Soluble Phosphate Production by Pseudomonas Aeruginosa in the Presence of Cadmium

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

[1]Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia
[2]Universidad de Sucre, Facultad de Ingeniería, Colombia
Email: alexander.perez@unisucre.edu.co

Abstract

The aim of the present study was to evaluate the tolerance capacity of Pseudomonas aeruginosa strains to different cadmium concentrations and to measure the in vitro solubilisation efficiency of soluble phosphate. Ps. aeruginosa was evaluated in vitro for maximum tolerance to CdCl2 and simultaneous solubilisation of soluble phosphate in NBRIND culture medium. The results show that P. aeruginosa has the ability to grow up to 500 mg/L CdCl2 and also solubilizes phosphate in vitro from an initial concentration of 231 mg/L after 2 hours of incubation to 1496 mg/L after 34 hours of incubation. Pseudomonas aeruginosa at different incubation times was found to be efficient in producing soluble phosphate which makes it a strategy to promote plant growth in cadmium-contaminated environments.

Keywords: Bacteria, cadmium, phosphate, remediation.

1. Introduction

According to (Paithankar et al., 2021), heavy metals are one of the major pollutants causing various problems for human and environmental health. Industries use various heavy metals and effluents contain a considerable amount of heavy metals. Most heavy metals are highly toxic in high concentrations to humans, animals and microbes. The treatment of heavy metals in the environment is significant because of their high toxicity to various living things.

Las bacterias tienen la capacidad de tolerar a mayores concentraciones de metales pesados y esta capacidad sería útil en el tratamiento de ambientes contaminados en las que estos microorganismos intervienen indirecta o directamente (Yang et al., 2021).

As expressed by (Mendoza, 2015), the advances shown in environmental biotechnology are allowing the remediation of soils contaminated by the different industries caused by man exploiting our human resources in an accelerated way, in this research we will remediate soils

contaminated by mining. In this context the use of microorganisms is very beneficial to recover soils contaminated by mining, the use of Pseudomona aeruginosa represents a promising and promising alternative application for any process of remediation of environments as in the case of heavy metals by applying a biosurfactant process.

According to (Garzón et al., 2017), the implementation of bioremediation allows obtaining quite results favorable in reducing available cadmium in the soil. In addition, microorganisms have a lower impact on the environment (in terms of contamination) and lower cost compared to other alternative technologies.

Given the problem of cadmium contamination of soils in Colombia, it was proposed as a strategy to isolate rhizospheric bacteria from contaminated environments and to evaluate the maximum capacity for cadmium tolerance and the efficiency of soluble phosphate production.

2. Materials and Methods

Isolation of rhizospheric bacteria from cadmium-contaminated environments. The procedure for the isolation of rhizospheric bacteria from cadmium-contaminated environments was carried out following the protocol as described in figure 1.

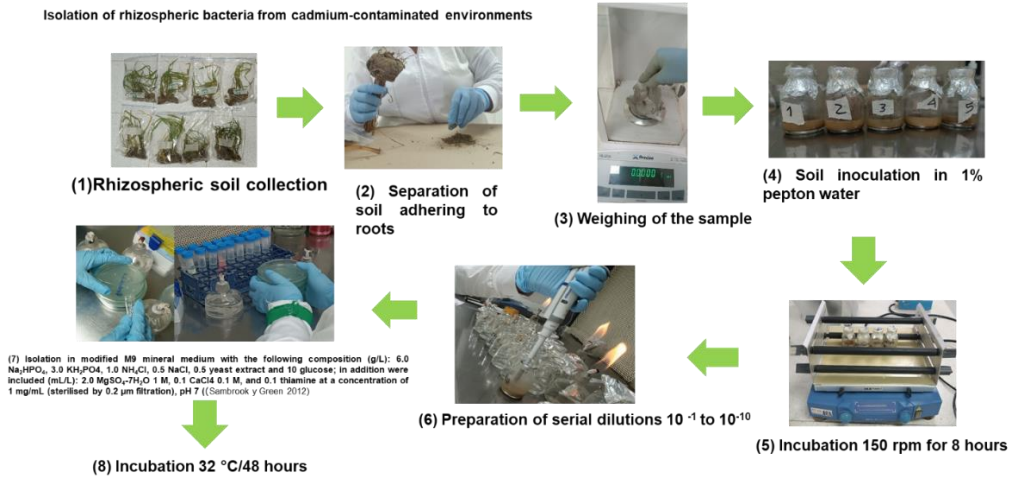

Figura 1. Técnica de aislamiento de bacterias rizosféricas de suelos contaminadas con cadmio según protocolo propuesto laboratorio de investigaciones microbiológica de la Universidad de Sucre.

