Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i14.6098

EFFICIENCY OF *BACILLUS CEREUS* IN THE PRODUCTION OF SIDEROPHORUS AT DIFFERENT CADMIUM CONCENTRATIONS

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

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

***Corresponding Author:** Alexander Pérez Cordero
*Universidad de Sucre, Facultad de Ciencias Agropecuarias, Sincelejo, Sucre, Colombia,
Email: alexander.perez@unisucre.edu.co

Submitted: 20 February 2023; Accepted: 29 April 2023; Published: 06 June 2023

ABSTRACT
Environmental contamination by cadmium has increased as a consequence of the increase in industrial activity that has taken place at the end of the 20th century and the beginning of the 21st century, progressively affecting different ecosystems and public health. The present study aimed to evaluate the efficiency of *Bacillus cereus* GU05811 to produce siderophores in the presence of different cadmium concentrations. The results infer the efficiency of this bacterium to produce siderophore at a concentration of 600 mg/L cadmium and it becomes a biological resource as a possible future use to contribute to reduce this metal in contaminated environments.

Keywords: Bacteria, cadmium, siderophore production, tolerance.

INTRODUCTION
Heavy metals, such as lead (Pb), cadmium (Cd) and zinc (Zn), have gained global attention due to recent research linking them to health risks when contaminated food is consumed (De Los Santos et al., 2019).
Soil remediation technology is based on physicochemical and biological approaches, the latter of which is called bioremediation because it uses the metabolic capabilities of living organisms such as bacteria and fungi to purify soil. The most commonly used bioremediation processes include adsorption, precipitation, leaching and evaporation of heavy metals (Hernández Caricio et al., 2022). Among the micro-organisms reported to be used in heavy metal bioremediation processes, bacteria are indicated as the main biological agents (Valls and De Lorenzo, 2002). These micro-organisms are a potential resource to enhance heavy metal phytoremediation. Various processes, such as methylation, sorption, leaching and precipitation, have the potential to increase or improve the rate of metal recovery in phytoremediation procedures (Ma et al., 2011; Rajkumar et al., 2012).

Among these strains, the genus Bacillus has been widely studied due to its high diversity and distribution in agricultural systems including soil, water and plants. Based on the above, it was proposed as a strategy to evaluate in vitro the ability of *Bacillus cereus* GU05811 strain to tolerate different cadmium concentrations.

RESULTS AND DISCUSSION

Strain used. The reference strain used was identified by sequencing technique as *Bacillus cereus* GU05811, isolated from soils contaminated with mercury and isolated from plant species *Paspalum arundinaceum*, which is stored in the genomic bank of the microbiological research laboratory of the University of Sucre.

In vitro evaluation of cadmium tolerance of *Bacillus cereus* GU05811. The in vitro evaluation of the cadmium tolerance capacity was carried out using the protocol proposed by Pérez et al. Bacterial isolates were seeded on nutrient agar supplemented with CdCl2, at concentrations of 100, 200, 300, 400, 400, 500, 600 mg/L; then, they were incubated at 32°C, for 120 hours, as suggested by (Sorkhoh et al. 2010).

Siderophore production by *Bacillus cereus* GU05811. Siderophore production was evaluated on chromium azurol-S (CAS) medium proposed by Schwyny and Neilands (1987) and adjusted by Torres et al. (2019). *Bacillus cereus* GU05811 was inoculated from the growth of the strain grown on the surface of gar nutrient supplemented with different concentrations of cadmium and incubated for 7 days at 30°C.

Molecular identification. Genomic DNA extraction and rRNA amplification with specific oligonucleotides for the bacterial domain of cadmium-tolerant bacteria was performed using the methodology proposed by Oliveira et al. (2013). The amplification products obtained were purified and sequenced at Macrogen Korea. The sequences obtained were compared with those stored in Genbank. Base alignment was performed in the Clustal w program, analysis and correction in the Mega 5 program, phylogenetic inferences were obtained by the maximum similarity method, based on the kimura-2-parameter model (Perez et al., 2016).

RESULTS AND DISCUSSION

The result of rRNA gene sequencing of endophytic bacteria resistant to mercury concentrations isolated from plant species according to the study carried out by Perez et al., (2016), on "Endophytic bacteria associated with the genera Cyperus and Paspalum in mercury-contaminated soils" is shown in figure 1.

