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# Production of biosurfactants by bacteria isolated from a mine tailing zone in Southern Mexico and their resistance to heavy metals

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Tailings generated through mining processes often create leachates containing high concentrations of heavy metals such as As, Fe, Mn, Zn and Pb. These high concentrations of heavy metals result in environmental damage such as contamination of soil, groundwater and air, which represents a huge problem for individuals living near mining areas. An alternative for soil metal removal is microbiological processes including the production of biosurfactants, possibly a survival mechanism for adverse conditions of mine tailings and leachates. Moreover, mine tailings are materials that have attracted interest among researchers, because they can be exploited by innovative techniques like phytomining. In this study, we sampled the leachates of the “El fraile” mine tailings and identified 103 bacteria capable of growth on these leachates. We observed that 11 bacteria produce a high amount of biosurfactants and developed the multi-metal tolerance with higher concentration gradient of Pb, Cd, Cu, Fe, Zn and As. We showed that the bacteria tolerate 853 nM of As and up to 12 nM of Pb, 17 nM of Cd, 10.6 nM of Cu, 22 nM of Fe and 10.5 nM of Zn. We determined that the bacterial isolates clustered within five phylogenetic groups that were very close: *Enterobacter*, *Klebsiella*, *Artherobacter*, *Pantoea* and *Solibacillus* groups. A bank of strains resistant to heavy metals and producers of biosurfactants was obtained for future studies on the mechanism of absorption or assimilation of heavy metals and light was shed on the alternative use of these bacteria in bioremediation of metal pollution.

**Key words:** Bacteria, metals, biosurfactants, mine tailings.

## INTRODUCTION

Mining is one of the most important economic activities of Mexico since the 15<sup>th</sup> Century and is currently active in many parts of the country. The Taxco mining district has been one

of the richest producers of silver and gold, among other metals, since colonial times (1523-1810). In this district, there are tailing impoundments of various ages and sizes

(La Concha, Santa Rosa, Taxco and El Fraile) with high levels of metals (As, Pb, Cd, Zn, Mn) (Armienta et al., 2003; Talavera et al., 2005). During mining activities, large amounts of material or mine wastes are removed and deposited into surrounding areas. Leaching of heavy metals from these dumps is common during the rainy season, which contaminates the surface water as well as ground water bodies (Dhakate and Singh, 2008). In the El Fraile impoundments, different types of acidic (pH=2.4-2.5) or near-neutral (pH=6.3-7.7) leachates were identified. Some environmental monitoring studies on these leachates have shown high levels of Ca-SO<sub>4</sub>, (Ca+Mg)-SO<sub>4</sub>, Mg-SO<sub>4</sub> and Ca-(SO<sub>4</sub>+HCO<sub>3</sub>) (Talavera et al., 2006). These deposits of mine tailings and their leachates are major sources of heavy metal contamination in and around the communities, because the leachates are sometimes used as alternative sources of domestic water.

Heavy metals are stable and persistent contaminants of the environment, since they cannot be degraded or destroyed (Sevgi et al., 2009) so, the accumulation of metals in soil represents a health risk for people (Moreno et al., 2010), and other living things in terrestrial and aquatic environments near mining areas. The use of micro-organisms for decontamination of heavy metals in the soil and water around industrial plants has gained growing attention (Doni et al., 2013; Gadd, 2004; Kavamura and Esposito, 2010). Tailings have very low concentrations of macronutrients for microbial growth, especially nitrogen, and conversely can contain high concentrations of heavy metals, which can be particularly problematic in tailings where the pH is low. In such instances, soil fertility and microbial communities are drastically reduced (Seaker and Sopper, 1988; Pepper et al., 2012). However, multiple investigations have been carried out with isolated microbial strains from soil, mud and water samples from metallurgically polluted environments for bioremediation of toxic heavy metals (Umrana, 2006; Das et al., 2013). Recent work indicated that most of the transitions between metal speciation forms are controlled by microbial activity (Johnson and Hallberg, 2004; Hall et al., 2005; Hall and Puhmann, 2004). The potential for natural attenuation of acid mine drainage by microbial populations, and the subsequent removal of key toxic metal, particularly the removal of copper and zinc and the interaction of the root-organism in the activation of such decontamination procedures, were clearly demonstrated (Doni et al., 2013; Whitehead and Prior, 2004; Mulligan et al., 1999). Therefore, microbial processes that facilitate the detoxification and metal mobility using resistant microbial strains can be exploited for bioremediation of heavy metals from waste water and effluents containing heavy metals.

