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

Saúl ESPINOSA-ZARAGOZA1, Ricardo SÁNCHEZ-CRUZ2, Diana SANZÓN-GÓMEZ3, Margarita C. ESCOBAR-SANDOVAL4, Gustavo YAÑEZ-OCAMPO5, Mario A. MORALES-CONSTANTINO6, Arnoldo WONG-VILLARREAL6*

1Facultad de Ciencias Agrícolas, Universidad Autónoma de Chiapas, Huehuetán, Chiapas, México.
2Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico.
3Departamento de Agronomía, División Ciencias de la Vida, Campus Irapuato-Salamanca, Universidad de Guanajuato. Irapuato, Guanajuato, México.
4UMR BioForA-INRAE Val de Loire Orléans, 2163 avenue de la Pomme de Pin CS 40001 Ardon, 45075 Orléans Cedex 2 France
5Laboratorio de Edafología y Ambiente, Universidad Autónoma del Estado de México, Instituto Literario # 100. Col. Centro. C.P. 50000. Toluca México
6División Agroalimentaria; Universidad Tecnológica de la Selva, Ocosingo, Chiapas, México.

*For correspondence: wova79@hotmail.com

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 (IAA), la solubilización de fosfato y el crecimiento en compuestos xenobióticos (fenantreno, benceno, antraceno o fenol). El 21 % de las cepas inhibió el crecimiento micelial de Alternaria solani y Fusarium sp., y el 13 % del oomyceto Phytophthora capsici. La producción de ácido indolacético...
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 microorganisms 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 seeds. Seed endophytes can form endospores, thus protecting from changing environmental 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 *Plantago asiatica*, could degrade pyrene *in vitro* and *in vivo*; similar behavior was reported in *Azospirillum* sp. strains and the endophyte *Pseudomonas stutzeri* isolated from *Hordeum sativum*, *Zea mays* L., and *Leymus arenarius*, which degraded anthracene, phenanthrene, and pyrene (Gałżka and Gałżka, 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_2(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; KH_2PO_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 HB_4O_2, 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 Patrichin medium with the following composition (g/L): succinic acid, 2.5; fructose, 2.5; KH_2PO_4, 6; KH.PO_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):

\[
\text{Mycelial inhibition} \, \% = \left[ 1 - \left( \frac{D_a}{D_b} \right) \right] \times 100
\]

Where:

- \( D_a \) = Diameter of the mycelial growth zone of treatments (mm)
- \( D_b \) = 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): KH_2PO_4, 0.4; KH.PO_4, 0.4; MgSO_4.7H_2O, 0.2; CaCl_2, 0.02; NaMoO_4, 0.002; FeCl_3, 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); MN073816 (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 rRNA genes in the GenBank database using BlasN 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
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 genus Bacillus. 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

| Code  | Benzene | Anthracene | Phenol | Phenanthrene | Indoleacetic acid production (µg/mL) | Phosphate solubilization halo size (mm) 7 days |
|-------|---------|------------|--------|--------------|-------------------------------------|-----------------------------------------------|
| 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                                        |
| Cedo21| +       | +          | -      | +            | 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
Endophytes of seeds with biotechnological characteristics

Acta Biol Colomb, 26(2):196-206, Mayo - Agosto 2021  - 201

*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 rpoB 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 odorata* 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...
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.

