd, 1990), there are few studies of the rhizospheric bacteria of metal accumulating and hyperaccumulating plants and their involvement in the tolerance to and uptake of heavy metals by the plants (Belimov *et al.*, 2005).

Free-living as well as symbiotic Plant growth promoting rhizobacteria (PGPR) can improve plant nutrition and growth, plant competitiveness and responses to external stress factors like drought and osmotic stress (Burd *et al.*, 2000; Egamberdiyeva and Hoflich, 2004; Mantelin and Touraine, 2004; Dell'Amico *et al.*, 2008). The rhizobacteria enhance the plant biomass and nutrients either by the solubilization of phosphate and other mineral complexes or by nitrogen fixation, the production of siderophores for the acquisition of trace metals or by the release of phytohormones for better root growth and controlling the effects of deleterious organisms (Sharma *et al.*, 2005; 2008; Zhuang *et al.*, 2007; Rau *et al.*, 2009). Many studies have been conducted to evaluate the role of PGPR in phytoremediation efficiency action on metal contaminated soils (Khan, 2005); these bacteria generate a stimulating effect on the growth of plants, giving to them a continuous nutrients and hormones supply through their metabolic activities (Glick *et al.*, 1998; Wu *et al.*, 2005). The soil bacteria have a broad kind of mechanisms for surviving in environments under elevated heavy metals concentrations (Bremer and Geasey, 1993); one mechanism involved the localization of metal ions bound to their outer cell surface (Churchill *et al.*, 1995), through the interaction with sulfhydryl groups of cysteine residues (Erbe *et al.*, 1995) or organic materials that cells synthesize and release (Clarke *et al.*, 1987).

Wu *et al.* (2009) mentioned that PGPR might have a potential to protect host plants from acute biotoxicity and make the phytoextraction of them more effective. The improvement of the interactions between plants and beneficial rhizospheric microbes can enhance biomass production and tolerance of the plants to heavy metals; thereby, isolation of microorganisms from contaminated environments with heavy metals makes the possibility of the isolation of metal resistant strains of importance for a given phytoextraction strategy (Ma *et al.*, 2009).

The aim of this study was to compare the relationships between the physiological *in vitro* characteristics of rhizobacteria isolated from different plant species and their distribution related with the heavy metals content in metal contaminated soils.

MATERIALS AND METHODS

Isolation of rhizobacteria from a heavy metals contaminated soils

Several rhizobacteria were isolated from roots of six plant species: *Acacia farnesiana* (L.) Willd., *Amaranthus hybridus, Brickellia veronicifolia* (Khunth) A., *Sporobolus indicus,* and *Viguiera dentata* (Cav.) Spreng grown in metal contaminated soils located in Villa de la Paz in the state of San Luis Potosí, México, with high concentration of As (548 to 4694 mg/kg of dry soil), Cu (505 to 1154 (mg/kg of dry soil) , Pb (476 to 754 mg/kg of dry soil) and Zn (1386 to 1766 mg/kg of dry soil) (Vásquez-Murrieta *et al.,* 2006; Franco-Hernández *et al.*, 2010). The soils were selected from three sites with a different heavy metals gradient (Table 1) and three representative specimen plants with their rhizospheric soils were collected in 1st of August 2009, from each site.

The rhizobacteria were isolated by placing 1 g of rhizospheric soil from each plant samples, in a 50 mL Erlenmeyer flasks containing five of 0.1cm diameter glass beads and 10 mL of sterile phosphate saline buffer (1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, 0.20 g KCl, 8 g/L NaCl, pH= 7.4) and agitating the flasks for 30 m at room temperature. After agitation, 0.1 mL of appropriate serial dilutions (10^{-1} to 10^{-7}) of each flask contents was placed onto agar Luria-Bertani (LB). The plates were incubated

at 28 ºC for 24 h and the bacterial number was expressed as Colony forming units (CFU)/g of soil. The isolated rhizobacteria strains were maintained and preserved on LB medium plates for the conventional bacterial analyses such as cell form and size, gram staining and colony pigmentation performed for the rhizobacteria strains isolated and identified by the determination of gene 16S rRNA sequences. Colony Polymerase chain reaction (PCR) for the *in vitro* amplification of DNA was performed from live cell cultured on agar LB medium plates. Cells were harvested after 24 h and processed for DNA isolation using the Allers and Linchen (2000) procedure. Using the purified genomic DNA, the molecular target gene 16 S rRNA was amplified using universal primer set fD1 and rD1 designed by Weisburg *et al.* (1991). Aliquots of PCR reaction products were electrophoresed in 1 % agarose gel and then stained with ethidium bromide. These PCR products of the DNA templates amplified were purified and sequenced by the Unidad de Biotecnología y Prototipos de la FES-Iztacala (UNAM). The sequences were then compared to similar sequences in the databases using BLAST analysis (Basic Logical Alignment Search Tool, BLAST at NCBI).

