Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8448

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2021) *9*(1), 7-11

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

Research Article

Root Promotion of *Acacia farnesiana* by PGPB in Mine Tailings

Patricio-Hernández A.[1], Ramírez-Peralta A.[2], Romero Ramírez Y.[1], Toledo Hernández E.[1], Urióstegui Acosta M.[3] and Toribio-Jiménez J.[1]*

[1]Laboratory of Molecular Microbiology and Environmental Biotechnology, Faculty of Chemical-Biological Sciences, Autonomous University of Guerrero, Mexico

[2]Laboratory of Microbial Patometabolism, Faculty of Chemical-Biological Sciences, Autonomous University of Guerrero, Mexico

[3]Laboratory of Immunotoxigenomics, Higher School of Natural Sciences, Autonomous University of Guerrero, Mexico

*Corresponding Author E-mail: jeiryjimenez2014@gmail.com

Received: 2.12.2020 | Revised: 10.01.2021 | Accepted: 18.01.2021

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 N_2*, 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.

Cite this article: Patricio-Hernández, A., Ramírez-Peralta, A., Romero Ramírez, Y., Toledo Hernández, E., Urióstegui Acosta, M., & Toribio-Jiménez, J. (2021). Root Promotion of *Acacia farnesiana* by PGPB in Mine Tailings, *Ind. J. Pure App. Biosci. 9*(1), 7-11. doi: http://dx.doi.org/10.18782/2582-2845.8448

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 (Ritcey, 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 (FeS_2), calcite ($CaCO_3$), jarosite ($KFe_3[SO_2]_2[OH]_6$) , anglesite ($PbSO_4$), and copper sulfate ($CuSO_4$) 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), Cu^{+2} ($CuSO_4$), Zn^{+2} ($ZnSO_4$), and Pb^{+2} [$Pb(NO_3)_2$] and some metalloids as As^{+5} (NaH_2AsO_4) and As^{+3} ($NaAsO_2$). 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 N_2, 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 OD_{600} 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 $As^{3+}>Ag^{1+}>Cd^{2+}>Zn^{2+}>Cu^{2+}>Pb^{2+}>Cr^{6+}$, *Enterobacter sp* to

$As^{3+}>Pb^{2+}>Cd^{2+}>Cr^{6+}>Ag^{1+}>Zn^{2+}>Cu^{2+}$, and *P. putida* JM1 of $As^{3+}>Pb^{2+}>Ag^{1+}>Cd^{2+}>Cr^{6+}>Zn^{2+}>Cu^{2+}$, in the detection of direct and indirect mechanisms of plant promotion. The IAA was observed only in 21.4% (6/28), from which 50% were *Enterobacter sp* (6/12). The N_2 fixation was observed in 57% (16/28) of the strains, being the highest *Bacillus sp* with 56% (9/16) and *Enterobacter sp* with 48% (7/16). 21% (6/28) of the strains solubilize phosphates, highlighting *Enterobacter sp* with 66.6% (4/6), *Bacillus sp* with 33.3% (2/6); in *P. putida* JM1 no IAA, fixed N_2, nor solubilizes phosphates were detected. Regarding the production of siderophores, only 57.14% (16/28) produce them, of these 62.5% are *Bacillus sp* (10/16). It was observed that 85% (24/28) of the strains is capable of producing amylases, lipases, and proteases.

Regarding the dry weight of the roots of *A. farnesiana* with the different consortiums and concentrations of tailings, it was determined if there were differences among them, finding that the weight of the roots of the plant from the seeds inoculated with consortium B was higher (1.83±0.15), which is statistically significant when they are germinated with a percentage of tailing substrate of 100% ($p=0.012$) as well as of consortium C (3±0, $p=0.001$) with a 90% substrate.

