Metallophores Production by Bacteria Isolated From Heavy Metal Contaminated Soil and Sediment at Lerma-chapala Basin

Jessica Maldonado-Hernández1
Instituto Politécnico Nacional: Instituto Politecnico Nacional
Brenda Román-Ponce
Universidad Politécnica del Estado de Morelos: Universidad Politecnica del Estado de Morelos
Ivan Arroyo Herrera
Instituto Politécnico Nacional: Instituto Politecnico Nacional
Joseph Guevara-Luna
Instituto Politécnico Nacional: Instituto Politecnico Nacional
Juan Ramos-Garza
Universidad del Valle de México: Universidad del Valle de Mexico
Salvador Embarcadero-Jiménez
Instituto Mexicano del Petróleo: Instituto Mexicano del Petroleo
Paulina Estrada de los Santos
Instituto Politécnico Nacional: Instituto Politecnico Nacional
En Tao Wang
Instituto Politécnico Nacional: Instituto Politecnico Nacional
María Soledad Vásquez Murrieta (murrieta@hotmail.com)
Instituto Politécnico Nacional https://orcid.org/0000-0002-3362-5408

Research Article

Keywords: soil bacteria, heavy metals, siderophores, metallophores, plant growth promoting

Posted Date: June 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-622126/v1

Version of Record: A version of this preprint was published at Archives of Microbiology on February 17th, 2022. See the published version at https://doi.org/10.1007/s00203-022-02780-6.

Abstract

Environmental pollution derived from heavy metals (HMs) is a worldwide problem and the implementation of eco-friendly technologies for remediation of the pollution are necessary. The metallophores are low-molecular weight compounds that have important biotechnological applications in agriculture, medicine and biorremediation. The aim of this work was to isolate the HM resistant bacteria from soils and sediments of Lerma-Chapala basin, and to evaluate their abilities to produce metallophores and to promote plant growth. A total of 320 bacteria were recovered, and the siderophores synthesis was detected in cultures of 170 of the total isolates. The Lerma-Chapala Basin bacteria also produced metallophores for all the tested metal ions and presented a greater production of As^{3+} metallophores. The members in genera *Delftia* and *Pseudomonas* showed siderophores production above 92 percent siderophore units (psu). In addition, the hydroxamate was the most common fuctional group among the analyzed siderophores. Also, the bacteria showed high HM resistance especially to Zn^{2+}, As^{5+} and Ni^{2+}. Our results evidenced that Lerma-Chapala basin bacteria or their metallophores could be employed in biorremediation process or may even have potential for applications in other biotechnological purposes.

1. Introduction

Soil is considered a fragile and non-renewable natural resource because it is difficult and expensive to restore or even improve its properties after deterioration. Industrial and mining activities are important for economic progress, especially in many developing countries. Nevertheless, these activities are the main cause of soil and sediment contamination by heavy metals (HMs) around the world. Unlike organic substances, HMs are essentially non-biodegradable and therefore accumulate in the environment (Ali et al., 2013). Accumulation of HMs in agricultural soils and water bodies possess a great threat to human health due to the risk of their entry into the food chain (Sarwar et al., 2010). Thus, a growing public concern has been raised over the soil and sediment contamination resulting from anthropogenic activities.

Therefore, biological methods have been developed for decontamination of HMs-polluted soils, sediments and aquifers in the last decade. Biological agents such as animals, plants and microorganisms offer advantages based on their economically feasible, practical, and without secondary pollutions. Furthermore, bioremediation alone can recover natural flora and fauna in contaminated lands to the original form (Sulaymon 2013). Bioremediation process included the use of biological resources (both microorganisms and plants). Among the microbes, bacteria have specific genetic mechanisms and interactions with the HM and play an important role in the mitigation of contaminants in environment. Some bacteria can establish synergism with plant and improve the HM phytoremediation through direct and indirect mechanisms (Ma et al., 2016). As the direct mechanisms, bacteria so called plant growth promoting bacteria (PGPB) improve the proliferation of their host plants by nitrogen fixation, mineral solubilization, and production of phytohormones, specific enzymes and siderophores (Glick 2004; Zahid 2013). As the indirect mechanisms, some of the PGPB can enhance the plant growth by reducing metal toxicity (Rajkumar et al., 2009), by inhibiting the phytopathogens to reduce the damage through biological control of the pathogens or induction of systemic resistance (ISR) of plants against their pathogens (Harish et al. 2008). Moreover, some bacteria can improve the HM removal, enhance the tolerance of plant to environmental stresses and reduce the bioavailability of HMs through metal (in)mobilization, metal biosorption/bioaccumulation, metal detoxification and defense mechanism against metal toxicity (Ma et al., 2016).

Metallophores are low-molecular-weight organic ligands that function to supply a metal ion as nutrient to an organism, in which the ligand biosynthesis and excretion is regulated based on the nutritional status with respect to the corresponding metal ion. The metallophores have been shown to bind (Mishra et al. 2009) and solubilize (Schenkeveld et al. 2014) not only the essential metals, but also the no essential metals. Therefore, the microbial production of metallophores could affect the rate of dissolution and transport of toxic metal concentrations in the environment, suggesting that metallophores can be used in bioremediation of environmental systems contaminated with toxic metal concentrations. According to the chemical nature of their functional groups, metallophores and siderophores are classified as catecholate, hydroxamate and mixed groups (Miethke et al. 2007). Currently, several studies have evidenced the metallophore production by bacteria. *Streptomyces acidiscabies* and *Streptomyces tendae* strains can produce metallophores to bind Cd and Ni, respectively, which protect the

cells from metal toxicity (Dimkpa et al. 2008; Dimkpa et al. 2009). Pyoverdine and pyochelin, two major siderophores excreted by *Pseudomonas aeruginosa*, are able to chelate 16 different metals (Braun et al. 2008). A metallophore-mediated metal uptake and detoxification in *Azotobacter vinelandii* was proposed by Kraepiel et al. 2009, while siderophores produced by *Pseudomonas azotoformans* are able to bind As (Nair et al. 2007). Recently, the arsenic-binding siderophores have been described in arsenic-tolerant Actinobacteria (Retamal-Morales et al. 2018), and arsenophores to bind As^{3+} and/or As^{5+} were evidenced in arsenic resistant endophytic bacteria isolated from mine tailings (Román-Ponce et al. 2018).

Considering the importance of metallophore production for bacterial environment adaptation and the potential in phytoremediation, we performed the present study. The objectives of this study were a) to isolate, screen and characterize HM resistant bacteria; b) to evaluate their metallophore production; and c) to determine the functional chemical groups of siderophores excreted by selected HM-resistant bacteria.

2. Materials And Methods

2.1 Site description, soil and sediment sampling

The collection of the samples was carried out at three sites, Ibarra (20°13' N 102°37' W, altitude 1530 m), La Palma (20°07' N 102°39' O, altitude 1530 m) and Maltaraña (20°13' N 102°41 O', altitude 1528 m) in the Lerma-Chapala Basin locating in the Ciénega of Chapala, Michoacán state, Mexico, where the mean annual temperature is 18ºC and average annual precipitation is 800 mm (Fig. S1). The contamination of Lerma-Chapala Basin was evidenced with the presence of HMs of As, Cd, Cr, Cu, Hg, Mn, Fe, Pb and Zn (Hasen et al. 1995; Trujillo-Cardenas et al. 2010; Torres et al. 2016), in which the concentrations of As, Cr, Cu, Hg, and Pb exceed the international benchmarks (Torres et al. 2016). In the present study, three soil samples and one sediment sample were collected from each site during the dry season (January 2014). Soil samples (500 g for each) without debris and rocks were collected from 0–20 cm in depth. The top sediment sample was collected from 5–10 cm in depth. After colletion, all the samples they were stored in polyethylene bags at 4ºC, during 24 h for further analysis.