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

| Code  | 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 subtilis*                     | 99           | MN073809        |
| Cedo4 | Bacillus       | *Bacillus cereus*                       | 99           | MN073825        |
| Cedo6 | Bacillus       | *Bacillus cereus*                       | 99           | MN073822        |
| Cedo7 | Bacillus       | *Bacillus subtilis*                     | 99           | MN073819        |
| Cedo8 | Bacillus       | *Bacillus subtilis*                     | 99           | MN073818        |
| Cedo9 | Bacillus       | *Bacillus cereus*                       | 99           | MN073806        |
| Cedo10| Bacillus       | *Bacillus cereus*                       | 89           | MN073807        |
| Cedo11| Bacillus       | *Bacillus subtilis*                     | 99           | MN073827        |
| Cedo12| Bacillus       | *Bacillus sp.*                          | 99           | MN073806        |
| Cedo13| Bacillus       | *Bacillus subtilis*                     | 99           | MN073830        |
| Cedo14| Bacillus       | *Bacillus sp.*                          | 99           | MN073824        |
| Cedo15| Bacillus       | *Bacillus subtilis*                     | 99           | MN073817        |
| Cedo16| Bacillus       | *Bacillus cereus*                       | 99           | MN073810        |
| Cedo17| Bacillus       | *Bacillus subtilis*                     | 99           | MN073811        |
| Cedo18| Bacillus       | *Bacillus cereus*                       | 99           | MN073820        |
| Cedo19| Bacillus       | *Bacillus cereus*                       | 99           | MN073822        |
| Cedo20| Bacillus       | *Bacillus subtilis*                     | 99           | MN073813        |
| Cedo21| Bacillus       | *Bacillus cereus*                       | 99           | MN073816        |
| Cedo22| Bacillus       | *Bacillus subtilis*                     | 99           | MN073812        |
| Cedo23| Bacillus       | *Bacillus cereus*                       | 99           | MN073814        |
| Cedo24| Bacillus       | *Bacillus subtilis*                     | 99           | MN073821        |
| Cedo25| Bacillus       | *Bacillus subtilis*                     | 99           | MN073828        |
| Cedo26| Bacillus       | *Bacillus cereus*                       | 99           | MN073808        |
| Cedo27| Bacillus       | *Bacillus cereus*                       | 99           | MN073819        |
| Cedo28| Bacillus       | *Bacillus cereus*                       | 99           | MN073817        |
| Cedo29| Bacillus       | *Bacillus cereus*                       | 99           | MN073816        |
| Cedo30| Bacillus       | *Bacillus cereus*                       | 99           | MN073812        |
| Cedo31| Bacillus       | *Bacillus cereus*                       | 99           | MN073814        |
| Cedo32| Bacillus       | *Bacillus cereus*                       | 99           | MN073821        |
| Cedo33| Bacillus       | *Bacillus cereus*                       | 99           | MN075223        |
| Cedo34| Bacillus       | *Bacillus cereus*                       | 99           | MN073819        |
| Cedo35| Bacillus       | *Bacillus cereus*                       | 99           | MN073808        |
| Cedo36| Bacillus       | *Bacillus cereus*                       | 99           | MN073814        |
| Cedo37| Bacillus       | *Bacillus cereus*                       | 99           | MN073821        |
Endophytes of seeds with biotechnological characteristics

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

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.
Gałżka, 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 thuringiensis 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.

REFERENCES

Banerjee G, Gorthi S, Chattopadhyay P. Beneficial effects of bio-controlling agent Bacillus cereus IB311 on the agricultural crop production and its biomass optimization through response surface methodology. An Acad Bras Cienc. 2018; 90(2): 2149-2159. Doi: http://dx.doi.org/10.1590/0001-3765201720170362

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, et al. Engineered endophytic bacteria improve phytoremediation of water soluble, volatile, organic pollutants. Nat Biotechnol. 2004; 22: 583-588. Doi: https://doi.org/10.1038/nbt960

Brader G, Companet S, Vescio K, Mitter B, Trognitz F, Ma LJ, Sessitsch A. Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. Annu Rev Phytopathol. 2017; 55:61-83. Doi: https://doi.org/10.1146/annurev-phyto-080516-035641

Cábblero-Mellado J, Onofre-Lemus J, Estrada-de los Santos P, Martínez-Aguilar L. The tomato rhizosphere, an environment rich in nitrogen-fixing Burkholderia species with capabilities of interest for agriculture and bioremediation. Appl Environ Microbiol. 2007; 73:5308-5319. Doi: https://doi.org/10.1128/AEM.00324-07

Companet S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A. Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb Ecol. 2011; 62: 188-197. Doi: https://doi.org/10.1007/s00248-011-9883-y

Díaz Herrera S, Grossi C, Zawoznik M, Groppa MD. Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of Fusarium graminearum. Microbiol Res. 2016; 186-187: 37-43. Doi: https://doi.org/10.1016/j.micres.2016.03.002

Estrada-Contreras I, Equihua M, Laborde J, Martínez Meyer E, Sánchez-Velásquez LR. Current and Future Distribution of the Tropical Tree Cedrela odorata L. in Mexico under Climate Change Scenarios Using MaxLike. PLoS ONE. 2016; 11: e0164178. Doi: https://doi.org/10.1371/journal.pone.0164178

Fazzeli H, Reza AM, Nasr EB, Khorvash F, Reza M. Development of PCR-based method for detection of Enterobacteriaceae in septicemia. J Res Med Sci. 2012; 17: 671-675.

