ACTA BIOLÓGICA COLOMBIANA
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Facultad de Ciencias
Departamento de Biología
Sede Bogotá

UNIVERSIDAD NACIONAL DE COLOMBIA

ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

MICROBIOLOGÍA

IDENTIFICATION OF ENDOPHYTIC BACTERIA OF SEEDS FROM *Cedrela odorata* L. (Meliaceae) WITH BIOTECHNOLOGICAL CHARACTERISTICS

Identificación de bacterias endófitas de semillas de *Cedrela odorata* L. (Meliaceae) con características biotecnológicas

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Received: 26th February 2020, **Returned for revision**: 01st July 2020, **Accepted**: 27th July 2020.
Associate Editor: Juan F. González.

Citation/Citar este artículo como: Espinosa Zaragoza S, Sánchez Cruz R, Sanzón Gómez D, Escobar Sandoval MC, Yañez Ocampo G, Morales Constantino MA, Wong Villarreal A. Identification of endophytic bacteria of seeds from *Cedrela odorata* L. (Meliaceae) with biotechnological characteristics. Acta Biol Colomb. 2021;26(2):196-206. Doi: http://dx.doi.org/10.15446/abc.v26n2.85325

ABSTRACT

In the present study, 62 endophytic bacterial strains of cedar seeds (*Cedrela odorata* L.), collected in the municipalities of Huehuetán, Motozintla, and Pijijiapan in the state of Chiapas, Mexico were isolated. The goal was to identify characteristics of biotechnological interest such as biocontrol, promotion of plant growth, and growth in aromatic compounds. The strains were identified by the partial sequence of the 16S ribosomal gene as belonging to the *Bacillus* genus. The biocontrol capacity of phytopathogenic fungi, production of indoleacetic acid (IAA), solubilization of phosphate, and growth in xenobiotic compounds (phenanthrene, benzene, anthracene, or phenol) were detected in 26 strains of the 62 isolates. 21 % of the strains inhibited the mycelial growth of *Alternaria solani* and *Fusarium* sp., and 13 % of the *Phytophthora capsici* oomycete. IAA production was detected in 24 isolates, phosphate solubilizing activity was identified in 18 isolates, while the ability to grow in the presence of phenanthrene and benzene was found in 26 isolates; 24 isolates grew in the presence of anthracene and only two isolates grew in phenol as the only carbon sources. This is the first report of the isolation and identification of endophytic bacteria from cedar seeds, where biotechnological characteristics were detected for biological control, promotion of plant growth, and growth in the presence of xenobiotic compounds.

Keywords: Cedar, Phytopathogenic, Aromatic Hydrocarbons, Indole acetic Acid.

RESUMEN

En el presente estudio se aislaron 62 cepas bacterianas endófitas de semillas de cedro (*Cedrela odorata* L.) colectadas en los municipios de Huehuetán, Motozintla y Pijijiapan en el estado de Chiapas, México, con el objetivo de identificar características de interés biotecnológicas como biocontrol, promoción del crecimiento vegetal y crecimiento en compuestos aromáticos. Las cepas se identificaron por la secuencia parcial del gen 16S ribosomal como pertenecientes al género *Bacillus*. En 26 cepas de las 62 aisladas se detectaron la capacidad de biocontrol de hongos fitopatógenos, la producción de ácido indolacético (AIA), la solubilización de fosfato y el crecimiento en compuestos xenobióticos (fenantreno, benceno, antraceno o fenol). El 21 % de las cepas inhibió el crecimiento miceliar de *Alternaria solani* y *Fusarium* sp., y el 13 % del oomiceto *Phytophthora capsici*. La producción de ácido indolacético

se detectó en 24 aislados y la actividad solubilizadora de fosfato se encontró en 18 aislados, mientras que la capacidad de crecer en presencia de fenantreno y benceno se manifestó en 26 aislados (24 aislados crecieron en presencia de antraceno y solo dos aislados crecieron en fenol como únicas fuentes de carbono). Es importante mencionar que este es el primer reporte del aislamiento e identificación de bacterias endófitas de semillas de cedro, en el que se detectaron características biotecnológicas para el control biológico, la promoción del crecimiento vegetal y el crecimiento en presencia de compuestos xenobióticos.

Palabras clave: Cedro, Fitopatógeno, Hidrocarburos aromáticos, Ácido indol acético.

INTRODUCTION

Mexico is one of the few mega-diverse countries on the planet. Despite having more than 23 000 species of vascular plants, few forest species have an industrial interest. Red cedar (*Cedrela odorata* L.) is the second most important tropical timber species in the forest industry in Mexico, only surpassed by mahogany (*Swietenia macrophylla*). It has a great economic potential for its excellent features and high commercial timber value (Estrada *et al*., 2016). Recent evaluations suggest that over 300 000 plant species are found worldwide and that every plant carries at least one endophyte (Smith *et al*., 2008). Indeed, endophytic microbes have been found in every plant species examined to date; Partida and Heil (2011), report that a plant without endophytes could only occur infrequently. It can be assumed that plants deprived of endophytes would be more vulnerable to environmental stress and pathogenic attacks (Khan *et al*., 2012; Leitão and Enguita, 2016; Suman *et al*., 2016; Brader *et al*., 2017).