2.2 Enrichment and isolation of HM-resistant bacteria

In order to recovered HM-resistant heterotrophic bacteria with potential application in further investigation the enrichment protocol was carried out using MES buffered minimal medium (MBMM) (Rathanayake et al. 2013) supplemented separately with different HMs salt dissolved in 10 mM of HCl (Table S1). The HM-resistant bacteria were enriched by transferring 0.5 g of soil or sediment sample in 20 mL of MBMM cointaining the mentioned HMs and incubated at 28°C with shaking at 120 rpm for 5 days. This process was repeated three times, and the final enriched culture was used for isolation of the HM-resistant bacteria. Approximately, 0.1 mL aliquot of enriched culture was previously homogenized and immediately dispersed on MBMM agar plates amended with the corresponding HMs and were incubated at 28°C in aerobiosis during 5 days. At the end of the incubation, colonies with different morphology were selected and purified by repeated streaking on TSA (trypticasein soy agar, DIFCO) plates.

The direct method for bacterial isolation in the original soils/sediments was carried out by diluting the samples to 10^{-5} with sterile distilled water. Aliquots of 0.1 mL from the three last dilutions were placed separately on TSA plates in triplicate. The plates were incubated in aerobiosis for 48 h at 28°C. Single colonies with different morphologies were selected and purified by repeated cross streaking until very similar colonies in the same isolate were obtained. The purified bacterial cells from both isolation techniques were preserved in 30% (w/v) glycerol at − 70°C.

2.3 Plant growth promoting characteristics

Plant growth promoting traits such as Indole-3-acetic acid (IAA) production (Glickmann and Dessaux 1995), mineral phosphate solubilization (Silva Filho and Vidor 2000), siderophore production (Schwyn and Neilands 1987; Alexander and Zuberer 1991) and production of polyamines (Cloete et al. 2009) were determined by following the standard protocols. All the determinations were performed in triplicates.

2.4 Screening for metallophores production

The assay was developed for detection of metal(loid) ion-complex formation [metal(loid)phores] based on Chrome Azurol S assay (Schwyn and Neilands 1987), supplemented with corresponding HMs salts (Table S1) to a final concentration of 56 ppm that was calculated from Fe^{3+} concentration in CAS assay (Nair et al. 2007). For metallophore production the metallic salt tested were the same as that used in the enrichment process. The plates were incubated at 28ºC for 24 to 48 h to observe the color change.

2.5 Determination of HM resistance by bacteria

The minimum inhibitory concentration (MIC) of metal was determined using 96-well plates. Each well was filled with 100 µL sterile MBMM and supplemented with different amounts to the metal salt dissolved in 10 mM of HCl (Table S1) at final concentration of 0.25, 1, 2, 4, 10, 20, 30 and 50 mM of As^{5+}, As^{3+}, Co^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}, respectively. Each of the isolate was overnight incubated in 5 mL of TSB (trypticasein soy broth, DIFCO) medium without HM at 28°C on a rotary shaker (120 rpm). Then 100 µL of bacterial culture (0.9 OD at 600 nm) was added in each well. Medium without HM was inoculated with bacteria as control, and medium with HM without bacteria was used as abiotic control. Microplates were incubated at room temperature (120 rpm) for 96 h and the bacterial growth was measured by using an ELISA reader (Multiskan FC, Thermo Scientific) at 620 nm. When the growth was apparently inhibited, the corresponding concentration was designed as MIC for the HM.

2.6 Siderophores quantification

The quantification of siderophores was carried out in CAS liquid medium according to the protocol of Schwyn and Neilands 1987. The medium was adjusted to pH 6.8 with 0.1 M PIPES buffer. The cultures were incubated until stationary phase in minimal medium 9 (MM9), which contained (g L^{-1}) 0.3 KH_2PO_4, 0.5 NaCl, 1.0 NH_4Cl, 6.0 NaOH, 30.24 PIPES, supplemented with 30 mL of deferred casamino acids (10%, w/v), 2.0 g of glucose, 1.0 mL of $MgCl_2$ (1 M) and 1.0 mL of $CaCl_2$ (100 mM). The reaction mixture, containing 0.5 mL of CAS reagent, 0.5 mL of culture supernadant and 10 µL of 5-sulfosalicylic acid (0.2 M), was allowed to settle for few minutes. The complex formation was recognized by a change in the reaction mixture from blue to yellow. The siderophores production was determined by measuring the optical absorbance at 630 nm. Medium without bacteria was used in each assay as a blank, whereas medium without bacteria aded with 5-sulfosalicylic acid was used as reference. The siderophore units were calculated with the equation: psu = [(Ar - As) / Ar] · 100 where Ar is the absorbance of reference and As is the absorbance of sample (Payne 1993).

2.6 Determination of siderophore functional groups

The Arnow test was performed to detect the catecholate-siderophore type based on the chemical properties (Arnow 1937). For iron removal, each bacterium was cultured in MM9 medium previously treated with 30% (w/v) of hydroxyquinone in chloroform. The identification assay was carried out in the following amount and order: 1.0 mL of bacterial supernatant, 1.0 mL of 0.5 N HCl, 1.0 mL of nitrite-molybdate reagent (the presence of catecholates will produce a yellowish color) and 1.0 mL of 1 N NaOH (the color turns to red). The color is stable for 1 h and the absorbance was measured at 510 nm.

For hydroxamic acid functional group the Csáky standard method was used, which is based on the oxidation of hydroxylamine to nitrite in the medium supplemented with acetic acid and iodine in presence of sodium arsenite (Csaky 1948). The produced nitrite was determined with colorimetric reaction with sulphanilic acid and α-naphthylamine.

2.7 Identification of the selected bacteria based on 16S rRNA gene sequence

Genomic DNA was extracted from each of the selected bacteria with the commercial Kit “Wizard® Genomic DNA Purification Kit” (Promega), following the instructions of the manufacturer. The isolated genomic DNA was used as template to amplify the 16S rRNA genes using the universal primers 27 F and 1492 R by PCR (Ancinas et al. 2004) with a thermocycler (Axygen Maxygen®). Amplicons (c.a.1500 pb) were sequenced under Big Dye™ terminator cycling conditions with the same primers using 730xl DNA Analyzer (Applied Biosystems®) in Macrogen (Korea). The sequences of reference strains were obtained from EzBiocloud platform (Chun et al. 2007) to carry out a phylogenetic analysis. A multiple sequence alignment was

performed with CLUSTAL X (2.0) software (Larkin et al. 2007) and acquired sequences were manually edited with SEAVIEW software (Galtier et al. 1996). The phylogenetic analysis was carried out with neighbor-joining method (NJ) in program MEGA version 5 (Tamura et al. 2011). Similarities among the sequences were obtained using EzBiocloud platform (Chun et al. 2007). Taxonomic assignment was obtained by using the Chun prokaryotes criteria (Chun et al. 2018).

2.8 Statistical analysis

All statistical analyses were performed using R package (R Development Core Team 2012, http://cran.r-project.org/). The data from triplicate (n = 3) of metallophores for the isolates and plant promoting characteristics were analyzed through a one-way analysis of variance (ANOVA) and Tukey post hoc (Tukey Honest Significant Difference). A heatmap was constructed with the CIMminer tool (http://discover.nci.nih.gov) to visualize the metallophores production across the isolated strains. The results of each strain were clustered using average linkage. The distance metric used was Euclidean distance (Scherf et al. 2000).

Principal Component Analysis (PCA) was used to visualize relationships between the MIC and metallophore production by the strains, and between plant growth promotion (PGP) features and metallophore production using FactoMiner package (Lê et al. 2008).

2.9 Accession number

In the present study, the accession number for the identified isolates comprised from MT197028 to MT197081 and from MT226497 to MT226502.

