Journal of Positive Psychology & Wellbeing
2023, Vol. 7, No. 1, 942 – 949

http://journalppw.com
ISSN 2587-0130

Production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) by Burkholderia cepacia as an indicator of cadmium contamination

Alexander Pérez Cordero[1*], Donicer E. Montes Vergara[2], Yelitza Aguas Mendoza[3]

[1] *Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia*
alexander.perez@unisucre.edu.co
https://orcid.org/0000-0003-3989-1747
[2] *Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia*
donicer.montes@unisucre.edu.co
https://orcid.org/0000-0002-2860-0505
[3] *Universidad de Sucre, Facultad de Ingeniería, Colombia*
yelitza.aguas@unisucre.edu.co
https://orcid.org/0000-0003-4880-4510
**corresponding author*: alexander.perez@unisucre.edu.co

ABSTRACT

The most common sources of cadmium are volcanic, biological and anthropogenic processes such as industry, manufacturing, metallurgy and fertilizer use. In Colombia, there is evidence that cadmium is introduced into the soil by the use of agrochemicals in agriculture, contact with waste water, the use of irrigation water containing this element or by the deposition on the surface of wet and dry particles that are carried by the air from industrial processes. This metal can be absorbed by plants in contaminated soils and its incorporation into the food chain, which motivated this study to isolate rhizospheric bacteria; to evaluate in vitro the capacity of these bacteria to tolerate different cadmium concentrations and the capacity to produce the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) in vitro. The cadmium values in the rhizosphere were 0.95 ± 2.21 mg/kg and, according to international reference values, correspond to soils in the highly toxic category. The results of the in vitro bacterial tolerance test showed that the maximum tolerance capacity was 500 ppm Cd. Furthermore, these morphotypes showed qualitative siderophore production activity. The results of the identification by sequencing of the 16S DNAr gene showed high homology with the bacterium Burkholderia cepacia, which according to several studies corresponds to a bacterium with the capacity to produce different heavy metals such as cadmium and lead and the capacity to promote growth in plant species that grow in environments contaminated with these metals.

Keywords: Cadmium, bacteria, rhizosphere, enzyme, tolerance, tolerance.

1. INTRODUCTION

According to Esquivel et al. (2013), the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme or ACC deaminase is a mechanism used by some plant growth-promoting bacteria (PGPR) to promote the growth of plants influenced by environmental stress, which brings them two important advantages: decreasing ethylene concentrations in the plant and increasing the availability of ammonium in the rhizosphere. Thus, the activity of the ACC deaminase enzyme improves plant nutrition and resistance to stress factors. Plant-associated rhizospheric bacteria play a fundamental role in the adaptation and defenses, contributing significantly to sustainable agriculture and its commercialization through cleaner technologies that help to conserve resources. Several studies infer that plant-associated rhizospheric bacteria play an important role in increasing plant biomass, carbohydrate and protein content, photosynthetic pigments, modulation of metabolite expression, and modulation of metabolite expression over exogenous hormones applied to improve traits associated with low yields caused by stress (Beneduzi et al., 2008).

Many of the bacteria are known as plant growth promoting rhizobacteria (PGPR) which includes bacteria belonging to genera Acinetobacter, Agrobacterium, Arthobacter, Azotobacter, Azospirillum, Burkholderia, Bradyrhizobium, Rhizobium, Frankia, Serratia, Thiobacillus, Pseudomonads and Bacillus (Velasco et al., 2020) the interactions established between root-rhizosphere and rhizobacteria have evolved over time in which the parties involved have developed abilities that allow for adaptive capacity (Velasco et al., 2020). The processes of colonization and stimulation by rhizobacteria are subject to host recognition mechanisms and molecular signaling processes between bacteria and host plant (Esquivel et al., 2013).

When a plant species comes into contact with toxic compounds (heavy metals), it produces a high concentration of endogenous ethylene. Ethylene is an essential hormone that is naturally produced in plants for the growth and senescence of plants, flowers and fruits, it is produced endogenously which induces important physiological changes in the plant, as it serves as a signaling molecule that activates the transcription of several genes that are associated with reproductive success and organ longevity, regulating the lifespan of plants (Belimov et al., 2005).