The production of biosurfactants by bacteria plays a role in the adaptation of these organisms to different environ-

ments (Toribio et al., 2011); therefore, this could be a mechanism by which the bacteria can absorb heavy metals and become resistant to growth in soils polluted by tailings. Biosurfactant producing bacteria are very diverse and have been isolated from a wide variety of environments, including contaminated soil or water (Bodour et al., 2003), but there are no reports of biosurfactant producing bacteria isolated from mining soils.

The present study is aimed at isolating, characterizing and monitoring biosurfactant producing bacterial isolates and their ability to resist heavy metal leachates from the mine El Fraile tailings located in the Taxco mining district in southern Mexico for their possible use in bioremediation in metal contaminated environments.

## MATERIALS AND METHODS

### Site description and tailing sampling

The present study was conducted near a mine site (El Fraile) located at Taxco, Guerrero, in southern Mexico (Figure 1). Thirty-two samples were randomly collected from heavy metal-contaminated mine tailings and leachates of the El Fraile and were put into aseptic screw capped bottles. The pH of the mine tailing and leachate was determined using a pH meter (Orion Research, Beverly, MA).

### Isolation of strains, media and growth conditions

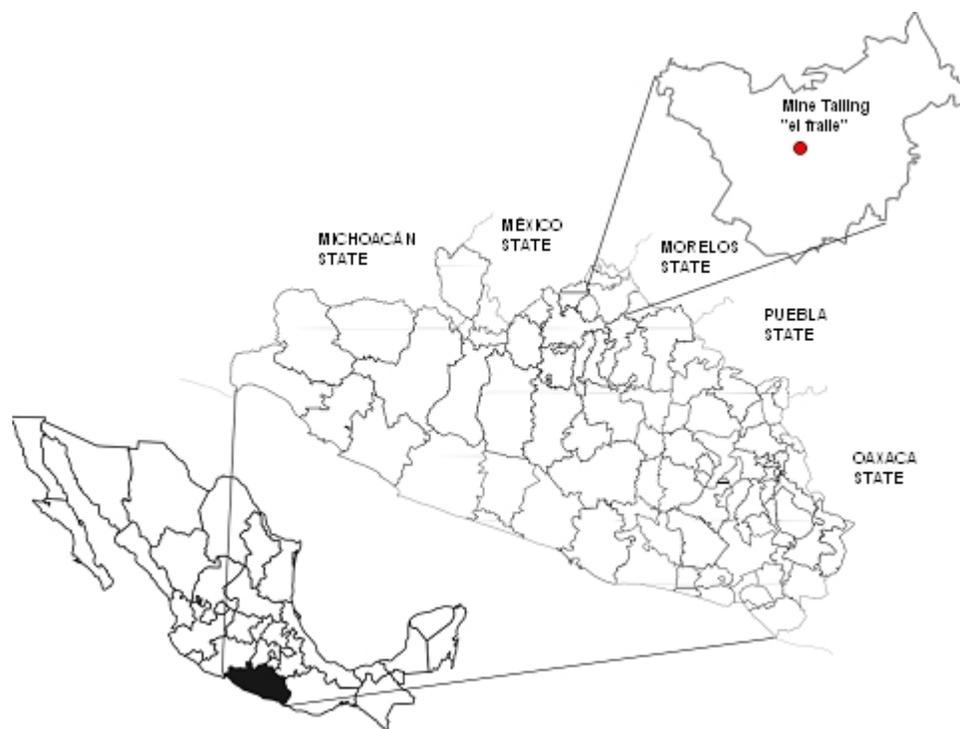
A quantity of 1 mL of leachate or 1 g of soil of El fraile mine tailings from each of the collected samples was mixed in 9 mL of sterile saline solution (NaCl 0.85%) and serial dilutions were made (1:1,000 and 1:10,000). 100 µl of each dilution was plated on Luria Bertani (LB) agar plates supplemented with 0.025 mM Pb (NO<sub>3</sub>)<sub>2</sub> and 0.0125 mM de Cd (NO<sub>3</sub>)<sub>2</sub>. Plates were incubated at 30°C for 48 h and colonies differing in morphological characteristics were selected.