3. Results And Discussion

3.1 Bacterial isolation and selection for further analysis

In the Lerma-Chapala Basin, contaminants of pesticides, industrial residues, oils, detergents, and heavy metals (chromium, lead, zinc, and mercury) have been detected from environmental samples (Jay and Ford 2001). In the present study, a total of 320 HM resistant bacterial isolates were obtained from soil and sediment of Lerma-Chapala Basin, including 92 from Ibarra site (30 from TSA medium and 62 from enrichment), 122 from Maltaraña (32 from TSA and 90 from enrichment) and 106 from La Palma (30 from TSA medium and 76 from enrichment). Approximately, 45.3% (145 isolates) were Gram-positive bacteria while 54.6% (175 isolates) were Gram-negative bacteria. The abundance of bacteria in soils and sediments from Lerma-Chapala Basin in dry season (January, 2014) was greater than that reported in rainy season (Arroyo-Herrera et al. 2021), evidencing that the seasonal changes are a factor to alternate the abundance, diversity and distribution of bacterial communities as reported previously (Yokobe et al. 2008).

3.2 Plant growth promoting traits of the HM-resistant bacteria

Previously, multiple HM-resistant bacteria with plant growth promotion (PGP) characteristics have been reported (Ma et al. 2019). This was also the case in our present study since out of the 320 isolates, 170 showed at least one plant growth promoting characteristics (Table S2), indicating that more than 50% of the Lerma-Chapala Basin bacteria were potential PGP microbes. Siderophores chelating ferric iron (Fe^{3+}) from the environment could improve the plant growth in soils with limited iron nutrient (Rajkumar and Freitas). In the present study, 10 of the 118 siderophore producing isolates showed high production index from 3.0 to 6.11, and the isolate MS2Zn-3 presented the highest production index; 48 isolates presented a moderate production index ranging from 2.0 to 2.75; while 60 bacteria showed low index from 1.06 to 1.95 (information available in Table S2). The index of siderophore production obtained in this study was similar with those reported previously for bacteria isolated from other sources (Zhang et al. 2012; Liu et al. 2014). The widely distribution of siderophore production in the bacteria from Lerma-Chapala Basin evidenced that the excretion of siderophores might be an adaptation trait for the surviving to HM contaminated sites. Nevertheless, the bacteria associated to hyperaccumulator plants, as *Pteris vittata*, showed arsenic resistance and siderophore synthesis, and they also produced enzymes involved in the As^{3+} oxidation that requires iron as cofactor (Silver and Phung 2005). In non plant-associating bacteria isolated from metal contaminated sites,

the sideropore synthesis was involved in the removal of others-metals (Volesky 2001). Therefore, the siderophore-producing bacteria have a great potential to be used in the metal removal processes.

The auxins are important phytohormones and IAA is the most studied auxin in PGPB, it improves the mineral and nutrient uptake and stimulates the plant biomass accumulation, cell division/elongation and differentiation, allowing the development of roots (Glick 2010). Here, the results of IAA production showed significant differences among the strains (data not show). There were more IAA producers among the strains isolated directly from the samples compared with those isolated after the enrichment process (Table S2). The values for IAA production obtained in the present study (0.59 to 53.74 μg mL^{-1}) are within the previously reported range (1.38 to 75.6 μg mL^{-1}) (Roy and Roy 2019; Wu et al. 2019), suggesting that Lerma-Chapala Basin bacteria can improve the plant growth through auxins production that may favor the removal of metals by phytoremediation.

Meanwhile, an eco-friendly alternative to provide P in the available forms to plants is possible by using phosphate-solubilizing bacteria (PSB) with various mechanisms, therefore the use of chemical phosphate fertilizers could be reduced (Khan et al. 2007). In the present study, only 26 Lerma-Chapala Basin bacteria showed phosphate solubilization, and only one of them was isolated from direct sample. The isolates MS1Co-2 and PS2As-3 showed the highest phosphate solubilization index of 4. The portion of P solubilizing bacteria in the present study (26/320) was similar to that in previously report for the rainy season (Arroyo-Herrera et al. 2021), but the phosphate solubilization index was higher. In addition, several studies suggested that phosphate immobilization caused by heavy metals (Moberly et al. 2010; Li and Ramakrishna 2011) could explain the low solubilization index observed from Lerma-Chapala Basin bacteria compared with the other bacteria recovered from uncontaminated sites (Pande et al. 2017; Paul and Shina 2017).

Polyamines are low molecular weight aliphatic nitrogenous bases, which are involved in diverse plants processes (Xu et al. 14), including flower development, embryogenesis, organogenesis, senescence, and fruit maturation (Xu et al. 2014; Mustafavi et al. 2018). Also, polyamines are implicated in plant responses to biotic and abiotic stresses (Mustafavi et al. 2018; de Oliveira et al. 2017). The bacterial polyamines have been considered as phytohormones for enhancing the plant growth under stress conditions as drought, salinity and heavy metals (Rangan et al. 2014). In the present study, the polyamine production was observed in 31 strains (Table S2), indicating that this trait may also contribute to the HM-resistant bacteria for helping the growth of plants under conditions of HM stress.

3.3 Bacterial identification

The 16S rRNA gene phylogenetic tree (Supplementary Fig. S2-S8) identified the HM-resistant bacteria obtained in the present study within 13 genera: *Achromobacter, Acinetobacter, Comamonas, Cupriavidus, Delftia, Enterobacter, Flavobacterium, Klebsiella, Ochrobactrum, Pseudomonas, Staphylococcus* and *Stenotrophomonas*, in which *Pseudomonas* and *Delftia* were the most abundant groups corresponding to 25% and 41% of the total isolates, respectively (Table S3). All the genera detected in the present investigation have been previously isolated from contaminated sites (Ma et al. 2009; Thatoi et al. 2014; Wu et al. 2016), evidencing them the bacteria rather adapted to the contaminated environments.

3.4 Screening of metallophore production and siderophore quantification

The metallophores are a unique class of low-molecular-weight organic ligands that function to supply metal ion nutrient to the organisms. Siderophores are the most studied metallophore exuded by plants and microbes to promote the iron uptake, but more types of metallophores are involved in chelation process of diverse metals including Ag, Al, As, Co, Cu, Mn, Mo, Ni, V, W and Zn (Kraemer et al. 2015; Román-Ponce et al. 2018; McRose et al. 2018; Deicke et al. 2019). In the present work, only 19% (60 of 320 isolates) of the bacteria isolated from Lerma-Chapala Basin showed metallophore synthesis and all of them came from enrichment samples (Table S3). Contrary to the analyzed metal(loid) ions, most of the bacterial isolates presented a metallophore production index > 3.0 up to 5.0. For arsenite (As^{3+}), ten isolates showed arsenophore production index > 5.0. For Pb, no isolates developed a metallophore production index > 5.0 (Fig. 1). The heatmap (Fig. 2) inferred using the clustering method of Euclidean distance showed that 60 tested bacterial isolates formed 18 clusters with a Euclidean distance less than

3. The clusters were conformed based on metallophore production profile with similarities in the value of the observed production indices. Some clusters included strains belonging to phylogenetically distant genera as *Pseudomonas* spp. IS1 Ni-1, MS2 As-2 and *Delftia* sp. PS2 Zn-2. Also, *Delftia* spp. PS4 Zn-1, PS4 Zn-1, PS4 Zn-3, PS4 Zn-4, formed a cluster suggesting that their ability to excrete metallophores could depend on common genetic information shared by these bacterial strains; other possibility is that they might be clones of the same strains. In general, the heatmap shows than metallophores production indices are not homogenous for all metals tested. The metallophore production range in the HM-resistant bacteria isolated in the present study was three times greater than that obtained previously (Arroyo-Herrera et al. 2021).

