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Tolerances Of Rhizospheric And Endophytic Bacteria Isolated From Paspalum Notatum In Mercury-Contaminated Environments

Alexander Pérez-Cordero[1*], Donicer E Montes-Vergara[2] and Diego Carrillo-González[2]

[1]Grupo Bioprospección Agropecuaria, Laboratorio de investigaciones microbiológicas, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Colombia.

[2] Departamento de Zootecnia, Facultad de Ciencias Agropecuarias Universidad de Sucre, Colombia.

* Correspondence: Author: alexander.perez@unisucre.edu.co

ABSTRACT

The aim of this study was to isolate rhizospheric and endophytic bacteria from mercury-contaminated soils in southern Bolivar (Colombia) and to evaluate in vitro their tolerance capacity to different mercury concentrations. A completely random sampling was carried out, collecting, at the same time, samples of soil, rhizosphere and plant tissues (root, stem and leaves) in a mercury-contaminated environment. The samples were sent to specialized laboratories for determination of mercury concentration by instrumental cold vapor atomic absorption spectrophotometry. The populations of rhizospheric and endophytic bacteria were correlated with the mercury values present in each environment. Finally, the sensitivity and tolerance of the bacteria to different mercury concentrations was evaluated in vitro. The average mercury contents in soil were 5.9, in rhizosphere 5.3 and 4.5 kg/ kg in P. notatum. The amount of bacteria ranged as follows: in soil 2 x 10^5, in rhizosphere 2.6 x 10^{11} CFU/ g soil and in tissue 5.45 x 10^7 CFU/ g tissue. A total of 40 bacterial morphotypes were isolated, five rhizosphere bacteria and 35 as endophytic bacteria from the three identified plant species. The mercury sensitivity test, related to the PNM4RizHgLIM isolate from rhizosphere of Paspalum notatum, as well as PNM10RHgLIM from root with the highest mercury tolerance percentages with 85.4% and 79.1% respectively at 500 mg/L concentrations. This was followed by the morphotypes PNM6RHgLIM with 77.1% at 400 mg/L, PN1RHgLIM with 72.8% at 350 mg/L and finally PNM3THgLIM with 68.7% at 300 mg/L. Sequencing analysis linked the PNM6RHgLIM isolate with sequence homology to Bacillus thuringiensis strain F14; PNM4RizHgLIM to Bacillus cereus strain ML259 and Bacillus mycoides O-1; PNM10RHgLIM with Bacillus cereus strain LB1016; PNM1HgLIM

of stem with species belonging to the genus Bacillus sp and PNM3HgLIM of stem with bacteria of the Pseudomonas sp. group. There is presence of bacteria in rhizosphere and as endophytes present in soils contaminated with high concentration of mercury in the south of Bolivar(Colombia) that showed capacity to tolerate high concentrations of mercury in vitro and possibly accompanied by other studies could contribute to remediate environments contaminated with this metal.

Keywords: Bacteria, rhizosphere, endophytes, pasture.

INTRODUCTION

Since the beginning of the industrial era, mercury (Hg) levels in the environment have increased considerably, reaching concentrations that affect ecosystems and human health. Mercury is a heavy, silvery, ubiquitous, liquid metal at room temperature. In its pure form it is known as elemental mercury (Hg0). It volatilizes easily, forming colorless and odorless vapors (Gaiolí et al., 2012). In this sense, mercury is established as one of the toxic metals that most affects the wellbeing of ecosystems and puts human health at risk.

When mercury reaches nature and some time has elapsed, it is transformed into methylmercury (CH3Hg+). The difference between simple mercury and methylmercury is very important, as this second molecule is an organic form, which accumulates in the organism, especially in the nervous system. Moreover, this substance synergizes with other substances, increasing their harmful effects. This process of transformation to organic mercury is called methylation, which results in the mobilization of mercury. Methylmercury, like other organometallic compounds, is fat-soluble, and consequently highly toxic, as it can easily pass through biological membranes and in particular the skin, and from here the incorporation of the metal into the trophic chain follows (Posada et al., 2006).

The contamination of water, sediments and soils by heavy metals arises as a consequence of human activities, both industrial and agricultural, and the inadequate processing and disposal of waste containing these materials. This problem is becoming increasingly serious because it has direct effects on ecosystems and on the health of populations. Metals are not susceptible to degradation in the same way as organic compounds, so they cannot be eliminated in the elemental sense, as only the concentration varies, as well as the chemical form in which they are found. Worldwide, it has been confirmed that exposure to high concentrations of mercury causes adverse health effects, such as central nervous system, cardiovascular, immune and renal disorders (Holmes et al., 2009).

A large number of metal-tolerant or metal-accumulating micro-organisms can be found in nature and the study of these will allow the development of more effective biological remediation technologies. The organisms used in this technology can be bacteria and fungi (bioremediation), algae (phyco-remediation) or plants (phytoremediation). More recently,

rhizoremediation has emerged as an alternative technology involving the joint action of rhizospheric microorganisms and plants (Paisio et al., 2012).

Currently, techniques such as phytoremediation are being used to mitigate the effects of metal contamination, which is an effective, economical and environmentally friendly technology that is receiving much attention worldwide. The success of phytoremediation depends on the plant's ability to tolerate high concentrations of metals and produce large amounts of biomass (Pérez et al, 2016).

