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Siderophore Production By Burkholderia Cepacea At Different Cadmium Concentrations

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ABSTRACT

Heavy metals are considered one of the most toxic pollutants in the environment as they have harmful effects throughout the food chain, especially Cd, Cr and Hg are relevant due to their persistence and toxicity. There is evidence of the synthesis of siderophores by bacteria when they grow in environments with the presence of toxic heavy metals; therefore, the aim of this study was to evaluate in vitro the production of siderophores at different concentrations of CdCl2. The bacterial species Bukholderia cepacea, strains of endophytic bacteria from the genomic bank of the microbiological research laboratory of the University of Sucre, isolated from environments contaminated with mercury, were selected. Lead tolerance tests were carried out at different concentrations (100, 200, 300, 400 and 500 ppm), and the capacity of these bacteria to produce siderophores was evaluated. The results obtained indicate that the endophytic strains evaluated produced siderophores at different concentrations of cadmium metal. The results indicate that these endophytic bacteria could contribute to the phytoremediation of plant species adapted to different environments contaminated with lead in the Colombian Caribbean.

Keywords. Cadmium, siderophore, endophytic bacteria, remediation.

1. INTRODUCTION

The interactions between bacteria and metals are well known and can occur extracellularly, on the bacterial surface or intracellularly. At the extracellular level, the role of micro-organisms in the mobilization and immobilization of metals (Chen et al., 1995; Ford and Ryan, 1995; Vajargah, 2021); and the secretion of low molecular weight organic compounds with high affinity for these elements (siderophores) (Schwyny Neilands, 1987; Lindsay and Riley, 1994).

Among the most important uses of siderophores is to help plants reduce the toxicity generated by heavy metals in contaminated environments and thus optimize phytoremediation processes by participating in the reduction of the inhibitory effect of some heavy metals on auxin synthesis (Rajkumar et al., 2009). Several studies indicate that bacteria that show resistentes a metales pesados such as Bacillus megaterium, Pseudomonas marginalis, Citrobacter freundii, Ralstonia metallidurans, Burkholderia cepacia and Pseudomonas putida (Levinson and Mahler 1998, Roane 1999, Borremans et al. 2001, Pérez et al. 2015). Some bacterial species can synthesise secondary metabolites such as indole-3-acetic acid (IAA), gibberellic acid (GA3), zeatin, and abscisic acid, among others, which have a positive effect on plant development (Karadeniz et al. 2006, Vega et al. 2016). Siderophore-producing

rhizobacteria provide nutrients, especially iron, to plants; furthermore, siderophores produced by these bacteria in the rhizosphere bind heavy metal ions, improving availability to plants (Nair et al. 2007, Rajkumar et al. 2010, Velásquez et al. 2011, Sessitsch et al. 2013).

Based on the problem of cadmium metal in agricultural soils in Colombia and its environmental impact, a strategy is proposed to evaluate in vitro the capacity of the endophytic bacteria Burkholderia cepacea to tolerate different concentrations of cadmium and contribute to reduce the concentration of this metal through the production of chelating agents such as siderophores in the presence of different concentrations of this metal.

2. MATERIALS AND METHODS

2.1 Ensayos de tolerancia a diferentes concentraciones de cadmio. Los ensayos in vitro de la tolerancia de Burkholderia cepacea, se llevó a cabo en medio mínimo tris-MMT propuesto por (Rathnayake et al., 2013), con 5 tratamientos (concentraciones) de cadmio en la forma de $CdCl_2$. La concentración inicial de plomo utilizada fue de 0.01 mg/mL y a partir de estas se prepararon los diferentes tratamientos: T1: 100 (0.1); T2: 200 (0.2); T4: 300 (0.3); T5: 400 (0.35); T4 400 (0.4); y T5:500 (0.5 mg/mL). Alícuotas de cada cepa en fase logarítmica fueron inoculadas en el medio MMT. Como control se utilizó medio MMT sin $CdCl_2$. El experimento se realizó teniendo en cuenta cada cepa, el cual se incubó en agitación a 150 rpm a 32 °C por 24 horas (Zhang et al., 2011). El crecimiento de cada bacteria fue determinado mediante turbidimetría a 600 nm cada hora por un día.