### Detection of biosurfactant production

The isolated bacteria were screened for their biosurfactant-producing ability by blood agar plate assay and PSVW agar plate assay. The screening was done culturing the bacteria on PSVW (PPGAS medium supplemented with 2.5 mg/ml of methylene blue, 200 mg/ml of the surfactant cetyl-trimethyl-ammonium-bromide (CTAB) and 1.5% agar) plates and blood agar (15 g/L enzymatic digest of casein, 4 g/L enzymatic digest of animal tissue, 2g/L yeast extract, 1g/L corn starch, 5g/L sodium chloride and 14g/L agar) plates (Will et al., 1997). Plates were incubated at 30°C for 72 h. Bacteria producing a blue halo around the colonies and hemolysis were cultured on PPGAS liquid medium (0.02 M NH<sub>4</sub>Cl<sub>2</sub>, 0.02 M KCl, 0.12 M Tris-HCl, 0.0016 M MgSO<sub>4</sub>, 0.5% glucose, and 1% peptone, pH 7.2; Zhang and Miller, 2002) and were incubated at 30°C for 7 or 15 days shaken at 225 rpm. Foam-forming activity, drop collapse assay and emulsification ability (Sen, 2010; Toribio et al., 2011) were also evaluated.

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**Figure 1.** Map of Taxco mining district showing the localization of the El Fraile tailings deposits. Elaborated based on the Geostatistical Framework National Institute of Statistics and Geography (INEGI).

### Biochemical characterization

Morphological and biochemical properties of the strains isolated were determined and compared by The Analytical Profile Index (API 20E) and VITEK2 Compact (bioMérieux). These systems use 8 conventional biochemical tests and 12 carbohydrate assimilation tests and were performed according to the manufacturer's instructions. Gram staining, catalase and oxidase test were done with standard protocols as described in MacFaddin's Manual of Identification of Bacteria (MacFaddin, 2000; Kannan, 2002).

### Minimum inhibitory concentration (MIC) determination

The MIC was used to define the lowest concentration of heavy metals and metalloids that inhibit the visible growth of bacteria (Andrews, 2001). The MIC values were determined using the agar dilution method (Kamala-Kannan and Krishnamoorthy, 2006). A log-phase culture of the isolates was inoculated onto LB agar plates supplemented with increasing concentrations (1-900 mM) of heavy metal salts and metalloids:  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{Cd}(\text{NO}_3)_2$ ,  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{Fe}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2$  and As IV. The plates were incubated at 30°C for a week, looking for bacterial growth.

### DNA extraction, sequencing and phylogenetic analyses

Genomic DNA was extracted by using a GeneJet™ Genomic DNA purification kit (Thermo Scientific) according to the manufacturer's instructions. Bacterial 16S rDNA was amplified by PCR using the universal bacterial 16S rDNA primers fd1 5'-AGAGTTTGATCCTGGCTCAG-3' and rd1 5'-AAGGAGGTGATCCAGCC-3' (Ndp et al., 2007; Rodicio et al.,

