

Isolation and characterization of endophytic bacteria from nickel hyperaccumulator plant *Leucocroton havanensis* (*Euphorbiaceae*)

Aislamiento y caracterización de bacterias endófitas de la planta hiperacumuladora de níquel *Leucocroton havanensis* (*Euphorbiaceae*)

Alexander Govin Sanjudo^{1,*}, Dubiel Alfonso González², Orquídea Coto Pérez¹, Jeannette Marrero Coto¹, Dayesi López Hernández¹, Giselle Torres Farradá¹

¹Departamento de Microbiología y Virología, Facultad de Biología, Universidad de La Habana, Calle 25 # 455 e/ I y J, La Habana, Cuba. C.P. 10400. ²Jardín Botánico Nacional, Universidad de La Habana, Carretera El Rocío, km 3½, Calabazar, Boyeros, La Habana, Cuba. C.P. 19230. *Autor para correspondencia: agovin@fbio.uh.cu

ABSTRACT

Endophytic bacteria from heavy metals hyperaccumulators can promote plant growth and the metal uptake, thereby they can assist the phytoremediation process. The aim of this work was to isolate and characterize morphological and biochemically endophytic bacteria from nickel hyperaccumulator *Leucocroton havanensis*, an endemic Cuban plant. The resistance to Ni(II) was evaluated and the isolates more resistant were selected for its characterization. We determined the growth promoting plant traits such as the production of indole acetic acid, siderophores and 1-aminocyclopropane-1-carboxylic acid deaminase, ammonia, as well as phosphorous solubilization and dinitrogen fixation. The solubilization of an insoluble nickel compound were determined in the selected isolates. We isolated 33 endophytic bacteria from leaves, roots and stems from *L. havanensis*. Gram-positive endospore-forming rods bacteria predominated. All isolates grew on media containing more than 0.5 mmol/L of Ni(II), but only ten of them grew on more than 10 mmol/L of Ni(II), which were selected to continue the characterization. The selected isolates were characterized biochemically and morphologically and they were identified as *Bacillus* sp. All bacteria produced indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid deaminase, ammonia and siderophores. Nine of them fixated nitrogen and two solubilized phosphorus. Moreover, they solubilized nickel from nickel carbonate insoluble compound. The endophytic bacteria from nickel hyperaccumulator *L. havanensis* are resistant to Ni(II), they present several traits for plant growth promotion and solubilize Ni(II), hence that they are attractive for to be applied in the improvement of phytoremediation.

Keywords: nickel resistant bacteria, nickel solubilization, phytoremediation, plant growth promotion

RESUMEN

Las bacterias endófitas de las plantas hiperacumuladoras de metales pesados pueden promover el crecimiento de la planta y la absorción del metal, por lo que es posible su empleo en el proceso de fitorremediación. El objetivo de esta investigación fue aislar y caracterizar morfológica y bioquímicamente bacterias endófitas de la planta hiperacumuladora de níquel *Leucocroton havanensis*, una planta endémica de Cuba. Se evaluó la resistencia de los aislados al Ni(II) y se seleccionaron los más resistentes para continuar su caracterización. Se determinó la producción de ácido indol acético (AIA), sideróforos y 1-aminociclopropano-1-carboxilato desaminasa, amoníaco, así como la solubilización de fósforo y la fijación biológica de dinitrógeno. Además, se determinó la capacidad de solubilizar Ni(II) a partir del carbonato de níquel. Se aislaron un total de 33 bacterias endófitas a partir de raíces, tallos y hojas de *L. havanensis*, donde prevalecieron los bacilos Gram positivos esporulados. Todos los aislados crecieron en presencia de concentraciones mayores de 0,5 mmol/L de Ni(II) y 10 de ellos resistieron concentraciones superiores a 10 mmol/L de este metal. Estos últimos se caracterizaron bioquímicamente y morfológicamente y se identificaron como *Bacillus* sp. Todos los aislados seleccionados mostraron la capacidad de producir ácido indolacético, 1-aminociclopropano-1-carboxilato desaminasa, amoníaco y sideróforos. Dos aislados solubilizaron fósforo inorgánico y nueve de ellos fijaron dinitrógeno atmosférico. Además, todos solubilizaron níquel a partir del carbonato de níquel. Las bacterias endófitas de la planta hiperacumuladora de níquel *L. havanensis* son resistentes a Ni(II) y presentan varias características relacionadas con la promoción del crecimiento vegetal por lo que pueden ser aplicadas en el mejoramiento de la fitorremediación.