In the case of the length of the *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. subtilis*, *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 mechanisms						
	$Ag(NO)_3$	$K_2Cr_2O_7$	$3CdSO_4$	$Zn(NO_3)_2$	$Pb(NO_3)_2$	PbO	Cu_2SO_4	$NaAsO_2$	Na_3AsO_4	K_2CrO_4	AIA (ug/l)	PO_{43-} solubility	Lip	Prot	Amy	N_2	Sid
Bacillus sp EAF 1	800	200	200	600	200	1600	200	200	600	200	ND	ND	+	ND	+	ND	ND
B. aryabhattai EAF 2	800	200	800	200	800	1600	200	1200	1200	200	ND	+	ND	ND	+	+	+
B. cereus EAF 3	800	200	200	400	200	800	200	200	600	200	ND	+	+	ND	+	+	+
B. aryabhattai EAF 8	400	200	800	400	200	1600	200	200	1800	200	ND	ND	ND	+	+	+	+
Bacillus sp EAF 11	400	200	800	400	200	1600	200	1200	1200	200	ND	ND	ND	ND	+	ND	ND
B. cereus EAF 15	400	200	200	400	200	800	200	200	1800	200	4,717	ND	+	ND	+	ND	ND
B. cereus EAF 18	400	200	600	400	200	800	200	200	600	200	ND	ND	+	+	+	+	ND
B.cereus EAF 20	800	200	800	400	200	1600	200	800	1800	200	ND	ND	+	ND	+	+	ND
B. aryabhattai EAF 22	400	600	120	400	200	1600	200	400	1800	200	ND	ND	+	+	+	+	+
Bacillus sp EAF 23	800	600	200	400	200	1600	200	200	1800	200	ND	ND	+	+	+	ND	+
Bacillus sp EAF 28	400	600	800	400	200	1600	200	800	1800	200	2,944	ND	ND	+	+	ND	+
B. megaterium EAF 29	800	200	200	400	200	1600	200	800	1200	200	ND	ND	ND	ND	+	ND	+
Bacillus sp EAF 31	800	600	800	200	800	1600	200	200	1800	200	ND	ND	ND	ND	+	ND	+
B.cereus EAF 41	800	200	200	400	200	800	200	200	600	200	ND	ND	+	ND	+	ND	+
B. cereus EAF 42	200	200	200	400	200	1600	200	200	1800	200	ND	ND	+	ND	+	+	ND
B. megaterium EAF 43	800	200	800	400	200	1600	200	200	1800	200	ND	ND	ND	ND	+	ND	ND
B. cereus EAF 48	800	200	800	400	200	1600	200	800	1800	200	ND	ND	+	ND	+	+	ND
B. megaterium EAF 50	800	200	200	400	200	1600	200	200	1200	200	ND	ND	ND	ND	+	+	+
B. licheniformis EAF 68	400	600	600	200	200	1600	200	1200	1200	200	ND	ND	ND	ND	+	ND	+
B. cereus EAF 73	400	600	800	400	200	800	200	1200	1800	200	ND	+	+	ND	+	+	+
B. megaterium EAF 75	800	200	800	200	200	1600	200	200	1200	200	ND	+	ND	+	ND	+	ND
E. hormaechei EAF 52	800	200	800	400	200	1600	400	1800	1800	200	8,107	ND	ND	ND	ND	ND	ND
E. cloacae EAF 60	400	600	1200	400	800	1600	400	1800	1200	200	15,934	ND	ND	ND	ND	+	+
Enterobacter sp EAF 63	400	200	800	400	200	1600	400	1200	1800	200	11,697	+	ND	+	+	+	+
E. hormaechei EAF 65	1200	200	800	400	200	1600	400	1800	1200	200	11,388	+		+	ND	+	+
E. cloacae EAF 67	400	200	1200	400	200	1600	200	1800	1800	200	17,220	ND	ND	ND	+	+	+
P. putida JM1	8000	600	800	400	800	1600	300	800	1200	200	ND	ND	ND	ND	+	ND	ND

All subtitles ND (not detected), PO_{43-} 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.

Acknowledgements

We thank the QBP. Brisa Luna Juarez and the MCB. Daniel Chávez Gonzales for the technical support of this project.