In the analysis of siderophore quantification, 95% (57/60) metallophore producing bacteria detected in the screening assay, the siderophore production was confirmed. According to the quantification results, the siderophore production was lower than 25 psu for 7% (4/57) of the metallophore producing bacteria; between 25 to 50 psu for 16% (9/57) of the tested bacteria; between 50 to 75 psu for 21% (12/57) of the bacteria, and > 75 psu for the major (58%, 33/57) tested isolates (Table 1). Both the screening and siderophore quantification results suggested that the bacteria from the Lerma-Chapala Basin have developed the metallophore production ability as a mechanism to survive in HM contaminated environments, as reported in other studies for bioremediation purposes (Ngom et al. 2016). In the present study, the isolates presenting high siderophore production were *Delftia* sp. PS4Zn-1, *Pseudomonas* spp. PS4Ni-3, MS4Zn-2, IS4Zn-1 and MS4Co-3 with 94.5, 94.1, 93.9 and 92.6 psu, respectively. Whereas the strains with low siderophore production were *Achromobacter* sp. PS3As-1, *Comamonas* sp. IS1As-2 and *Flavobacterium anhuiense* MS1As-3 with 14.9, 14.8 and 11.5 psu, respectively. Among these genera, *Delftia* and *Psedudomonas* have reported as widely distributed metallophore producers (Nair et al. 2007; Lhospice et al. 2017; Mastropasqua et al. 2017). In addition, the units of siderophore production obtained from *Delftia* and *Pseudomonas* strains were higher than those previously reported (Kumar et al. 2017). As far as our knowledge, the siderophore production by *Comamonas* and *Flavobacterium* has not been reported previously.

Table 1
Plant growth promoting characteristics and siderophore excretion from Lerma-Chapala Bacteria.

Isolates	Metal(loid)phore	IAA production	Siderophore	PO_4^{3-}	Polyamines	siderophore production (psu)	Functional group
IS1As-2 (*Comamonas* sp.)	+	-	1.44	-	+	14.82	H
IS1Co-3 (*Comamonas* sp.)	+	-	2.75	-	-	91.89	H
IS1Ni-1 (*Pseudomonas* sp.)	+	-	1.21	-	-	93.47	H
IS2As-3 (*Pseudomonas* sp.)	+	-	1.87	2.3	+	89.73	H
IS2Co-2 (*Ochrobactrum* sp.)	+	-	1.53	1.6	-	90.45	ND
IS2Co-3 (*Ochrobactrum* sp.)	+	-	1.06	1.6	+	47.94	H
IS2Co-4 (*Ochrobactrum* sp.)	+	-	-	-	+	-	-
IS2Zn-1 (*Delftia* sp.)	+	-	2.16	-	+	88.94	H
IS2Zn-2 (*Stenorophomonas* sp.)	+	-	1.75	-	+	71.57	C
IS2Zn-3 (*Pseudomonas* sp.)	+	-	1.7	-	+	49.04	ND
IS2Zn-4 *(Delftia* sp.)	+	-	2.18	-	+	27.03	ND
IS3As-1 (*Pseudomonas* sp.)	+	-	2.22	2.3	-	89.78	H
IS3As-2 (*Pseudomonas* sp.)	+	-	2.2	2	-	91.67	ND
IS3Cu-2 *(Delftia* sp.)	+	-	-	-	+	75.96	H
IS3Zn-1 (*Delftia* sp.)	+	-	2.42	-	+	83.51	H
IS4As-4 (*Pseudomonas* sp.)	+	-	3.16	1.75	-	89.45	H
IS4Cr-1 *(Acinetobacter* sp.*)*	+	-	-	-	+	-	-

H, hydroxamic acid; C, catecholate ; ND, not determinate;

Isolates	Metal(loid)phore	IAA production	Siderophore	PO_4^{3-}	Polyamines	siderophore production (psu)	Functional group
IS4Cu-3 (*Cupriavirus* sp.)	+	-	1.95	+	+	46.92	ND
IS4Pb-2 (*Pseudomonas* sp.)	+	-	-	-	-	62.16	H
IS4Zn-1 (*Pseudomonas* sp.)	+	-	1.8	2	-	92.58	ND
IS4Zn-2 (*Pseudomonas* sp.)	+	-	1.17	-	-	91.57	ND
MS1As-1 (*Pseudomonas* sp.)	+	-	-	-	+	-	-
MS1As-2 (*Pseudomonas* sp.)	+	-	2.25	-	-	90.08	H
MS1As-3 (*Flavobacterium anhuiense*)	+	-	-	-	+	11.49	H
MS1Co-1 (*Pseudomonas* sp.)	+	-	1.62	2	-	72.49	ND
MS1Cr-5 (*Klebsiella* sp.)	+	-	-	-	-	55.11	H/C
MS1Cu-1 (*Cupriavirus* sp.)	+	-	2.37	-	+	53.95	H
MS1Cu-3 (*Cupriavirus* sp.)	+	-	3.25	-	+	71.28	H/C
MS1Ni-3 (*Klebsiella* sp.)	+	-	-	-	-	35.15	H/C
MS1Zn-2 (*Pseudomonas* sp.)	+	-	2.2	-	-	91.32	ND
MS1Zn-3 (*Enterobacter* sp.)	+	-	2.1	-	+	18.01	H
MS2As-1 (*Pseudomonas* sp.)	+	-	1.55	1.4	-	77.82	ND
MS2As-2 (*Delftia* sp.)	+	-	3	2	+	39.87	H
MS2As-3 (*Pseudomonas* sp.)	+	-	2.12	-	-	70.82	ND
MS2As-4 (*Delftia* sp.)	+	-	2	-	+	87.06	H/C

H, hydroxamic acid; C, catecholate ; ND, not determinate;

Isolates	Metal(loid)phore	IAA production	Siderophore	PO_4^{3-}	Polyamines	siderophore production (psu)	Functional group
MS3Co-1 (*Pseudomonas* sp.)	+	-	1.74	-	+	32.29	ND
MS3Co-2 (*Pseudomonas* sp.)	+	-	1.23	-	-	75.28	H
MS3Cu-4 *(Cupriavirus* sp.)	+	-	-	-	+	72.19	H
MS4Co-3 (*Pseudomonas* sp.)	+	-	1.25	1.6	-	92.58	ND
MS4Ni-2 (*Pseudomonas* sp.)	+	-	2.33	-	-	92.1	ND
MS4Zn-1 (*Pseudomonas* sp.)	+	-	2	-	-	90.15	ND
MS4Zn-2 (*Pseudomonas* sp.)	+	-	2.25	-	-	93.89	ND
PS1Co-1 *(Pseudomonas* sp.)	+	-	1.8	2.3	-	90.39	ND
PS1Co-2 (*Comamonas* sp.)	+	-	2	2.3	-	83.53	ND
PS1Co-3 (*Comamonas* sp.)	+	14.74	-	-	-	81.64	ND
PS2As-3 (*Pseudomonas* sp.)	+	-	1.87	4	-	91.85	H
PS2Zn-1 (*Delftia* sp.)	+	4.91	1.5	-	+	86.9	H
PS2Zn-2 (*Delftia* sp.)	+	-	1.71	-	+	54.28	H
PS2Zn-3 (*Delftia* sp.)	+	-	1.37	-	+	65.69	H/C
PS2Zn-4 (*Delftia* sp.)	+	-	1.85	-	+	90.86	H/C
PS3As- 1 *(Achromobacter* sp.)	+	-	1.77	-	+	14.89	H
PS3As- 3 *(Achromobacter* sp.)	+	-	-	-	+	78.14	H
PS3As-4 *(Achromobacter* sp.)	+	-	2	-	+	57.84	H

H, hydroxamic acid; C, catecholate ; ND, not determinate;

Isolates	Metal(loid)phore	IAA production	Siderophore	PO_4^{3-}	Polyamines	siderophore production (psu)	Functional group
PS3Co-2 *(Pseudomonas* sp.)	+	-	1.41	-	-	38.31	H
PS4As-3 *(Pseudomonas* sp.)	+	-	-	-	+	91.61	H
PS4Cr-1 *(Acinetobacter* sp.)	+	-	1.62	-	+	74.81	H
PS4Ni-3 *(Pseudomonas* sp.)	+	-	1.5	-	-	94.08	ND
PS4Zn-1 (*Delftia* sp.)	+	7.7	2.16	-	-	94.55	H
PS4Zn-2 *(Staphylococcus* sp.)	+	3.54	2.42	-	+	92.14	H
PS4Zn-3 (*Delftia* sp.)	+	7.07	2.25	-	+	43.6	H
PS4Zn-4 *(Delftia* sp.)	+	-	2	-	-	75.22	H
H, hydroxamic acid; C, catecholate ; ND, not determinate;							