Nowadays, phytoremediation is an effective, economical and environmentally friendly technology that is receiving much attention worldwide, bringing with it great benefits as opposed to the traditional technology of accumulating heavy metals from the soil. Such advantages are its low cost and negligible impact on humans and ecosystems (Glick, 2010; Sheng et al., 2008). The success of phytoremediation depends on the plant's ability to tolerate high concentrations of metals and produce large amounts of biomass (Ma et al., 2011). The efficiency of heavy metal phytoremediation is dependent on the performance of the plant and its ability to accumulate metal ions, the microorganisms associated with these plant species provide benefits to the plant as they can provide nutrients and reduce the harmful effects caused by heavy metals (Belimov et al., 2002; Ma et al., 2011).

Similarly, these microorganisms also have effects on plant development by promoting plant growth and increasing biomass through the production of phytohormones such as indole acetic acid, while improving the nutritional status of plants through nitrogen fixation, phosphate solubilization and production of siderophore for the uptake of essential nutrients in their development (Sessitsch et al., 2013). Endophytic bacteria living in the internal tissues of plants favors the efficiency of the phytoremediation process and increase the production of plant biomass, through three mechanisms: increased root surface area and the production of root hairs; increased availability of metals and increased transfer of soluble metals from the rhizosphere to the plant, some of the endophytic bacteria studied (Pérez et al., 2016).

Accordingly, the research focuses on the following question: Are rhizospheric and endophytic bacterial species associated with plant tissues growing in mercury-contaminated soils tolerant to mercury in situ? The problem of mercury in soil and its impact on the environment can be reduced through the use of biological techniques by studying populations of rhizospheric and endophytic bacteria adapted to mercury-contaminated environments.

MATERIALS AND METHODS

Collection of the study material. Sampling was carried out in the department of Bolivar(Colombia), in which a random zig-zag sampling was carried out to take soil and tissue samples of plant species, collecting complete plants (root, stem and leaves). For the collection and selection of plant material, we selected those species with good and bad

phytosanitary status and without the presence of symptoms of mercury phytotoxicity. Soil and plant tissue samples were labelled with georeferencing of the sampling site and sent to specialized laboratories for determination of mercury concentration by cold vapors atomic absorption spectrophotometry. The other part of the samples was sent to the microbiological research laboratory of the University of Sucre for the respective analyses.

Determination of mercury levels in tissues, soil and rhizosphere. The plant samples were divided into different root, stem and leaf tissues, which were washed with distilled water to eliminate mineral particles adsorbed on their surface. Each tissue was then placed in paper bags and dried in an oven at 60°C for 24 hours. To determine the total mercury in these samples, 0.5 g of dry material was taken and an acidic HNO3/H2O2 mixture (5+2mL) was added. On the other hand, from the previously dried soil, 0.5g was taken and 10mL of 65% HNO3 was added. Both soil and plant samples were processed in a Milestone ETHOS TOUCH 127697 series microwave oven and total mercury was analysis by cold vapors atomic absorption spectrophotometry, according to procedures described in Marrugo-Negrete et al. (2015).

Isolation of rhizospheric bacteria. For the isolation of rhizospheric bacteria, root soils were taken from plants present in mercury-contaminated environments, removing the soil adhering to the roots and washed with 100 mL of sterile distilled water, then shaken for 30 min. Once sediment, an aliquot of the suspension was taken and serial dilutions were prepared from 104 to 108, and then inoculated on nutrient agar and incubated at 30±1°C for 72 h. All dilutions were performed in triplicate. All dilutions were performed in triplicate. Morphotypes were identified according to their characteristics and preserved on nutrient agar slants at 4°C (Salgado et al., 2012).

Isolation of endophytic bacteria. Plant samples collected from mercury-contaminated environments in southern Bolivar were separated by tissue (root, stem and leaves) and subjected to the process of isolation of endophytic bacteria described by Perez et al. (2016) with modifications. The protocol consisted of weighing one gram of each plant tissue and subjecting it to surface disinfection and then macerating each tissue until a homogeneous mixture was obtained. Each homogenate was added to nine mL of sterile peptonized water and incubated at 35±1°C for 24 h, after which, serial dilutions of each homogenate were prepared and an aliquot was inoculated by surface diffusion technique on R2A agar and incubated at 33±1°C for 72 h. The population density of endophytic bacteria was expressed as CFU/g tissue, and was estimated by direct counting of colonies on plates. During counting, colonies were observed and selected for shape, surface appearance, color and size (Pérez et al., 2016).

Mercury sensitivity test. All isolated bacterial morphotypes were used to evaluate their sensitivity to different mercury concentrations of 20 mg/L, 50 mg/L, 100 mg/L, 150 mg/L,

200 mg/L and 250 mg/L on MacConkey agar for rhizospheric morphotypes and on R2A agar for endophytes. Once inoculated on the surface of the medium, they were incubated at 33±1°C for 7 days. The assays were performed in triplicate and the sensitivity was qualitatively assessed by the growth of the morphotypes on the culture media with the different concentrations of mercury used.