2.2 Siderophore production by Burkholderia cepacia. The production of siderophores in B. cepacea was carried out in the specific gelled CAS-agar medium proposed by Schwyn and Neilands (1987). The CAS-agar medium is the combination of four independently prepared solutions: a) Fe-CAS indicator solution (solution 1): 10 mL $FeCl_3$ 1 mM dissolved in 10 mM HCl, 50 mL of a CAS solution (1.21 mg/mL), the latter was slowly added to 40 mL of a solution (1. 82 mg/mL) of hexadecyltrimethylammonium bromide (HDTMA); (b) buffer solution (solution 2): dissolution of 30.24 g PIPES (piperazine-N,N'-bis[2-ethanesulfonic acid]) in 750 mL of a saline solution: 0. 3 g KH_2PO_4, 0.5 g NaCl and 1.0 g NH_4Cl at pH 6.8, made up to 800 mL, adding 15 g agar; c) solution 3: 2 g glucose, 2 g mannitol, 493 mg $MgSO_4$, 11 mg $CaCl_2$, 1.17 mg MnSO4, 1.4 mg H_3BO_3, 0.04 mg $CuSO_4$, 1.2 mg $ZnSO_4$ and 1. 0 mg Na_2MoO_4 in 70 mL of distilled water; d) the solutions were sterilized at 15 psi, then mixed and at the end 30 mL of a filtration-sterilized 10 % (w/v) casamino acid solution (solution 4) (Alexander and Zuberer 1991).

2.3 Identification of cadmium-resistant and siderophore-producing 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. RESULTS AND DISCUSSION

Figure 1 shows the growth curve of the endophytic bacterium B. cepacea growing in different concentrations of $CdCl_2$. The figure shows the ability of the bacterium to grow and withstand up to 500 ppm $CdCl_2$. The tolerance

of the bacteria to different concentrations of the metal was carried out up to 19 hours after the start of the experiment. Results found by Kamika and Momba (2013) argue that the interaction between metal and bacteria may be a key mechanism contributing to metal bioavailability and toxicity (Kamalakannan and Krishnamoorthy, 2006). Further studies will determine that in vitro ecological factors may influence the growth and resistance of endophytic bacteria to different concentrations of Cd.

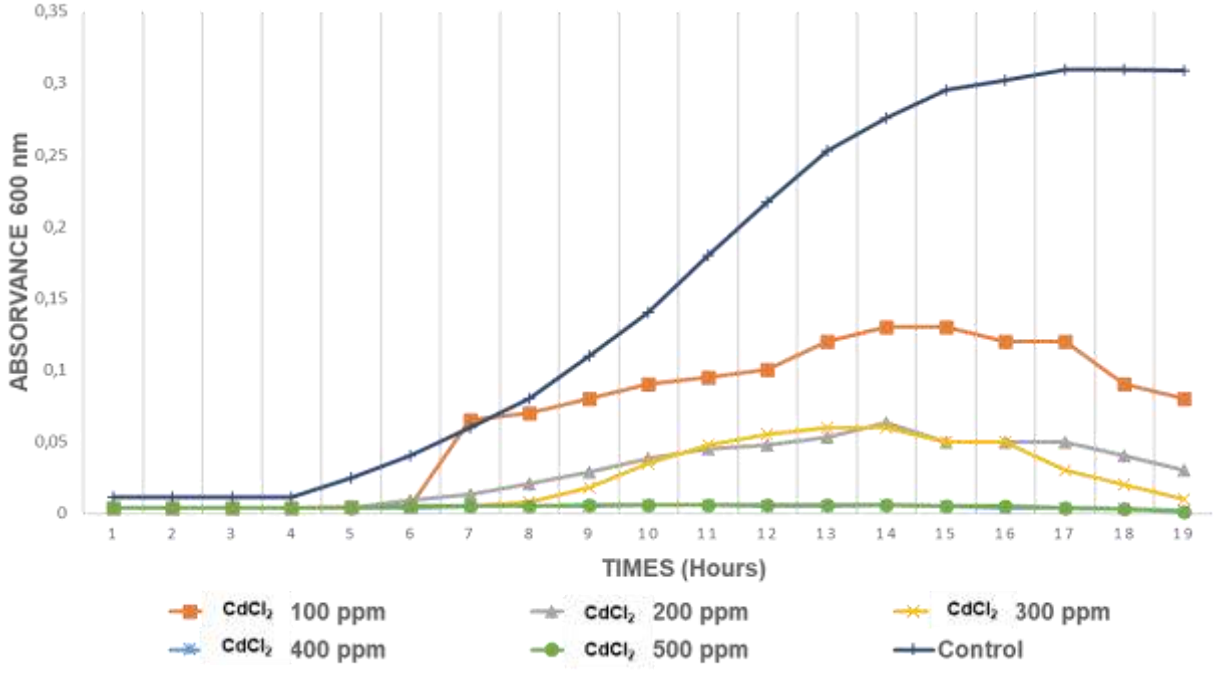

Figure 1. Growth behaviour of Burkholderia cepacea in different concentrations of $CdCl_2$.

Cd is a non-essential element and not very abundant in the earth's crust, at low concentrations it can be toxic to all living organisms (Weisberg et al., 2003). Environmental contamination by Cd has increased due to the increase in industrial activity, progressively affecting different ecosystems and public health (Sheng et al., 2008). The environmental deterioration caused by heavy metals is based on their high toxicity, which is aggravated by their marked persistence in ecosystems and bioaccumulation in organisms, as they are non-biodegradable compounds (Guerra et al., 2014).