Endophytic microorganisms are primarily bacteria and fungi that live within plants for at least part of their life cycle without causing apparent harm to the host. The various seed-borne bacterial endophytes found in plant tissues utilize either direct or indirect mechanisms to improve plant growth, development and enhance tolerance to biotic and abiotic stresses. In many cases, the microbes protect the plant hosts against diseases and insect pests (Santoyo *et al*., 2016; Shahzad *et al*., 2017a;b).

The internal environment of the seed changes during maturation, which consequently affects the seed endophytic community. The ability to inhabit a seed and adapt to severe environmental conditions are special characteristics of seed endophytes that are rarely found in endophytes isolated from roots, shoots, or other plant tissues. Seed endophytes can form endospores, thus protecting from changing conditions inside the seed (Compant *et al*., 2011; Kane, 2011). Seed-borne bacterial endophytes also participate in modulating endogenous phytohormones (Shahzad *et al*., 2016). Also, some plant growth-promoting bacterial endophytes can lower ethylene levels by synthesizing a catalyst, ACC deaminase (1-aminocyclopropane-1-carboxylate), of an ethylene precursor in higher plants (Shahzad *et al*., 2018). Verma *et al*. (2017), reported that *Enterobacter asburiae*, *Pantoea dispersa*, and *Pseudomonas putida* strains are endophytes of rice seeds, can produce indole acetic acid, solubilization of phosphates, and inhibit the growth of *Fusarium oxysporum*. They influence the growth and development of seedlings. biotechnological characteristics such as the production of indole acetic acid, siderophores, phosphate solubilization, nitrogen fixation, and ACC deaminase activity have also been reported in *Bacillus* strains isolated from seeds of four commercial varieties *Lycopersicum esculentum* Mill. (Xu *et al*., 2014).

Endophytic bacteria degrading polycyclic aromatic hydrocarbon (PAH) have been reported (Liu *et al*., 2016). The use of endophytes may offer advantages because the host plant provides a stable environment without interference from the native microflora and its ability to promote plant growth and reduce PAH content directly in plant tissues. Zhu *et al*. (2017) reported that *Serratia* sp. PW7, an endophyte isolated from P*lantago asiatica*, could degrade pyrene *in vitro* and *in vivo*; similar behavior was reported in *Azospirillum* sp. strains and the endophyte *Pseudomonas stutzer* isolated from *Hordeum sativum*, *Zea mays* L., and *Leymus arenarius*, which degraded anthracene, phenanthrene, and pyrene (Gałązka and Gałązka, 2015).

However, there is a lack of studies about the diversity of endophytic bacteria in *Cedrela odorata* L., seeds, which can promote the growth of plants that are of importance in biotechnology. Therefore, in this study, the objective was to isolate, characterize, and perform growth on aromatic compounds tests, growth promotion, and antagonisms of endophytic isolates of cedar (*Cedrela odorata* L.) seeds collected from trees in different regions of the state of Chiapas, Mexico.

MATERIALS AND METHODS

Seed collection

Seeds of *Cedrela odorata* L. were collected In the months of January and February 2019 from trees located in the municipalities of Huehuetán (15°01′ N, 92°23′ W), Motozintla (5°21′ N, 92°14′ W), and Pijijiapan (15°41′ N, 93°12′ W), Chiapas, Mexico.

Seed endophytes isolation

Endophytic bacteria were isolated from 100 seeds of *Cedrela odorata* L. as described below: the epiphytic microorganisms were eliminated by superficial disinfection of the seeds, washing with distilled water one min, ethanol

70 % one min, and sodium hypochlorite 3 % (v/v) three min; the excess sodium hypochlorite was removed by rinsing with sterile distilled water. Subsequently, the seeds were crushed and resuspended in 9 mL of a sterile 10 mM magnesium sulfate solution. Finally, serial dilutions of each sample were made and they were seeded in Petri dishes on peptone agar, and yeast extract (PY), with the following composition (g/L): 10, peptone; 5, yeast extract; and 18 agar, which were incubated at 28 °C for three days; the colonies that grew were selected. The effectiveness of disinfection was checked by placing seeds in Petri dishes with PY agar and incubated at 28 °C for five days.

Phosphate solubilization

Bacterial strains were inoculated into the PY liquid medium and incubated at 28 °C for 24 h with shaking at 200 rpm to obtain a pre-inoculum. The bacterial cultures were centrifuged at 10 000 g for five minutes and the optical density was adjusted to 0.2 absorbance units at 600 nm. The biomass collected was seeded in triplicate in NBRIP culture medium with the following composition (%): glucose, 1; $Ca_3(PO_4)_2$, 0.5; $(NH_4)_2SO_4$, 0.01; $MgSO_4.7H_2O$, 0.025; KCl, 0.02; $MgCl_2$. $6H_2O$, 0.5; Congo red, 2.5 mg/mL; and agar, 1.8, and incubated at 28 °C for seven days. After this incubation period, the presence of translucent halos around the bacterial colonies was sought (Caballero *et al.*, 2007).