Palabras claves: bacterias resistentes a níquel, solubilización de níquel, fitorremediación, promoción del crecimiento vegetal

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INTRODUCTION

Industrial operations such as mining, smelting, manufacturing of alkaline storage batteries, combustion of fossil fuel and sewage sludge, cause accumulation of metals or metalloids in natural resources such as soil, water and air and they are considered contaminant source (Ma & al. 2016a). Metals such as nickel (Ni), cobalt (Co), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and molybdenum (Mo) are considered

trace and essential elements for growth; however, the higher concentrations of metal cations are toxic and produce toxic effects on organisms (Ali & al. 2019). Moreover, metallic ions such as chromium (Cr), cadmium (Cd), lead (Pb) and mercury (Hg) with no known function in organisms are toxic (Yan & al. 2020). Heavy metals can be accumulated in ecosystem and they can be incorporated to organisms through the food chains due to biomagnification phenomenon (Ashraf & al. 2017).

Heavy metals contamination is a serious worldwide concern due to its toxicity and mutagenicity (Manoj & al. 2020). Various physicochemical and biological methods have been developed to remove the metals from ecosystems. Conventional technologies such as excavation and soil washing are frequently expensive and environmentally invasive because affect the soil microbiota (Hasan & al. 2019). Hence it is necessary search affordable solutions to improve the environmental health.

Phytoremediation involves the use of plants to reduce pollutants in ecosystem (Yan & al. 2020). This is a low cost, sustainable and environmentally friendly technology (Xu & al. 2016). Hyperaccumulators can contain more than 1000 mg/kg dry weight of nickel, copper or lead, or greater than 10,000 mg/kg dry weight of zinc or manganese or more than 300 mg/kg dry weight of cobalt or chromium (van der Ent & al. 2013, Hasan & al. 2019) and they are considered ideal candidates for the phytotechnologies. Despite the advantages of phytoremediation, it is considered as time-consuming form of remediation because in general plants are slow to grow. Moreover, when plants are exposed to high concentrations of metallic ions, they pose a slow growth and low biomass yield, also most of the metallic ions have low mobility in soil and are not easily absorbed by plant roots, hence it may limit the efficiency of phytoremediation (Ma & al. 2016a, Pan & al. 2016). Therefore, is very important to explore the possibilities of improve the biomass yield of metal accumulator plants using plant growth-promoting bacteria as inoculants (Visioli & al. 2015, Ma & al. 2016b).

Endophytes are microbes that reside within the plant endosphere without causing any damages (Mukherjee & al. 2018). Endophytic bacteria are of particular interest owing to they can produce phytohormones such as indole acetic acid (IAA) and gibberellic acid, moreover they can produce enzymes such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which inhibits ethylene synthesis (Visioli & al. 2015). Furthermore, endophytic bacteria can improve bioavailability and increase the uptake and bioaccumulation of metal by means of the production of siderophores (uptake of iron), solubilization of nutrients as phosphorus, potassium and solubilization of metallic cations (Sheng & al. 2008, Mukherjee & al. 2018). Endophytic microorganisms have the capacity to control pathogens and promote plant establishment under adverse conditions and enhance plant growth and development. A better understanding of the characteristics of heavy metal-resistant endophytic bacteria is a critical pre-requisite for the development of effective phytoremediation of heavy metal-contaminated soils. In recent years, the use of metal resistant endophytic bacteria from hyperaccumulators in phytoremediation of contaminated soils with metallic ions has attracted the attention of the scientific community (Visioli & al. 2014, Pan & al. 2016).

