Root Promotion of Acacia farnesiana by PGPB in Mine Tailings

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ABSTRACT
The mining activity has generated a large amount of waste called tailings; unfortunately, these contain EPT that affect environmental and human health. Phytoremediation assisted with PGPB is one of the strategies used to mitigate its toxic effects. We evaluated the effect of the PGPB tolerant to heavy metals on the Acacia farnesiana rhizosphere in substrates with different concentrations of tailings. For this, 28 bacterial strains of the A. farnesiana rhizosphere were identified and the MIC to heavy metals and metalloids was determined. In the same way, the plant promotion mechanisms were detected, and the effect capacity in the weight and length of the A. farnesiana rhizosphere was determined with three consortiums (A, B, and C, all including various combinations of Bacillus sp, Enterobacter sp and P. putida JM1) in different concentrations of tailings and amendments for 98 days. All the strains were tolerant to heavy metals and were able to produce IAA, fix N2, solubilize phosphates, siderophores, and lytic enzymes. Additionally, only the consortiums A and B were statistically significant in the dry weight of the root with 90 and 100% of substrate with tailings; in length, only a statistically significant difference was observed when the seed inoculated with consortium A was planted with 80% of tailing substrate. The data provide information on the plant-microorganism relationship to use mineral-based phytoremediation strategies with PGPB.

Keywords: Mine tailings, PGPB, A. farnesiana, Phytoremediation, Rhizosphere.

INTRODUCTION
mining is an important economic activity worldwide. In Mexico, it has allowed economic and technological development, contributing with a large part of the raw materials; however, the exploitation of mineral resources leads to the generation of high amounts of mining waste called tailings.

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These tailing are byproducts of grinding and crushing the ore after the minerals of economic interest have been recovered, in the end the waste is deposited in the open air (Ricey, 1989, & Arcega-Cabrera et al., 2009), representing a risk to human health and the environment, due to the generation of potentially toxic elements (PTEs) (Moreno et al., 2010). The composition of this mining waste is heterogeneous, finding potentially toxic metals (PTMs), such as, Pb, Cu, Zn, Fe, and primary and secondary minerals such as: pyrite (FeS2), calcite (CaCO3), jarosite (KFe3[SO4]2(OH)6), anglesite (PbSO4), and copper sulfate (CuSO4) among many others (Romero et al., 2008, & Ramos-Gómez et al., 2012). A viable strategy for its remediation has been the use of plants that have the ability to stabilize PTEs in their rhizosphere; however, the high level of contamination in these sites and the very low survival rate of the plants make their restoration difficult (Chibulike & Obiora, 2014). A. farnesiana resists water stress, accumulates ≥80% of heavy metals in the rhizosphere, adapts to extreme temperatures and pH levels, grows on and around the mine tailings, controls soil erosion, and improves its fertility through symbiosis with rhizobacteria (Maldonado-Magaña et al., 2011). Rhizobacteria, or plant growth promoting bacteria (PGPB) with tolerance to heavy metals, represent a viable and cheap option for the stabilization of metals in the rhizosphere of plants able to grow on or around tailings. The most studied genera as PGPB are; Bacillus sp, Serratia sp, Pseudomonas sp, Enterobacter sp, among others (Ramos-Gómez et al., 2012). Based on the above, we evaluated the capacity of radicular promotion of A. farnesiana using consortiums of PGPB in combination of tailings and amendments.

MATERIALS AND METHODS
roots from at least 30 A. farnesiana plants living around El Fraile, Guerrero, Mexico, were isolated and identified by sequencing the 16S rRNA gene (Chavez-Gonzales, 2017; & Naveed et al., 2014) of 28 bacterial strains that corresponded to the of Bacillus sp (22), Enterobacter sp (5) and Pseudomonas sp (1) genera. In each strain, the Minimum Inhibitory Concentration (MIC) to heavy metals was determined, in Minimum Saline Medium (MSM) supplemented with different concentrations of metallic salts (200-1200 mg/L), Cu2+ (CuSO4), Zn2+ (ZnSO4), and Pb2+ [Pb (NO3)2] and some metalloids as As5+ (NaH2AsO4) and As3+ (NaAsO2). In the same way, the production of direct and indirect secondary metabolites that promote plant growth was determined; indole-3-acetic acid (IAA), solubilization of inorganic phosphorus, and fixation of N2, siderophores, and lytic enzymes, such as lipases, proteases, and amylases (De Souza et al., 2015, Sánchez-López, & Pérez Pazos, 2018). At the same time, approximately 100 kg of substrate representative of the El Fraile tailings were collected, these were selected based on studies and criteria carried out by Flores-Mundo (2002).

In addition, 300 seed pods were collected from A. farnesiana that live around the tailings. The seeds were scarified and disinfected according to Lindsey et al. (2017). Later, they were placed in 56-cavity seedbeds that contained different concentrations of 100, 90, 80, and 70% tailings and the rest of amendment (commercial); the containers were placed under shade cloth. In the following five days, the formed consortia were inoculated, 5 ml each, with the best strains of tolerance to heavy metals and plant promotion; consortium A) included P. putida JM1, Enterobacter sp EAF63, and Bacillus sp EAF2, consortium B), P. putida JM1, Enterobacter sp EAF65, and Bacillus sp EAF2, and finally consortium C), P. putida JM1, Enterobacter sp EAF63, Enterobacter sp EAF65 and Bacillus sp EAF2. All the strains were mixed at a ratio of 1:1 v/v in LB broth at an OD600 nm of 1.3; they were evaluated for 98 days. Later, the seedlings were sacrificed and the differences in the growth and biomass of the root of A. Farnesiana were obtained for each treatment.

