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In Vitro Evaluation of the Remediation Capacity of Burkholderia Cepacia at different Arsenic Concentrations

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Abstract

Arsenic (As) contamination in different water sources and soils represents a threat to human health, environment and biodiversity on the planet. The most frequent and most toxic forms of arsenic in nature are arsenate (V) and arsenite, the latter being the most mobile and toxic. The aim of the present study was to evaluate in vitro the tolerance capacity of endophytic bacteria isolated from yam crop tissues and identified as Burkholderia cepacia to different concentrations of AsCl3 and to determine qualitatively the ability to produce siderophores. B. cepacia grew up to 500 mg/L AsCl3 and also produced siderophore at different concentrations of Arsenic. The presence of B. cepacia associated with plant species in arsenic-contaminated environments may contribute to the removal and management of arsenic to reduce its toxicity.

Keywords: *Arsenic, contamination, remediation, endophytic bacteria.*

1. INTRODUCTION

Arsenic is one of the metals that causes the greatest havoc worldwide, due to the high toxicity of its accumulation in water, air and soil (Han et al., 2003). This metal can be absorbed as particulate matter in its reduced chemical form arsenite (As III) and its oxidized form arsenate (As V) (Mandal and Suzuki, 2002). Moreover, due to its metabolic effect, after prolonged exposure to high doses it can be lethal (Flanagan et al., 2012).

Arsenic contamination of different water sources and soils represents a threat to human health, the environment and the development of biodiversity on the planet. The most frequent and most toxic forms of arsenic in nature are arsenate (V) and arsenite, the latter being the most mobile and toxic. As is known, in order to achieve an effective removal of arsenic from a contaminated environment, it must be taken into account that a suitable method will be one that in addition to removing or transforming it will be environmentally friendly, which means that it must not cause the formation of new contaminants and must be feasible in terms of costs for its viability (Choque, 2019).

Several studies have isolated microorganisms from arsenic-contaminated environments that are able to assimilate arsenic through their metabolic pathways, achieving a detoxifying effect (Kruger et al., 2013), and recent studies have focused on increasing the ability of microorganisms to resist arsenic through the use of genetic engineering (Lorenzo et al., 2016), so that more efficient microorganisms could be designed as a bioremediation strategy. Although bacteria with the ability to metabolize arsenic exist, genetically modified

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organisms can detoxify arsenic in a more efficient and environmentally friendly way (Rangel-Montoya and Balagurusamy, 2015; Yang et al., 2016).

Burkholderia cepacia has been demonstrated in vitro to tolerate different concentrations of metals, promote plant growth and exert biocontrol against phytopathogens. Based on the scientific evidence of the contributions of Burkholderia cepacia in productivity, sustainability, crop protection and in the recovery of environments contaminated with toxic compounds, it was proposed to isolate and evaluate the benefits of B. cepacia as a biocontrol agent and biological resource to help plant species to adapt to the presence of heavy metals.

Based on the report and evidence of the ability of the endophytic bacterium Burkholderia cepacia to remediate heavy metals and promote plant growth, the objective of the present study was to evaluate in vitro the ability of Burkholderia cepacia to tolerate different concentrations of arsenic and the production of siderophore.

2. MATERIALS AND METHODS

Isolation, quantification and purification of endophytic bacteria. Samples collected from different yam plant tissues were used. Ten separate root, stem and leaf tissues from each yam plant sample were used and placed in 50 mL erlenmeyer flasks for surface disinfection by washing with distilled water and neutral detergent for one minute, followed by four rinses in sterile distilled water. The washed roots were transferred to new flasks containing sterile water for isolation of endophytic bacterial communities.

For the isolation of endophytic bacteria, each tissue was subjected to surface sterilization process (Pérez et al., 2010, Barboza et al., 2023), which consisted of the following steps: two washes of the root in sterilized distilled water, followed by shaking for 15 min in potassium phosphate buffer solution 0.05 mol L-1, pH 7.0; immersion for 1 min in 70% alcohol; shaking for 5 min in 5% sodium hypochlorite solution and Tween 80%; again immersion for 1 min in 70% alcohol followed by shaking for 15 min in potassium phosphate buffer 0.05 mol L-1, pH 7.0 and, finally, washing four times in sterilized distilled water. The process was repeated twice. To confirm sterilization of the root surface, an aliquot of the last wash was spread on a plate containing nutrient agar culture medium and incubated at 28 °C for 72 hours. The roots were then transferred to a tube containing nutrient broth and incubated at 28 ºC for 72 hours, for certification of the absence of microorganisms on the surface of the roots to be used for the isolation of culturable endophytic bacteria.