Ni-rich soils, often described as "serpentine", house a characteristic flora. They present a high endemic level of hyperaccumulators that are adapted for tolerating high heavy metals concentration. Cuba accounts with 7 % of the land

area of serpentine soils and about 920 of the country's 6,375 species are serpentine-endemic (Reeves & al. 1999). Nickel hyperaccumulators are one of the most representative in Cuban serpentine soils. *Leucocroton havanensis* Borhidi (*Euphorbiaceae*) is a nickel hyperaccumulator, it accumulates up to 19,000 µg/g dry matter in the leaves (Reeves & al. 1996). It is a shrub, endemic to Cuba, and is geographically restricted to xeromorphic scrublands on serpentine soils of La Coca, La Habana, and Lomas de Galindo, Mayabeque. Previous studies showed that *L. havanensis* accumulate nickel in *in vitro* conditions and it can potentially be used for phytoremediation and phytomining (Alfonso & Matrella 2014). However, the endophytic bacterial community from *L. havanensis* are still not studied and the knowledge about microbiota associated to Cuban nickel hyperaccumulator is limited. The selection of endophytic bacteria resistant to nickel and with potentialities as plant growth promoting, is a powerful tool for enhancing the phytoremediation. In the present study we focused in the isolation and characterization of the endophytic bacteria from *L. havanensis*.

MATERIALS AND METHODS

Sampling and isolation of endophytic bacteria

Roots, stems and leaves were collected from nickel hyperaccumulator *Leucocroton havanensis* plants growing on serpentine soils at "Loma de la Coca" (23°4'57,04" lat. N; 82°6'43,68" long. W; 39 m asl), "Habana del Este", La Habana, Cuba. Plant samples were washed with tap water followed by three rinses with sterile deionized water; these were disinfected by immersion in 3 % sodium hypochlorite solution for 1 min, after three sequential immersions in 75 % ethanol alternated with immersion in sterile deionized water (Luo & al. 2011). In order to confirm that the surface disinfection process was successful, the water from the final rinse was plated out on Petri dishes plates of Luria-Bertani's (LB) agar. Vegetal samples were cut into pieces of 2 cm long and put on Petri plates of LB agar. Plates were incubated at 28 °C during five days. Bacteria displaying different morphologies were re-streaked on new plates (LB medium) to obtain axenic monocultures. The bacterial isolates were labelled by H (bacteria from leaves), T (bacteria from stem) and R (bacteria from roots) followed by a roman number. Bacterial isolates were characterized according to its microscopic morphology and stain.

Bacterial resistance to nickel

Ni(II) resistance was evaluated by means of the growth on Tris minimum medium (TMM) following the methodology of Mergeay & al. (1985). For each isolate, the lowest concentration which inhibited visible growth at 28 °C within five days was recorded as minimum inhibitory concentration (MIC). The most nickel resistant bacteria were selected for continuing the study.

Identification of endophytic bacteria

The identification of selected endophytic bacteria was carried out through biochemical tests: oxidase, catalase, nitrate reduction, indole test, arginine hydrolase, starch hydrolysis, gelatin hydrolysis, β glucosidase and β galactosidase production

and assimilation of different carbon source (urea, L-arabinose, D-mannose, D-mannitol, N-acetyl glucosamine, D-maltose, D-glucose, fructose, sucrose, gluconate)

Evaluation of plant growth promoting activities

Production of IAA by bacteria, was determined in LB broth amendment with 1 mg/mL of tryptophan. After 96 h, 1 mL of cell suspension was mixed thoroughly with 1 mL Salkowski's reagent (Bric & *al.* 1991), then they were incubated at room temperature for 30 minutes until a stable pink color was developed. The absorbance at $\lambda = 530$ nm was measured for IAA quantification.