Tolerance of bacteria to mercury. From the results of the sensitivity test, the morphotypes that grew in the highest concentration of mercury were taken and subjected to tolerance tests of the minimum and maximum growth concentration for which aliquots of log-phase suspensions of endophytic bacteria were inoculated on Tris-Low Phosphate Buffer (TLP) medium proposed by Rathnayake et al. (2013) at concentrations of 100; 150; 200; 250; 250; 300; 350, 400, 450 and 500 mg/L mg/L $HgCl_2$. TLP medium without $HgCl_2$ 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., 2014). The growth of endophytic bacteria was determined by turbidimetry at 600 nm every hour for four days. Siderophore production. The siderophore production capacity was carried out in chromium azurol-S (CAS) medium proposed by Schwyn and Neilands (1987), for those isolates that showed a greater capacity for mercury tolerance. For this purpose, 60.5 mg CAS was dissolved in 50 ml of distilled water and combined with 10 ml of an iron(III) solution (1 mM FeCl3.6 H2O and 10 mM HCl). Under stirring, this solution was mixed with 72.9 mg of HDTMA dissolved in 40 ml of water. The resulting blue liquid was sterilized at 121°C for 15 minutes. In another vessel, a mixture of 750 ml of water, 15 g of agar, 30.24 g of pipes, and 12 g of a 50% (w/w) solution of NaOH was sterilized to reach a pH of 6.8. To the medium, 4 g of glucose is added as a carbon source. The strains are incubated for 7 days at 30°C. The ability of the bacteria to produce siderophores is evidenced by the formation of a ring.

Identification of rhizospheric and endophytic bacteria with tolerance to different mercury concentrations. The isolates of endophytic bacteria that showed the highest tolerance to different mercury concentrations were used for molecular identification. Before starting the DNA extraction process, morphotypes were differentiated by Gram staining. Genomic DNA extraction was performed according to the protocol described by (Oliveira et al., 2013). Bacterial isolates were purified and activated in LB medium for 18 h; after this time, 1 mL of the bacterial suspension was taken and centrifuged at 12,000 rpm to obtain a bacterial precipitate; this precipitate was resuspended in 0.5 M EDTA and centrifuged at 12,000 rpm for 20 min; the precipitate was treated with lysis buffer (0.5 M EDTA and 0.25% SDS) and the mixture was incubated at 60°C for 1 h. After this time, 1 mL of the bacterial suspension was added to the bacterial suspension. After this time, 5M NaCl was added, incubated at 4°C for 5 min, and the samples were centrifuged at 12,000 rpm. To the resulting suspension, an equal volume of phenol-chloroform-isoamyl alcohol 25:24:1 was added and centrifuged at 12,000 rpm, and half a volume of isopropanol was added to the suspension and incubated for 14 h. Finally, the samples were added to the suspension.

Finally, the samples were added ethanol to dry the DNA and then resuspended in 0.5X TE buffer. Subsequently, amplification of 16S rDNA from endophytic bacterial communities was performed by PCR technique. The amplification of rDNA fragments was carried out with the use of specific oligonucleotides for eubacterial groups as listed in table 1. The conditions under which PCR was performed are also described.

The PCR products were sent for sequencing to Macrogen (Seoul, South Korea) on an automated 3730XL capillary sequencer. The nucleotide sequence entities obtained were compared with those stored in the National Center For Biotechnology Information (NCBI) database. Base alignment was performed by means of the clusteal W program and analysis and correlation with the MEGA 6® program. Phylogenetic inferences were obtained by distance and maximum parsimony Neighbor-joining with bootstrap test (1,000 replicates). The trees for the phylogenetic analysis of the sequences were reconstructed with the MEGA 6.0® program.

RESULTS AND DISCUSSION

Collection of plant material from mercury-contaminated environments. The collection was carried out at the site identified with coordinates N 8° 56' 17.61" and W 74° 2' 27.98", N 8° 56' 16.79" and W 74° 2' 41.13", N 8° 56' 9.54" and W 74° 2' 37.6" corresponding to the site called Mina Santa Cruz, Sur de Bolívar. The plant species adapted to the conditions of high mercury concentrations according to the morphology and expertise of the inhabitants of the area was reported as pitchfork grass (Paspalum notatum).

Paspalum notatum is a summer perennial grass, C4 type, native and one of the most frequent species in the natural fields of the Colombian Caribbean region. It is an aggressive forage plant in its colonization, adapted to a wide range of soil types, from poorly drained clayey to sandy soils with low fertility and high aluminum saturation. This species has high viable seed production and has stoloniform rhizomes accompanied by a deep root system, has as its center of origin South America, being widely cultivated in Australia and mainly in the United States, occupying more than 1.5 million hectares (Wallau et al., 2019).

Determination of mercury in soil, rhizosphere and plant tissues. The average mercury value in soil was 5.9, in rhizosphere 5.3 and in tissues 4.5 mg/kg (Figure 1). P. notatum had an average mercury concentration of 4.9 mg/kg in roots, 4.5 mg/kg in stems and 4.1 mg/kg in leaves (Figure 2). The analysis of variance, which indicated significant statistical differences (p-value<0.05) between mercury levels (mg/kg) in soil, rhizosphere and tissues and between the interaction of these factors. Tukey's test showed statistically significant differences (p-value<0.05) between mercury levels (mg/kg) in the different environments evaluated (Figure 1).