3.5 Determination of functional groups of siderophores of bacteria from Lerma-Chapala Basin

Based on the oxygen ligands for metal ion coordination, the siderophore and metallophore can be classified into tree main categories, namely, hydroxamates, catecholates and carboxylates (Miethke and Marahiel 2007). Hydroxamates are the most common group of siderophores produced by bacteria and fungi in the nature. Also hydroxamates bind the metal ion Fe^{3+} with a binding constants in the range of 10^{22} to 10^{32} M^{-1} (Winkelmann 2007). The siderophores of ferrobactin and desferrioxamine B hydroxamates produced by *Pseudomonas fluorescens* and *Streptomyces pilosus*, respectively (Maurer et al. 1968; Saharan and Nehra 2011). The second group are catecholates that are secreted by a variety of bacteria (Dave et al. 2006), including enterobactin from *Escherichia coli* and *Streptomyces* sp. (Saharan and Nehra 2011; Fiedler et al. 2011), pryroverdin produced by *Pseudomonas aeruginosa* (Peek et al. 2012), ibriobactin from *Vibrio cholerae* (Saharan and Nehra 2011), salmochelins by *Salmonella enterica* (Hantke et al. 2003), bacillibactin secreted by *Bacillus* spp. (Saharan and Nehra 2011) and petrobactin from *Bacillus cereus* (Wilson et al. 2006). Finally, the group of carboxylates also namely mixed siderophores can bind to metal ion Fe^{3+} through carboxyl and hydroxyl groups (Dave et al. 2006). Rhizobactin synthesized by *Rhizobium melitoli* DM4 is the best characterized carboxylate siderophore, but this compound is also produced by several microorganism including fungi (Thieken and Winkelmann 1992). Other carboxylates produced by *Sthapylococcus* sp. have been identified and named as staphyloferrin A and B (Brasley et al. 2011). In the present study (Table 1), 54% (31/57) of the siderophore producing strains with hydroxamic acid as their functional chemical group, belonged to the genera *Comamonas, Pseudomonas, Ochrobactrum, Delftia, Cupriavidus, Enterobacter* and *Achromobacter.* Whereas for 35% (20/57) of the producers, it was not possible to determine the functional group. Only one strain (*Stenotrophomonas* sp. IS2Zn-2) produced the catecholate and 10% (6/57) of the strains synthesized metallophores with both the hydroxamic acid and catecholate functional groups, including strains of *Cupriavidus, Delftia* and *Klebsiella*. The results obtained here agreed that hydroxamate siderophores are the most common siderophores. However, it is essential to make an effort to determine the functional groups

of all the siderophores to evaluate their biological activity since in recent years new applications of siderophores have been reported in several fields including microbial ecology, agriculture, bioremediation, biosensor and medicine.

3.5 Determination of HM resistance

HM pollution generates adverse effects on the microbial communities that make them develop resistance mechanisms to survive in the habitats with high HM concentrations. Here, we evaluated the HM resistance for 60 strains presenting metallophore production. As results, all strains showed high degree of resistance to multiple HMs, especially for Zn^{2+}, As^{5+} and Ni^{2+} (Table S4). As shown in Fig. 3, 26, 27, 36, and 28 strains presented MIC values from 10–20 mM for As^{3+}, Co^{2+}, Cr^{6+} and Cu^{2+}, respectively. The MICs ranged from 30 to 40 mM for As^{5+} and Ni^{2+} in 29 and 26 strains, respectively. For Pb^{2+}, all strains presented MICs greater than 1 mM. Finally, for Zn^{2+} 33/60 strains showed a MIC of 50 mM. The order of toxicity of the HMs was Pb^{2+}>Cu^{2+}>Co^{2+}>As^{3+}>Cr^{6+}>As^{5+} and Zn^{2+} (Fig. 2). These data suggest that the Lerma-Chapala Basin bacteria are extremely HM resistant (Nies 2000) and their mechanism for HM resistance seems to be efficient, however further studies are needed in order to clarify what mechanisms have developed among them, in addition to the metallophore production.

3.6 Principal component analysis

A first PCA was carried out to visualize the relationship between the studied strains and their capacity to produce metallophores and plant growth promotion (PGP) characteristics. The first two principal components (PC) explained only a 54.9 % of the data variation. The PCA biplot (Fig. 4) shows no relation between the metallophores productions and the PGP characteristics in the identified strains. However the strains *Pseudomonas* sp. PS2As-3 and *Delftia* sp. MS2As-2 were correlated with the siderophores production and phosphate solubilization. A second PCA showed that the MIC and metallophores production explained only the 49.4% of the data variation. No relationship was observed between metal tolerance ability and the production a special type of metallophore. Interestingly, *Pseudomonas* spp. MS4Zn-1, IS3As-1, MS4Zn-2 and *Delftia* sp. IS3Zn-1 showed relation with the resistance of metals such as Cr, Cu, Ni and Zn. The strains *Pseudomonas* sp. IS1Ni-1 and *Delftia* IS2Zn-4 were associated with As^{3+}, while some strains of *Comamonas*, *Delftia* and *Pseudomona*s were associated with As^{5+} resistance. For resistance to Pb and Co, a relation between the strains *Achromobacter* sp. PS3As-4, *Acinetobacter* sp. PS4Cr-1 and *Commamonas* sp. Is1Co-3 was observed. On the other hand, strains of the genus *Delftia* sp. IS3Cu-2 and *Pseudomonas* spp. IS2Zn-3, IS4Pb-2, IS4Zn-2, MS1Zn-2 and PS1Co-1 were related to the production of metallophores with affinity for the metals Cu and Zn (Fig. S9).

4. Conclusions

In the present study, many of the Lerma-Chapala Basin bacteria can be considered as potential plant growth promoting bacteria since they showed plant growth promoting traits, mainly the siderophores synthesis and IAA production. *Delftia* and *Pseudomonas* were the most common genera identified in the soils and sediments from Lerma-Chapala Basin. Also, the local bacteria showed high HM resistance especially for Zn^{2+}, As^{5+} and Ni^{2+}, demonstrating that the Lerma-Chapala Basin bacteria have adapt to their habitats with high HM concentrations and developed resistance mechanisms such as metallophore production. The strains of *Delftia* and *Pseudomonas* presented siderophore production above 92 psu. Our results indicate that the Lerma Chapala Basin bacteria have a great potential in study and/or application in areas of ecology, agriculture, bioremediation, biosensor and medicine. However, further investigation is needed to elucidate the chemical structures of their metallophores and other possible HM-resistant mechanisms.

Declarations

Acknowledgments

This study was financially supported by the projects SIP20140353, SIP20140354, SIP20150311 and SIP20150358. J Maldonado-Hernández, I. Arroyo-Herrera and J. Guevara-Luna received scholarships from the Consejo Nacional de Ciencia y Tecnología (CONACyT) and BEIFI-IPN. B. Román-Ponce appreciates the support of Sistema Nacional de Investigadores-

CONACyT. P. Estrada-de los Santos, E. T. Wang and M. S. Vásquez-Murrieta appreciate the scholarships of Comisión de Operación y Fomento de Actividades Académicas-IPN and Estímulos al Desempeño de los Investigadores-IPN and Sistema Nacional de Investigadores-CONACyT.