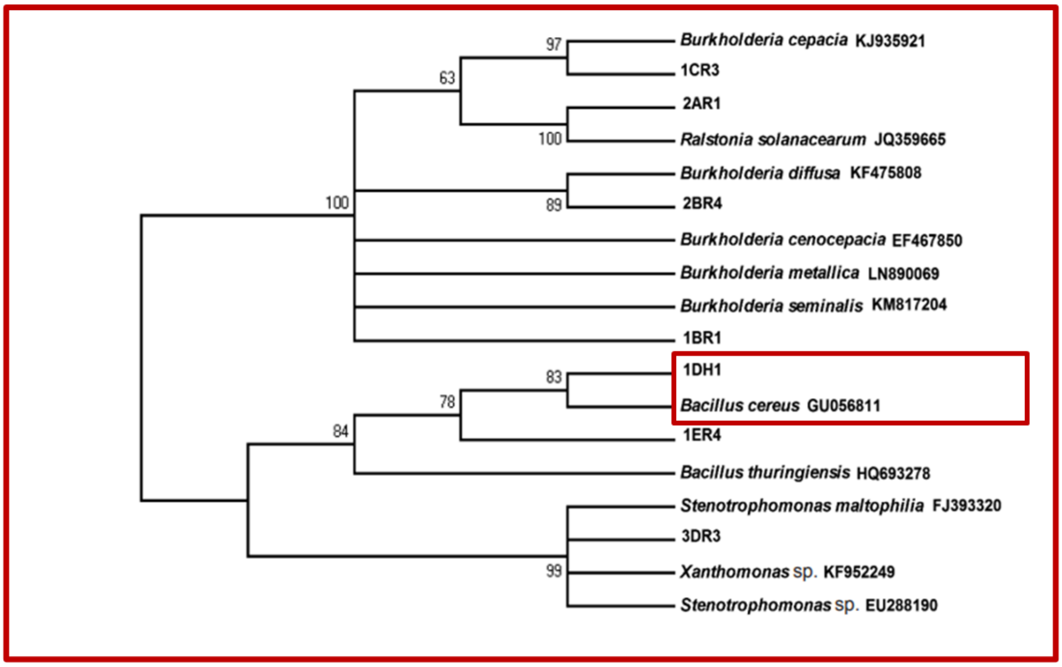

Figure 1. Phylogenetic analysis of 16S rRNA gene of endophytic bacteria isolated from plant tissues grown in mercury-contaminated soils and which is part of the genome bank of the microbiological research laboratory of the University of Sucre. Source: Pérez et al., (2016).

The maximum in vitro cadmium tolerance capacity of *Bacillus cereus* GU05811 was 800 mg/L and the siderophore production capacity was 600 mg/L as shown in figure 2.