Evaluation of the IAA production of the isolated rhizobacteria

The rhizobacteria isolated, were analyzed by their Indole acetic acid (IAA) production (Sheng and Xia, 2006; Zaidi *et al.*, 2006) using the Salkowski reagent according to the method of Bric *et al.* (1991). Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxins as secondary metabolites. Plant morphogenic effects may also be a result of different ratios of these plant hormones produced by roots as well as by rhizosphere bacteria, which may exert pronounced effects on plant growth and establishment (Ahmad *et al.,* 2005). Auxin production by the rhizobacterial strains was analyzed in the presence and absence of L-tryptophan (IAA precursor) and determined by colorimetric analysis. The assays were done taking 4.9 mL of sterile LB liquid media, added to culture tubes (10 x 15 cm) and supplemented with L-tryptophan at final concentrations of 1, 2 and 5 mg/L. The culture tubes were inoculated with 0.1 mL of each rhizobacteria inoculum (5 x 10^7 cells/mL in sterile distilled water) and incubated at 28 ºC for 120 h. After the incubation, the cultures were centrifuged at 3,500 rpm, at 25 ºC for 45 min to discard the bacteria pellets and to recover the supernatant where the auxins were excreted; 2 mL of each supernatants were mixed with 2 mL of Salkowski's coloring reagent and the development of a pink color indicates IAA production and was quantified reading its absorbance at 535 nm and the concentration was estimated by a standard IAA curve. The assays with and without L-tryptophan were performed by triplicate.

Evaluation of the siderophores production of the isolated rhizobacteria

The production of siderophores of the rhizobacterial isolates were assayed by the universal method of Schwyn and Neilands (1987) with Chrome azurol S. The assays were done using blue agar plates with LB medium containing the dye Chrome azurol S (LB-CAS) (Sheng *et al.*, 2008), divided equally into 24 sectors and spot inoculated with a sterile toothpick and each colony of the rhizobacteria isolated separately. The plates were incubated at 28 ºC for 24 h and the development of yellow halo around the growth was considered as positive for siderophore production. The siderophore levels produced by the strains were recorded as the diameter of the yellow halo produced around the colonies. The assays were performed by triplicate.

Statistical analysis

All the results were analysed by ANOVA test and Tukey-Kramer method using the statistics program

Table 1: Content of the most abundant heavy metals founded in the analyzed rhizospheric soil samples

Sites	Altitude (masl)	Latitude	Longitude	As	Cu	Pb	Zn
				(mg/kg of dry soil)			
S1	1786	23º 14''	100º 25'	548 bc	505 b	476 ab	1691 a
S2	1770	23º 25''	100º 12'	8420 a	1154 a	754 a	1386 a
S3	1638	23º 26''	100º 54'	4694 ab	727 b	409 b	1766 a

The different letters shows statistic difference (P<0.05). masl: meters above sea level

Graph Pad Instat Ver. 2.03 (Aceves, 2003). A linear regression equation between the IAA and siderophores production (halo diameter measurement) was introduced to establish the relationship between those plant promoting traits of the isolated rhizobacteria.

A numerical comparative analysis of the physiological traits of the rhizobacteria was done; a distance matrix was built using the conventional standard distance coefficient, a phenogram was build using the Unweighted pair group method of arithmetic averages (UPGMA) method and correlation coefficient of Pearson was obtained (Sneath and Sokal, 1973), using the NTSyS-PC version 2.11T (Numerical Taxonomy and Multivariate Analysis System) software (Rohlf, 2004).