2004). PCR was performed with a 25 µl reaction mixture containing 1 µl (10 ng) of DNA as a template, each primer at a concentration of 5 µM, 2.5 mM  $\text{MgCl}_2$  and dNTP's at a concentration of 2.5 µM, as well as, taq polymerase and buffer used as recommended by the manufacturer (Thermo Scientific). The PCR conditions consisted of an initial denaturing step at 95°C for 7 min, 30 cycles of 95°C for 40 s, 55°C for 40 s, 72°C for 2 min and a final extension at 72°C for 15 min. The PCR products were electrophoresed and purified from 1% agarose gels (0.56 TAE buffer; 2.42 g Tris-HCl, 0.57 ml glacial acetic acid, 1 ml 0.5 M EDTA (pH 8.0) and 1 L distilled and deionized water) using the GeneJET™ PCR Purification Kit (Thermo Scientific). DNA sequences were determined by the dideoxy chain termination method (Sanger et al., 1977) with a Perkin Elmer/Applied Biosystems DNA Sequencer. The 16S rDNA sequences were aligned and compared with other 16S rDNA genes in Ribosomal Database Project II (RDP) by using SEQMATCH (Wang et al., 2007) and GeneBank by using NCBI basic local alignment search tools BLASTn program (Benson and Karsch-Mizrachi, 2000). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test, has 1000 replicates (Felsenstein, 1985). The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969). The rate variation among sites was modeled with a gamma distribution. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). GenBank accession numbers for *Enterobacter cancerogenus* 16S rDNA sequences are KF811178, KF811179, KF811181, KF811185, KF811186, KF811187 and KF811188, for *Klebsiella pneumoniae* 16S rDNA gene sequence is KF811183. That for *Solibacillus silvestris* 16S rDNA gene sequence is KF811182; for *Arthrobacter luteolus* 16S rDNA gene sequence is: KF811177 and for *Pantoea vagans* 16S rDNA gene sequence is KF811192.

**Table 1.** Microbiological characterization.

Isolate	Samples pH	Gram staining	Oxidase assay	Catalase assay
<b>Leachates</b>				
M14D1-12	2.0	Bacillus Gram -	-	+
M14D2-13	2.0	Bacillus Gram -	-	+
M14-1	6.0	Bacillus Gram +	-	+
M14A-1	2.0	Bacillus Gram -	+	+
M14B	2.0	Bacillus Gram -	+	+
<b>Mine tailings</b>				
M2B-1	8.4	Bacillus Gram -	-	+
M2B-4	8.0	Bacillus Gram +	-	+
M15A-4	7.0	Bacillus Gram -	+	+
M4D-2	7.4	Bacillus Gram -	-	+
M10-7	8.4	Bacillus Gram -	-	+
PAO	8.4	Bacillus Gram -	-	+

All determinations were done in duplicate. (-), No activity, (+), activity.

### Statistical analysis

The experiments were set up in duplicate. Data sets of resistance to metal among individual isolates were analysed by principal component analysis (PCA) using a statistical package STATA v 11.

## RESULTS

### Isolation, identification and characteristics of biosurfactant-producing bacteria

This study represents an attempt to identify the biosurfactant producing bacteria within mine tailings and leachates from El Fraile. Serial dilution plating of mine tailing suspensions and leachates on LB plates resulted in the isolation of a total of 103 morphologically distinct bacteria (23 isolates of leachates and 80 of mine tailings). Screening of the bacterial isolates for biosurfactant production resulted in the isolation of 11 strains (5 of leachates and 6 of mine tailings) which gave positive results for most of the screening assays, indicating that they are efficient biosurfactant producers. Strains were subjected to Gram staining to determine their structure and morphology. We found that nine of these bacteria are Gram negative bacillus and two are Gram positive bacillus. We also carried out the oxidase and catalase assays in order to determine the metabolism of the 11 strains isolated. Table 1 indicates the results obtained. All bacteria were catalase positive and most were oxidase negative.

### Study of the emulsifying capacity of biosurfactants produced by the bacterial isolates

The 11 isolates were tested for biosurfactant production and activity (Table 2). In all assays and tests *Pseudomonas*

