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Lead Resistance by *Bacillus cereus* 1DH1LIM Isolated from Contaminated Environments with Mercury

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Abstract

Objective: Evaluated resistance capacity *in vitro* of *Bacillus cereus* 1DH1LIM at different concentrations of lead in the form of Pb $(NO_3)_2$. **Materials and Statistical Analysis:** *B. cereus* was purified, aliquots of suspensions in log phase were inoculated into minimal medium tris-MMT with different concentrations of lead in the form of Pb $(NO_3)_2$ and incubated by stirring at 150 rpm at 32 °C for 120 hours; growth was determined by turbidimetry at 600 nm every hour for four days. Siderophore production was determined by growth on médium azurol-S (CAS). **Findings:** *B. cereus* 1DH1LIMIt shows statistical difference with respect to adaptation time and concentration of lead metal present in the medium. At concentrations of 100, 150 and 200 ppm of Pb an adaptation phase of 4 hours, with respect to concentrations of 250 to 400 ppm which lasted 7 hours was observed. The highest growth of *B. cereus* 1DH1LIMIt was observed at 100 ppm, 150 ppm and 200 ppm and less at400. In CAS medium the bacterial culture exhibited siderophore production. **Applications:** The findings of this study expand the knowledge to use this endophytic bacteria asa biological resource to remedy lead-contaminated environments.

Keywords: Bacterium, Heavy Metal Remediation Growth

1. Introduction

Modern agricultural practices and uncontrolled increase in human activities have introduced the environment high levels of toxic materials contaminated with heavy metals that have significant environmental impacts and therefore affect human health through the chain alimenticia[1]. The presence of high concentrations of metals also has negative effects on the communities of microorganisms in the soil, reducing the functionality of these in ecosistema[2].The ion lead (Pb) is reported by several studies as the second heavy metal contaminants dangerous to the environment because it causes toxicological effects on humans, plants and animals, and exposure pathways ingestion of contaminated food, contaminated soil and dust, but inhalation as a route of entry can also be significant[3].

The presence of metal Pb in the last decade in the environment has increased over 1000 persists in the environment and slowly increases until reaching the Biomagnifications to different levels of the tropicin food chains and therefore, it is known as poison acumulativo[4-6]. Pb contaminated soil, sediment and water, which creates an extreme condition for both growth and to the survival of micro-organisms in said environment, because they cause damage to DNA, proteins and lipids and also replacing metal ions essential as the Zn, Ca and Fe of enzimas[7]. Remediating contaminated with this metal flooring uses traditional or conventional techniques, such as destructive chemical precipitation, filtration, ion exchange, reverse osmosis, membrane technology, and the electrochemical treatment, which can be exorbitant and the soil[8].

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Certain species of indigenous bacteria use a variety of protective mechanisms to survive and adapt to high lead levels, without causing any effect on their growth and metabolism as strategies. Strategies that employ bacteria to resist high concentrations are: efflux mechanisms, extracellular sequestration, biosopcion, precipitation, and change in cellular morphology, increased production of siderophores and bioaccumulation intracelular[9-12]. Bacteria with these characteristics, becomes a new tool for bioremediation of contaminated environments Pb. But it is important, knowledge of the mechanism of resistance to Pb using bacteria,

Latterly for biological techniques implemented as alternatives for the remediation of Pb, using plants and/or microorganisms that have been shown to tolerate different mechanisms or detoxify these substances into less toxic compounds for the environment and vivos[13] beings. In studies where native bacteria contaminated with this metal sites are used, it was concluded that more likely to find strains that develop different survival strategies, which resist the high metal concentrations and contribute to detoxification metal exists in these sites with which coexist. The objective of this study was to evaluate the in vitro capacity of tolerances Pb *Bacillus cereus*1DH1, isolated of the plant species *Paspalum arundinaceum*, from contaminated soils high mercury concentration.