Siderophores secretion was detected by the universal method of Schwyn & Neilands (1987), using blue agar plates containing the dye Chrome azurol S (CAS). Orange halos around the colonies on agar were indicative of siderophores excretion. The ACC deaminase activity was determined by the bacterial growth on Dworkin Foster (DF) broth followed the considerations from Jacobson & *al.* (1994).

Phosphate solubilization was determined on National Botanical Research Institute's Phosphate (NBRIP) medium after 120 hours of incubation. The presence of translucent halo was considered as positive test. Fixation nitrogen by bacterial isolates was determined in a nitrogen free medium, following the methodology applied by Xinxian & *al.* (2011).

Production of ammonia was evaluated in each bacterium in peptone water tube and incubated at 28 °C for 4 days and 1 mL of Nessler's reagent was added in each tube. Tubes were observed for presence of a yellow to brownish color from production of ammonia (Brígido & *al.* 2019).

In vitro solubilization of nickel

The bacterial isolates were grown in LB broth for 24 h and subsequently were inoculated in 100 mL volumes to SMS flasks (in g/L: sucrose, 10.0; $(\text{NH}_4)_2\text{SO}_4$, 1.0; K_2HPO_4 , 2.0; MgSO_4 , 0.5; NaCl, 0.1; yeast extract, 0.5; CaCO_3 , 0.5; pH 7.2) amended with insoluble nickel compound NiCO_3 (0.3 g/L), at 30 °C for seven days in shaking conditions at 150 r/min (Xinxian & *al.* 2011). The nickel concentration in the solution was determined at 24, 96, 144, 192 and 240 hours using the Merkoquant's indicator rods.

Statistical Analysis

The IAA concentration in culture was determined using a calibration curve of pure IAA as a standard following the linear regression analysis. The comparison of averages was determined by means one-way ANOVA and after a Tukey's test with $p < 0.005$. All statistical analyses were performed using *Statistic v.8* (StatSoft 2007).

RESULTS

Isolation of endophytic bacteria from nickel hyperaccumulator and resistance to Ni(II)

No contamination was found on LB medium employed for confirming the disinfection process. Thirty-three endophytic bacteria were isolated from roots (21), stems (9) and leaves

(3) of *Leucocroton havanensis* (Table I). We observed the prevalence of Gram positive sporulate rod-shaped bacteria (26). Nevertheless, five-bacteria were Gram positive rod that did not form endospores. Only two endophytes showed bacillary morphology and negative at Gram's stain and one-isolated showed coccus morphology and was Gram positive.

All bacteria grew at nickel concentration above 0.5 mmol/L of Ni(II) (Table I). The 54.5 % of them grew in medium containing up to 1.0 mmol/L, however 15.1% resisted up to 3.0 to 10 mmol/L and 30.3 % (10) showed high resistance to nickel (more than 10 mmol/L). The most resistant bacteria (NR) were isolated from stems and roots (T8, T9, R1, R3, R5, R7, R8, R10, R11 and R18) and they were selected to continue the study.

Identification of NR endophytic bacteria

The biochemical characteristics of the isolates are listed in the Table II. All NR bacteria showed negative response to oxidase and indole test and they were positive to catalase test, starch hydrolysis, gelatin hydrolysis and nitrate reduction test. Nevertheless, the NR bacteria showed different patterns of carbon source assimilation. The integral analysis of this results (Table I and II) allowed to identify the ten NR isolates as *Bacillus* sp.

Evaluation of plant growth promoting activities

The ten NR strains could promote plant growth due to the production of siderophores, ammonium and ACC deaminase. The 90 % of the NR bacteria fixated atmospheric nitrogen (Table III). The R8 and R18 strains were the only ones that solubilized inorganic phosphorus, they showed all the evaluated characteristics for the plant growth promoting activity

All NR strains produced IAA, in LB medium supplemented with tryptophan (Figure 1). NR bacterium R5 showed highest concentration of IAA (18.4 mg/mL), followed by strains R7 (16.3 mg/mL), R8 (16.2 mg/mL) and R1 (16.0 mg/mL).