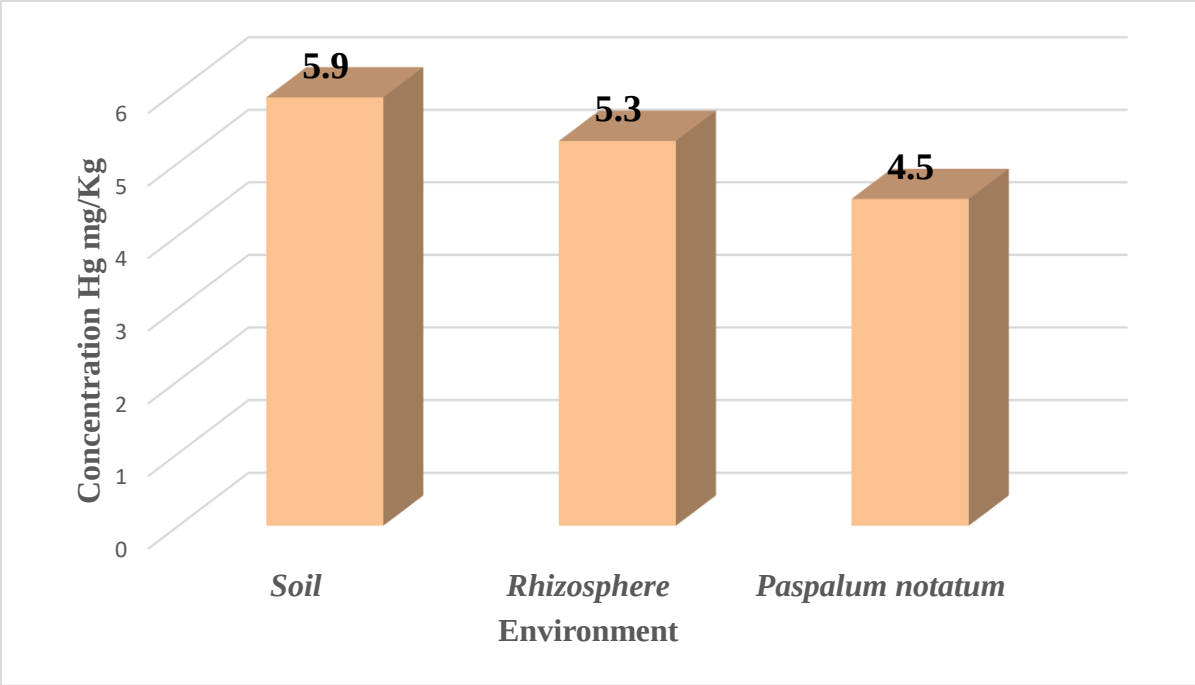

Figure 1. Mercury concentration in the soil environment, rhizosphere and plant tissue of P. notatum.

The average mercury levels found in soil taken from the mercury-contaminated area near the Santa Cruz Mine, Sur de Bolivar, Colombia are 5.9 mg/kg soil, which is above the permissible values at which it can cause adverse effects on human health. Investigations on heavy metals in this critical area of the Colombian Caribbean have also shown the presence of heavy metals in sediments with mercury levels of 7.67 µg/g, which is above the permissible standard (0.5 µg/g). The maximum contamination levels permitted by the Ministry of Environment and Sustainable Development for the particular case of mercury (0.02 mg/L) are much higher (MADS, 2015) than the values permitted for Hg 0.00003 mg/L by the United States Environmental Protection Agency (USEPA) (Nguyen et al., 2013).

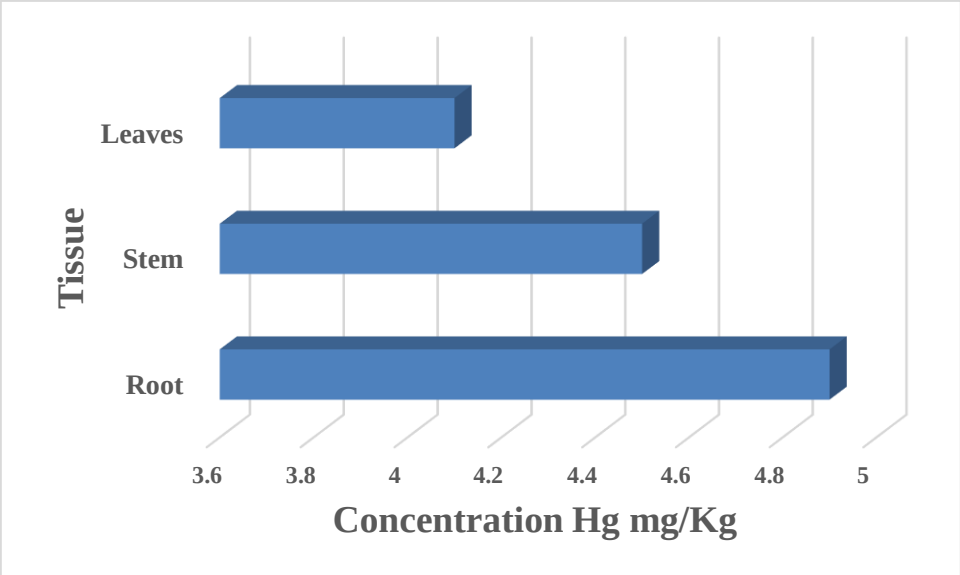

Figure 2. Concentration of mercury per tissue in Paspalum notatum.

Figure 2 shows the results of the Tukey test for mercury levels by tissue showing significant statistical differences (p-value<0.05) between the average concentrations in the plant tissues. The lowest mercury concentration was reported for the leaf tissue with 4.5 mg/kg. While the root tissue presented the highest mean mercury concentration with 4.9 mg/kg.

Figure 3 shows the presence of isolated bacteria by environment (soil, rhizosphere and endophytes). The quantity of bacteria ranged as follows: in soil 2 x 105; in rhizosphere 2.6 x 1011 CFU/ g soil and in tissue 5.45 x 107 CFU/ g tissue (Figure 3). The results show a higher presence of rhizosphere and endophyte bacteria in relation to the amount present in the soil.

Figure 3. Number of bacteria per environment (soil, rhizosphere and endophyte).