Author contributions

JMH, BRP, JRG, PES, EW and MSVM were involved in the design of the project. JMH, BRP and JRG standardized the methodology. JMH, IAH, and BRP evaluate the metallophore production and PGP characteristics. BRP, JGL and SEJ analyzed the obtained data. BRP, PES, EW and MSVM wrote the manuscript. All the authors read and approved the final manuscript. MSVM supervised each stage of the experiment.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Acinas SG, Klepac-Ceraj V, Hunt DE, et al. (2004) Fine-scale phylogenetic architecture of a complex bacterial community. Nature 430:551.
2. Alexander DB, Zuberer DA (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil soils 12:39–45.
3. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881.
4. Arnow LE (1937) Colorimetric determination of the components of 3, 4-dihydroxyphenylalanine-tyrosine mixtures. J biol Chem 118:531–537.
5. Arroyo-Herrera I, Román-Ponce B, Reséndiz-Martínez AL, et al. (2021). Heavy-metal resistance mechanisms developed by bacteria from Lerma–Chapala basin. Arch Microbiol. 1-17.
6. Beasley FC, Marolda CL, Cheung J, et al. (2011) *Staphylococcus aureus* transporters Hts, Sir, and Sst capture iron liberated from human transferrin by Staphyloferrin A, Staphyloferrin B, and catecholamine stress hormones, respectively, and contribute to virulence. Infect Immun 79:2345–2355.
7. Braun V, Pramanik A, Gwinner T, et al. (2009) Sideromycins: tools and antibiotics. Biometals 22:3.
8. Chun J, Lee JH, Jung Y, et al. (2007) EzTaxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol 57:2259–2261. https://doi.org/10.1099/ijs.0.64915-0.
9. Chun J, Oren A, Ventosa A, et al. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466.
10. Cloete KJ, Valentine AJ, Stander MA, et al. (2009) Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* (Berg.) Pillans. Microb Ecol 57:624–632.
11. Csaky TZ (1948) On the estimation of bound hydroxylamine in biological materials. Acta chem scand 2:450–454.
12. Dave BP, Anshuman K, Hajela P (2006) Siderophores of halophilic archaea and their chemical characterization.
13. Deicke M, Mohr JF, Roy S, et al. (2019) Metallophore profiling of nitrogen-fixing *Frankia* spp. to understand metal management in the rhizosphere of actinorhizal plants. Metallomics 11:810–821.
14. de Oliveira LF, Elbl P, Navarro B V, et al. (2017) Elucidation of the polyamine biosynthesis pathway during Brazilian pine (*Araucaria angustifolia*) seed development. Tree Physiol 37:116–130.
15. Dimkpa CO, Merten D, Svatoš A, et al. (2009) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696.
16. Dimkpa C, Svatoš A, Merten D, et al. (2008) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54:163–172.

17. Fiedler H-P, Krastel P, Müller J, et al. (2001) Enterobactin: the characteristic catecholate siderophore of *Enterobacteriaceae* is produced by *Streptomyces* species. FEMS Microbiol Lett 196:147–151.
18. Galtier N, Guoy M, Gautier C (1996) SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogenies. Bioinformatics 12:543.
19. Glick BR (2004) Bacterial ACC deaminase and the alleviation of plant stress. Adv Appl Microbiol 56:291–312.
20. Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374. https://doi.org/10.1016/j.biotechadv.2010.02.001.
21. Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61:793–796.
22. Hansen AM, Leon-Zavala A, Bravo-Inclan L (1995) Sources of pollution and metal enrichment in sediments of the Lerma-Chapala Basin. Hydraul Eng Mex 55–69.
23. Hantke K, Nicholson G, Rabsch W, Winkelmann G (2003) Salmochelins, siderophores of *Salmonella enterica* and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IroN. Proc Natl Acad Sci 100:3677–3682.
24. Harish S, Kavino M, Kumar N, et al. (2008) Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. Appl soil Ecol 39:187–200.
25. Jay JA, Ford TE (2001) Water Concentrations, Bioaccumulation, and Human Health Implications of Heavy Metals in Lake Chapala. The Lerma-Chapala Watershed 123–136. https://doi.org/10.1007/978-1-4615-0545-7_5.
26. Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agron Sustain Dev 27:29–43.
27. Kraemer SM, Duckworth OW, Harrington JM, Schenkeveld WDC (2015) Metallophores and trace metal biogeochemistry. Aquat geochemistry 21:159–195.
28. Kraepiel AML, Bellenger JP, Wichard T, Morel FMM (2009) Multiple roles of siderophores in free-living nitrogen-fixing bacteria. BioMetals 22:573–581.
29. Kumar V, Menon S, Agarwal H, Gopalakrishnan D (2017) Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. Resour Technol 3:434–439.
30. Larkin MA, Blackshields G, Brown NP, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404.
31. Lê S, Josse J, Husson F (2008) FactoMineR: An R package for multivariate analysis. J Stat Softw 25: 1-18. https://doi.org/10.18637/jss.v025.i01.
32. Lhospice S, Gomez NO, Ouerdane L, et al. (2017) *Pseudomonas aeruginosa* zinc uptake in chelating environment is primarily mediated by the metallophore pseudopaline. Sci Rep 7:17132.
33. Li K, Ramakrishna W (2011) Effect of multiple metal resistant bacteria from contaminated lake sediments on metal accumulation and plant growth. J Hazard Mater 189:531–539.
34. Liu W, Yang C, Shi S, Shu W (2014) Effects of plant growth-promoting bacteria isolated from copper tailings on plants in sterilized and non-sterilized tailings. Chemosphere 97:47–53.
35. Ma Y, Rajkumar M, Freitas H (2009) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manage 90:831–837.
36. Ma Y, Rajkumar M, Oliveira RS, et al. (2019) Potential of plant beneficial bacteria and arbuscular mycorrhizal fungi in phytoremediation of metal-contaminated saline soils. J Hazard Mater 120813.
37. Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manage 174:14–25.
38. Mastropasqua MC, D'orazio M, Cerasi M, et al. (2017) Growth of *Pseudomonas aeruginosa* in zinc poor environments is promoted by a nicotianamine-related metallophore. Mol Microbiol 106:543–561.

39. Maurer B, Muller A, Kellersc W, Zahner H (1968) Ferribactin a siderochrome from *Pseudomonas fluorescens* migula. Arch Mikrobiol 60:326.
40. McRose DL, Seyedsayamdost MR, Morel FMM (2018) Multiple siderophores: bug or feature? JBIC J Biol Inorg Chem 23:983–993.
41. Miethke M, Marahiel MA (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71:413–451.
42. Mishra A, Fischer MKR, Bäuerle P (2009) Metal-free organic dyes for dye-sensitized solar cells: From structure: Property relationships to design rules. Angew Chemie Int Ed 48:2474–2499.
43. Moberly JG, Staven ARI, Sani RK, Peyton BM (2010) Influence of pH and inorganic phosphate on toxicity of zinc to *Arthrobacter* sp. isolated from heavy-metal-contaminated sediments. Environ Sci Technol 44:7302–7308.
44. Mustafavi SH, Badi HN, Sękara A, et al. (2018) Polyamines and their possible mechanisms involved in plant physiological processes and elicitation of secondary metabolites. Acta Physiol Plant 40:102.
45. Nair A, Juwarkar AA, Singh SK (2007) Production and characterization of siderophores and its application in arsenic removal from contaminated soil. Water Air Soil Pollut 180:199–212.
46. Ngom M, Oshone R, Diagne N, et al. (2016) Tolerance to environmental stress by the nitrogen-fixing actinobacterium *Frankia* and its role in actinorhizal plants adaptation. Symbiosis 70:17–29.
47. Nies DH (2000) Heavy metal-resistant bacteria as extremophiles: molecular physiology and biotechnological use of *Ralstonia* sp. CH34. Extremophiles 4:77–82.
48. Pande A, Pandey P, Mehra S, et al. (2017) Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. J Genet Eng Biotechnol 15:379–391.
49. Paul D, Sinha SN (2017) Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. Ann Agrar Sci 15:130–136.
50. Payne SM (1993) Iron acquisition in microbial pathogenesis. Trends Microbiol 1:66–69.
51. Peek ME, Bhatnagar A, McCarty NA, Zughaier SM (2012) Pyoverdine, the major siderophore in *Pseudomonas aeruginosa*, evades NGAL recognition. Interdiscip Perspect Infect Dis 2012:
52. Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160.
53. Rajkumar M, Freitas H (2008) Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. Bioresour Technol 99:3491–3498.
54. Rangan P, Subramani R, Kumar R, et al. (2014) Recent advances in polyamine metabolism and abiotic stress tolerance. Biomed Res Int 2014:
55. Rathnayake IVN, Megharaj M, Krishnamurti GSR, et al. (2013) Heavy metal toxicity to bacteria–Are the existing growth media accurate enough to determine heavy metal toxicity? Chemosphere 90:1195–1200.
56. Retamal-Morales G, Mehnert M, Schwabe R, et al. (2018) Detection of arsenic-binding siderophores in arsenic-tolerating *Actinobacteria* by a modified CAS assay. Ecotoxicol Environ Saf 157:176–181.
57. Román-Ponce B, Ramos-Garza J, Arroyo-Herrera I, et al. (2018) Mechanism of arsenic resistance in endophytic bacteria isolated from endemic plant of mine tailings and their arsenophore production. Arch Microbiol 200:883–895.
58. Roy S, Roy M (2019) Characterization of plant growth promoting feature of a neutromesophilic, facultatively chemolithoautotrophic, sulphur oxidizing bacterium *Delftia* sp. strain SR4 isolated from coal mine spoil. Int J Phytoremediation 21:531–540.
59. Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21:30.
60. Sarwar N, Malhi SS, Zia MH, et al. (2010) Role of mineral nutrition in minimizing cadmium accumulation by plants. J Sci Food Agric 90:925–937.