RESULTS AND DISCUSSION

Isolated rhizobacteria characteristics from the plant species analyzed

There were ten isolated rhizobacteria from the plant species listed in Table 2; these rhizobacteria showed prolific growth having different morphological appearance on LB medium (Table 3). Most of the bacilli are gram negative (90 %) with a predominance of beige color, colony diameter size between 0.1 to 0.5 cm and rounded. All the rhizobacteria isolated were identified based on their 16 S rDNA sequence homology analysis (Table 2).

Total rhizobacteria counts (culturable) were between 2.8×10^3 to 4.1×10^5 CFU/g of soil: *Acacia farnesiana* (4.1×10^5 CFU/g of soil) and *Amaranthus hybridus* (6.2×10^4 CFU/g of soil) from soil site 1, followed by plant species from site 2: *Acacia farnesiana* (1.8×10^4 CFU/g of soil), *Viguiera dentata* (4.9×10^3 CFU/g of soil) and *Brickellia veronicifolia* 2.7×10^3 CFU/g of soil), also affecting soil microbial communities (Epelde *et al.*, 2010). The present study agrees with Epelde *et al.* (2010) analysis of the microbial communities founded in their mine soil analyzed with total metal concentration of 48, 26, 328 and 113, 620 mg/kg DW soil for Cd, Pb and Zn, respectively, with a low number of rhizobacteria present in the rhizosphere of the plant species analyzed; they found only 1 to 4 microbial species, even though positive effects of microbes on plant growth are common in nutrient poor ecosystems like mine soils, where they enhance the supply of growth limiting nutrients making microbial activity of vital importance for plant development.

Characterization of the IAA producers

The rhizosphere is defined as the zone of soil in which microbes are influenced by the root system and many microbes isolated from the rhizosphere have root growth-stimulating or growth inhibiting properties. The results of the physiological characteristics evaluated, like IAA and siderophores production, are present in Table 4; the ten isolated rhizobacteria were screened for their ability to produce plant growth regulator the IAA, recording with different concentrations of tryptophan (0, 1, 2 and 5 mg/L) that influence the concentration of it. The IAA production of the isolates without tryptophan was 7.6 to 18.7 µg/mL and in all isolated rhizobacteria there was a little increase in the production of the auxin in the presence of tryptophan; for 1 mg/L: 9.5 to 20.9 µg/mL, for 2mg/L: 10.6 to 25 µg/mL and for 5 mg/L: 10.5 to 23 µg/mL.

Ahmad *et al.* (2005; 2008) mentioned that there are numerous soil microflora involved in the synthesis of auxins in pure culture and soil (Barazani *et al.*, 1999) and some microorganisms produce auxins in the presence of a suitable precursor such as L-tryptophan; in general, the IAA produced by the rhizobacteria promotes root growth by directly stimulating plant cell elongation or cell division (Glick and Penrose, 1998). Ma *et al.* (2009) and Zaidi *et al.* (2006) mentioned that IAA production plays an important role in plant–bacterial interactions and plant growth in metal contaminated soils. Khalid *et al.* (2004) categorized the *in vitro* production of IAA by rhizobacteria in three principal groups: lower producers (1 to 10 µg/mL), medium producers (11 to 20 µg/mL) and higher producers (21 to 30 µg/mL). According to Khalid *et al.* (2004), the isolated rhizobacteria could be classified as follows: *Pseudomonas sp. strain* C4 and *Enterobacter sp. strain* Sp4C as lower producers (7.6 and 9.9 µg/mL, respectively) and the seven rhizobacteria: *Bacillus sp. strain* C3, *Pseudomonas sp. strain* C8, *Pseudomonas sp. strain* Sp7D, *Serratia sp. strain* Sp3B, *Achromobacter sp. strain* C1, *Pseudomonas sp. strain* Sp7E and *Acinetobacter sp. strain* C7 as medium producers (10 to 14.5 µg/mL).