Figure 4 shows the number of endophytic bacteria per tissue of P. notatum. In the case of the plant tissue factor, the Tukey test shows significant statistical differences (p-value<0.05), with the leaves having the highest average density of endophytic bacteria, followed by the stem tissue and finally the roots with the highest population density of endophytic bacteria. Similarly, the levels of mercury present in soil, rhizosphere and endophytes were correlated, finding that soil presented the highest levels of mercury with 5.9 mg/kg and the lowest levels of bacterial population density with 12.0x1015 CFU/g soil. With respect to mercury levels and bacterial population density per tissue, higher densities of endophytic bacteria were observed in those tissues where mercury concentrations were lower. However, lower mercury concentrations were found in the roots compared to soil levels. Likewise, the same trend was found, where it is evident that at low mercury concentrations the population density is high.

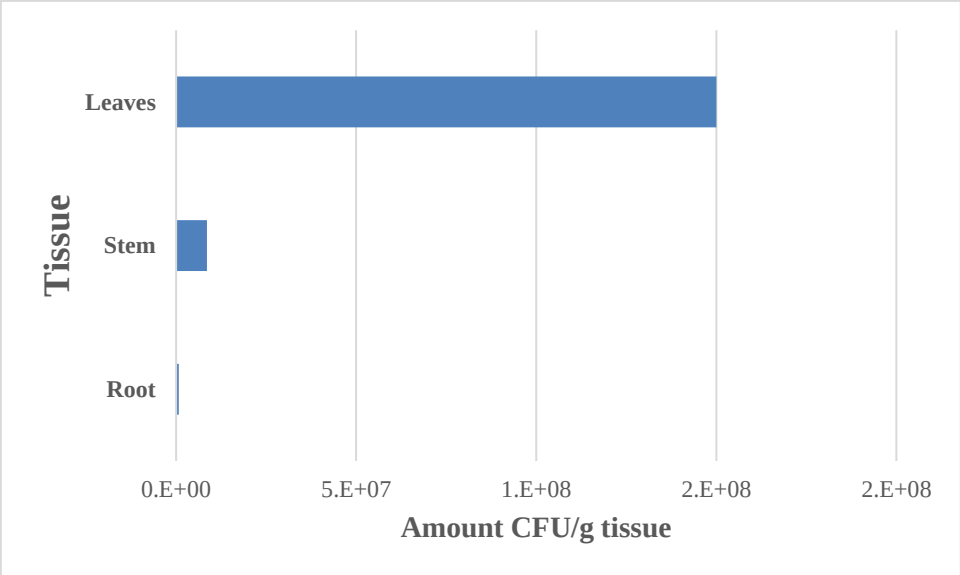

Figure 4. Number of endophytic bacteria per tissue from Paspalum notatum.

The correlation between population density and mercury levels evaluated shows that high mercury concentrations have a negative influence on the population density of bacteria, with the rhizospheric soil showing the highest mercury levels with 5.9 mg/kg and therefore the lowest average bacterial population density. The results found indicate that there is an inverse relationship between rhizospheric bacterial population density and mercury concentration.

Evaluation of the sensitivity of endophytic and rhizospheric bacteria to mercury. A DCA was carried out to evaluate the percentage of sensitivity to mercury of the bacterial morphotypes isolated from plant tissues, indicating that there are significant statistical differences (p-value<0.05) between the susceptibility of the bacterial morphotypes to the different concentrations of mercury, with the PNM4RHgLIM morphotype together with PNM10RHgLIM showing the highest percentages of tolerance to mercury with 85.4% and 79.1% respectively at 500 mg/L concentrations. This was followed by the morphotypes PNM6RHgLIM with 77.1% at 400 mg/L, PN1RHgLIM with 72.8% at 350 mg/L and finally PNM3THgLIM with 68.7% at 300 mg/L (Figure 5).