2. Materials and Methods

2.1 Identification of *Bacillus cereus* 1DH1LIM

Microbial species used in this study corresponds to *Bacillus cereus* 1DH1LIM, belonging to the collection genomics research laboratory at the University of Sucre, which was reported as an isolated endophytic root of the plant species *Paspalum arundinaceum* Adapted to soils with high mercury content in the municipality Mina Santa Cruz, of San Martin de Loba, located in southern Bolivar, Colombia. DNA extraction of endophytic bacteria, it was performed using protocol the proposed by[14]. The set of oligonucleotides BLS342F (325-342), CAGCAGTAGGGAATCTTC and 1392R (1392-1406), ACGGGCGGTGTGTACA, belonging to the class firmicutes was used for amplification of the 16S rRNA gene, using cycles, temperature and time given[15]. Amplified products were purified and sequenced were sent to the company Macrogen. The sequences obtained were compared with those stored in the Genbank. The alignment of the bases was conducted in the Clustal W program, phylogenetic inferences were obtained by the maximum similarity method based on the Kimura 2-parameter-MEGA 7 program model.

2.2 Resistence Test *Bacillus cereus* 1DH1LIM to Lead

The in vitro assay of tolerance of *B. cereus* 1DH1LIM at different concentrations of metal ion Pb, in minimum tris-MMT was performed in half reported by[16] with five different concentrations of lead in the form of Pb $(NO_3)_2$. The initial concentration of Pb used was 0.01 mg / mL and from these concentrations of 100 were prepared (0.1 mg / mL), 150 (0.15 mg / mL), 200 (0.2 mg / mL, 250 (0250 mg / mL), 300 (0.3), 350 (0.35) and 400 ppm (0.4 mg / mL). Aliquots of suspensions of *B. cereus* 1DH1LIM log phase were inoculated into the medium MMT. As control means MMT was used Pb $(NO_3)_2$ the experiment was performed in triplicate, which was incubated under stirring at 150 rpm at 32 ° C for 120 horas[17]. The growth of *B. cereus* 1DH1LIM was determined by turbidimetry at 600 nm every hour for four days.

2.3 Siderophore Production

Qualitative siderophore production was determined on the culture medium azurol-S (CAS) proposed by[18]. Pure colonies of *B. cereus* 1DH1LIM log phase were inoculated on the culture medium, which was incubated at 30 °C for 5 days. The ability of bacteria to produce siderophores was evidenced by the formation of a clear halo around the colonies.

2.4 Analysis of Data

Data were organized in figures for better understanding of the results. Analysis of variance and multiple ranges Tukey test was used to establish significant differences between the variables analyzed. Assays were performed in triplicate and results expressed in half. Data were analyzed in the InfoStar software.

3. Results and Discussion

Bacillus cereus 1DH1LIM, was identified as an endophyte isolated from roots of the plant species of *Paspalum arundinaceum*I adapted soils contaminated with mercury located

in themine Santa Cruz, municipality of San Martin de Loba, located in southern Bolivar, Colombia, by teams of researchers from the research laboratory at the University of Sucre.

By meeting the criteria of ANOVA, we proceeded to the analysis of variance, which indicates significant statistical differences (p-value <0.05) among the times in hours of exposure of the bacterium *B. cereus* lead as well as different concentrations of lead in the form of shaped Pb $(NO_3)_2$. The results of the Tukey test shows no statistically significant difference (p-value> 0.05) between the exposure times 0,1,2,3 and 4 hours, which demonstrate that the adaptation phase of this microbial spice lasted until four hours into the experiment; from the fifth hour, the bacteria enter the log phase of maximum growth and between time of 11, 12 and 13 hours of exposure to metal, statistically significant differences regarding the growth was not found, indicating that due to I stress caused by the metal species of bacteria entered the stationary phase (Figure 1).