Case apart is the rhizobacteria *Pseudomonas sp. strain* C2 as the only higher producer with 25 µg/mL with 2 mg/L of Trp, instead of its production without Trp (18.7 µg/mL). The results obtained from these rhizobacteria of present study were lower than the reported by Ma *et al.* (2009); their highest producer of IAA (87.7 µg/mL) isolated from the rhizosphere of *Alyssum serpyllifolium* and *Phleum phleoides*, and compared to the results obtained by Ahmad *et al.* (2005)

Table 2: CFU/g of soils of the rhizobacteria isolated from the rhizospheric soil of each plant species from contaminated soils with heavy metals

Soil site	Plant species	CFU/g of rhizospheric soil	Identified rhizobacteria	Identity (%)
1	*Amaranthus hybridus*	6.2×10^4	*Serratia sp.strain* Sp3B	91
	Acacia farnesiana (L.) Willd.	4.1×10^5	*Bacillus sp. strain* C3	91
	Acacia farnesiana (L.) Willd.	1.8×10^4	*Enterobacter sp. strain* Sp4C	92
			Pseudomonas sp. strain C4	99
2	*Brickellia veronicifolia* (Khunth) A. Gray	2.7×10^3	*Acinetobacter sp. strain* C7	83
			Pseudomonas sp. strain C8	98
	Viguiera dentata (Cav.) Spreng.	4.9×10^3	*Pseudomonas sp. strain* Sp7D	95
			Pseudomonas sp. strain Sp7E	94
3	*Sporobolus indicus*	2.8×10^3	*Achromobacter sp. strain* C1	98
			Pseudomonas sp. strain C2	96

Table 3: Colony and microscopy morphological characteristics of the ten isolated rhizobacteria from heavy metals contaminated soils

Rhizobacteria	Soil Site	Form	Size	Color	Edge	Surface	Aspect	Consistence	Elevation	Reflect light	Gram behavior
Sp3B	1	Round	0.5 cm	Red	Complete	Smooth	Wet	Soft	Convex	Brilliant	Bacilli gram -
C3	1	Round	0.4 cm	Beige	Complete	Smooth	Dry	Soft	Crater form	Mate	Bacilli gram +
C4	2	Round	0.3 cm	Beige	Complete	Smooth	Wet	Soft	Pulvinate	Brilliant	Bacilli gram -
C7	2	Round	0.2 cm	Beige	Complete	Smooth	Wet	Soft	Convex	Brilliant	Coc-Bac gram -
C8	2	Amoeboid	Irregular	Beige	Undulate	Smooth	Wet	Mucous	Elevate	Brilliant	Bacilli gram -
Sp4C	2	Round	0.2 cm	Beige	Complete	Smooth	Wet	Soft	Convex	Brilliant	Bacilli gram -
Sp7D	2	Round	0.3 cm	Beige	Complete	Smooth	Wet	Soft	Convex	Brilliant	Bacilli gram -
Sp7E	2	Round	0.2 cm	Beige	Complete	Smooth	Wet	Soft	Convex	Brilliant	Bacilli gram -
C1	3	Amoeboid	Irregular	Beige	Undulate	Smooth	Wet	Mucous	Elevate	Brilliant	Bacilli gram -
C2	3	Round	0.1 cm	Clear yellow	Complete	Smooth	Wet	Soft	Convex	Brilliant	Bacilli gram -

Table 4: Phytohormones and siderophores production by the ten isolated rhizobacteria from heavy metals contaminated soils

		Indol acetic acid produced (µg/mL)				Siderophores production		
Rhizobacteria	Soil site	Basal	1 mg/L Trp	2 mg/L Trp	5 mg/L Trp	Colony diameter (cm)	Yellow halo diameter (cm)	Siderophores production (Yellow halo %)
Serratia sp.strain Sp3B	1	13.2 ± 1.6	10.2 ± 4.7b	18.4 ± 3.3	12.7 ± 4.5	0.44 ± 0.05abc	0.14 ± 0.05c	14
Bacillus sp. strain C3	1	10.0 ± 0.4a	9.5 ± 0.5b	13.0 ± 1.5a	10.5 ± 1.1a	0.39 ± 0.05abc	0.29 ± 0.06c	29
Pseudomonas sp. strain C4	2	7.6 ± 0.1b	10.7 ± 0.1a	10.6 ± 0.0b	10.7 ± 0.1a	0.47 ± 0.04c	0.15 ± 0.05c	15
Acinetobacter sp. strain C7	2	14.5 ± 2.1	11.5 ± 1.6a	14.6 ± 1.9	12.4 ± 3.2	0.44 ± 0.05abc	0.44 ± 0.05c	44
Pseudomonas sp. strain C8	2	10.2 ± 0.6a	12.6 ± 2.9	17.5 ± 5.4	15.4 ± 1.4	0.44 ± 0.05abc	0.51 ± 0.07ac	51
Enterobacter sp. strain Sp4C	2	9.9 ± 1.9a	16.2 ± 4.5	16.4± 1.3	15.3 ± 3.3	0.44 ± 0.05abc	0.17 ± 0.06bc	17
Pseudomonas sp. strain Sp7D	2	11.7 ± 0.9	14.7 ± 0.3	12.5 ± 1.7a	13.2 ± 2.8	0.49 ± 0.05abc	0.27 ± 0.06bc	27
Pseudomonas sp. strain Sp7E	2	14.5 ± 1.5	10.2 ± 0.7b	14.2 ± 6.2	15.8 ± 0.2	0.42 ± 0.04c	0.73 ± 0.1c	73
Achromobacter sp. strain C1	3	13.3 ± 5.9	20.9 ± 4.9ab	18.1 ± 4.9	16.6 ± 9.8	0.35 ± 0.05ac	0.42 ± 0.05ac	42
Pseudomonas sp. strain C2	3	18.7 ± 4.1ab	17.1 ± 2.7	25.0 ± 6.2al	23.0 ± 4.2a	0.40 ± 0.02ac	0.59 ± 0.06c	59