Determination of auxin production

The strains were grown in liquid NFB medium with the following composition (g/L): malic acid, 5; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2$, 0.02; $FeSO_4$, 0.015; Na_2MoO_4, 0.0025; $MnSO_4$, 0.01; KOH, 4.8; NH_4Cl, 0.2; yeast extract, 0.3; and H_3BO_4, 0.01; they were incubated for 18 h at 28 °C at 200 rpm. Subsequently, the cultures were adjusted to an optical density of 0.2 units at 600 nm. Finally, 100 µL of the cultures were inoculated in the Jain and Patriquin medium with the following composition (g/L): succinic acid, 2.5; fructose, 2.5; K_2HPO_4, 6;KH_2PO_4, 4; NH_4Cl, 1; $MgSO_4$, 0.2, NaCl, 0.1; $CaCl_2$, 0.02; $FeCl_3$, 0.01; $NaMoO_4$, 0.002; and KOH, 2.1; with and without tryptophan (0.1 g/L) and incubated at 28 °C for 24 and 48 h at 200 rpm. One mL aliquots of the cultures were centrifuged for 5 minutes at 5000 x g, and 0.5 mL aliquots of the supernatant were taken and mixed with 0.5 mL of Salkowski reagent (Glickmann and Dessaux, 1995). The mixture was incubated in the dark at room temperature for 20 minutes; the absorbance at 530 nm was subsequently measured using a SmartSpec Plus spectrophotometer (Bio-Rad Laboratories Inc., Hercules, CA, USA). The presence of IAA was detected according to the method modified by Rahman *et al*. (2010). IAA concentrations were determined using a standard IAA curve (Sigma-Aldrich Corp.) from 0 to 50 µg/mL.

Antifungal activity

The antifungal activity of the endophytic strains was evaluated against the phytopathogenic fungi *Fusarium* sp., *Alternaria solani*, and the oomycete *Phytophthora capsici*. These phytopathogens were grown for seven days at 28 °C in PDA medium with the following composition (%): 0.4, potato extract; 2, dextrose; and 1.5, agar. Subsequently, 7 mm blocks containing mycelia were cut with a sterile scalpel and placed in the middle of a Petri dish with PDA medium. Endophytic cultures were scratched at two ends of the plate and incubated at 28 ± 2 °C for 48-96 h to evaluate the zone of fungal growth inhibition. The control consisted only of placing the 7 mm blocks of the fungus on PDA agar. The diameter of the mycelial growth was measured with a vernier to calculate the percentage of inhibition using the equation of Guo *et al.* (2006):

$$\text{Mycelial inhibition (\%)} = [1 - (Da / Db)] * 100$$

Where:

Da = Diameter of the mycelial growth zone of treatments (mm)

Db = Diameter of the mycelial growth zone of the control (mm)

Growth on solid medium with aromatic compounds

Bacterial strains were grown in BSE liquid medium for 18 h at 28 °C with shaking 200 rpm. The cultures were centrifuged at 10 000 g, the supernatant was removed and the biomass was adjusted to an optical density of 0.2 to 600 nm. 100 µL of these cultures were inoculated in duplicate in SAAC medium with the following composition (g/L): K_2HPO_4, 0.4; KH_2PO_4, 0.4; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.02; Na_2MoO_4, 0.002; $FeCl_2$, 0.01; $(NH_4)_2SO_4$, 0.5; bromothymol blue, 0.075; agar, 18; as the sole carbon source, 0.1 % phenol, 0.05 % benzene, 0.05 % phenanthrene, or 0.05 % anthracene. The plates were incubated at 28 °C for four days.

Molecular identification of the bacterial strains

Molecular identification was carried out on 26 strains that were selected based on the presence of one or more characteristics of biotechnological interest such as the production of indoles, phosphate solubilization, growth in aromatic compounds, and inhibition of phytopathogens. Genomic DNA of each bacterial strain was extracted using the ZR Fungal/Bacterial DNA Kit ™. The 16S ribosomal gene was amplified using the oligonucleotides rD1 and fD1 and the conditions described by Weisburg *et al*. (1991). The amplification products were purified from gel using the GeneJET kit (Thermo Scientific) and were sequenced at

the Instituto de Biotecnología, Universidad Nacional Autónoma de México. The sequences obtained were deposited in the GenBank of the National Centre for Biotechnology Information (NCBI) under accession numbers: MN073815 (Cedo1); MN073829 (Cedo2); MN073809 (Cedo3); MN073825 (Cedo4); MN073826 (Cedo6); MN073818 (Cedo8); MN073806 (Cedo10); MN073807 (Cedo11); MN073827 (Cedo12); MN073830 (Cedo13); MN073824 (Cedo14); MN073817 (Cedo16); MN073823 (Cedo17); MN073810 (Cedo18); MN073811 (Cedo19); MN073820 (Cedo21); MN073822 (Cedo22); MN073813 (Cedo23); MNO73816 (Cedo25); MN073812 (Cedo26), MN073814 (Cedo28), MN073828 (Cedo31); MN073808 (Cedo33); MN073821 (Cedo35); MN075223 (Cedo36); and MN073819 (Cedo37). The 16S rDNA sequences were compared with the 16S rDNA genes in the GenBank database using BlastN and the phylogenetic analysis was performed using the program MEGA 6 (Tamura *et al*., 2013). The phylogenies were constructed using the neighbor-joining method (Saitou and Nei, 1987) based on 600 nucleotides for 16S rDNA, using the distance matrix of Jukes and Cantor (1969). The trees topology was bootstrapped 1000 times.