However, when the plant is drastically exposed to abiotic stress conditions ethylene levels increase having a detrimental effect on the plant (Etesami et al., 2015; Jha & Saraf, 2015). Rhizospheric bacteria have been reported to produce the microbial derived enzyme 1-amino cyclopropane-carboxylate deaminase (ACC), which is key in the metabolism of α-ketobutyrate and ammonia, thereby decreasing high ethylene levels in host plants and providing resistance to various stresses (Glick, 2014). This hormone is widely distributed in the general Achromobacter sp, Alcaligenes sp, Azospirillum sp, Bacillus sp, Burkholderia sp (Onofre-Lemus et al., 2009), Rhizobium, Rhodococcus; as well as Klepsiella oxytoca, Methylobacterium fujisawaense, Pseudomonas putida and Sinorhizobium meliloti (Jorquera et al., 2012). Study by Pramanik et al. (2018) reports rhizobacteria capable of resisting heavy metals (cadmium, lead, arsenic, nickel and mercury) by promoting plant growth through phosphate solubilization, IAA production, ACC deaminase and nitrogen fixation.

Among the metals, cadmium (Cd) is of great importance due to its strong impact on human health (Jarup, 2003; Weisberg et al., 2003), as it causes damage to vital biological activities that can be irreversible in different organisms (Tietzel and Parsek 2003). For example, Cd_2^+ causes severe lung, kidney and bone damage (Sinott, 2001), as well as damage to the nervous system (Navarro et al., 2006), and causes carcinogenic, embryotoxic, teratogenic and mutagenic effects (Majumder et al., 2003). Based on the above, the present study was carried out to evaluate in vitro the production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) by rhizospheric bacteria with the ability to tolerate high concentrations of cadmium in the form of $CdCl_2$.

2. MATERIALS AND METHODS

2.1 Cadmium concentration in soil and plant samples. To determine the total cadmium by tissues, 0.5 g of dry material was taken and an acidic HNO_3/H_2O_2 mixture (5+2 mL) was added to it. On the other hand, 0.5 g of previously dried soil was taken and 10 mL of 65% HNO3 was added. Both soil and plant samples were processed in a Milestone ETHOS TOUCH 127697 series microwave oven and total cadmium was analyzed by cold vapor atomic absorption spectrophotometry according to procedures described in [20].

2.2 Isolation of rhizospheric bacteria. Ten g of rhizospheric soil were dissolved in 90 ml of sterilized water in an Erlenmeyer of 250 ml volume were shaken for approximately 2 hours by

120 rpm, after this time dilutions of 10^{-1} to 10^{-10} were prepared, in which 0.1 mL (100 µL) of each dilution was inoculated in three replicates on the surface of the following semi-specific culture media for each group of rhizobacteria proposed by Argüello-Navarro et al, al (2016) as follows: a) NFb (semi-specific for Azospirillum spp); b) JMV (semi-specific for Burkholderia spp); c) LGI (semi-specific for Gluconacetobacter spp); d) JNFb (semi-specific for Herbaspirillum spp) and King B Agar (semi-specific for Pseudomonas spp.).

2.3 In vitro remediation tests. Tolerance of rhizospheric bacteria to different concentrations of $CdCl_2$ will be performed in tris-MMT minimal medium (Rathnayake et al., 2013). The initial concentration of Cd and as used in the present study will be 10 mg/L and from these, metal concentrations up to 500 MG/mL were prepared. Aliquots of log-phase rhizospheric bacterial suspensions were inoculated onto MMT medium. MMT medium without CdCl2 and as was used as a control. The experiment was performed in triplicate, which was incubated in shaking at 150 rpm at 32 °C for 120 hours (Zhang et al., 2011). Bacterial growth was determined by turbidimetry at 600 nm every hour for four days.

2.4 In vitro activity of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) enzyme. Stretch seeding was performed in Dworkin and Foster (DF) minimal medium (Belimov et al., 2001, El- tarabily, 2008), supplemented with 0.3 g/L 1-aminocyclopropane carboxylic acid (ACC) as the sole nitrogen source. Incubation was carried out for 5 days at 30°C. Boxes showing bacterial growth were considered as ACC deaminase producers (Andy et al., 2020).