*aeruginosa* was used as reference biosurfactant producing bacteria. We used the hemolytic activity assay as the first method for screening biosurfactant producers. In this assay, we looked for a clear zone around the wells produced by hemolysis. Although we did not observe hemolytic activity of the isolates in this assay, when we used PSVW plates to detect extracellular glycolipid production, the formation of dark blue halos around all the isolates was observed, and the halos produced by the isolates M14D1-12, M14D2-13 and M14-1 were similar to those produced by *P. aeruginosa* (Table 2). Biosurfactant activity was measured by the drop collapse test, by comparing an oil droplet treated with sterile medium as the control to an oil droplet treated with the culture (Table 2). The oil droplet collapsed when treated with cultures of all isolates, especially with cultures of the isolates M14D2-13 and M14-1 (Table 2). In addition, biosurfactant activity was also confirmed by the emulsifying activity of biosurfactant in the supernatant. The emulsifying capacity was evaluated by the E<sub>24</sub> emulsification index using *P. aeruginosa* PAO1 as a control. Amongst the isolates from the mine tailings, the emulsifying capacity was higher with the isolates M10-7 and M4D-2, with a 52.27 and 26.09% respectively, whereas with the isolates from the leachates it was M14D1-12, with 44% (Table 2). No substantial emulsification was achieved with M14D2-13 (isolate from leachates) and M2B-4, M15A-4 and PAO (isolates from mine tailings). Foam formation was evaluated in the isolates on PPGAS medium, for a period of 7 to 15 days. We observed that the isolates from leachates that showed the higher production were M14D2-13, M14D1-12 and M14A-1, whereas the isolates M2B-1, M2B-4, M15A-4 and M4D-2 from the mine tailings showed the same production, except for strain M10-7 that produced less and the strain PAO who's production was similar to that of the control strain (Table 2).

**Table 2.** Detection of biosurfactant production assays.

Strain	Hemolytic activity	PSVWagar test	Drop collapse test	E <sub>24</sub> (%)	Foam-forming
<b>Control</b>					
<i>Pseudomonas aeruginosa</i> PA01	++++	++++	++++	60	++++
<b>Leachates</b>					
M14D1-12	-	+++	+	44	+++
M14D2-13	-	+++	+++	10	+++
M14-1	-	+++	++++	17	++
M14A-1	-	+	+	12	+++
M14B	-	+	+	12	+++
<b>Mine tailings</b>					
M2B-1	-	+	+	11.9	++
M2B-4	-	+	+	6.98	++
M15A-4	-	+	+	8.70	++
M4D-2	-	+	+	26.09	++
M10-7	-	+	+	52.27	+
PAO	-	+	+	0	++++

All determinations were done in duplicate using *P. aeruginosa* PAO1 as a control. (-), No produced hemolysis, (+), Foam formation and stability.

### Effect of growth of bacteria on heavy metal

Diverse studies have shown that bacteria isolated from mine tailings show metal tolerance (Pepper et al., 2012; Koc et al., 2012). To determine the extent of tolerance through MIC values, the isolated bacteria were grown on LB agar plates containing different concentrations of heavy metals and metalloids (Pb, Cd and As). The results of the metal sensitivity tests (inhibition zone diameters) for the isolates and the control strain (*P. aeruginosa* PAO1), on Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Fe(NO<sub>3</sub>)<sub>2</sub>, Zn(NO<sub>3</sub>)<sub>2</sub> and As IV, are provided in Table 3. The cut-off value of MIC was taken to be 17 mM for Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Fe(NO<sub>3</sub>)<sub>2</sub>, Zn(NO<sub>3</sub>)<sub>2</sub> and 853 for As. All the isolates showed high tolerance to the various heavy metals and metalloids tested. The MIC levels were significantly higher as compared to the control (Table 3). Isolates from leachates exhibited MIC values of 11 mM on Fe(NO<sub>3</sub>)<sub>2</sub>, up to 17 mM on Cd(NO<sub>3</sub>)<sub>2</sub>, 12 mM on Pb(NO<sub>3</sub>)<sub>2</sub>, 10.5 and 10.6 mM on Zn(NO<sub>3</sub>)<sub>2</sub> and Cu(NO<sub>3</sub>)<sub>2</sub> respectively; whereas on As IV, the MIC value was 853 mM. For the isolates from the mine tailings, we observed that the MIC values on Pb(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub> and Zn(NO<sub>3</sub>)<sub>2</sub> were similar to the values of isolates from the leachates, whereas the maximum MIC values on Fe(NO<sub>3</sub>)<sub>2</sub> and Cd(NO<sub>3</sub>)<sub>2</sub> were 22 and 8.5 mM, respectively, instead of 11 and 17 mM. Of these isolates from the mine tailings, only M15A-4 and M10-7 displayed a high MIC value on As IV (853 mM (Table 3). PCA was performed to compare resistance to metal among individual isolates. We observed the higher tolerance to Pb ascribed to the variability between the