Mean values ± S.D. from three replicates for IAA production and seventy five replicates for siderophores production. The different lower-case letters show the significant differences between experiments ($p < 0.05$). Trp: Tryptophan

for their 21 isolates of *Azotobacter* and *Pseudomonas* sp. with different tryptophan concentrations.

Fig. 1 shows the phenogram with the associated groups according with the IAA production; in this figure two groups forming at first: group I made only by *Pseudomonas sp. strain* C2 with the highest production of IAA and the rest of the rhizobacteria comprise the group II; in this group *Achromobacter sp. strain* C1 is separate of the rest (group IIa) and the other rhizobacteria forming the group IIb constitute the medium (group IV) and lower (group III) IAA producers.

Characterization of the siderophores producers

The siderophores are another important metabolites released by the plant growth promoting rhizobacteria

that indirectly alleviate heavy metal toxicity by increasing the supply of iron to the plant (Burd *et al.*, 1998, 2000; Ma *et al.*, 2009). Heavy metals in soils could stimulate the production of bacterial siderophores (Whithing *et al.*, 2001) that, in turn, alleviate metal toxicity to in plants (Burd *et al.*, 2000; Dell' Amico *et al.*, 2005). The isolated rhizobacteria from these plant species and soil samples with heavy metals contaminants, showed a direct relationship between the heavy metal content in soils and the higher production of siderophores. Based on the yellow diameter produced (lower siderophores producers < 0.2 cm, medium siderophores producers between 0.2 to 0.5 cm and higher siderophores producers > 0.5 cm), the isolated siderophore producer rhizobacteria were classified (Fig. 2) and two groups were obtained from Fig. 2. It may be observed that group I associate the higher siderophores producers formed by group Ia: *Pseudomonas sp. strain* Sp7E (0.73 cm of halo diameter), group Ib: *Achromobacter sp. strain* C1 and *Acinetobacter sp. strain* C7 (0.42 and 0.44 cm of halo diameter, respectively) and group Ic: *Pseudomonas sp. strain* C2 and *Pseudomonas sp. strain* C8 (0.59 and 0.51 cm of halo diameter, respectively); group II constitutes the rest of the rhizobacteria by group IIa (the medium siderophores producers): *Bacillus sp. strain* C3 and *Pseudomonas sp. strain* Sp7D (0.29 and 0.27 cm of halo diameter, respectively) and group IIb (the lower siderophores producers): *Serratia sp. strain* Sp3B, *Pseudomonas sp. strain* C4 and *Enterobacter sp. strain* Sp4C (0.14, 0.15 and 0.17 cm of halo diameter, respectively). According to this study and its agreement with the results reported by Ma *et al.* (2009), siderophores produced by these rhizobacteria can help in plant root proliferation and enhancement of soil minerals uptake. Many of the rhizobacteria isolated (*Serratia liquefaciens, Pseudomonas tolaasii, Pseudomonas fluorescens, Ralstonia taiwanenses)* were capable of producing IAA and siderophores; significant differences between these rhizobacteria were also observed in the amount of IAA and siderophores produced: *Variovorax paradoxus* (AY196950) with 0.4 µg of IAA/mg and 29 mM of deferoxamine mesylate/mg, *Variovorax paradoxus* (AY196998) with 6 µg of IAA/mg and 27 mM of deferoxamine mesylate/mg, *Pseudomonas* sp. with 3 µg of IAA/mg and 90 mM of deferoxamine mesylate/mg (Belimov *et al.*, 2005). Fig. 3 shows the correlation between the IAA production and the siderophores production (yellow halo diameter) of the isolated rhizobacteria: The rhizobacteria with higher IAA and siderophores production was *Pseudomonas sp. strain* C2, the rhizobacteria with higher siderophores production and medium IAA production was *Pseudomonas sp. strain* Sp7E, the rhizobacteria with higher siderophores production and lower IAA production was *Pseudomonas sp. strain* C8, the rhizobacteria with higher IAA production and medium siderophores production were *Achromobacter sp. strain* C1 and *Acinetobacter sp. strain* C7 and a big group of rhizobacteria with medium IAA production and lowest siderophores production were: *Serratia sp. strain* Sp3B > *Pseudomonas sp. strain* Sp7D > *Enterobacter sp. strain* Sp4C > *Bacillus sp. strain* C3 > *Pseudomonas sp. strain* C4. Finally, the results of this study showed that the heavy metals present as contaminants in the rhizosphere of the plant species analyzed, decrease the microbial biomass, in agreement with the report of Li *et al.* (2005) and Rau *et al.* (2009) on heavy metal contaminated soils. Nutrient acquisition has been considered as the primary factor to determine the ecological success of a species at the stressed sites and the cell shape has physiological significance in determining the ability of bacterial species to take up the nutrients (Young, 2006). Rau *et al.* (2009) mentioned that the dominance of bacilli cell shape provides ecological advantage to maximize the nutrient uptake and the dominance of both gram-negative and gram-positive rhizobacteria has been reported at metal contaminated sites by Frostegård *et al.* (1993) and Abaye *et al.* (2005). Even metals exert their toxic effects on microorganisms through various mechanisms; the metal-tolerant bacteria could survive in these habitats and could be isolated and selected for their potential application in the bioremediation of contaminated sites (Piotrowska-Seget *et al.*, 2005; Ma *et al.*, 2009).