61. Schenkeveld WDC, Oburger E, Gruber B, et al. (2014) Metal mobilization from soils by phytosiderophores–experiment and equilibrium modeling. Plant Soil 383:59–71.
62. Scherf U, Ross DT, Waltham M, et al. (2000) A gene expression database for the molecular pharmacology of cancer. Nat Genet 24:236.
63. Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56.
64. Silva Filho GN, Vidor C (2000) Solubilização de fostatos por microrganismos na presença de fontes de carbono. Rev Bras Ciência do Solo 24:311–319.
65. Silver S, Phung LT (2005) Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol 71:599–608.
66. Sulaymon AH, Abdulmajeed BA, Salman AB (2015) Electrochemical removal of cadmium from simulated wastewater using a smooth rotating cylinder electrode. Desalin Water Treat 54:2557–2563.
67. Tamura K, Peterson D, Peterson N, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739.
68. Thatoi H, Das S, Mishra J, et al. (2014) Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: A review. J Environ Manage 146:383–399.
69. Thieken A, Winkelmann G (1992) Rhizoferrin: a complexone type siderophore of the mocorales and entomophthorales (*Zygomycetes*). FEMS Microbiol Lett 94:37–41.
70. Torres Z, Mora MA, Taylor RJ, Alvarez-Bernal D (2016) Tracking Metal Pollution in Lake Chapala: Concentrations in Water, Sediments, and Fish. Bull Environ Contam Toxicol 97:418–424. https://doi.org/10.1007/s00128-016-1892-6.
71. Trujillo-Cárdenas JL, Saucedo-Torres NP, Zárate del Valle PF, et al. (2010) Speciation and sources of toxic metals in sediments of Lake Chapala, Mexico. J Mex Chem Soc 54:79–87.
72. Volesky B (2001) Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy 59:203–216.
73. Wilson MK, Abergel RJ, Raymond KN, et al. (2006) Siderophores of *Bacillus anthracis, Bacillus cereus*, and *Bacillus thuringiensis*. Biochem Biophys Res Commun 348:320–325.
74. Winkelmann G (2007) Ecology of siderophores with special reference to the fungi. Biometals 20:379.
75. Wu W, Huang H, Ling Z, et al. (2016) Genome sequencing reveals mechanisms for heavy metal resistance and polycyclic aromatic hydrocarbon degradation in *Delftia lacustris* strain LZ-C. Ecotoxicology 25:234–247.
76. Wu Z, Kong Z, Lu S, et al. (2019) Isolation, characterization and the effect of indigenous heavy metal-resistant plant growth-promoting bacteria on sorghum grown in acid mine drainage polluted soils. J Gen Appl Microbiol 2011–2018.
77. Xu D, Guo J, Xu L, et al. (2014a) The relationship between polyamine oxidase activity and lignin deposition and chrysanthemum flower bud differentiation. Acta Agric Boreali Sin 29:164–169.
78. Yokobe T, Hyodo F, Tokuchi N (2018) Seasonal effects on microbial community structure and nitrogen dynamics in temperate forest soil. Forests 9:153.
79. Zahid M (2015) Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). Front Microbiol 6:207.
80. Zhang W, Huang Z, He L, Sheng X (2012) Assessment of bacterial communities and characterization of lead-resistant bacteria in the rhizosphere soils of metal-tolerant *Chenopodium ambrosioides* grown on lead–zinc mine tailings. Chemosphere 87:1171–1178.

Figures

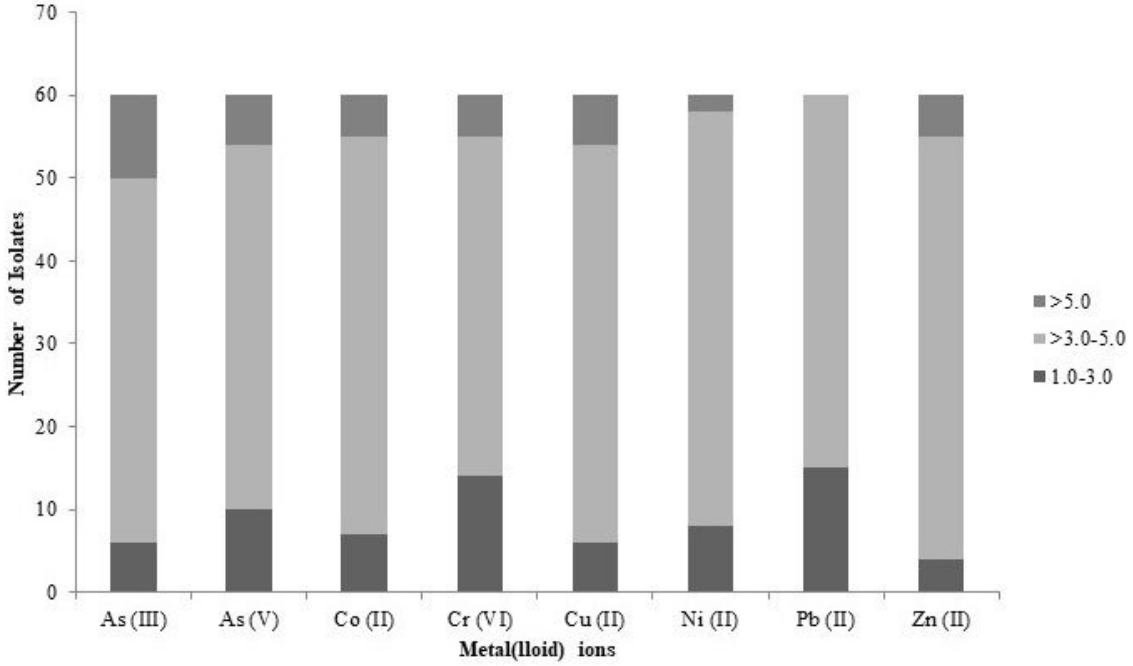

Fig. 1.

Figure 1

Metallophore synthesis by Lerma-Chapala Basin bacteria after 96 h assays. Bar represent numbers of positive strains against halo production.