strains isolated, variability was not observed in the resistance to Cd, As, Fe and Zn, even though the As showed the highest resistance concentrations, but has no variability as the Pb resistance. So, according to data from the principal component analysis (PCA), the strains isolates showed higher tolerance to Pb followed by Cd and As and less tolerance to Fe and Zn, the analysis results were consistent with the values of the MICs.

### 16S rRNA sequence analysis

To further characterize the isolated bacteria, their 16S rDNA sequences were obtained by amplifying and sequencing their DNA fragments. The results from the analysis using the Ribosomal Database project II (RPD II), showed that the 16S rDNA sequences of all isolates group in five distant clusters (Figure 2). The first one belongs to the *Enterobacter* group and contains seven isolates showing a unique 16S rDNA sequence (M14D2-13, M14-A1, M2B-1, M14B, M15A-4, M4D-2 and PAO). The second one (M14D1-12) belongs to the *Pantoea* group and displays the same 16S rDNA sequence as *P. vagans*. The third (M10-7) belongs to the *Klebsiella* group, displaying the same 16S rDNA sequence as *K. pneumoniae*. The fourth one (M14-1) belongs to the *Solibacillus* group; displaying the same 16S rDNA sequence as *S. silvestris*, and the fifth one (M2B-4) belongs to the *Arthrobacter* group, displaying the same 16S rDNA sequence as *A. luteolus*. The seven *Enterobacter* strains, sharing a 100% identity of the 16S rDNA sequence between them, were closer to

**Table 3.** MICs of the isolates.

Isolate	Pb(NO <sub>3</sub> ) <sub>2</sub>	Cd(NO <sub>3</sub> ) <sub>2</sub>	Cu(NO <sub>3</sub> ) <sub>2</sub>	Fe(NO <sub>3</sub> ) <sub>2</sub>	Zn(NO <sub>3</sub> ) <sub>2</sub>	As IV
<b>Control</b>						
<i>Pseudomonas aeruginosa</i> PA01	3	4	5	5	5	-
<b>Leachates</b>						
M14D1-12	12	17	10.6	11	10.5	853
M14D2-13	12	17	10.6	11	5.3	853
M14-1	12	8.5	10.6	11	5.3	853
M14A-1	12	17	10.6	11	5.3	853
M14B	12	17	10.6	11	5.3	853
<b>Mine tailings</b>						
M2B-1	6	8.5	10.6	22	10.5	426
M2B-4	6	4.2	10.6	22	10.5	426
M15A-4	6	8.5	10.6	22	10.5	853
M4D-2	6	4.2	10.6	22	5.3	426
M10-7	12	8.5	10.6	22	10.5	853
PAO	6	4.2	10.6	22	5.3	426

All determinations were done in duplicate in cells grown for a week on LB agar plates supplemented with increasing concentrations (1–900 mM) of heavy metal salts and metalloids: Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Fe(NO<sub>3</sub>)<sub>2</sub>, Zn(NO<sub>3</sub>)<sub>2</sub> and As IV. (–), No MIC hence no growth. *P. aeruginosa* PA01 Was used as a control, the results are expressed as MIC mM.