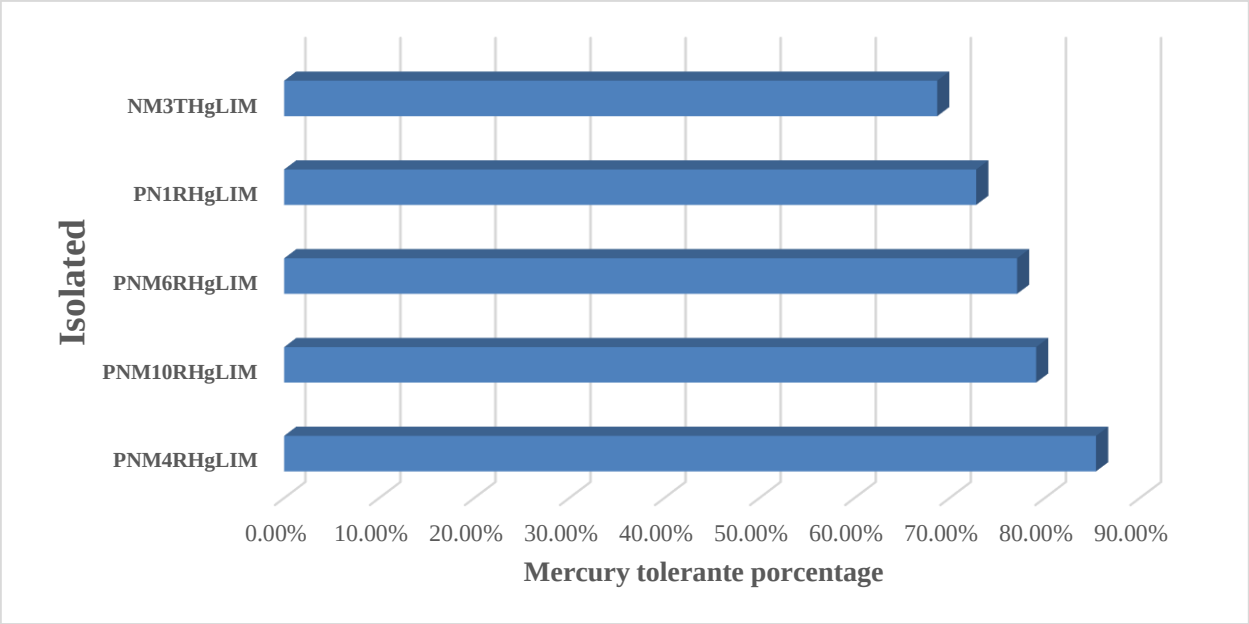

Figure 5. Bacterial tolerance test at different mercury concentrations

Growth curve. The tolerance growth curve of the five morphotypes of endophytic bacteria to different concentrations of mercury. The morphotypes identified as SOM4RHgLIM and SRM10RHgLIM according to the graph showed tolerance up to 500 mg/L, respectively. The morphotypes SOM6RHgLIM tolerated up to 400; MPM1HgLIM to 350 and SOM3HgLIM up to 300 mg/mL mg/L. Mercury is a highly reactive metal when in cationic form or bound to other compounds; biochemically they possess affinity towards functional groups (e.g. sulphhydryl groups) present in enzymes that catalyzed critical reactions in an organism, metal ions have been found to interact with cellular components such as DNA and proteins, causing damage and conformational changes that can alter the cell cycle (Tchounwou et al., 2012).

Siderophore production. With respect to the qualitative test assays for siderophore production in chromium azurol-S (CAS) medium proposed by Schwyn and Neilands (1987), in three (PNM10RHgLIM, PNM4RizHgLIM and PNM6RHgLIM) of the five isolates found, the production of this compound was evidenced. The results of the siderophore production assays are shown in figure 6. When the microorganism produces a chelator such as siderophores, iron is removed from the complex and the medium forms an orange halo (García et al., 2012).

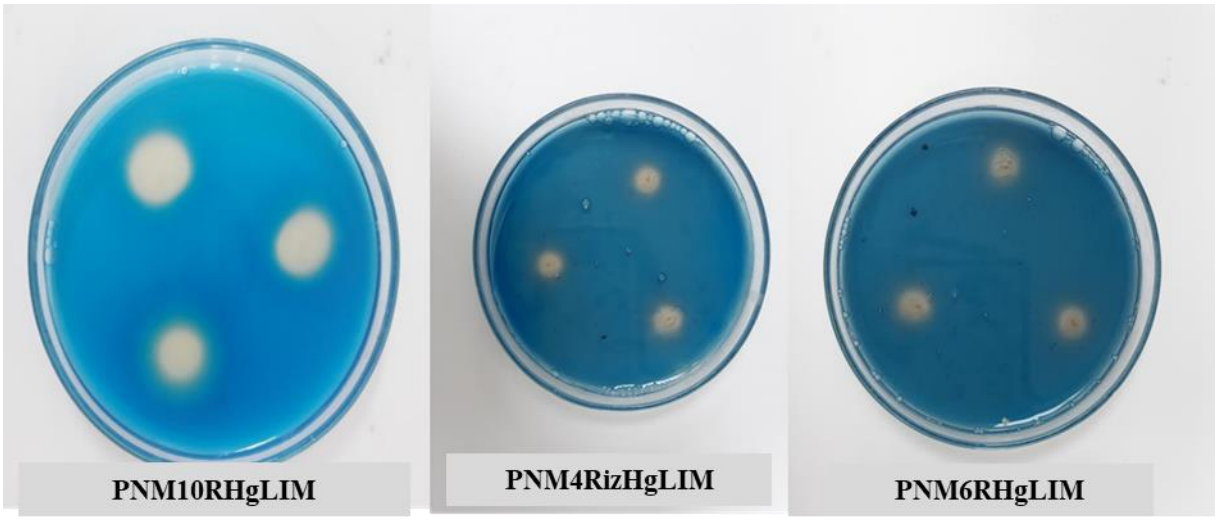

Figure 6. Assays of medium chromium azurol-S (CAS) siderophore production activity by bacterial morphotypes isolated from mercury-contaminated environments in southern Bolivar(Colombia).

Plant growth-promoting bacteria that have the ability to produce siderophores sequester iron by forming Fe^{3+} -siderophore complex via specific receptor, located on the bacterial membrane, which makes iron unavailable to other microorganisms that lack the specific assimilation system to recognize this complex (Kramer et al., 2020). Thus, by using all or most of the iron available in the soil, it suppresses or inhibits the growth of other pathogenic microorganisms present in the rhizosphere (Compant et al., 2005; Schroth and Hancock, 1982). According to Rajkumar et al. (2009) siderophores contribute to reducing the toxicity caused by heavy metals by binding metal ions having iron-like chemistry such as Al, Cd, Cu, Ga, In, Pb and Zn, which are of great concern to the environment (Braud et al., 2009) thereby making them unavailable to the plant and also supplying the need for iron as an essential nutrient, thus stimulating plant development under heavy metal production stress.

Molecular identification of mercury-tolerant endophytic bacteria. The sequences of the isolated morphotypes obtained by sequencing technique were compared with sequences present in the NBCI library. Phylogenetic analysis of 16S rDNA gene of endophytic bacteria with the ability to tolerate different concentrations of mercury showed similarity with Bacillus thuringiensis, Bacillus cereus, Bacillus sp and Pseudomona sp species (Figure 7).