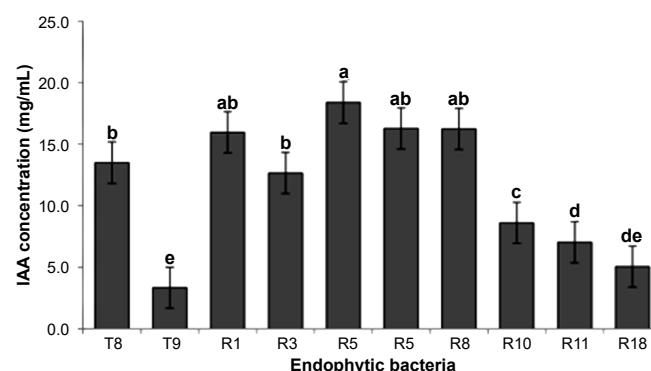


Fig. 1. Production of IAA by endophytic bacterial isolates nickel resistant from *Leucocroton havanensis* in LB medium amendment with tryptophan. The cultures were incubated at 28 °C, 150 r/min at 96 hours. Different letter represents of significant differences by Tukey test ($n = 3$, $p < 0.05$).

Fig. 1. Producción de AIA por los aislados bacterianos endófitos resistentes a níquel de *Leucocroton havanensis* en medio LB suplementado con triptófano a las 96 horas, 28 °C, 150 r/min. Letras diferentes representan diferencias significativas según la prueba de Tukey ($n = 3$, $p < 0,05$).

In vitro solubilization of nickel

We detected soluble Ni (II) in the bacterial culture media indicating that all strains solubilized the nickel present in NiCO₃ (Figure 2). R1 showed the best capacity for nickel solubilization (92 %). Eight strains (T8, T9, R1, R3, R5, R8, R10 and R18) solubilized more than 50 % (w/v) of the NiCO₃ present in the culture medium. The abiotic control did not produce soluble nickel at all.

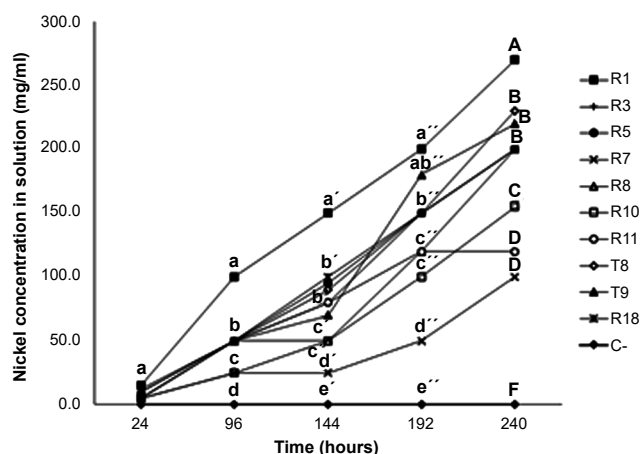


Fig. 2. Variation of nickel concentration in solution of the broth medium amended with insoluble nickel (nickel carbonate) after 240 hours of incubation at 150 r/min. (C-: abiotic control). The different letter represents of significant differences by Tukey test ($n = 3$, $p < 0.05$).

Fig. 2. Variación de la concentración de níquel en solución del medio mínimo líquido suplementado níquel insoluble (carbonato de níquel) 240 horas, 150 r/min. (C-: control abiótico). Las letras diferentes representan diferencias significativas según la prueba de Tukey ($n = 3$, $p < 0,05$).