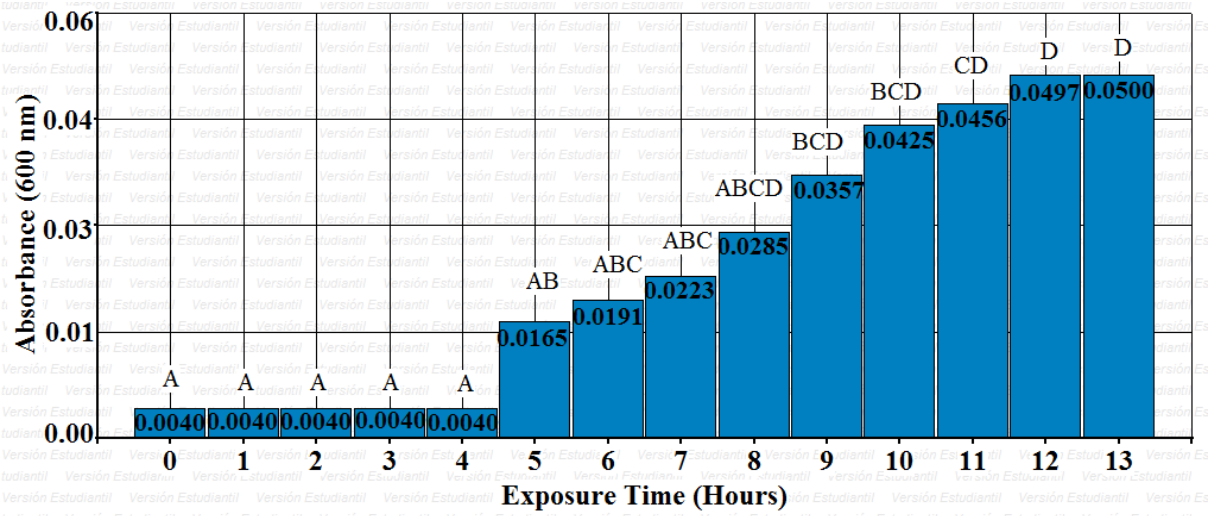

Figure 1. *Bacillus cereus* 1DH1LIM growth according to exposure time (in hours) to different concentrations of lead in the form of Pb $(NO_3)_2$

With respect to the different levels of lead, statistical differences (p-value <0.05) concentration of 100 ppm and 400 ppm higher averages observed, the latter showing the slower growth rates of the bacteria, and 100 ppm with 0.0443 OD at 600 nm. However it did not reveal a different concentration between 100 and 150 ppm (Figure 2).

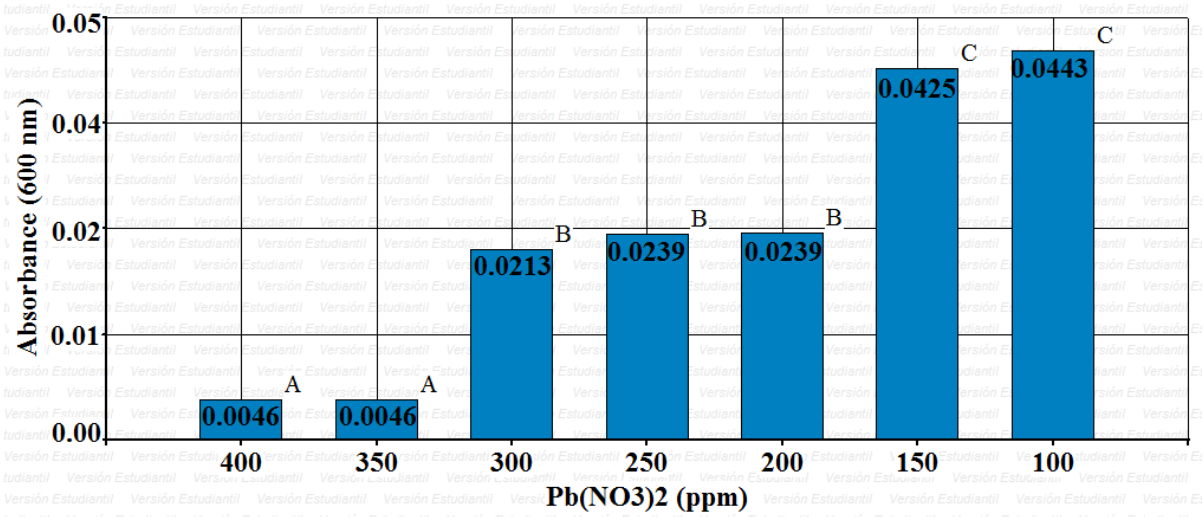

Figure 2. *Bacillus cereus*1DH1LIM growth at different concentrations of lead in the form of Pb $(NO_3)_2$