CONCLUSION

This work analyzed the different associated rhizobacteria with plant species distributed in different soils with high heavy metals concentration. Gram-negative rhizobacteria (90 %) and gram-positive rhizobacteria (10 %) were islolated and characterized by their plant growth promoting traits like IAA and siderophores production that help plants and may protect them against the contaminant environment. Fifty percent of isolated rhizobacteria have both traits,

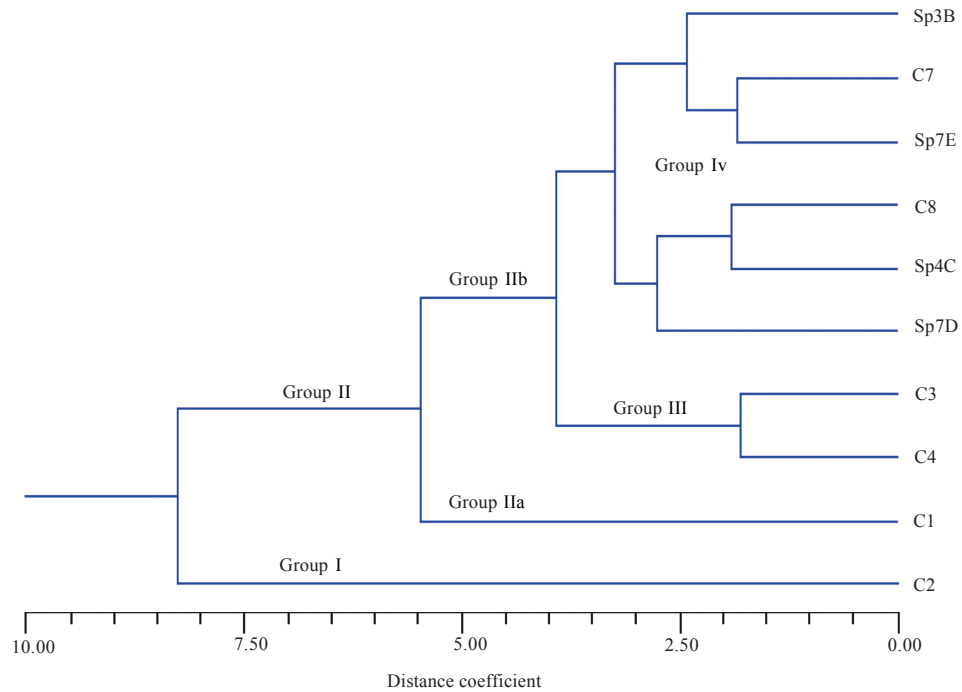

Fig. 1: Phenogram of the isolated rhizobacteria related with their IAA production trait (r= 0.89)