In vitro cadmium tolerance test for Pseudomonas aeruginosa. Bacterial isolates were seeded on nutrient agar supplemented with $CdCl_2$ at concentrations of 100, 150, 200, 250, 300, 350, 400 up to 1000 mg/L; then incubated at 29°C for 7 days (Sorkhoh et al., 2010).

Phosphate solubilisation capacity by Pseudomonas aeruginosa. Preliminary evaluation of phosphate solubilizing activity, the solid culture medium according to NBRID was used: 10 g Glucose, 5 g $Ca_3(PO_4)2$, 5 g $MgCl_2$ * $6H_2O$, 0.25 g $MgSO_4$ * $7H_2O$, 0.2 g KCl, (NH_4) SO_4 in 1000 ml distilled water (Dawwam et al., 2013). This culture medium contains calcium phosphate salts and bromocresol purple as pH indicator. Approximately 6 days after sowing, bacterial colonies were selected and formed a transparent halo, indicative of the acidification process of the medium (turning from red to yellow due to the pH change), which indicated phosphate solubilizing activity.

Effectiveness of phosphate solubilisation by Pseudomonas aeruginosa. Once the halo production was observed, the efficiency of phosphate solubilisation capacity in the NBRID culture medium was determined by indirect measurement of dissolved phosphate (Rodriguez et al., 2006). For this, the bacteria were incubated in NBRID medium enriched with tribasic calcium phosphate for 72 hours at 30°C at 150 rpm; after this time the samples were centrifuged at 8000rpm for 20 minutes. Then 1 ml of the supernatant was taken and resuspended in 7 ml of distilled water; 2 ml of Vaneate Molybdate reagent was added to this suspension and the samples were incubated for 30 minutes. After this time, absorbance readings were taken at 540 nm, the data were analyzed using the standardized standard curve (Lara et al., 2013).

Molecular identification of Pseudomonas aeruginosa. The extraction of genomic DNA from cadmium-tolerant rhizospheric bacteria was performed using the protocol proposed by Oliveira et al. (2013). For the amplification of the 16S rRNA gene, three specific primer sets were used: F BLS342 (5‘CAGCAGTAGGGAATCTTC 3’) and R 1392 (5‘ ACGGGGGCGGTGTGTGTACA3’), for the class Firmicutes; F 948β (5‘ CGCACAAGCGGTGGTGGATGA 3’) and R 1492 (5‘TACGG(C/T) TACCTTGTTACGACTT 3’), for the class Betaproteobacteria; FD2 (5‘ AGAGTTTGATCATGGCTCAG 3’) and RP1 (5‘ ACGGTTACCTTGTTACGCTT 3’), for the gammaproteobacteria class.

The conditions used in each amplification reaction were based on the protocol described by Oliveira et al. (2013), using a mastercycler nexus eppendorf thermal cycler. 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.

3. Results and Discussion

Figure 2 shows the growth of strain C85LIM in medium supplemented with 500 mg/L CdCl2 and phosphate solubilisation in N-BRIND MEDIUM.