RESULTS

Isolation of endophytic bacterial strains of cedar seeds

From the cedar seeds of the selected trees, 62 strains were isolated which were named Cedo1 to Cedo62; they were evaluated to detect activities associated with growth promotion, antagonism against phytopathogenic fungi, and growth in xenobiotic compounds as described below.

Phosphate solubilization activity

Phosphate solubilization activity by microorganisms is very important because this activity can solubilize non-soluble phosphorus in soil. The phosphate solubilization activity of 62 endophytic bacterial strains was evaluated, resulting in 20 strains that could solubilize phosphate with diameter halos of 20-26 mm after seven days of incubation. The greatest diameter halo of phosphates solubilization was obtained with bacterial strain Cedo31 (Table 1).

Quantification of Indoleacetic acid

A direct mechanism that promotes plant growth is the production of auxins, mainly indoleacetic acid. Of the endophytic bacteria isolated from cedar seeds, 24 bacterial strains could produce IAA in a range of 3 to 97.6 µg/mL at 48 hours (Table 1). Bacterial strains Cedo3, (97.6 µg/mL), Cedo11, (96.5 µg/mL), and Cedo 37 (81 µg/mL) had the highest production of this hormone (Table 1).

Antagonistic activity against different phytopathogens

An indirect mechanism proposed to promote plant growth is the control of phytopathogenic fungi, which affects crop yields or even kills plants. With this as background, the ability of endophytes to inhibit the mycelial growth of phytopathogens, *Fusarium* sp., *Alternaria solani*, and the oomycete *Phytophthora capsici* was evaluated (Fig. 1). Of the 62 bacterial strains evaluated, 24 had antagonistic activity against at least one phytopathogen (Table 2). The strain Cedo13 inhibited the mycelial growth of the three phytopathogens, in 72 % for *Alternaria solani*, 62 for % *Phytophthora capsici*, and 54 for % *Fusarium* sp. This behavior was also presented by the strain Cedo22 inhibiting 75 % of the mycelial growth of *Alternaria solani*, 68 % of *Phytophthora capsici*, and 50 % of *Fusarium* sp.. The bacterial strain with the highest percentage of inhibition of mycelial growth against *Alternaria solani* and *Fusarium* sp. was Cedo36, with 76 % and 59 % of inhibition, respectively, while for *Phytophthora capsici* it was strain Cedo22 with 68 % (Table 2).

Growth in aromatic compounds

In this work, the ability of endophytic strains to grow in culture media containing benzene, phenol, anthracene, or phenanthrene as the only carbon source was evaluated. 26 strains grew in the presence of benzene and phenanthrene; 24 bacterial strains in anthracene and two in phenol, and only strain Cedo22 grew in the presence of the four aromatic compounds (Table 1).

Molecular Identification

The 16S ribosomal gene sequences of the 26 selected strains were analyzed by the Blast algorithm, where all strains were observed to have a high similarity with species of the *Bacillus* genus (Table 3). The phylogenetic tree was constructed using fragments of the sequences of the 16S ribosomal genes to confirm the identity of the isolates at the genus level. The cladogram shows that strains Cedo1, Cedo2, Cedo3, Cedo4, Cedo6, Cedo11, Cedo12, Cedo14, Cedo18, Cedo23, Cedo26, Cedo28, Cedo31, Cedo33, Cedo35, and Cedo37 are probably related to *B. cereus*, while strains Cedo8, Cedo10, Cedo13, Cedo16, Cedo17, Cedo19, Cedo21, Cedo22, Cedo25, and Cedo36 are probably a group of new species of *Bacillus* (Fig. 2).

DISCUSSION

The endophytic microorganisms present in seeds are of great importance because they have a primary role in the control of pathogens, and they contribute to conserve and facilitate germination (Shahzad *et al*., 2018). In this study, 62 endophytic bacterial strains were isolated from *Cedrela*

Table 1. Endophytic bacterial strains growth in aromatic compounds and biotechnological characteristics