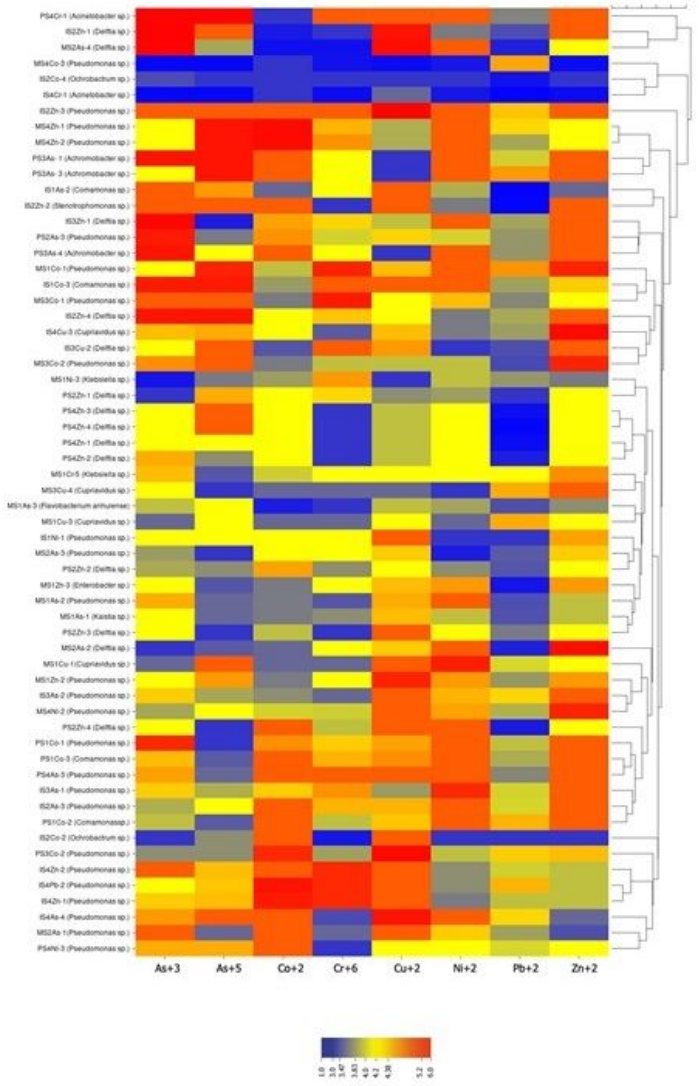

Fig. 2.

Figure 2

Hierarchically clustered heatmap of the bacterial metalloid and metallophores indexes studied in this work. The metallophore index of each metal were depicted by color intensity with the legend indicated at the under of the figure, ranging from dark blue (low value) to red (high value).

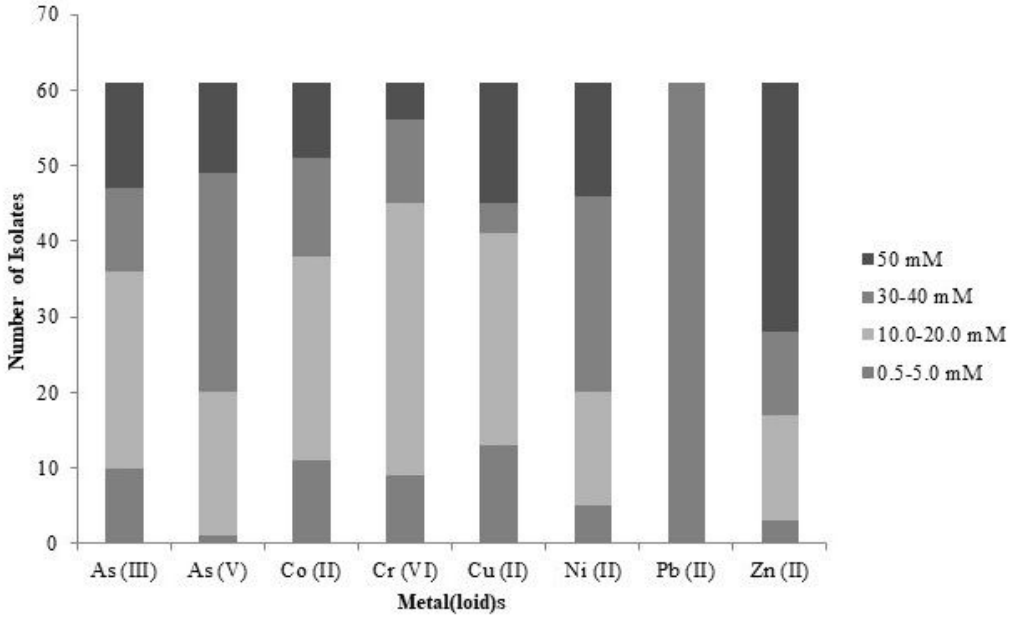

Fig. 3

Figure 3

Minimum inhibitory concentration (MIC) from Lerma-Chapala after 96 h assays. Bars represent numbers of isolates against metal(loid) concentration.

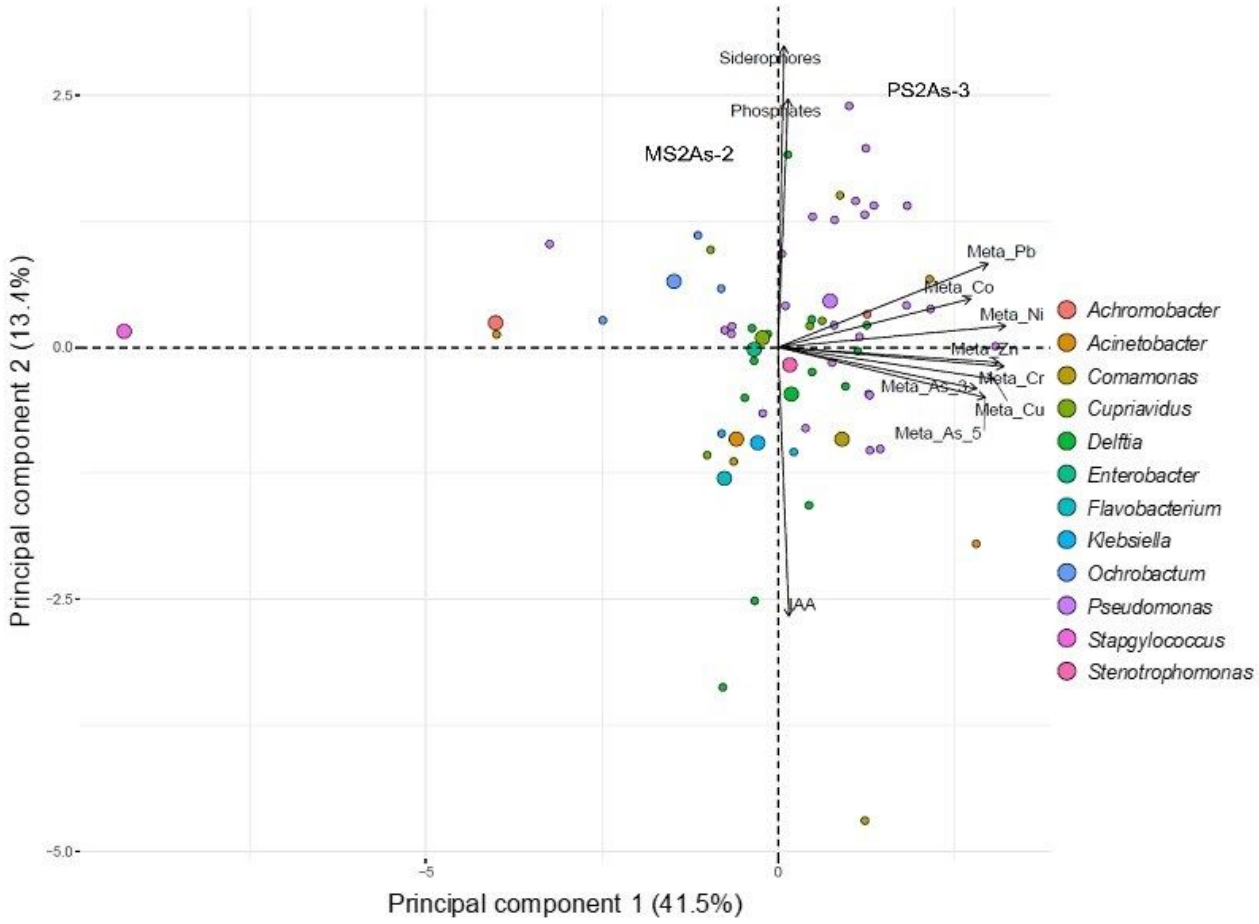

Fig. 4

Figure 4

Principal component analysis (PCA) of the relation between metallophores production and plant growth promoting characteristics of the identified strains.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTablesR1.docx
- Supplementaryfiguresfinal.pdf