2.5 Identification of Cd-tolerant bacteria. Genomic DNA extraction was performed according to the protocol described by (Oliveira et al., 2013). Universal primers of the 16S rDNA region of the gene encoding the 16S rRNA small ribosomal subunit molecule were used to identify bacteria with Cd-tolerance activity. The specific primers used for each of the classes belonging to the bacterial domain (alpha, beta, gamma proteobacteria and Firmicutes) corresponded to those proposed by (Oliveira et al., 2013). The amplification products were sent for purification and sequencing to Macrogen Korea. Once the nucleotide sequences were obtained, the homologous sequences were searched against the sequences stored in the National Center for Biotechnology Information (NCBI) database. Base alignment was performed with Clustal W software and analysis and correction with Mega 4.0. (Tamura et al., 2007). Using the same programme, the method used to evaluate phylogenetic inferences was determined.

3. RESULTADO Y DISCUSIÓN

As for cadmium values per plant tissue, values ranging from 0.95 to 2.21 mg/kg of tissue were found (Figure1), with the highest amount of this metal found in the root (2.21 mg/kg) and the lowest in the fruit (0.95 mg/kg). At the international level for vegetables, the permitted values correspond to 0.05 - 0.5 mg/kg of tissue, which indicates that the values found in the different tissues are above those permitted at the international level according to the standard in force for each specific situation (Kabata and Pedia, 2001).

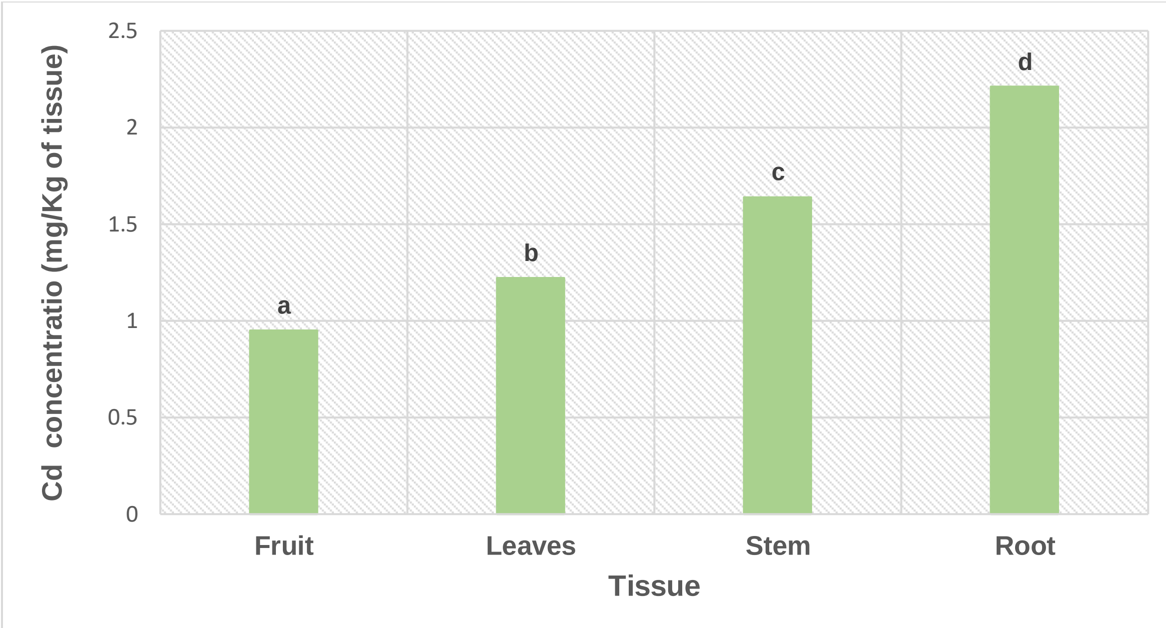

Figure 1. Cadmium concentration values per plant tissue.

Figure 2 shows the activity of the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) by the rhizospheric bacterium *Burkholderia cepacia* at a minimum Dworkin and Foster (DF).