Code	Growth in aromatic compounds				Indoleacetic acid production (µg/mL)	Phosphate solubilization halo size (mm) 7 days
	Benzene	Anthracene	Phenol	Phenantrene		
Cedo1	+	+	-	+	6.0±0.9	0
Cedo2	+	+	-	+	31.7±2.6	20±2.2
Cedo3	+	+	-	+	97.6±5.7	0
Cedo4	+	+	-	+	48.6±2.5	17±1.4
Cedo6	+	+	-	+	14.5±1.5	0
Cedo8	+	+	-	+	0	18±1.9
Cedo10	+	+	-	+	23.8±1.7	25±2.3
Cedo11	+	+	-	+	96.5±4.9	20±2.5
Cedo12	+	+	-	+	17.9±1.4	16±1.4
Cedo13	+	-	-	+	23.0±3.1	16±1.2
Cedo14	+	-	-	+	26.0±3.9	23±2.7
Cedo16	+	+	-	+	0	0
Cedo17	+	+	-	+	17.5±1.9	19±1.1
Cedo18	+	+	-	+	2.7±0.6	20±2.7
Cedo19	+	+	-	+	13.5±1.6	17±2.3
Cedo 21	+	+	-	+	6.5±1.1	0
Cedo22	+	+	+	+	29.8±1.9	19±1.3
Cedo23	+	+	-	+	39.8±4.3	15±1.7
Cedo25	+	+	-	+	19.4±2.9	15±2.0
Cedo26	+	+	-	+	19.1±2.2	13±1.6
Cedo28	+	+	-	+	41.3±4.8	0
Cedo31	+	+	-	+	55.6±7.7	26±2.9
Cedo33	+	+	-	+	22.3±2.9	20±2.1
Cedo35	+	+	-	+	27.5±3.6	10±1.7
Cedo36	+	+	-	+	48.2±3.9	17±1.3
Cedo37	+	+	-	+	81.0±5.9	17±1.9

(+) indicated growth, (-) indicated no growth

odorata L. seeds, collected in different geographical regions of the state of Chiapas, Mexico; 26 strains were shown to produce IAA and/or solubilize phosphate, which could promote plant growth, and 24 strains the ability to *in vitro* biocontrol phytopathogenic fungi. Phylogenetic analysis of the 16S ribosomal gene shows that the 26 strains selected from the 62 isolated in this work are related to the *Bacillu*s genus. It is very interesting in this study that the characteristics of biotechnological interest were only detected in strains identified as belonging to the genus *Bacillus*. Another factor that could be related to the isolation of species of the genus *Bacillus* as endophytes of cedar seeds is that the PY culture medium was used, which favored the growth of one group of microorganisms over others, as reported by Lee *et al*. (2016), authors that used different culture media to selectively isolate microorganisms from the rhizosphere of *Solanum lycopersicum*. In the cladogram we can see that strains Cedo1, Cedo2, Cedo3, Cedo4, Cedo6, Cedo11, Cedo12, Cedo14, Cedo18, Cedo23, Cedo26, Cedo28, Cedo31, Cedo33, Cedo35, and Cedo37 are related to