Figure 2 shows the production of siderophores by B. cepacea bacteria under normal conditions and at different cadmium concentrations.

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 siderophore-producing.

The Burkholderia cepacia complex (BCc) consists of bacteria with a wide distribution in the environment, with resistance to antibiotics and antiseptics, able to survive in nutrient-poor environments and to metabolize some antimicrobial compounds as a carbon source. Some species of the B. cepacia complex exhibit beneficial activities, such as bioremediation, biocontrol and plant growth promotion. However, due to their role in human infections, their use in agriculture is restricted. The B. cepacia complex is a constant subject of study due to its impact on the health sector and its potential in agriculture.

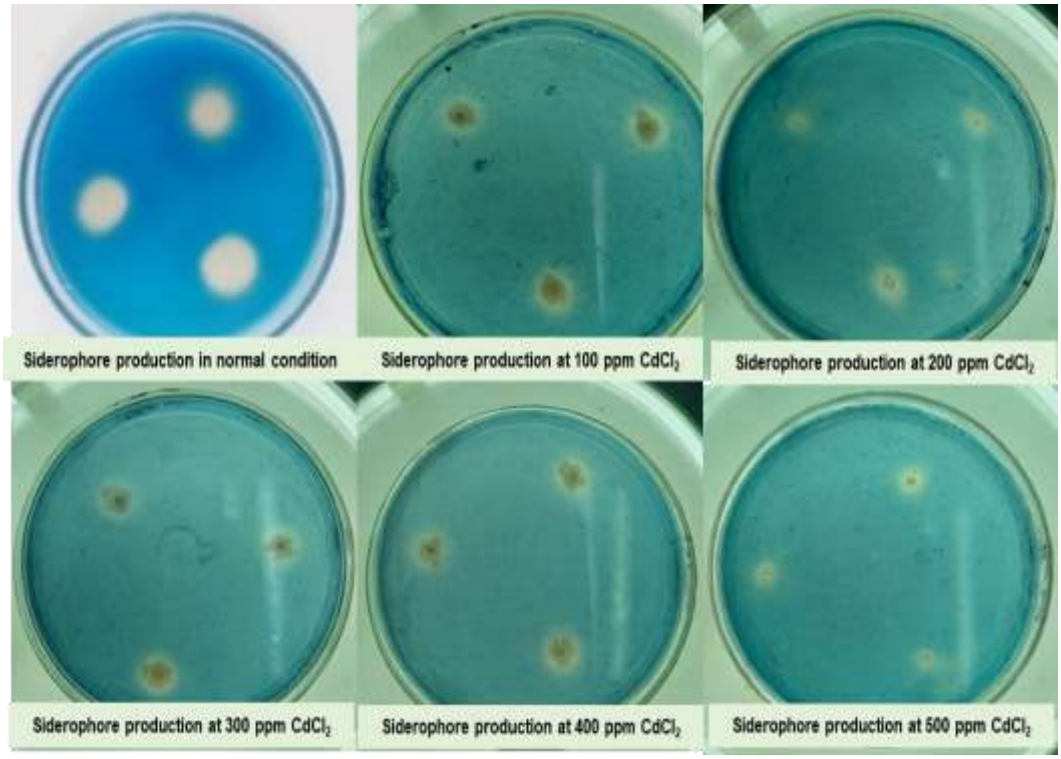

Figure 2. Production of siderophores by Burkholderia cepacea in different cadmium concentrations in specific gelled CAS-agar medium.

Burkholderia cepacia is considered a bacterium found as rhizospheric and endophytic in different plant species, it is important in the integrated management of plant diseases (Hernandez et al., 1997), because they have the ability to grow colonizing plant organs, such as roots and other tissues, and produce a wide variety of secondary metabolites toxic to fungi and phytopathogenic bacteria, among which siderophores, antibiotics and quinolysidine alkaloids stand out. Studies carried out in recent years have concluded that the bacterium species Burkholderia cepacia is of vital importance in studies related to agriculture, mainly due to the production of a wide range

of active metabolites, which positively influence the growth and healthy development of plants (Bevivino et al., 2000).

On the other hand, studies carried out by Pérez et al., (2016), on the presence of endophytic bacteria associated with pasture species in environments contaminated with mercury in the south of the department of Bolívar-Colombia, identified the species Burkholderia cepacia KJ935925 from plant tissue of Paspalum arundinaceum, with the capacity to resist in vitro to high concentration of mercury.

4. CONCLUSION

The results obtained in the present study show that the bacterium Burkholderia cepaceae is capable of tolerating high concentrations of cadmium, as well as promoting plant growth through the production of siderophores. This allows us to conclude that this bacterium becomes a biological alternative in the removal of metals in environments contaminated with them.

5. AUTHORSHIP CONTRIBUTIONS

All authors have jointly and equally contributed to the argumentation and writing of the manuscript.

6. FUNDING.

None.

7. CONFLICT OF INTEREST

None.

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