DISCUSSION

Endophytic microorganisms colonize host plant tissues without causing damage. In addition, they establish an intimate relationship with their host and stimulate growth through various mechanisms such as phytohormone and ammonium production, phosphate solubilization, nitrogen fixation, and siderophore production (Xinxian & al. 2011). Application of endophytic bacteria in phytotechnologies have received special attention in the last decade because they can improve plant response to multiple stresses and increase resistance of plants against metal ions (Wang & al. 2020). Endophytes can significantly enhance chlorophyll content, shoot and root length, and biomass, as well as, they could improve phytoextraction efficiency (Mukherjee & al. 2018). Characterization researches of endophytic bacteria from hyperaccumulators are essential to achieve success in phytotechnologies.

The higher density of endophytic bacteria in the roots was reported in an isolation of endophytic bacteria (83) from roots, stems and leaves of the nickel hyperaccumulator *Alyssum bertolonii* Desv. which 58 % of the bacteria were isolated from the roots, 19 % from the stems and 23 % from the leaves (Barzanti & al. 2007). In a study of isolation of endophytic bacteria from *Sedum alfredii* Hance, a Zn/Cd hyperaccumulator plant, it was observed that between the roots, stems and leaves

the bacterial density was significantly different, the highest density of endophytes was isolated from the roots and its concentration could range from 2,000 to 816 000 CFU/g (Xinxian & al. 2011). Similar results have been reported in arsenic-accumulating plants like, *Pteris multifida* Poir. (Zhu & al. 2014), in manganese hyperaccumulator *Phytolacca americana* L. (Zhang & al. 2015) and arsenic hyperaccumulator *Pteris vittata* L. (Xu & al. 2016).

We found a predominance of endospore-forming Gram-positive rod (Table I), these characteristics belong to bacteria from Firmicutes phylum (Logan & de Vos 2007). The phylum Firmicutes, Proteobacteria and Actinobacteria are more frequent and abundant in the endophytic bacteria of metal accumulating plants (Zhang & al. 2015, Gu & al. 2018). The ten strains were identified as *Bacillus* sp. The presence of this genus had been frequently reported in endosphere of nickel hyperaccumulator plants such as *Alyssum bertolonii* (Barzanti & al. 2007), *Noccaea caerulescens* (J.Presl & C.Presl) F.K.Mey. (Visioli & al. 2014), as well as the endophytic bacteria of the arsenic hyperaccumulator plants *Pteris vittata* and *Pteris multifida* (Zhu & al. 2014) and endophytic bacteria from Mn hyperaccumulator *Phytolacca americana* (Zhang & al. 2015).

Ni-hyperaccumulators can provide a specific environment for bacterial endophytes to adapt to high Ni concentrations. All endophytic bacteria of *Leucocroton havanensis* were able to tolerate larger concentrations of Ni indicating an adaptation of these endophytic bacteria to the high levels of this ion metal. The isolates from roots and stems were more resistant to Ni than leaves endophytes (Table I). Although Ni concentrations in hyperaccumulators is higher in leaves than in stems and roots, its bioavailability is lower due to that it is mainly accumulated in leaf vacuoles while in stem and root tissues is mainly present in the xylem sap (Deng & al. 2018, van de Pas & Ingle 2019). Xylem inhabiting bacteria could likely cope with the higher free Ni concentration than those living in parenchymatic tissues of leaf (Barzanti & al. 2007). Endophytic bacteria have several mechanisms, through which they alleviate the toxicity of metal ions (efflux of metal ions, conversion to less toxic forms, sequestration on the cell surface or in intracellular polymers, and precipitation, adsorption/desorption) (Ma & al. 2016a).