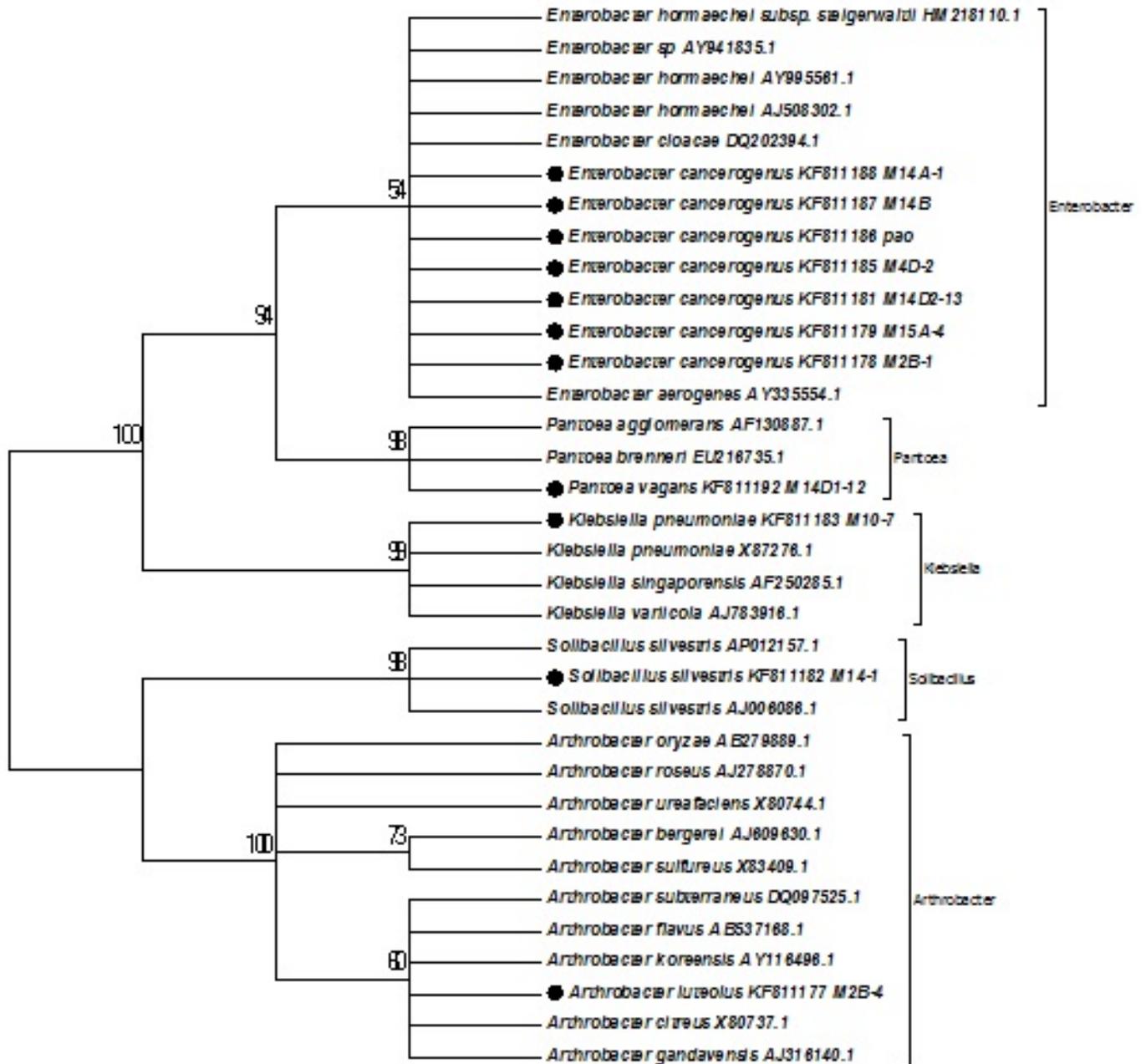
*E. cancerogenus*, than to *E. asburiae*.

## DISCUSSION

The mining activity in Taxco, Guerrero, Mexico has resulted in the discharge of large amounts of mine wastes (Talavera et al., 2005, 2006). These deposits are major sources of heavy metal contamination affecting the surrounding area. We have interest in finding bacteria capable of absorbing or assimilating heavy metals as an alternative to bioremediation. We isolated eleven novel strains capable of growing in mine tailings and leachates, in addition to producing biosurfactants. Biosurfactant applications in environmental applications are promising due to their biodegradability, low toxicity and effectiveness in enhancing the biodegradation and solubilization of low solubility compounds (Soberón-Chávez G and Maier, 2011; Mulligan et al., 1999). A number of studies have demonstrated that biosurfactants produced by bacteria, accelerate the bioremediation of hydrocarbon contaminated soils and organic contaminants (Moldes et al., 2001; Mahjoubi et al., 2013; Lawniczak et al., 2013). It has also been demonstrated that the production of biosurfactants greatly contributes to the metal removal from contaminated soils (Wang and Mulligan, 2009). It has been proposed that the rhamnolipids can remove arsenic and heavy metals through the formation of complex micelles with the metals, reducing the interfacial tension (Wang and Mulligan, 2009). However, the presence