The density of bacteria per root, in CFU. g. of roots-1, was estimated by direct colony counting on plates. During counting, colonies that were distinguishable in shape, surface appearance, color and size were observed and selected. Selected morphotypes were purified and maintained on nutrient agar for further analysis and identification (Pérez et al., 2010, Barboza et al., 2023).

In vitro evaluation of tolerance to AsCl3. In vitro evaluation of the tolerance of Burkholderia cepacia to different concentrations of the metal ion was carried out in tris-MMT minimal medium (Rathnayake et al., 2013) prepared with five concentrations of Arsenic in the form of AsCl3. The initial concentration of as used in the present study was from these concentrations of 100, 200, 300, 400 and 500 /L were prepared. Aliquots of log-phase endophytic bacterial suspensions were inoculated onto MMT medium. MMT medium without AsCl3 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). The growth of endophytic bacteria was determined by turbidimetry at 600 nm every hour for four days.

Phosphate solubilization. In vitro determination of siderophore production capacity was determined using chromium azurol-S (CAS) medium (Schwyn and Neilands, 1987). The ability of the bacteria to produce siderophores was evidenced by the formation of a transparent halo around the colonies.

Molecular identification. Genomic DNA of endophytic bacterial strains was carried out using a protocol proposed by Oliveira et al. (2013). Universal primers that amplify the small subunit of 16S rRNA were used. The amplified products were purified and sent for sequencing to Macrogen. The sequences obtained were compared with those stored in Genbank. Base alignment was performed in the Clustal W program; phylogenetic inferences were obtained by the Neighbor Joining method based on the kimura-2-parameter model with bootstrap 1,000 test in the MEGA X program.

3. RESULTS AND DISCUSSION

The results of the in vitro tolerance test to different concentrations of arsenate chloride show that Burkholderia cepacia have the capacity to grow up to 500 mg/L $AsCl_3$ up to 20 hours after the start of the experiment. Figure 1 shows that at 100 mg/L $AsCl_3$, B. cepacia had its maximum growth up to 19 hours, compared to the behaviour of the control used where the bacteria grew without the presence of the metal.