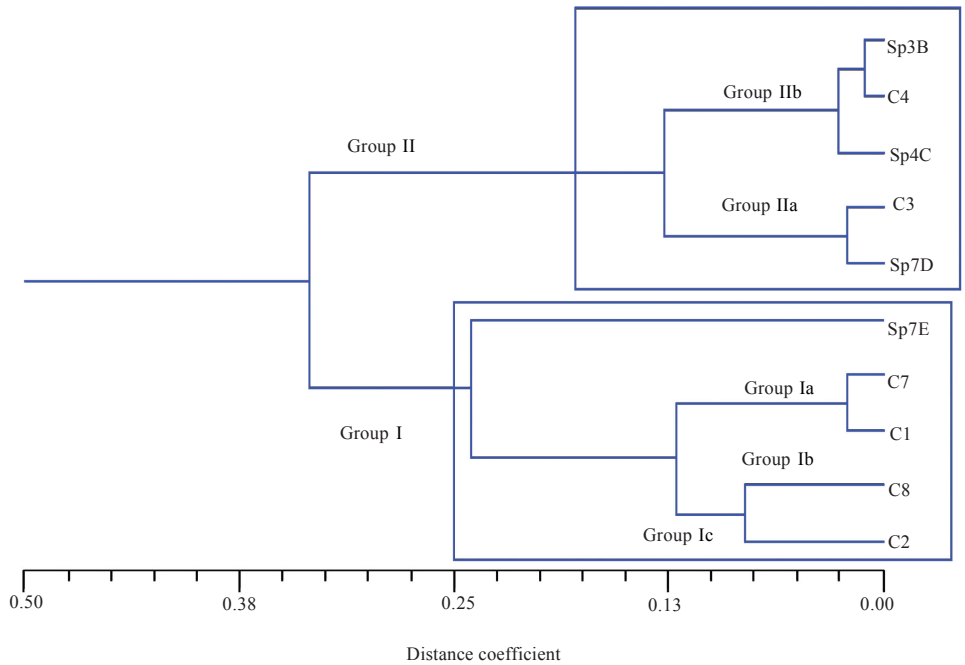

Fig. 2: Phenogram of the isolated rhizobacteria related with their siderophores production trait (r= 0.76)

are higher IAA and siderophore producers, and all of them are metal-resistant rhizobacteria related with the soil samples where they were isolated with high heavy metals concentration. There are further works with these isolated rhizobacteria that complete the effects of them as inoculants for the development of plant-

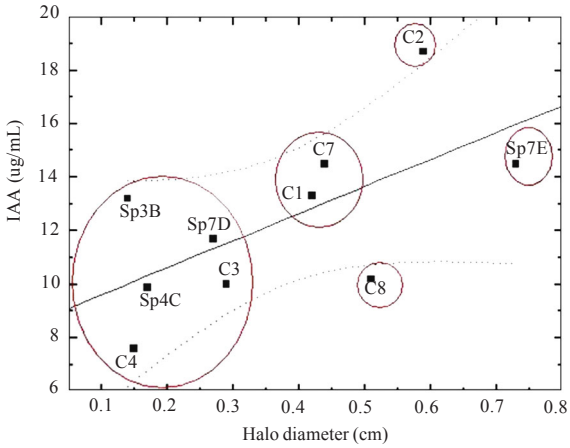

Fig. 3: Linear regression curve showing the relationship between IAA production (µg/mL) and sidrophores production (halo diameter (cm)), Strains are shown in the plot. Dotted lines show regression confidence area ($p<0.05$, $r= 0.63$)

microbe systems like experiments of seed inoculation. Therefore, inoculation with these rhizosphere microorganisms with metal-tolerating ability and plant growth promoting activities can be recommended with a practical importance for both metal contaminated environment and plant growth promotion.

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