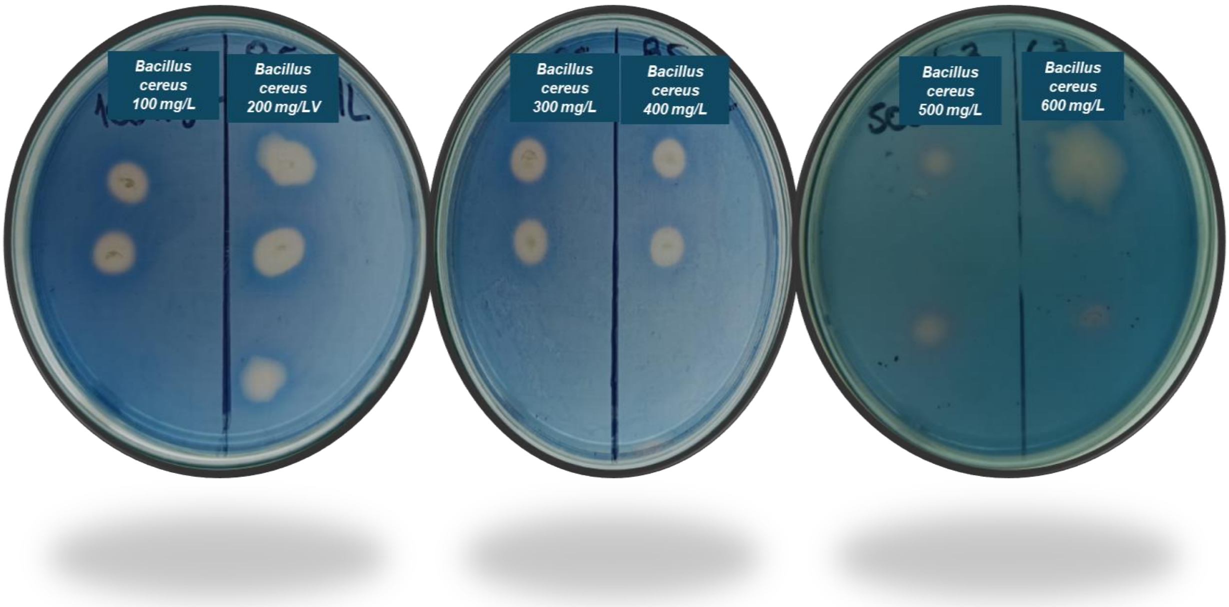

Figure 2. In vitro production of siderophore by *Bacillus cereus* GU05811 at different cadmium concentrations.

Some bacteria produce and excrete siderophores which are iron complexing molecules, many of which have a high affinity for heavy metals, in *Pseudomonas aeruginosa* and *Alcaligenes eutrophus* the synthesis of siderophores is induced by heavy metals in the presence of high iron concentrations (Gilis et al, 1996). On the other hand, some bacteria are capable of decreasing the solubility of metals and their mobility, reducing their phytotoxicity (Lasat, 2000), perhaps this characteristic is attributed to or related to the production of biosurfactants.

According to Luo et al. (2011), the tolerance to high concentrations of metals shown by endophytes isolated from plants growing in contaminated environments is due to the fact that they are adapted to living under conditions of constant metal stress.

According to Rajendran et al, (2003), metals bind to the cell surface through mechanisms that include electrostatic interactions, Van de Waals forces, covalent bonding, redox interactions, extracellular precipitation or a combination of these processes; the negatively charged groups (carboxyl, hydroxyl and phosphoryl) of the bacterial cellular network adsorb the metal ions and these are retained.

According to the evidence found, Bacillus has been reported as an endophytic bacterium, resistant to metals, such as cadmium, in association with the plant species Solanum nigrum (Luo et al. 2011) and nickel, in Oriza sativa (Pérez et al. 2015).

On the other hand, work by Li et al. (2020), in evaluating the tolerance and removal of chromium by isolating two strains of *Bacillus cereus*, *B. cereus* D and 332, respectively, showed that *B. cereus* D achieved 87.8% removal of Cr(VI) over a 24-hour period, starting from an initial load of 2 mM Cr(VI).

Bacillus cereus GU05811 is an endophytic bacterium isolated from roots of the plant species Paspalum arundinaceum adapted to soil with high mercury concentrations in soils of southern Bolivar in Colombia. Evidence from in vitro evaluation demonstrated the ability of this strain to tolerate up to 400 ppm of mercury in the form of $HgCl_2$ (Perez et al., 2016). The evaluation carried out with this same bacterium also demonstrates its ability to tolerate up to 800 mg/L cadmium and produce siderophore up to 600 mg/L $CdCl_2$.

CONCLUSION

By in vitro evaluation of the cadmium tolerance efficiency of *Bacillus cereus* GU05811, it was observed that this bacterium tolerates a maximum of 800 mg/L cadmium in the form of $CdCl_2$ and is able to produce siderophore up to 600 mg/L in the presence of cadmium.

ACKNOWLEDGEMENTS

The authors would like to thank the Microbiological Research Laboratory of the University of Sucre for carrying out this study.

AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

REFERENCES

1. De Los Santos Ramón, Candelario, Barajas Fernández, Juan, Pérez Hernández, Germán, Hernández Rivera, Miguel Ángel y DÍAZ FLORES, Laura Lorena. 2019. Adsorción de cobre (II) y cadmio (II) en suspensiones acuosas de $CaCO_3$ biogénico nanoestructurado. Boletín de la Sociedad Española de Cerámica y Vidrio, vol. 58, no. 1, DOI 10.1016/J.BSECV.2018.05.003.
2. Gilis A, Khan M, Cornelis P, Meyer J, Mergea M, van der Lelie D. 1996. Siderophore –mediated iron uptake in *Alcaligenes eutrophus* CH34 and identification of aleB encoding ferric-alcaligin E receptor. J. Bact, 178:5499-5507.
3. Hernández-Caricio, Carmelo, Ramírez, Verónica, Martínez, Javier, Quintero-Hernández, Verónica, Baez, Antonino, Munive, José-Antonio y Rosas-Murrieta, Nora, 2022. Los metales

pesados en la historia de la humanidad, los efectos de la contaminación por metales pesados y los procesos biotecnológicos para su eliminación: el caso de Bacillus como bioherramienta para la recuperación de suelos [en línea]. 25 septiembre 2022. S.l.: s.n. [consulta: 7 diciembre 2023]. Disponible en: https://hdl.handle.net/20.500.12371/16410.