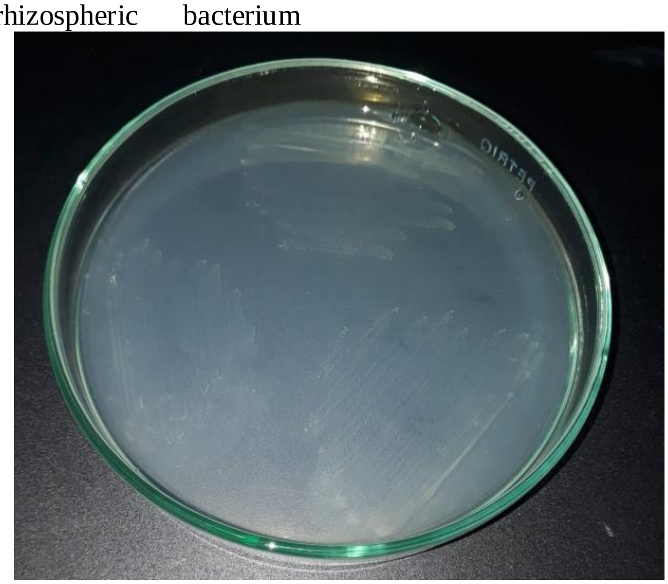

Figure 2. In vitro production of the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) by the rhizospheric bacterium *Burkholderia cepacia* at a minimum Dworkin and Foster (DF) (Belimov et al., 2001, El- tarabily, 2008).

Sequencing results from PCR product sequencing of the 16S DNAr gene using eubacterium-specific oligonucleotides identified *Burkholderia cepacia* as the species of rhizospheric bacteria with the highest cadmium tolerance capacity and was the species that showed in vitro production of the enzyme. 1-aminocyclopropane-1-carboxylic acid deaminase (ACC).

Figure 3 shows the growth curve of the rhizospheric bacterium Burkholderia cepacia at different concentrations of cadmium in the form of $CdCl_2$. By evaluating the minimum and maximum growth at $CdCl_2$, it was found that B. cepacea has the ability to tolerate different concentrations of $CdCl_2$ between 100 and 500 ppm.

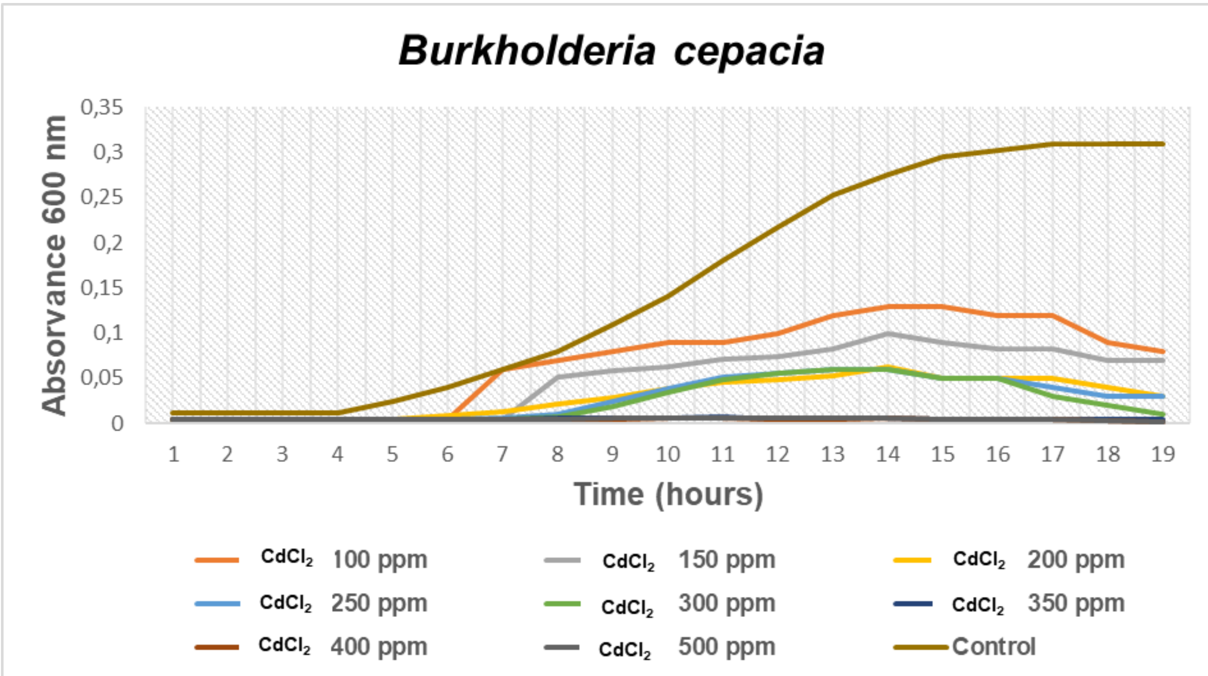

Figure 3. Growth curve assay of B. cepacea in different concentrations of $CdCl_2$.