of biosurfactant producing bacteria isolated from metal contaminated soils has not been documented; therefore, these bacteria are the focus of attention in this work because the production of biosurfactants could possibly be a mechanism that the bacteria isolates of leachates of mine tailings use to resist high levels of heavy metals. Therefore, in this study, we characterized biosurfactant producing bacteria capable of growth on high levels heavy metals with the objective of creating a collection of strains for future studies on the role of biosurfactants on removal of heavy metals from the mine tailings and the possible mechanisms involved.

The high value of MIC that the bacterial isolates present, indicate their capacity to resist high concentrations of heavy metals. This should be taken into consideration if these bacteria are to be used on the remediation of arsenic and heavy metal contaminated mine tailings or soils. We observed that the best BS producing strain isolated from leachates was the M14D1-12, exhibiting an index of emulsification of 44%, whereas that the best BS producing strain isolated from the mine tailing was the M10-7, with an index of emulsification of 52.27%, with respect to the control strain *P. aeruginosa* PA01 (Table 2). This strains were capable of tolerating more value of Cd(NO<sub>3</sub>)<sub>2</sub> and Fe(NO<sub>3</sub>)<sub>2</sub>, therefore we observed the correlation between the production of biosurfactant, with the ability for growth in high concentrations of heavy metals. The RDP II 16S rRNA gene sequence analysis was carried out to identify the dominant bacterial population. The data showed that the most



**Figure 2.** Molecular phylogenetic analysis by neighbor-joining method on 16s gene sequences. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

persistent bacterial populations were members of dominant soil bacterial group in distant clusters: *Enterobacter*, *Klebsiella*, *Arthrobacter*, *Silobacillus* and *Pantoea*, the strain isolated from leachates M14D1-12 was identified as *P. vagans*, whereas the strain isolated from the mine tailing M10-7 was identified as *E. cancerogenus* (Figure 1). Using denaturing gradient gel electrophoresis (DGGE)

bacteria isolates were found in heavy metal contaminated soils belonging to the genus *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Brochothrix*, *Comamonas*, *Cytophaga*, *Deinococcus*, *Enterobacter*, *Hafnia*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Rathayibacter*, *Rhodococcus*, *Salmonella*, *Serratia*, *Staphylococcus*, *Variovorax* and *Xanthomonas* (Ellis et al., 2003).

However, the existence of these microorganisms can be influenced by several factors such as the presence of nutrients, pH and specially the humidity and temperature. In this study, we found the genus: *Enterobacter*, *Silobacillus*, *Klebsiella*, *Arthrobacter* and *Pantoea* and we observed that they were capable of growth in a mineral-rich environment therefore these bacteria are remarkably tolerant to a wide range of soluble metal ions (Table 3). The capacity of these bacteria to tolerate high concentrations of heavy metals and the production of BS can be exploited for the remediation of heavy metal pollution.

Experiments are in progress to characterize the optimal conditions for biosurfactant production on heavy metal and to determine the type and the potential role biosurfactants may play in heavy metal metabolism by the producing organism. The current study showed that bacteria of genus: *Enterobacter*, *Silobacillus*, *Klebsiella*, *Arthrobacter* and *Pantoea* isolated from mine tailings and leachates of the "El fraile" mine tailing are capable of resisting high concentrations of heavy metal salts and metalloids:  $Pb(NO_3)_2$ ,  $Cd(NO_3)_2$ ,  $Cu(NO_3)_2$ ,  $Fe(NO_3)_2$ ,  $Zn(NO_3)_2$  and As IV and produce biosurfactants (BS). This is the first study on bacteria isolated from mine tailing and leachates that showed the production of BS which can be a key factor for living in these ecosystems.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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