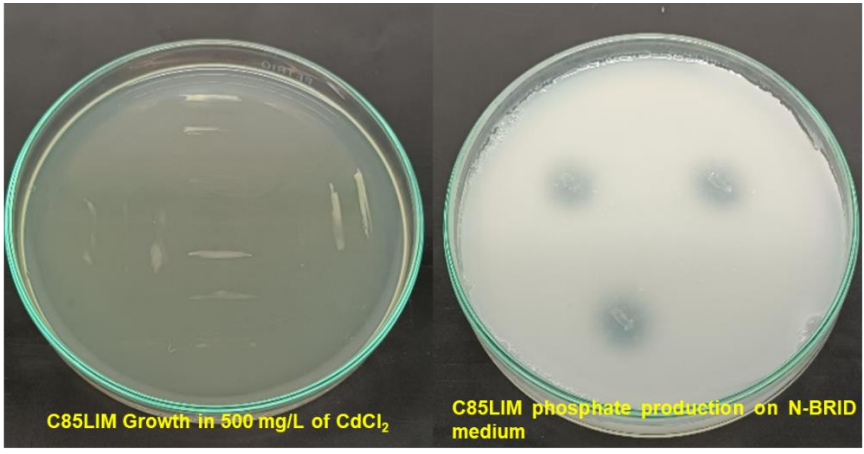

Figure 2. Growth of strain C85LIM in 500 mg/L and phosphate solubilisation in N-BRIND medium.

Figure 3 shows the production of phosphate ion by the bacterium Pseudomonas aeruginosa. In the figure, we observe that from 2 hours onwards, the bacteria produced phosphate ion (231 mg/L) and increased until the maximum production at 34 hours of incubation (1496 mg/L). Similarly, an inverse relationship with acidification of the medium is observed when, as phosphate production increases, a decrease in the pH values in the medium is observed.

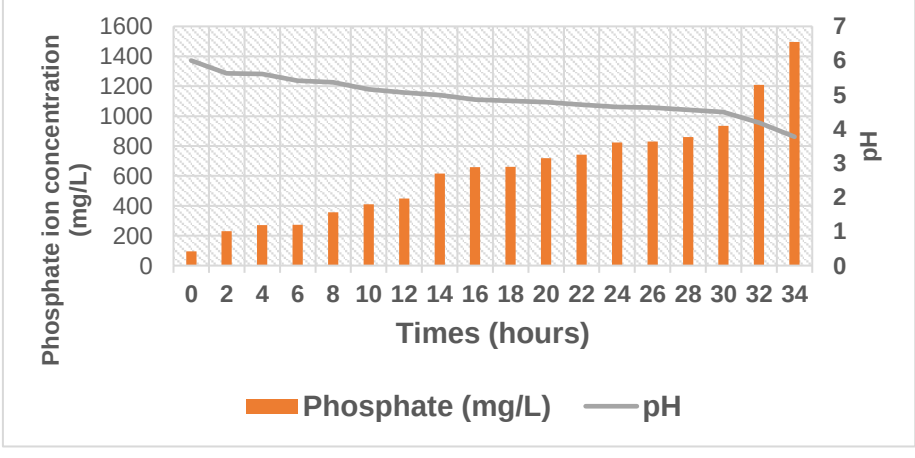

Figure 3. Concentration in mg/L of soluble phosphate produced by Pseudomonas aeruginosa isolated from cadmium-contaminated soil.

On the other hand, at the time of the test, the initial pH of the NBRIP medium was measured before being inoculated with the endophytic bacteria to be evaluated, and then the initial pH

value was compared with the pH value at the end of the test, observing a decrease in pH over time (34 hours) where the highest concentration of soluble phosphate was present.

The identification of phosphate solubilizing bacteria has increased significantly. Phosphate solubilizing bacteria can be free-living in the soil or establish symbiotic relationships with some plants, they are able to adapt, colonize and persist in the rhizosphere of the plant and favor its growth or development by solubilizing inorganic phosphate from different compounds such as dicalcium phosphate, tricalcium phosphate and phosphate rocks (Patiño, 2010).

Several studies show that the bacterial genera: Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aerobacter, Flavobacterium, Mesorhizobium, Azotobacter, Azospirillum, Pantoea and Erwinia (Rodríguez and Fraga, 1999; Orberá et al, 2005; Poonguzhali and Madhaiyan, 2008; Cordero et al., 2008; Sharan and Shikha, 2008), these bacteria have the ability to solubilize phosphate. Bacteria transform insoluble phosphates to soluble forms by different direct or indirect mechanisms. These include: The action of organic acids produced by microorganisms; chelation of the elements responsible for the insolubility of the phosphates present (Paredes and Espinosa, 2010) and direct assimilation of insoluble phosphates by microorganisms that accumulate it in their cells and subsequently release them (Puente et al, 2006).

Figure 4, shows the dendogram derived from sequencing analysis of 16 rDNA related sequences obtained from NCBI of rhizospheric bacteria isolated from cadmium-contaminated environments. The same figure shows that strain C85LIM has 100% homology with sequences of the bacterium Pseudomonas aeruginosa.