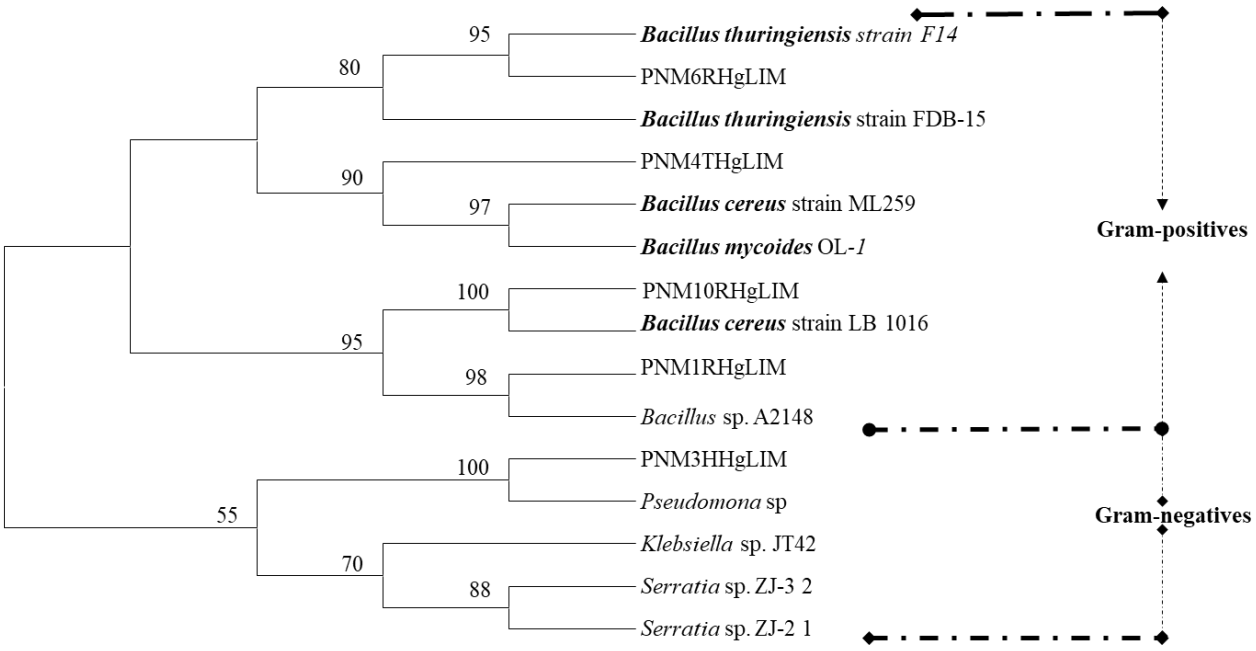

Figure 7. Phylogenetic tree of maximum similarity derived from the analysis of 16S rDNA gene sequences of endophytic bacteria isolated from Paspalum notatum from mercury-contaminated environments. At the base of each clade, the branch support is shown, expressed as the percentage of times the analysis produced the same association between sequences. PN: Paspalum notatum; M: morphotype; Riz: rhizosphere; R: root; T: stem; Hg: mercury; LIM: Laboratório Investigaciones Microbiológicas.

The PNM6RHgLIM root isolate (morphotype) has high homology with the sequences of Bacillus thuringiensis strain F14; PNM4RizHgLIM rhizosphere isolate with Bacillus cereus strain ML259 and Bacillus mycoides O-1; PNM10RHgLIM from root with Bacillus cereus strain LB1016; PNM1HgLIM from stem with species belonging to the genus Bacillus sp and PNM3HgLIM from stem with species belonging to the genus Pseudomonas.

Heavy metal tolerance in bacteria has been extensively studied worldwide. Several bacterial species have been reported for metal resistance. Most of the bacterial species that claim to be possible candidates for heavy metal bioremediation belong to the genera Bacillus, Pseudomonas and Streptomyces (Uslu and Tanyol, 2006).

The genus Bacillus belongs to the family Bacilliaceae, a genus that today includes more than 60 species of bacilli. This genus consists of Gram-positive, endospore-forming, chemiheterotrophic bacillary microorganisms that are normally motile and surrounded by periotic flagella. They are anaerobic or facultative aerobic, catalase positive. This genus is commonly found in soils and plants where they play an important role in the carbon and nitrogen cycle. They are common inhabitants of fresh and stagnant waters, and are particularly active in sediments. They have also been found in soils contaminated with heavy

metals using Denaturing Gradient Gel Electrophoresis (DGGE) and bacteria belonging to the genera: Arthrobacter, Bacillus, Brevibacterium, Brochothrix, Comamonas, Cytophaga, Deinococcus, Enterobacter, Hafnia, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Rathayibacter, Rhodococcus, Salmonella, Serratia, Staphylococcus, Variovorax and Xanthomonas (Ellis et al, 2003). Some metal resistance systems have been found to be encoded by chromosomal genes in some microorganisms such as Bacillus sp. which showed resistance against mercury. Kavaruma and Esposito (2010) carried out studies with Bacillus subtilis in order to study the genes that are expressed by exposure to metals, identifying that different types of affected genes were regulated by metallo-regulatory proteins known as Fur, MntR, Per R, ArsR and CueR.

Lin and Harichund (2011) studied the removal of heavy metals from chemical industry effluent and found that Bacillus sp. was able to remove As by 20.3% and Hg by 16.7% effectively from the effluent. Nanda et al. (2011a,b) reported different bacteria for the efficient removal of heavy metals in effluent from the pharmaceutical industry and found bacteria of the genus Bacillus sp. in the removal of Hg (45%) and Cu (62%). Pseudomonas sp. was able to remove 56% of Cd, 34% of As and 53% of Co.