The results of the growth curve of *Bacillus cereus* 1DH1LIM in different concentrations of $Pb(NO_3)_2$ it shows growth behavior varied with respect to the control. When *B. cereus* 1DH1LIM, was subjected to concentrations from 100 to 200 ppm Pb $(NO_3)_2$ showed an adaptation phase until 4 hours while 250 to 300 ppm was the adaptation phase until 7 hours. At concentrations of 350 and 400 ppm an adaptation phase was observed until 10 hours and after this time showed a low light growth (Figure 3). Low growth of the bacteria at concentrations of 350 and 400 ppm, can be explained by the effects of lead toxicity, which cause changes in metabolic and physiological characteristics of the bacteria. The observed results demonstrate that as the metal concentration increases, the amount of bacterial cells and growth retardation was presented and requiring more time for the bacteria adapt and recover their ability to grow in the medium contaminated with lead, this also being supported by[19].

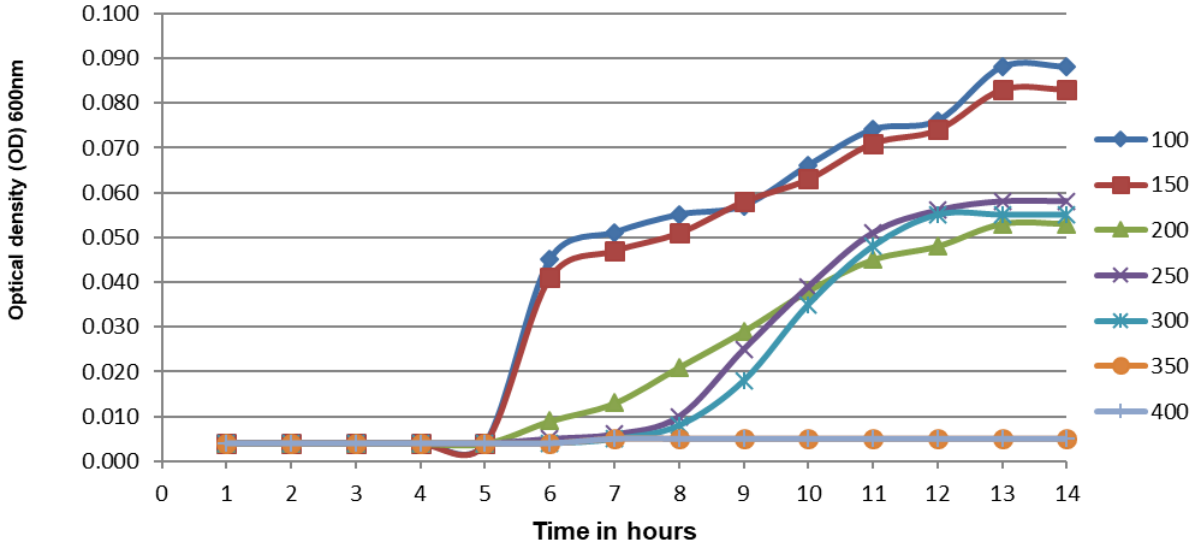

Figure 3. Growth of *Bacillus cereus* 1DH1LIM at different concentrations (ppm) of lead in the form of $Pb(NO_3)_2$.

In the experiment the maximum growth for *B. cereus* 1DH1LIM (OD: 0.08 nm) was observed at 100 ppm Pb $(NO_3)_2$ until 14 hours into the experiment, followed by concentration of 150 ppm 0083 nm where growth was observed at 14 hours, after this time the bacteria growth declined. With respect to the concentrations 200, 250 and 300 ppm and intermediate growth were observed whereas at concentrations of 350 and 400 ppm rate lowest growth for the bacteria (0.005nm) was found. As expresaron 7, who concluded that the metal ion lead in high concentrations present in different environments such as soil, sediment and water create an extreme environment for growth and microbial survival as are known to cause damage to DNA, proteins and lipids and replace the essential metal ions such as Zn, Ca and Fe of enzymes.