Figure 3 shows that B. cepacea showed tolerance up to 150 ppm up to 18 hours into the in vitro test compared to the control, while at concentrations of 200 to 500 ppm $CdCl_2$ showed lower tolerance up to 17 hours into the experiment.

Cadmium is a heavy metal that has a marked tendency to bioaccumulate in plants, causing imbalances in nutrition and water transport processes (Singh and Tewari, 2003). The ability of plants to capture cadmium is influenced by the concentration of the metal in the soil and its bioavailability, which depends on the presence of organic matter, pH, redox potential, temperature and the concentrations of other elements. In the case of cadmium, it competes with nutrients such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni) by competing for the same carrier protein (Di Toppi et al., 1999).

According to scientific reports, endophytic bacteria associated with copper-tolerant plants (Pantoea agglomerans Jp3-3 and Pseudomonas thivervalensis Y1-3-9) increase plant growth and copper accumulation of Brassica napus (Zhang et al., 2011). The genetically transformed bacterium Pseudomonas asplenii AC increases germination and plant growth of reed (Phragmites australis, Poaceae) plants in the presence of copper and aromatic hydrocarbons such as creosote, an important fact for phytoremediation because reed is a plant traditionally used to purify wastewater as it is tolerant to heavy metals (Reed et al., 2005). Transgenic tomato plants expressing the enzyme ACC deaminase (controlled by the PRB-1b promoter) can accumulate a large amount of metal within the plant tissue and decrease the deleterious effect of heavy metals such as Cd, Co, Cu, Mg, Ni, Pb or Zn on plant development compared to non-transgenic plants (Grichko et al., 2000).

Several studies concerning the use of bacteria that in association with plants can resist heavy metals such as cadmium, have contributed to the selection of different strains capable of carrying out this activity, some of them are Bacillus subtilis (Zaidi et al., 2006), Pseudomonas aeruginosa (Ganesan, 2008), Burkholderia cepacia (Li et al., 2007), Brevibacterium halotolerans (Abou et al., 2006), Bradyrhizobium sp., Pseudomonas sp., Ochrobactrum cytisi (Dary et al., 2010), Pseudomonas fluorescens ACC9 (Dell'Amico et al., 2008). Madhaiyan and Poonguzhali (2007), showed that the bacterium Burkholderia sp reduces the accumulation of cadmium and lead in roots and shoots of tomato seedlings as well as the metal available in the soil and this is due to the uptake and bioaccumulation of the metal by the bacterium.

Rhizospheric bacteria are able to enhance plant growth by several mechanisms including: siderophore production, 1-aminocyclopropane-1-carboxylic deaminase (ACC), indole-3-acetic acid (IAA) and phosphate (P) solubilisation and nitrogen fixation (Rajkumar et al., 2009; Estrada et al., 2002; Dutta and Gachhui, 2006; Dawwam et al., 2013).

On the other hand, in vitro studies on bacterial resistance to cadmium (Cd) showed that bacterial species of the Burkholderia sp. group possess the ability to solubilize in vitro metals such as Pb and Cd in different concentrations and to accumulate these two metals in maize and tomato plant tissues, and the subsequent stimulation of the growth of these plants in vivo (Chun et al., 2008). The genus of Burkholderia bacteria represents a group of eighteen related species that are currently of interest because of their extraordinary versatility as plant pathogens, saprophytes, biocontrol agents, bioremediation and human pathogens. These bacteria are naturally abundant in soil, water and on the surface of different plant species, and have the ability to metabolize a wide range of organic compounds as a source of carbon and energy.

4. CONCLUSION

The results of the identification showed a high homology with the bacterium Burkholderia cepacia, which according to several studies corresponds to a bacterium with the capacity to grow in different environments contaminated with heavy metals such as cadmium and lead, it also has the capacity to promote growth in plant species that grow in environments contaminated with these metals, and possibly the tolerance to different concentrations of cadmium is due to the presence of the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACC).