4. LI, Ming Hao, GAO, Xue Yan, LI, Can, YANG, Chun Long, FU, Chang Ai, LIU, Jie, WANG, Rui, CHEN, Lin Xu, LIN, Jian Qiang, LIU, Xiang Mei, LIN, Jian Qun y PANG, Xin. 2020. Isolation and identification of chromium reducing bacillus cereus species from chromium-contaminated soil for the biological detoxification of chromium. International Journal of Environmental Research and Public Health, vol. 17(6). DOI 10.3390/ijerph17062118.
5. Lasat M. 2000. Phytoextraction of metals from contaminated soil: a review of plan/soil/metal interaction and assessment of pertinent agronomic issues. J. Hazard Subst Res, 2: 5-25.
6. Luo, S.; Chen, L.; Chen, J.; Xiao, X.; Xu, T.; Wan, Y.; Rao, C.; Liu, C.; Liu, Y.; Lai, C.; Zeng, G. 2011. Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd-hyperaccumulator Solanum nigrum L. and their potential use for phytoremediation. Chemosphere. 85:1130-1138.
7. Oliveira, M.; Santos, T.; Vale, H.; Delvaux, J.; Cordero, P.; Ferreira, A.; Miguel, P.; Totola, M.; Costa, M.; Moraes, C.; Borges, A. 2013. Endophytic microbial diversity in coffee cherries of Coffea arabica from southeastern Brazil. Can. J. Microbiol. 59:221-30.
8. Ma, Y., Prasad, M.N.V., Rajkumar, M. Y Freitas, H. 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnology Advances, vol. 29, no. 2. ISSN 0734-9750. DOI 10.1016/J.BIOTECHADV.2010.12.001.
9. Pérez, A.; Arroyo, E.; Chamorro, A. 2015. Resistencia a níquel en bacterias endófitas aisladas a partir de Oriza sativa en Colombia. Rev. Soc. Venez. Microbiol. 35:20-25.
10. Pérez, A.; Martínez, D.; Barraza, Z.; Marrugo, J. 2016. Bacterias endófitas asociadas a los géneros Cyperus y Paspalum en suelos contaminados con mercurio. Rev. U.D.C.A Act. & Div. Cient. 19(1): 67-76.
11. Rajendran P, Muthukrishnan J, Gunasekaran P. 2003. Microbes in heavy metal remediation. Indian Journal of Experimental Biology, 41: 935-944.
12. Rajkumar, M., Sandhya, S., Prasad, M.N.V. y Freitas, H. 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnology Advances, vol. 30, no. 6. ISSN 0734-9750. DOI 10.1016/J.BIOTECHADV.2012.04.011.
13. Schwyny, B., & Neilands, J.B. (1987). Universal CAS assay for the detection and determination of siderophores. *Analytical Biochemistry*, *160*, 47-56.
14. Sorkhoh, N.; Ali, N.; Dashti, N.; Al-Mailem, D.; Al-Awadhi, H.; Eliyas, M.; Radwan, S. 2010. Soil bacteria with the combined potential for oil utilization, nitrogen fixation and mercury resistance. Int. Biodeterior. Biodegr. 64:226-231.
15. Torres Perez, M.P; Vitola Romero, d.; Pérez Cordero, A. 2019. Biorremediación de mercurio y níquel por bacterias endófitas de macrófitas acuáticas. Revista Colombiana de Biotecnología, vol. XXI, núm. 2, pp. 36-44. Instituto de Biotecnología, Universidad Nacional de Colombia. https://www.redalyc.org/journal/776/77662596005/html/.
16. Valls, Marc y De Lorenzo, Víctor.2002. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiology Reviews [en línea], vol. 26, no. 4, 2002. [consulta: 6 diciembre 2022]. ISSN 0168-6445. DOI 10.1111/J.1574-6976.2002. TB00618.X. Disponible en: https://dx.doi.org/10.1111/j.1574-6976.2002.tb00618.x