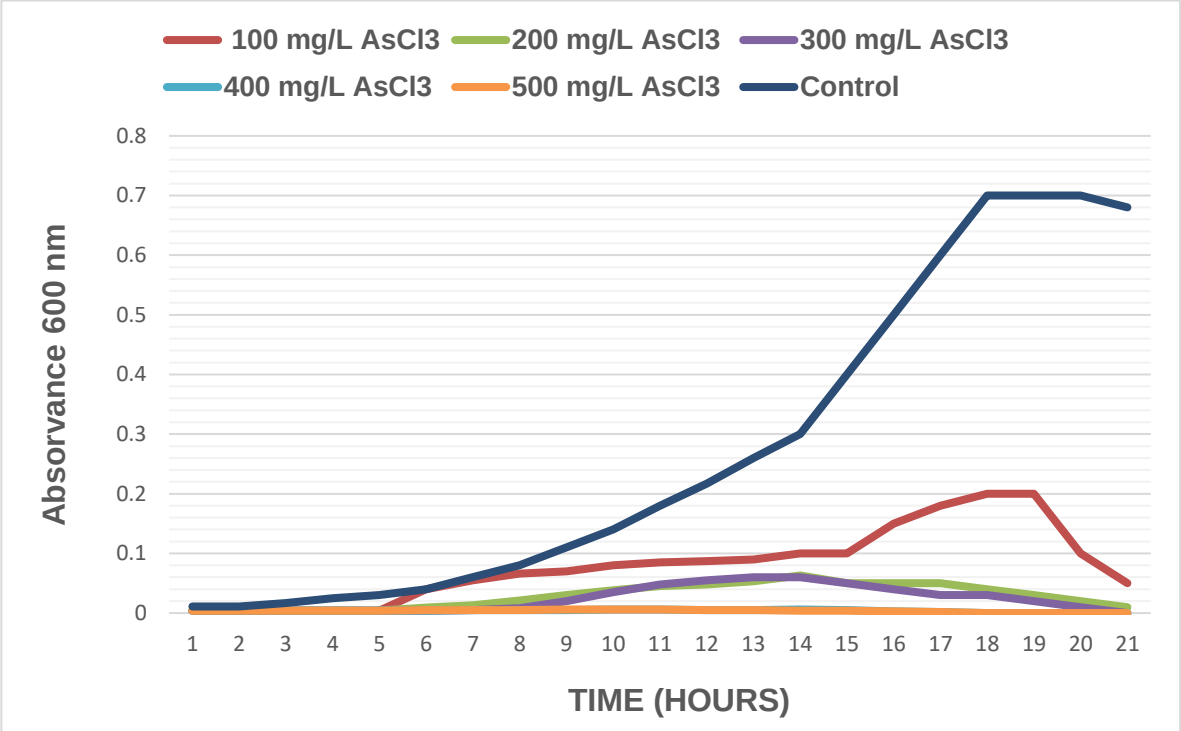

Figure 1. Growth behaviour of Burkholderia cepacia at different concentrations of arsenic in the form of $AsCl_3$.

It is possible that Burkholderia cepacia as endophytes employ different cellular protection mechanisms to adapt to environments contaminated with high concentrations of lead; among these are: efflux, extracellular sequestration, absorption on cell surfaces and in intercellular spaces, precipitation, alteration of cell morphology, increased production of siderophores and intracellular bioaccumulation, among others (Naik and Dubey, 2013).

Once the tolerance capacity of B. cepacia to different concentrations of $AsCl_3$ was determined, the production of siderophores was determined at the same concentrations. The results of the assay, as shown in figure 2, show that B. cepacia produces siderophores from 100 to 500 mg/L $AsCl_3$.

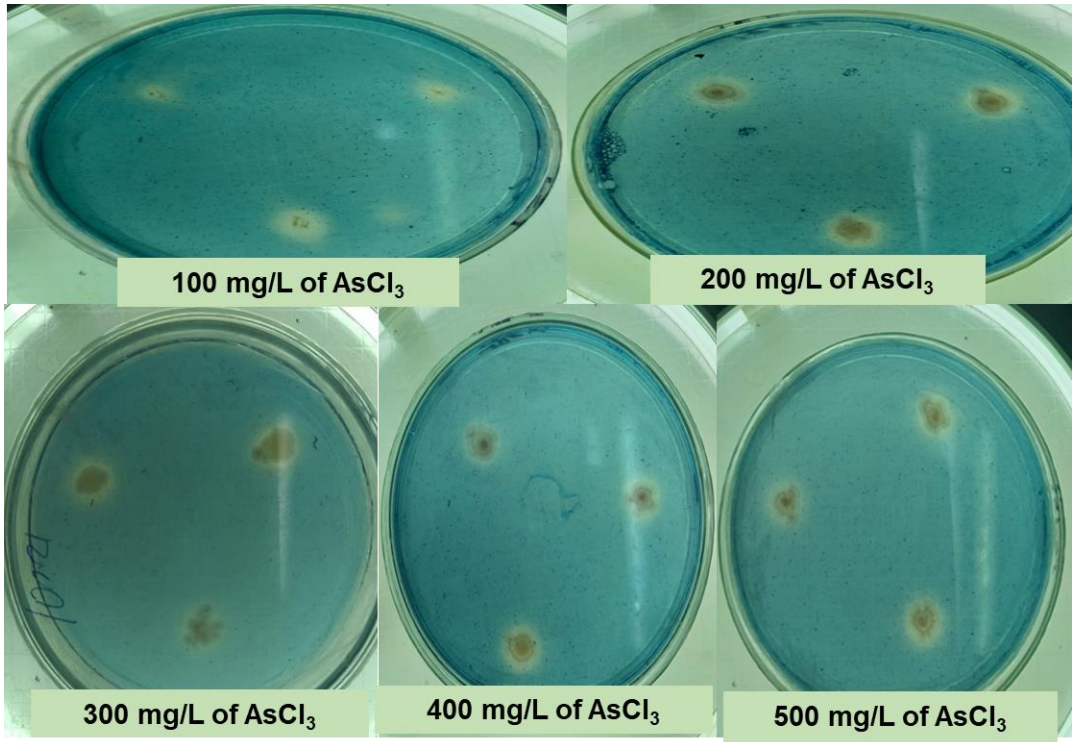

Figure 2. Production of siderophore by Burkholderia cepacia at different concentrations of $AsCl_3$.