Phytoremediation is a technique to clean up of contaminant environment with heavy metals. However, phytoremediation efficiency is limited due to the phytotoxicity at high toxic ion concentrations and small biomass production (Manoj & al. 2020). In this sense, the endophytic bacteria can promote plant growth using the same mechanism of the plant growth promoting bacteria (PGPB). These are traits that contribute to the improvement of the growth of hyperaccumulators. We evaluated the potentiality of the 10 bacterial isolates to promote plant growth (Table II). The production of siderophores and IAA are characteristics frequently researched in endophytic bacteria isolated from hyperaccumulators. Siderophores are organic molecules that show high affinity for Fe (III) ions, but they can also form complexes with other bivalent heavy metal ions that can be assimilated by the plant. Bacterial sidero-

TABLA I

Morphologic characteristics and minimum inhibitory concentration to nickel of bacterial isolates from *Leucocroton havanensis*

TABLA I

Características morfológicas y concentración mínima inhibitoria a níquel de los aislados bacterianos de *Leucocroton havanensis*

Isolate code	Plant organ	Cell shape	Gram reaction	Endospore formation	Ni(II) resistance (mmol/L)
H1	Leaves	Rods	+	+	1.0
H2	Leaves	Rods	+	+	1.0
H3	Leaves	Rods	+	+	1.0
T1	Stem	Rods	+	-	1.0
T2	Stem	Rods	+	-	1.0
T3	Stem	Rods	+	+	1.0
T4	Stem	Rods	+	+	1.0
T5	Stem	Rods	+	+	1.0
T6	Stem	Rods	+	+	4.0
T7	Stem	Rods	+	+	1.0
T8	Stem	Rods	+	-	15.0
T9	Stem	Rods	+	-	13.0
R1	Root	Rods	+	+	20.0
R2	Root	Rods	+	+	1.0
R3	Root	Rods	+	+	15.0
R4	Root	Rods	+	+	1.0
R5	Root	Rods	+	+	15.0
R6	Root	Rods	+	+	1.0
R7	Root	Rods	+	+	15.0
R8	Root	Rods	+	+	15.0
R9	Root	Cocci	+	-	1.0
R10	Root	Rods	+	+	15.0
R11	Root	Rods	+	+	15.0
R12	Root	Rods	-	-	5.0
R13	Root	Rods	+	+	1.0
R14	Root	Rods	+	+	4.0
R15	Root	Rods	+	+	1.0
R16	Root	Rods	+	+	7.5
R17	Root	Rods	-	-	3.0
R18	Root	Rods	+	+	20.0
R19	Root	Rods	+	+	1.0
R20	Root	Rods	+	+	1.0
R21	Root	Rods	+	+	1.0

TABLE II

Biochemical characteristics of NR endophytic isolates from *Leucocroton havanensis*

TABLA II

Características bioquímicas de los aislados endófitos resistentes a níquel de *Leucocroton havanensis*

Biochemical test	T8	T9	R1	R3	R5	R7	R8	R10	R11	R18
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+
Indole test	-	-	-	-	-	-	-	-	-	-
Arginine di hydrolase	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-
β glucosidase	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
β galactosidase	-	-	-	-	-	-	-	-	-	-
L-arabinose	-	-	+	+	+	-	-	+	-	+
D-mannose	+	+	+	+	+	+	+	+	+	+
D-mannitol	+	+	+	+	+	+	+	+	+	+
N-acetyl glucosamine	+	-	-	+	+	-	-	-	+	-
D-maltose	-	-	+	+	+	+	-	-	+	-
D-glucose	+	+	+	+	+	+	+	+	+	+
Fructose	-	+	-	-	-	-	+	-	+	-
Sucrose	+	+	-	-	+	-	-	-	-	+
Gluconate	-	-	-	-	-	-	-	-	-	-

TABLE III

Plant growth promoting activities of the ten nickel resistant endophytic bacterial isolates from *Leucocroton havanensis*

(+) positive activities, (-) negative activities.

TABLA III

Actividades de promoción del crecimiento vegetal de los diez aislados bacterianos resistentes a níquel endófitos de *Leucocroton havanensis*

(+) actividad positiva, (-) actividad negativa.