RESULTS AND DISCUSSION
in all the strains, the metal tolerance profiles were: Bacillus sp of As3+>Ag1+>Cd2+>Zn2+>Cu2+>Pb2+>Cr6+, Enterobacter sp to
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As$^{3+}$>Pb$^{2+}$>Cd$^{2+}$>Cr$^{6+}$>Ag$^{+}$>Zn$^{2+}$>Cu$^{2+}$. and *P. putida* JM1 of *A. farnesiana* roots, only a statistically significant difference was observed when the seed inoculated with consortium A was planted with 80% of tailing substrate (1.72±0.30, p=0.006).

It should be noted that no previous studies were found where the positive relationship on the rhizosphere of *A. farnesiana* with PGPB in mine tailing substrates was demonstrated, so this study provides information on the importance of plant-microorganism symbiosis for phytoremediation assisted with native PGPB of mining sites. He et al. (2010) describe *B. cereus, Flavobacterium sp.*, and *P. aeruginosa* capable of stimulating the elongation of the root in *Orychophragmus violaceus* seedlings in the presence of Zn.

Table 1: Determination of the MIC and ability to promote plant growth in the strains isolated from the rhizosphere of *A. farnesiana* in the El Fraile, Guerrero, Mexico mine

| Cepa          | Minimum Inhibitory Concentration (mg/L) | Plant growth promotion mechanism |
|---------------|----------------------------------------|---------------------------------|
|               | Ag(NO$_3$)  | K$_2$O$_3$ | H$_2$SO$_4$ | Zn(NO$_3$)$_2$ | Pb(NO$_3$)$_2$ | PbO | Cu$_3$O$_4$ | Na$_2$SO$_4$ | Na$_2$SO$_3$ | K$_2$O$_3$ | AIA (mg/L) | PO$_4$- solubility | Lip | Prot | Amy | N$_2$ | Sid |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 600 | 200 | 1600 | 200 | 1200 | 1200 | 200 | ND | ND | + | ND | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |
| Bacillus sp EAF 1 | 800 | 200 | 200 | 400 | 200 | 800 | 200 | 200 | 600 | 200 | ND | + | + | ND | + | + | ND | ND | ND | ND |

All subtitles ND (not detected), PO$_4$- solubility (phosphate solubility); Lip (lipases), Prot (proteases), Amy (amylases), N$_2$ (fixation of nitrogen), and Sid ( sideroforos).
Table 2: Dry weight of *A. farnesiana* roots in different concentrations of tailings and consortiums

| Concentration Mine tailings (%) | Control         | Consortium A | Consortium B | Consortium C | p*    |
|---------------------------------|-----------------|--------------|--------------|--------------|-------|
| 100                             | 0.7 ± 0.36      | 1.34 ± 0.28  | 1.83 ± 0.15  | 1.62 ± 0.72  | 0.012 |
| 90                              | 0.5 ± 0.26      | 2.64 ± 0.71  | 1.5 ± 0.2    | 3 ± 0        | 0.001 |
| 80                              | 1.06 ± 0.45     | 1.44 ± 0.46  | 1.4 ± 0.57   | 1.26 ± 0.48  | 0.743 |
| 70                              | 0.45 ± 0.26     | 1.98 ± 0.66  | 1.22 ± 0.67  | 2.1 ± 1.75   | 0.078 |

* Obtained by Student's t test, *p* < 0.05 is considered as statistically significant

Table 3: Length of the roots of *A. farnesiana* in different concentrations of tailings and consortiums

| Concentration Mine tailings (%) | Control         | Consortium A | Consortium B | Consortium C | p*    |
|---------------------------------|-----------------|--------------|--------------|--------------|-------|
| 100                             | 1.94 ± 0.57     | 1.58 ± 0.37  | 1.4 ± 0.26   | 1.47 ± 0.41  | 0.321 |
| 90                              | 1.83 ± 0.15     | 1.72 ± 0.43  | 0.73 ± 0.45  | 1 ± 0        | 0.037 |
| 80                              | 0.53 ± 0.20     | 1.72 ± 0.30  | 0.82 ± 0.68  | 1.16 ± 0.25  | 0.006 |
| 70                              | 1.32 ± 0.75     | 1.90 ± 0.55  | 1.1 ± 0.91   | 1.06 ± 1     | 0.404 |

* Obtained by Student's t test, *p* < 0.05 is considered as statistically significant

CONCLUSION

The consortium of *Bacillus sp.*, *Enterobacter sp.*, and *P. putida* is a viable alternative for the development of the *A. farnesiana* root in tailing concentrations of 90 and 80% respectively, which would be a friendly strategy to stabilize sites contaminated with heavy metals.

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