Fürnkranz M, Lukesch B, Müller H, Huss H, Grube M, Berg G. Microbial diversity inside pumpkins: microhabitat-specific communities display a high antagonistic potential against phytopathogens. Microb Ecol. 2012; 63: 418-428. Doi: https://doi.org/10.1007/s00248-011-9942-4

Gałżka A, Gałżka R. Phytoremediation of Polycyclic Aromatic Hydrocarbons in Soils Artificially Polluted Using Plant-Associated-Endophytic Bacteria and Dactylis glomerata as the Bioremediation Plant. Pol J Microbiol. 2015;64(3):241-252. Doi: https://doi.org/10.5604/01.3001.0009.2119

Germaine KJ, LiuX, Garcia Cabellos G, Hogan JP, Ryan D, Dowling DN. Bacterial endophyte enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol. 2006; 57(2): 302-310. Doi: https://doi.org/10.1111/j.1574-6941.2005.10012.x

Glickmann E, Dessaux Y. A critical examination of the specificity of the salkowski reagent for indolic compounds produced in vitro. Carbohydr Res. 2006; 34(3): 351-354. Doi: https://doi.org/10.1016/j.carres.2005.11.002

Guo Z, Chen R, Xing R, Liu S, Yu H, Wang P, et al. Novel derivatives of chitosan and their antifungal activities in vitro. Carbohydr Res. 2006; 34(3): 351-354. Doi: https://doi.org/10.1016/j.carres.2005.11.002

Jiang J, Liu H, Li Q, Gao N, Yao Y, Xu H. Combined remediation of Cd–phenanthrene co-contaminated soil by Pleurotus cornucopiae and Bacillus thuringiensis FQ1 and the antioxidant responses in Pleurotus cornucopiae. Ecotox Environ Safe. 2015;120:386-39. Doi: https://doi.org/10.1016/j.ecoenv.2015.06.028
Jukes TH, Cantor CR. Evolution of Protein Molecules. In: Munro HN, editor. Mammalian Protein Metabolism. New York: Academic Press; 1969. p. 21-132. Doi: https://doi.org/10.1016/B978-0-12-370080-0.00009-7

Kane KH. Effects of endophyte infection on drought stress tolerance of Lulium perenne accessions from the Mediterranean region. Environ Exp Bot. 2011; 71(3): 337-344. Doi: https://doi.org/10.1016/j.envexpbot.2011.01.002

Kazunga C, Aitken MD. Products from the incomplete metabolism of pyrene by polycyclic aromatic hydrocarbon-degrading bacteria. Appl Environ Microbiol. 2000; 66(5): 1917-22. Doi: https://doi.org/10.1128/aem.66.5.1917-1922.2000

Khan AL, Hamayun M, Kang S-M, Kim Y-H, Jung H-Y, Lee J-H. Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of Paecilomyces formosus LHL10. BMC Microbiol. 2012; 12: 3-14. Doi: https://doi.org/10.1186/1471-2180-12-3

Khan N, Zandi P, Ali S, Mehmoood A, Adnan Shahid M, Yang J. Impact of Salicylic Acid and PGPR on the Drought Tolerance and Phytoremediation Potential of Helianthus annuus. Front Microbiol. 2018; 2507. Doi: https://doi.org/10.3389/fmicb.2018.02507

Lee SA, Park J, Chu B, Kim JM, Joa JH, Sang MK, et al. Comparative analysis of bacterial diversity in the rhizosphere of tomato by culture-dependent and -independent approaches. J Microbiol. 2016; 54: 823-831. Doi: https://doi.org/10.1007/s10292-016-6410-3

Leitão AL, Enguita FJ. Gibberellins in Penicillium strains: challenges for endophyte plant host interactions under salinity stress. Microbiol Res. 2015; 183: 8-18. Doi: https://doi.org/10.1016/j.micres.2015.11.004

Liu S-H, Zeng G-M, Niu Q-Y, Liu Y, Zhou L, et al. Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: A mini review. Bioresour Technol. 2017; 224:25-33. Doi: https://doi.org/10.1016/j.biortech.2016.11.095

Martínez-Álvarez JC, Castro-Martínez C, Sánchez-Peña P, Gutiérrez-Dorado DR, Maldonado-Mendoza IE. Development of a powder formulation based on Bacillus cereus sensu lato strain B25 spores for biological control of Fusarium verticillioides in maize plants. World J Microbiol Biotechnol. 2016; 32:75. Doi: https://doi.org/10.1007/s11274-015-2000-5

Park J-M, Radhakrishnan R, Kang S-M, Lee I-J. IAA producing Enterobacter sp. I-3 as a potent bio-herbicide candidate for weed control: a special reference with lettuce growth inhibition. Indian J Microbiol. 2015; 55: 207-212. Doi: https://doi.org/10.1007/s12088-015-0515-y

Partida-Martínez LP, Heil M. The microbe-free plant: fact or artifact?. Front Plant Sci. 2011; 2:100. Doi: https://doi.org/10.3389/fpls.2011.00100