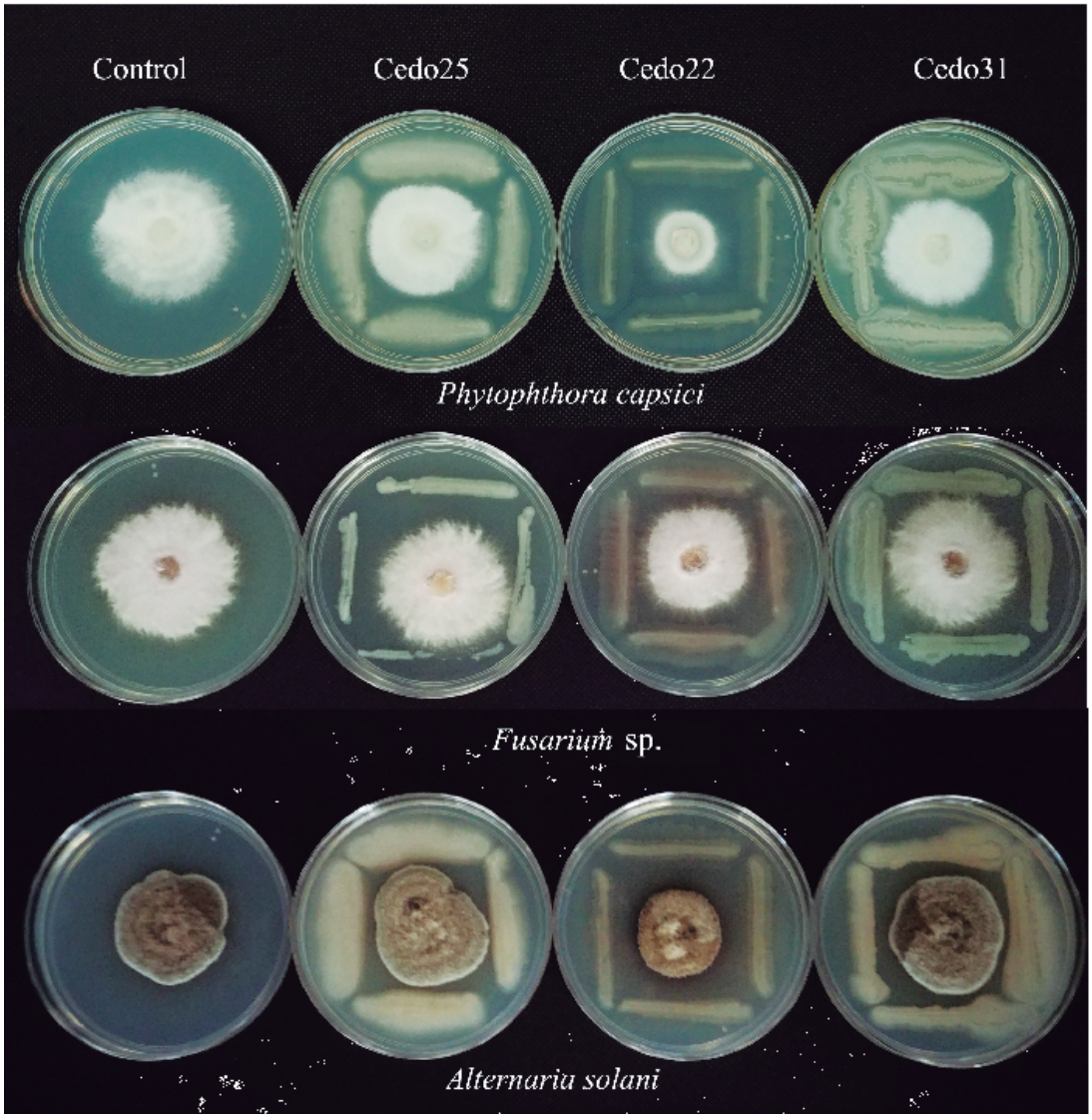

Figure 1. Antagonism of three isolates against pathogenic fungi. Control columns means that the fungus was inoculated without a phytopathogen. Row1: *Phytophthora capsici*; row 2: *Fusarium* sp.; row 3: *Alternaria solani*.

B. cereus, whereas bacterial strains Cedo8, Cedo10, Cedo13, Cedo16, Cedo17, Cedo19, Cedo21, Cedo22, Cedo25, and Cedo36 are probably a group of new species of *Bacillus* (Fig. 2). However, it is necessary to use another molecular marker such as the *rpo*B gene to identify them at the species level (Fazzeli *et al*., 2012).

According to our review, this is the first report of strains probably related to isolated *B. cereus* as endophytes of *Cedrela odarata* seeds. However, isolates of species of the *Bacillus* genus have been reported as endophytes in seeds of *Arachis hypogaea*, *Phaseolus vulgaris*, *Lycopersicum esculentum*, *Zea mays*, *Cucurbita pepo*, *Vitis vinifera*, *Triticum aestivum*, and *Oryza sativa* (Compant *et al*., 2011; Fürnkranz *et al*., 2012; Rosenblueth *et al*., 2012; Sobolev *et al*., 2013; Shahzad *et al*., 2016).

A characteristic detected in endophytic strains was the production of indoleacetic acid, the main auxin involved in cell division, while its function in seeds and tubers is to stimulate germination. In this work, the production of this auxin was quantified and it was found that strains Cedo3 (97.6 µg/mL), Cedo11 (96.5 µg/mL), and Cedo37 (81 µg/mL) had the highest production (Table 1). These IAA concentrations are higher than those reported by Sánchez *et al*. (2019) in endophytic strains of *Mimosa pudica* nodules (Spaepen and Vanderleyden, 2011). IAA production has also been reported in endophytes isolated of *Oryza sativa*, *Triticum aestivum*, and *Phragmites australis* (Díaz *et al*., 2016; Verma *et al*., 2017; White *et al*., 2017). Although IAA production is important for seed germination, root growth, and nodulation, in high concentrations it can act as a bioherbicide, as reported by Park *et al*. (2015) in *Enterobacter* sp. I-3.

Phosphate solubilization is a mechanism that microorganisms use to transform phosphorus from insoluble

Table 2. Antagonistic activity of endophytic bacterial strains isolated from *Cedrela odorata* L. seeds.

Code	Pathogen growth inhibition (%)		
	Alternaria solani	***Phytophthora capsici***	***Fusarium sp.***
Cedo1	-	-	52
Cedo3	72	-	-
Cedo6	59	-	-
Cedo8	75	-	50
Cedo10	-	50	-
Cedo11	-	44	-
Cedo12	56	-	-
Cedo13	72	62	54
Cedo14	-	50	-
Cedo16	75	-	50
Cedo17	71	-	-
Cedo18	-	-	37.5
Cedo19	70	-	-
Cedo21	72	-	46
Cedo22	75	68	50
Cedo23	-	-	37
Cedo25	75	-	47.5
Cedo26	-	51	-
Cedo28	-	65	-
Cedo31	-	-	54
Cedo33	-	-	25
Cedo35	-	-	47.5
Cedo36	76	-	59
Cedo37	-	56	50

Table 3. Possible genus of bacteria isolated from *Cedrella odorata* L. seeds using amplified 16S rDNA sequences.