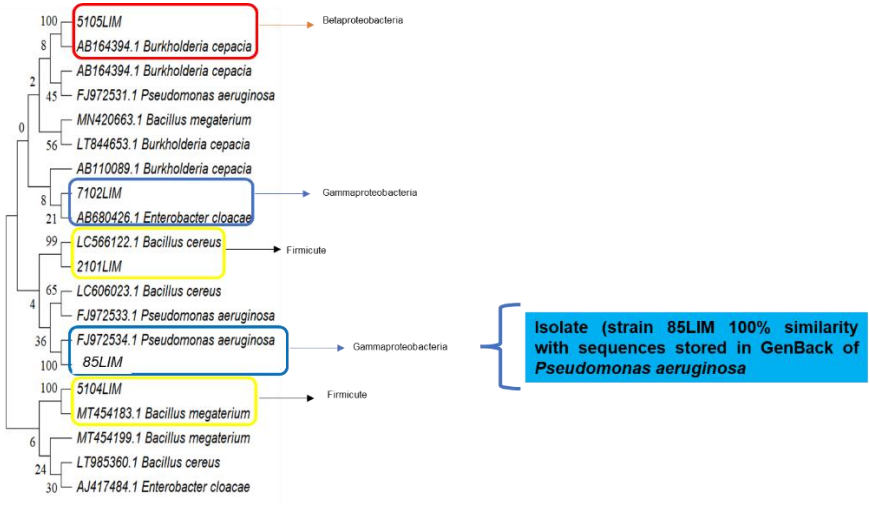

Figure 4. Dendogram derived from sequencing analysis of 16 rDNA related sequences obtained from NCBI of rhizospheric bacteria isolated from cadmium-contaminated environments.

The results obtained in the present study, which demonstrated the ability of Pseudomonas aeruginosa to solubilize soluble phosphate at different times during its growth, show that the microbial genera most frequently involved in the phosphate solubilisation process are: Pseudomonas, Bacillus, Rhizobium, Enterobacter and Azospirillum. Pseudomonas stands out for its wide genetic versatility and for using several solubilisation mechanisms, including the production of organic acids and high levels of phosphatase enzymes (Yu et al., 2011).

Pseudomonas aeruginosa is an environmental bacterium involved in mineralization of organic matter. It is also an opportunistic pathogen able to cause serious infections in immunocompromised hosts. As such, it is exposed to xenobiotics including solvents, heavy metals, and antimicrobials (Perron et al., 2004). Despite being mainly associated with health effects (Luján et al., 2008), several of its strains have been reported to detoxify certain organic and inorganic soil pollutants (Mayz and Manzi, 2017) and are especially useful in combating heavy metal contamination (Bojórquez et al., 2016).

According to (Luján, 2019), P. aeruginosa has an important role to play in bioremediation due to its high catabolic capacity, undemanding abiotic requirements and easy adaptation to adverse conditions. This allows it to actively participate in the degradation of pollutants such as hydrocarbons and heavy metals. Current research on this species is providing new evidence regarding its participation in this biotechnological stream and the possibility of its use in different environmental scenarios.

In a study carried out by (Bohórquez et al., 2016) on the adsorption and absorption capacity of cadmium and lead (Cd and Pb) using Pseudomonas aeruginosa and Enterobacter cloacae bacteria, it was concluded that both bacterial species adsorption and absorption percentages for Cd were between 14.4 and 16.5 % of the initial concentration, with no differences between strains. In the case of Pb, both strains are efficient, since the percentages retained by the two processes in both P. aeruginosa and E. cloacae add up to 82.47 and 72.16 % of the initial concentration, respectively. The uptake in the bacterial biomass was significantly higher in the P. aeruginosa cultures.

4. Conclusion

In the present study, Pseudomonas aeruginosa FJ972534 was identified as a rhizospheric rice culture bacterium adapted in soil with high cadmium concentration. The results show that P. aeruginosa has the ability to grow up to 500 mg/L $CdCl_2$ and also solubilizes phosphate in vitro from an initial concentration of 231 mg/L after 2 hours of incubation to 1496 mg/L after 34 hours of incubation

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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 conflictof interest.

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