Bacterial Isolates	Fixation of N ₂	Phosphorus inorganic solubilization	Production of NH ₃	Production of siderophores	ACC deaminase activity
T8	+	-	+	+	+
T9	+	-	+	+	+
R1	+	-	+	+	+
R3	-	-	+	+	+
R5	+	-	+	+	+
R7	+	-	+	+	+
R8	+	+	+	+	+
R10	+	-	+	+	+
R11	+	-	+	+	+
R18	+	+	+	+	+

phores production may be stimulated by the presence of heavy metals and can help the plant to reduce heavy-metal toxicity by increasing the supply of iron to the plant (Barzanti & al. 2007). All isolates produced IAA and siderophores, similar results have been informed in Ni-resistant strains isolated from *Thlaspi goesingense* Halácsy (Idris & al. 2004), *Noccaea caerulescens* (Visioli & al. 2014) and *Alysum bertolonii* (Barzanti & al., 2007). Moreover, this trait showed endophytic bacteria were isolated from arsenic hyperaccumulator plant *Pteris vittata* (Gu & al. 2018), Mn-hyperaccumulator *Phytolacca americana* (Zhang & al. 2015) and selenium hyperaccumulator plants (Sura-de Jong & al. 2015).

Endophytes protect plants from oxidative stress and alleviate metal toxicity (Jan & al. 2019). High concentrations of heavy metal ions in plants induce the synthesis of ethylene, whose precursor is 1-aminocyclopropane-1- carboxylic acid (ACC), causing the inhibition of root elongation, lateral root growth and root hair formation. Certain endophytic bacteria could alleviate the stress by enzymatic hydrolysis of ACC by production of ACC desaminasa. This characteristic is common in endophytic bacteria isolated from hyperaccumulator *Brassica napus* (Sheng & al. 2008), *Noccaea caerulescens* (Visioli & al. 2014, Visioli & al. 2015), and *Phytolacca americana* (Zhang & al. 2015).

Endophytic bacteria can also indirectly contribute to the tolerance of plants to metals ions, by providing them with additional nutrients such as N, P and Fe. Plants growing in mineralized soils, generally suffer from macro and micro-nutrient deficiencies (He & al. 2013). The mobility and bioavailability of metal ions in soils is a critical factor affecting the success of metals uptake by plants. In general, ions are bound to organic and inorganic compound, or alternatively, present as insoluble precipitates, which are not available for plants. Plant growth-promoting bacteria enhance the bioavailability and solubility of heavy metals; hence they increase the accumulation of metal ion by plant (Ashraf & al. 2017). All bacteria were able to solubilize insoluble NiCO₃ compound (Figure 2). Solubilization of NiCO₃ could occur by secreting organic acids which increase in the H⁺ concentration of the medium (Xinxian & al. 2011). Endophytic bacteria can alter heavy metal bioavailability and enhance the Ni uptake by plants; hence they are promising for phytotechnologies (Idris & al. 2004, Visioli & al. 2014, 2015).

CONCLUSIONS

Microbiota from hyperaccumulator plants is a promising field of research to improve phytotechnologies and to better understand the plant- microbe interactions. This study contributes to the knowledge of the microbiota associated to the hyperaccumulator plants in Cuba. We isolated and characterized 33 endophytic bacteria from roots, stems and leaves from the Ni hyperaccumulator *Leucocroton havanensis*. All endophytic bacteria showed resistance to Ni (II) and the highly resistant one (10) were characterized. All selected isolates belong to *Bacillus* genus, showed plant growth-promoting traits and solubilize Ni, thereby they may play an important role in effec-

tive phytoremediation. The nickel-resistant endophytic bacteria could be used as inoculant for the effective phytoremediation of Ni-contaminated soil.

AUTHOR'S CONTRIBUTIONS

A. Govin conceived the original idea, designed the research, collected the samples, and performed the experiments. D. Alfonso wrote the first version of the manuscript and participated in the collection of the samples. G. Torres also wrote the first version of the manuscript. O. Coto and J. Marrero designed the research. D. López performed the experiments. All authors contributed to the writing, discussion of the results, and critical revision of the manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: All authors have carried out fieldwork and data generation ethically, including obtaining appropriate permitting.

Consent for publication: All authors have consented to publishing this work.

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