Code	Possible genus	Related strains (from GenBank sequences)	Identity (%)	Access Number
Cedo1	*Bacillus*	*Bacillus cereus*	99	MN073815
Cedo2	*Bacillus*	*Bacillus cereus*	99	MN073829
Cedo3	*Bacillus*	*Bacillus* sp.	99	MN073809
Cedo4	*Bacillus*	*Bacillus cereus*	99	MN073825
Cedo6	*Bacillus*	*Bacillus cereus*	99	MN07382
Cedo8	*Bacillus*	*Bacillus subtilis*	99	MN073818
Cedo10	*Bacillus*	*Bacillus subtilis*	99	MN073806
Cedo11	*Bacillus*	*Bacillus cereus*	89	MN073807
Cedo12	*Bacillus*	*Bacillus* sp.	99	MN073827
Cedo13	*Bacillus*	*Bacillus subtilis*	99	MN073830
Cedo14	*Bacillus*	*Bacillus* sp.	99	MN073824
Cedo16	*Bacillus*	*Bacillus subtilis*	99	MN073817
Ced017	*Bacillus*	*Bacillus* sp.	99	MN073823
Cedo18	*Bacillus*	*Bacillus cereus*	99	MN073810
Cedo19	*Bacillus*	*Bacillus subtilis*	99	MN073811
Cedo21	*Bacillus*	*Bacillus subtilis*	99	MN073820
Cedo22	*Bacillus*	*Bacillus subtilis*	99	MN073822
Cedo23	*Bacillus*	*Bacillus cereus*	99	MN073813
Cedo25	*Bacillus*	*Bacillus subtilis*	98	MN073816
Cedo26	*Bacillus*	*Bacillus* sp.	98	MN073812
Cedo28	*Bacillus*	*Bacillus paramycoides*	99	MN073814
Cedo31	*Bacillus*	*Bacillus cereus*	99	MN073828
Cedo33	Bacillus	Bacillus cereus	99	MN073808
Cedo35	*Bacillus*	*Bacillus* sp.	99	MN073821
Cedo36	*Bacillus*	*Bacillus* sp.	98	MN075223
Cedo37	*Bacillus*	*Bacillus cereus*	97	MN073819

to soluble forms by participating in the biogeochemical cycle processes of this element. This ability to solubilize phosphate has been reported in endophytic bacteria isolated from *Oryza sativa*, *Triticum aestivum*, and *Phragmites australis* (Díaz *et al*., 2016; Verma *et al*., 2017; White *et al*., 2018). In this paper, 20 endophytic bacterial strains isolated from cedar seeds show the ability to solubilize phosphate, so these strains could promote plant growth through this mechanism. The largest solubilization halo (26 mm) was observed in the bacterial strain Cedo31; it was greater than strains of genus *Pseudomonas* isolated from *Jatropha curcas* rhizosphere with 4.5-9.6 mm diameter of solubilization halo, reported by Wong *et al*. (2015) (Table 1).

The ability to contribute to the promotion of plant growth and stress tolerance in host plants from the action of seed endophytes, as well as reducing or preventing damage by fungi, bacteria, viruses, and in some cases even damage caused by insects and nematodes is very important (Shahzad *et al*., 2018). Of the 62 bacterial strains isolated from cedar seeds that were evaluated against the three phytopathogenic fungi, *Alternaria solani*, *Phytophthora capsici*, and *Fusarium* sp., only 24 strains inhibited at least mycelial growth from one of the phytopathogens evaluated; their inhibition range was between 37 to 87 %. The bacterial strains Cedo13 and Cedo22 inhibited mycelial growth of the three phytopathogens (Table 2). This characteristic of antifungal activity has also been reported in other isolated endophytes