5. AUTHOR CONTRIBUTION

Alexander Pérez Cordero: planning and execution of the experimental part. Donicer Montes V and Yelitza Aguas M, data analysis, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

6. CONFLICT OF INTEREST

The authors of the manuscript declare that there is no conflict of interest in the presentation and publication.

7. REFERENCIAS

[1] Abou A, M., Abu Z, I., y El Shakh A, E. (2011). Accumulation of heavy metals in crop plants from Gaza Strip, Palestine and study of the physiological parameters of spinach plants. Journal of the Association of Arab Universities for Basic and Applied Sciences 10: 21 - 27.

[2] Andy, A. K., Masih, S. A., & Gour, V. S. (2020). Isolation, screening and characterization of plant growth promoting rhizobacteria from rhizospheric soils of selected pulses. *Biocatalysis and Agricultural Biotechnology* 27: 101685.

[3] Belimov, A. A., Safronova, V. I., Sergeyeva, T. A., Egorova, T. N., Matveyeva, V. A., Tsyganov, V. E., Dietz, K. J. 2001. Characterization of plant growth promoting

rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology* 47(7):642-652.

[4] Beneduzi A., D. Peres, P.B. da Costa, M.H.B. Zanettini, L.M.P. Passaglia,(2008). Genetic and phenotypic diversity of plant-growth-promoting bacilli isolated from wheat fields in southern Brazil, *Research in Microbiology* 159: 244e250.

[5] Belimov, A., Hontzeas, N., Safronova, V., Demchinskaya, S., Piluzza, G., Bullitta, S.; Glick B. (2005). Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica junceaLCzern). ***Soil** Biol Biochem* 2 (37):241–250.

[6] Chun, Y.J., XIA-FANG, S., MENG, Q., QING-YA, W. (2008). Isolation and characterization of a heavy metal resistant Burkholderia sp. from heavy metal contaminated paddy feld soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. *Chemosphere* 72(2):57-64.

[7] Dawwam, G., Elbeltagy, A., Emara, H., Abbas, I., y Hassan, M. (2013). Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Annals of *Agricultural Sciences* 58(2): 195-201.

[8] Dary, M., Chamber, P., Palomares, A. J. y Pajuelo, E. (2010). In situ phytostabilisation of heavy metal polluted soils using Lupinus luteus inoculated with metal resistant plant-growth promoting rhizobacteria. *J. Hazard. Mater* 177(1-3): 323-330.

[9] Dell'Amico, E., Cavalca, L. y Andreoni, V. (2008). Improvement of Brassica napus growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biology and Biochemistr*y 40(1): 74-84.

[10] Di Toppi, L. S. y Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environmental and Experimental Botany* 41(2):105-130.

[11] Dutta, D., y Gachhui, R. (2006). Novel nitrogen-fixing Acetobacter nitrogenifigens sp. nov., isolated from Kombucha tea. *International Journal of Systematic and Evolutionary Microbiology* 56(8):1899-1903.

[12] El-Tarabily, K. A. 2008. Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. *Plant and Soil* 308(1-2): 161-174.

[13] Esquivel-Cote, Rosalba, Gavilanes-Ruiz, Marina, Cruz-Ortega, Rocío, & Huante, Pilar. (2013). Importancia agrobiotecnológica de la enzima ACC desaminasa en rizobacterias, una revisión. *Revista fitotecnia mexicana* 36(3): 251-258. Recuperado en 17 de febrero de 2023, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802013000300010&lng=es&tlng=es

[14] Estrada, P., Mavingui, P., Cournoyer, B., Fontaine, F., Balandreau, J. y Caballero J. (2002). A N2-fixing endophytic Burkholderia sp. Associated with maize plants cultivated in Mexico. *Canadian Journal of Microbiology* 48 (4): 285–294.

[15] Grichko V P, B Filby, B R Glick (2000). Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. J. *Biotechnol* 81:45-53.

[16] Kabata, A., y Pendias, H. (2001). Trace Elements in Soils and Plants. New York Washington, D.C. CRC Press LLC.

[17] Järup, L., y Akesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology* 238(3): 201 -208.

[18] Li, W.C., Ye, Z.H., y Wong, M.H. (2007). Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyperaccumulating plant, Sedum alfredii. *Journal of Experimental Botany* 58(15-16): 4173-4182.