Sinha et al. (2012) immobilized a strain of Bacillus cereus in calcium alginate, with which they obtained high removal efficiencies of Hg(II) through bioadsorption to the bacterial biomass (80% removal of initial 20 mg/L Hg(II), in 120 h), in both batch and continuous systems.
Highly mercury-resistant bacteria (Bacillus thuringiensis) have been isolated from coastal marine sediments in India and are able to tolerate up to 50 ppm mercury chloride (Dash et al., 2013). Furthermore, mercury-resistant aerobic bacteria were isolated from sediments of the mercury-contaminated area of the Tagus estuary in Portugal that were mostly Bacillus spp. and were able to tolerate up to 10 ppm Hg2+ and can reduce Hg2+ to Hg0 (Figueiredo et al., 2016). In addition, a strain of Bacillus thuringiensis PW-05 was isolated from the Odisha coast of India and was found to resist 50 ppm Hg (Dash et al., 2013). Bacillus cereus, has been reported as an endophytic bacterium from plants of the genera Cyperus and Paspalum with in vitro ability to tolerate up to 400 ppm (400 mg/L) mercury in the form of HgCl2 and furthermore from rice plants with ability to tolerate up to 400 ppm Pb in the form of Pb (NO3)2 and to produce siderophore (Perez et al., 2016; Perez et al., 2018).

On the other hand, the genus Pseudomonas is the most heterogeneous and ecologically important group of known bacteria. Because the nutritional requirements of species of this genus are very simple, representatives have been detected in virtually all natural habitats and tend to be predominant among bacteria associated with the rhizosphere of plants (Arora, 2015; De Oliveira et al., 2015). The role of Pseudomonas in Bioremediation is a consequence of their environmental importance and metabolic diversity thanks to their ability to degrade

a wide range of organic compounds as demonstrated by many authors in their research where they have shown to be efficient in the bioaccumulation of heavy metals, this process has gained importance in recent years due to its good performance, low cost, specificity and easy reusability (Ahu¬ja et al, 2001).

Wagner-Döbler (2003) summarizes the results obtained after two years of work in a pilot plant built to treat effluents from a chlor-alkali electrolysis industry in the Czech Republic. In this work, a packed bed bioreactor with a capacity to treat 100 m3 of effluent per day (containing between 2 and 10 mg/L Hg) was used and operated continuously for 8 months with excellent results. This bioreactor was inoculated with a bacterial biofilm, consisting of seven strains of Pseudomonas immobilized on pumice, which has the capacity to reduce Hg(II). The optimization of this system involved the testing of different culture conditions, immobilization matrices, biofilm types and bioreactor operating conditions.

Pepi et al., (2011), used biofilm-producing bacteria belonging to the genera Pseudomonas and Psychrobacter, both free and immobilized on a pumice matrix, to volatilize organic and inorganic Hg with high efficiency (up to 190 ng/mL in 5 min). Regarding the removal of MeHg.

Lee et al. (2012), described that Pseudomonas balearica reduced 97% of MeHg (20 μg/L) in 3 h while Cabral et al. (2012) indicated that Pseudomonas putida V1 volatilized 77% of MeHg (2.5 μM), in only 24 h. A recent contribution to the understanding of bacterial MeHg detoxification mechanisms was made by Adelaja and Keenan (2012), who reported for the first time that strains of Pseudomonas fluorescens, Enterobacter cloacae, Citrobacter braakii and Alcaligenes faecalis use MeHg as their sole carbon and energy source.

Deng and Wang (2012) reported that a bacterial strain of Pseudomonas sp. isolated from marine sediments removed and accumulated more Hg2+ on the cell surface. The bio-capture of mercury was probably through functional groups attached to the bacterial cell wall, such as carboxyl, phosphate, hydroxyl, thiol and pyridine groups that contributed to the uptake of Hg2+, and carboxyl groups were the most important in this action.

CONCLUSIONS

The soil of the study area showed mercury values of 5.9 mg/kg; in the rhizosphere of 5.3 mg/kg and in the plant tissue of 4.5 mg/kg in Paspalum notatum, which were found to be above the internationally permitted levels and the soil was considered to be in the toxic category. The average population density of bacteria showed a higher presence in the rhizosphere and plant tissue environments where Hg concentrations were relatively lower compared to the soil environment where the highest average values of the heavy metal were reported. The susceptibility test of the bacteria isolates to the different concentrations of mercury indicated that PNM4RHgLIM (Bacillus cereus strain ML259 and Bacillus mycoides

O-1); PNM10RHgLIM (Bacillus cereus strain LB1016) presented the highest percentages of tolerance to mercury with 85.4% and 79.1% respectively at concentrations of 500 mg/L. This was followed by the morphotypes PNM6RHgLIM (Bacillus thuringiensis strain F14) with 77.1% at 400 mg/L, PNM1RHgLIM (Bacillus sp) with 72.8% at 350 mg/L and finally PNM3THgLIM (Pseudomonas sp) with 68.7% at 300 mg/L. The presence of rhizospheric and endophytic bacteria in the soil conditions with high mercury concentration in southern Bolivar showed the ability to tolerate high mercury concentrations in vitro and possibly accompanied by other studies could contribute to remediate environments contaminated with this metal. The isolates PNM10RHgLIM, PNM4RizHgLIM and PNM6RHgLIM, which showed the capacity to tolerate 500 and 400 mg/L of HgCl2, respectively, and the capacity to produce siderophores in vitro, this compound being possibly responsible for reducing the toxicity caused by mercury.

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