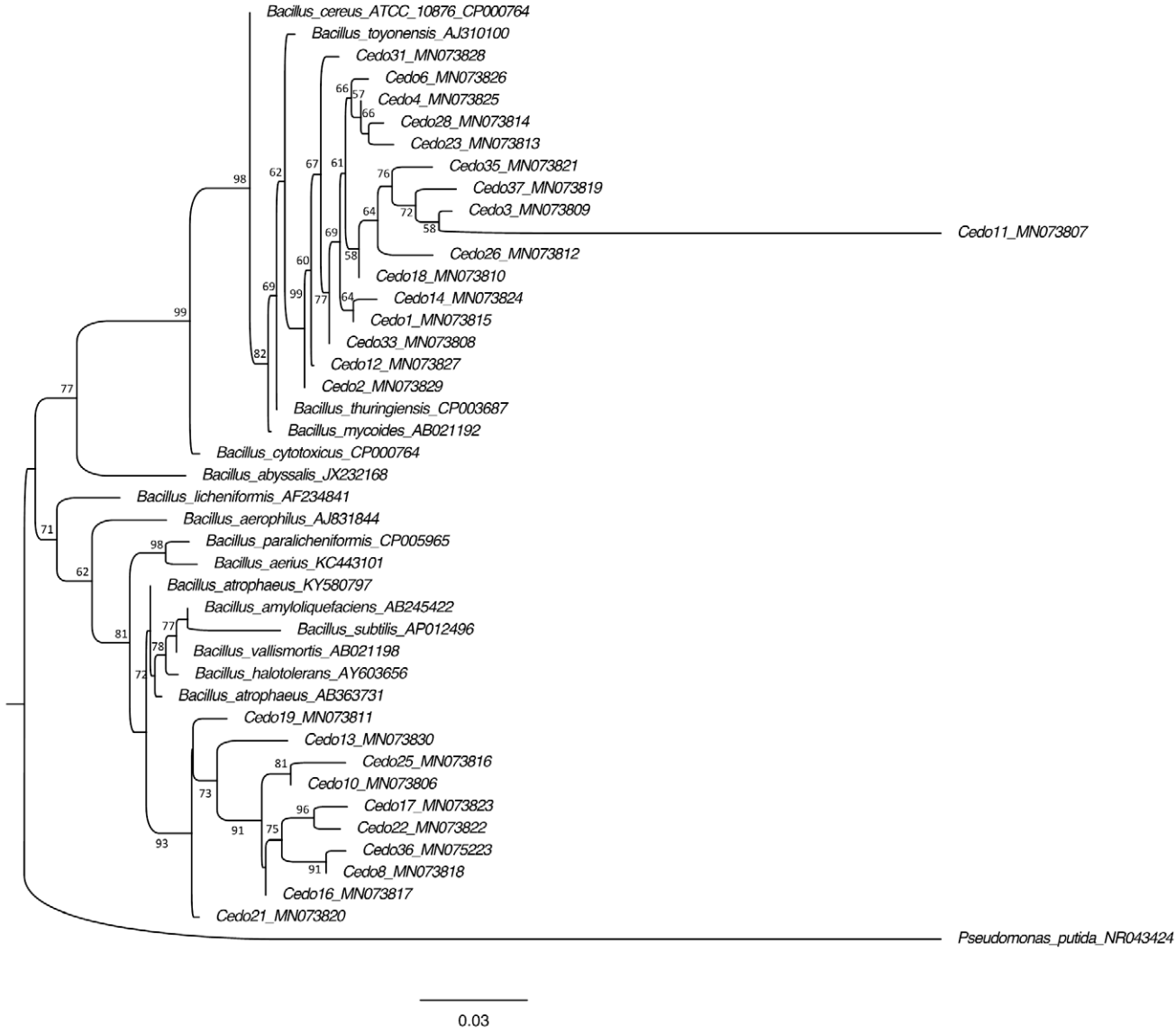

Figure 2. Phylogenetic analysis based on 16S rDNA gene sequences of *Cedrela odorata* L. seed isolates. The access numbers of the reference sequences are shown after the name of the species.

of *Mimosa pudica*, *Paullinia cupana*, *Phragmites australis*, *Arachis hypogaea*, *Cucurbita pepo*, and *Zea mays* (Fürnkranz *et al*., 2012; Sobolev *et al*., 2013; Santos *et al*., 2016; Verma *et al*., 2017; White *et al*., 2017; Sánchez *et al*., 2019). It has also been reported that *Bacillus cereus* B25 has antifungal activity against *Fusarium verticillioides* in maize plants (Martínez *et al*., 2016). Banerjee *et al*. (2017) reported *Bacillus cereus* IB311 with an antagonistic effect against *Pseudomonas syringae* and *Agrobacterium tumefaciens* on plants of *Arachis hypogaea* var. Koushal G201 and *Sesamum indicum* var. Kanak.

A relevant feature found in this work is the ability of 26 endophytic strains to grow in the presence of some aromatic compounds such as phenol, benzene, phenanthrene, or anthracene as the only carbon source in the culture medium, while only strain Cedo22 grew in the presence of these four compounds (Table 1). What is very interesting about this strain is that in addition to growing in the presence of these aromatic compounds, it also produces IAA, solubilizes phosphate, and inhibits the growth of phytopathogenic fungi. These results open the possibility of using this strain not only as a promoter of plant growth, but also in bioremediation processes, as reported from some isolated endophytic strains of *Elymus dauricus*, *Populus*, *Salix*, *Lupinus luteus* L., *Plantago asiatica*, *Hordeum sativum*, *Zea mays* L., and *Leymus arenarius,* which can degrade toluene, benzene, anthracene, phenanthrene, pyrene, and other volatile organic compounds (Siciliano *et al*., 1998; Barac *et al*., 2004; Taghavi *et al*., 2005; Germaine *et al*., 2006; Gałązka and

Gałązka, 2015; Zhu *et al*., 2017). It has also been reported that the inoculation of *B. cereus* strains in *Helianthus annuus* stimulates the accumulation of Cd and Ni in the leaves of this plant (Khan *et al*., 2018). This growth characteristic in aromatic compounds has also been reported in *Bacillus thuringensis* FQ1, which degrades 95 % of phenanthrene when added with *Pleurotus cornucopiae* in soils contaminated with Cadmium, and *Bacillus cereus* as a pyrene degrader (Kazunga and Aitken, 2000; Jiang *et al*., 2015).

CONCLUSIONS

In this research, endophytic bacteria were isolated from *Cedrela odorata* seeds from different geographic regions of the state of Chiapas, Mexico. This is the first report of isolates of the genus *Bacillus* on cedar seeds. Approximately 40 % of the isolates could solubilize phosphate, produce indole, grow on xenobiotic compounds, and had antifungal activity. The phylogenetic analysis shows that strains identified by 16S ribosomal gene sequence are probably related to *Bacillus cereus* species, while a group of these strains are probably new *Bacillus* species. These strains are good candidates to be evaluated as plant-growth promoters, biological control agents against phytopathogens, and also grown in presence of xenobiotic compounds.

ACKNOWLEDGMENT

We thank Mayna Esther Domínguez González for the review and corrections of the English of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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