[19] Madhaiyan, M.; Poonguzhali, S.; Sa, T. (2007). Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (Lycopersicon esculentum L.). *Chemosphere* 69(2): 220-228.

[20] Majumder, S., Ghoshal, K., Summers, D., Bai, S., Datta, J., y Jacob, S. (2003).

Chromium (VI) down regulates heavy metal induced metallothionein gene transcription by modifying transactivacion potential of the key tran-scription factor, metal res-ponsive transcription factor 1. *Journal of Biologycal Chemistry* 278(28): 26216-26226.

[21] Marrugo, J., Durango, J., Pinedo, J., Olivero, J.; Díez, S. Phytoremediation of mercury-contaminated soils by Jatropha curcas. *Chemosphere* 2015 (127): 58-63.

[22] Navarro, J., Aguilar, A., y López, J. (2007). Aspectos bioquímicos y genéticos de la tolerancia y acumulación de metales pesados en plantas. *Ecosistemas* 16 (2): 10-25.

[23] Oliveira, M. N.; Santos, T. M.; Vale, H. M.; Delvaux, J. C.; Cordero, A. P.; Ferreira, A. B.; Moraes, C. A. (2013). Endophytic microbial diversity in coffee cherries of Coffea arabica from southeastern Brazil. *Can J Microbiol* 2013(59): 221-230.

[24] Rajkumar, M., Noriharu, A. y Freitas, H. (2009). Endophytic bacteria and their potential to enhance heavy metal phytoextraction. *Chemosphere* 77(2): 153-160.

[25] Rathnayake, I.V.N.; Mallavarapu, M.; KrishnamurtI, G.S.R.; Bolan N.S., y Naidu R. (2013). Heavy metal toxicity to bacteria – Are the existing growth media accurate enough to determine heavy metal toxicity. *Chemosphere* 90:1195-1200.

[26] Reed M L E, B G Warner, B R Glick (2005). Plant growth-promoting bacteria facilitate the growth of the common reed *Phragmites australis* in the presence of copper or polycyclic aromatic hydrocarbons. *Curr. Microbiol* 51:425-429.

[27] Singh, P. K., y Tewari, R. K. (2003). Cadmium toxicity induced changes in plant water relations and oxidative metabolism of Brassica juncea L. plants. Journal of *Environmental Biology* 24(1): 107-112.

[28] Shin, M., Shim, J., You, Y., Myung, H., Bang, K.S., Cho, M., Kamala-Kannan, S., y Oh, B.T. (2012). Characterization of lead resistant endophytic Bacillus sp. MN3-4 and its potential for promoting lead accumulation in metal hyperaccumulator Alnus firma. *Journal of Hazardous Materials* 199-200: 314-320.

[29] Tamura, K.; Dudley, J.; Nei, M.; Kumar, S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007(24):1596-1599.

[30] Tietzel, G., y Parsek, M. (2003). Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. *Applied and Environmental Microbiology* 69(4): 2313-2320.

[31] Velasco-Jiménez, Antonio, Castellanos-Hernández, Osvaldo, Acevedo-Hernández, Gustavo, Aarland, Rayn Clarenc, & Rodríguez-Sahagún, Araceli. (2020). Bacterias rizosféricas con beneficios potenciales en la agricultura. *Terra Latinoamericana 38*(2): 333-345. Epub 20 de junio de 2020.https://doi.org/10.28940/terra.v38i2.470

[32] Weisberg, M., Joseph, P., Hale, B., y Beyersmann, D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology* 192(2-3): 95-117.

[33] Zaidi, S., Usmani, S., Singh, BR., y Musarrat, J. (2006). Significance of Bacillus subtilis strain SJ-01 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea. *Chemosphere* 64(6): 991–997.

[34] Zhang, Y.F.; He, L.Y.; Chen, Z.J.; Zhang, W.H.; Wang, Q.Y.; Qian, M.; Sheng, X.F. 2011. Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape Journal of Hazardous *Materials* 186:1720–1725.

[35] Zhang Y F, L Y He, Z J Chen, Q Y Wang, M Qian, X F Sheng (2011). Characterization of ACC deaminase-producing endophytic bacteria isolated from copper-tolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus*. *Chemosphere* 83(1):57-62.