REFERENCES

Arcega-Cabrera, F., Armienta, M. A., Daesslé, L. W., Castillo-Blum, S. E., Talavera, O., & Dotor, A. (2009). Variations of Pb in mine-impacted tropical river, Taxco, Mexico: Use of geochemical, isotopic and statical tools'. Applied Geochemistry. 24. Pp. 162-171. doi: 10.1016/j.apgeochem.2008.09.015.

Chávez-Gonzales, J. D. (2017). Evaluación de la fitoestabilización de As, Cd y Pb asistida por rizobacterias aisladas en los jales El Fraile de Taxco, Guerrero 'Tesis de grado no publicada'

Chibulike y Obiora, (2014). Heavy metal polluted soils: Effect on plants and bioremediation methods. Hindawi Publishing Corporation Applied and Environmental Soil Science. Pp. 12. Doi: 10.1155/2014/752708.

de Souza, R., Meyer, J., Schoenfeld, R., & da Costa, P. (2015). Characterization of plant growth-promoting bacteria associated with rice cropped in iron-stressed soils. *Annals of Microbiology*. *65*(2), pp. 951-964. doi: 10.1007/s13213-014-0939-3.

Flores-Mundo, N. (2002). Caracterización Químico-Minerlógica de los jales El Fraile, Taxco de Alarcón Guerrero, 'Tesis de Licenciatura no publicada' UAG. Taxco el Viejo, Gro.

He, C. Q., Tan, G. E., Liang, X., Du, W., Chen, Y. L., Zhi, G. Y., & Zhu, Y. (2010). Effect of Zn-tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. *Appl Soil Ecol*, *44*, pp. 1-5. doi: 10.1016/j.apsoil.2009.07.003.

Lindsey, B. (2017). Standardized Metod for High-throughput Sterilization of *Arabidosis* Seeds. *Journal of Visualized Experiments*. *128*, *doi: 10.3791/56587*.

Maldonado-Magaña, A. (2011). Lead bioaccumulation in *Acacia farnesiana* and its effect on lipid peroxidation and glutathione production.' *Pland and sold*. *339*, pp. 377-389. doi: 10.1007/s11104-010-0589-6.

Moreno, (2010). Biomonitoring of metal in children living in a mine tailings zone in Southern Mexico: A pilot stud. *International Journal of Hygiene and*

Environmental Health. *Elsevier GmbH*, *213*(4), pp. 252–258. doi: 10.1016/j.ijheh.2010.03.005.

Naveed, M., Mubeeb, S., Khan, S., Ahmed, I., Khalid, N., Rasul-Suleria, H. A., Bano, A., & Mumtaz, A. S. (2014). Identification and characterization of rhizospheric microbial diversity by 16S ribosomal RNA gene sequencing. *Brazilian Journal of Microbiology*, *45*(3), pp. 985-993.

Ramos-Gómez, M., Avelar, J., Medel-Reyes, A., Yamamoto, L., Godínez, L., Ramírez, M., Guerra, R., & Rodríguez, F. (2012). Movilidad de metales en jales procedentes del distrito minero de Guanajuato, *México. Rev. Int. Contam. Ambie*. *28*(1), pp. 49-59.

Ritcey, G. M. (1989). Tailings management, problems and solutions in the mining industry. Waste Management and Research. *Firts published january 1*, 1991. doi: 10.1177/0734242X9100900110.

Romero, F. M., Armienta, M. A., Gutiérrez, M. E., & Villaseñor, G. (2008). Factores geológicos y climáticos que determinan la peligrosidad y el impacto ambiental de jales mineros. *Revista Internacional de Contaminación Ambiental*, *24*(2), pp. 43–54.

Sánchez-López, B.Y., Pérez Pazos, J. V. (2018). Caracterización y evaluación de PGPRs sobre el crecimiento de plántulas de *Dioscorea rotundata in vitro*. *Agronomía Costarricense 42*(2), 75-91.