The results research carried out by[20] on identifying resistant lead endophytic bacteria isolated from rice plants, found by in vitro tests of tolerance *Pseudomonas putida* (M1TFm) and *Burkholderia cepacia* (M2TF733), at different concentrations of lead, they concluded according to the growth curve of these two species bacteria have the ability to resist lead as Pb (NO3) 2 to 10 ppm. on the other hand works carried out by[21] on tolerance and reduction of chromium (VI) *Bacillus cereus* B1, isolated from wastewater from a tannery, concluded that *B. cereus*, have the ability to tolerate up to 8000 ppm and remove 100% of the pollutant after 9, 34, 50 and 96 hours, when the initial chromium concentration was 10, 30, 50 and 100 ppm respectively. Studies conducted by[22], in endophytic bacteria associated with the genera Cyperus and Paspalum in soils contaminated with mercury, showed that the results of the resistance test for *Bacillus cereus* GU056811 show that this endophytic bacteria has the capacity to withstand 400 ppm (0.4 mg / L) of mercury in the form of $HgCl_2$.

The qualitative test siderophore production (Figure 4) on the medium surface azurol-S (CAS) B. cereus 1DH1LIM after being subjected to growth on 400 ppm Pb as Pb $(NO_3)_2$, states that this bacterium as well as being a promoter of growth resilience could be

related to the production of this compound. Various studies report that bacteria capable of withstanding heavy metals improve the growth and development of plants contaminated with these metals environments, which can be explained by the production of beneficial metabolites for the growth of these plants as: siderophores; acid, indole 3-acetic acid (IAA); 1-aminocyclopropane deaminase, 1- carboxylate (ACC) and solubilizing fosfatos[23-24]. As manifested by[25,26] the bacterial siderophores that are chelating agents of iron contribute in the plants to reduce the toxicity caused by the presence of heavy metals and also supply the need for iron as an essential element, promoting the development and growth of plants in contaminated environments.

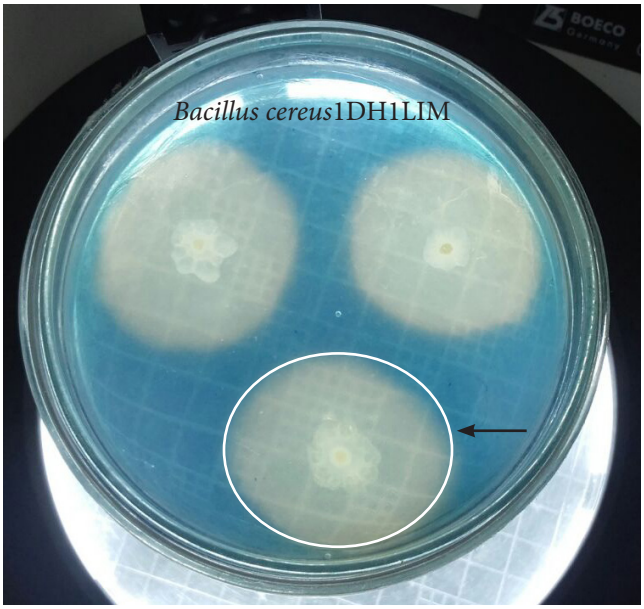

Figure 4. Siderophore production by *Bacillus cereus* 1DH1LIM in médium azurol-S (CAS).

4. Conclusion

Bacillus cereus 1DH1LIM is an endophytic bacteria of the species of grass *Paspalum arundinaceum* which grows in soil contaminated with mercury (4.7mg $kg^{-1)}$; That according to international standards is considered as a toxic soil category. This endophytic bacterium is able to withstand up to 400 ppm simultaneously in form of Pb $(NO_3)_2$ and mercury as $HgCl_2$ and contributes to the adaptation of plants to tolerate contaminants through siderophore production as observed *in vitro* experimentally.

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Conflict of interest: This manuscript is original and was prepared, revised with the participation of all authors, who declare that there is no conflict of interest that jeopardizes the validity of the results.

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