The results of the phylogenetic analysis using the maximum similarity method of the amplified sequences with primers F948β and R1492, specific for the beta proteobacteria class, show that the isolated bacteria form a clade with bacteria of the Burkholderia cepacia complex, with a branch support of 98%, indicating that the study samples belong to the Burkholderia cepacia complex and show higher homology with the species Burkholderia ambifaria LN889999, Burkholderia diffusa CP013364 and Burkholderia territorio CP013366, taken from the Genkbank database and described in the literature (Coenye et al, 2001; Vanlaere et al, 2008; De Smet et al, 2015) as bacteria of the complex (figure 3).

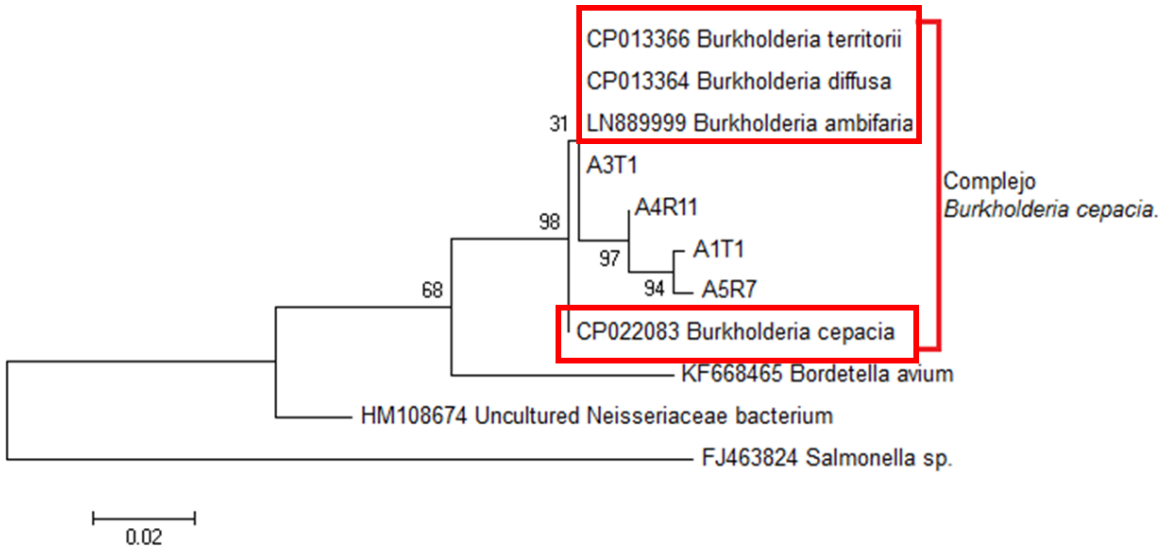

Figure 3. Phylogenetic tree derived from 16S rDNA gene sequencing analysis using F948β and R1492 primers of endophytic bacterial strains with homologous sequences obtained

from NCBI. Bar scale is 0.02 substitutions per nucleotide position. Source: Doncel-Manrique; Pérez-Cordero, 2017.

Burkholderia. cepacia complex species are beneficial in bioremediation, biocontrol and plant-growth promotion. However, because the B. cepacia complex is involved in human infection, its use in agriculture is restricted. B. cepacia complex is being constantly studied due to the health problems that it causes and because of its agricultural potential.

The distribution of the B. cepacia complex in the environment is wide; it has been indicated that due to its large genomes and the presence of multiple insertion sequences, its members can colonize natural environments such as soil, water, rhizosphere or seeds, and recently it was discovered that they can also form nodules on the legume Stylosanthes, which is found in the Amazon. Previously, B. cepacia genomovar VI (now Burkholderia dolosa, isolated from nodules of Alysicarpus glumaceus (VAHL) DC in Senegal, was identified, but was not shown to form nodules on this plant species (Vandamme et al., 2002; Drevinek et al., 2010; Da Silva et al., 2016).

Biological methods, unlike physicochemical methods, do not achieve high removal rates but have the advantage of being environmentally friendly as they are based on taking advantage of the metabolic potential of microorganisms to clean contaminated environments (Covarrubias et al., 2015).

4. CONCLUSION

Burkholderia cepacia is a Gram-negative endophytic bacterium isolated from yam plant roots. In vitro tests indicate that it is able to tolerate up to 500 mg/L $AsCl_3$ and induces the production of siderophore-like compounds at different concentrations of this metal (100, 200 and 300 mg/L). The results obtained in this study infer the possibility of using this endophytic bacterium for remediation and safe production of plant species in arsenic-contaminated soils.

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