Rahman A, Sitepu I, Tang SY, Hashidoko Y. Salkowski’s reagent test as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium strongly acidic tropical Peat soil. Biosci Biotech Biochem. 2010; 74: 2202-2208. Doi: https://doi.org/10.1271/bbb.100360

Rosenblueth M, López-López A, Martínez J, Rogel MA, Toledo I, Martínez-Romero E. Seed bacterial endophytes: common genera, seed-to- seed variability and their possible role in plants. Acta Hortic. 2012; 938: 39-48. Doi: https://doi.org/10.17660/ActaHortic.2012.938.4

Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4: 406-425. Doi: https://doi.org/10.1093/oxfordjournals.molbev.a040545.

Sánchez-Cruz R, Tapia Vázquez I, Batista-García R, Méndez-Santiago EW, Sánchez-Carbente M, et al. Isolation and characterization of endophytes from nodules of Mimosa pudica with biotechnological potential. Microbiol Res. 2019; 218: 76-86. Doi: https://doi.org/10.1016/j.microbes.2018.09.008

de Silva MCS, Polonio JC, Quecine MC, de Almeida TT, Bogas AC, Pamphile JA, et al. Endophytic cultivable bacterial community obtained from the Paullinia cupana seed in Amazonas and Bahia regions and its antagonistic effects against Colletotrichum gloeosporioides. Microb Pathog. 2016; 98:16-22. Doi: https://doi.org/10.1016/j.micpath.2016.06.023

Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda MC, Glick BR. Plant growth-promoting bacterial endophytes. Microbiol Res. 2016; 183: 92-99. Doi: https://doi.org/10.1016/j.microbes.2015.11.008

Shahzad R, Khan AL, Bilal S, Asaf S, Lee IJ. Plant growth promoting endophytic bacteria versus pathogenic infections: an example of Bacillus amyloliquefaciens RWL-1 and Fusarium oxysporum f. sp. lycopersici in tomato. PeerJ. 2017a; 5:e3107. Doi: https://doi.org/10.7717/peerj.3107

Shahzad R, Khan AL, Bilal S, Waqas M, Kang SM, Lee IJ. Inoculation of abscisic acid producing endophytic bacteria enhances salinity stress tolerance in Oryza sativa. Environ Exp Bot. 2017b; 136: 68-77. Doi: https://doi.org/10.1016/j.envexpbot.2017.01.010

Shahzad R, Khan AL, Bilal S, Asaf S, Lee IJ. What Is There in Seeds? Vertically Transmitted Endophytic Resources for Sustainable Improvement in Plant Growth. Front Plant Sci. 2018; 9:1-10. Doi: https://doi.org/10.3389/fpls.2018.00024

Shahzad R, Khan AL, Waqas M, Asaf S, Khan MA, Kang SM. Seed-borne endophytic Bacillus amyloliquefaciens RWL-1 produces gibberellins and regulates endogenous phytohormones of Oryza sativa. Plant Physiol Biochem. 2016; 106: 236-243. Doi: https://doi.org/10.1016/j.plaphy.2016.05.006
Siciliano SD, Goldie H, Germida JJ. Enzymatic activity in root exudates of dahiruan wild rye (Elymus dauricus) that degrades 2-chlorobenzoic acid. J Agric Food Chem. 1998; 46(1): 5-7. Doi: https://doi.org/10.1021/jf9708195

Smith SA, Tank DC, Boulanger LA, Bascom-Slack SC, Eisenman K, Kingery D, et al. Bioactive endophytes warrant intensified exploration and conservation. PLoS ONE. 2008; 3:e3052. Doi: https://doi.org/10.1371/journal.pone.0003052

Sobolev VS, Orner VA, Arias RS. Distribution of bacterial endophytes in peanut seeds obtained from axenic and control plant material under field conditions. Plant Soil. 2013; 371: 367-376. Doi: https://doi.org/10.1007/s11104-013-1692-2

Spaepen SJ, Vanderleyden J. Auxin and plant-microbe interactions. In: Estelle M, Weijers D, Leyser O, Ljung K, editor. Cold Spring Harbor perspectives in biology. NY: Cold Spring Harbor Laboratory Press; 2011. p. 1-13. Doi: https://doi.org/10.1101/cshperspect.a001438

Suman A, Yadav AN, Verma P. Endophytic Microbes in Crops: Diversity and Beneficial Impact for Sustainable Agriculture. In: Singh D, Singh H, Prabha R, editors. Microbial Inoculants in Sustainable Agricultural Productivity. New Delhi: Springer; 2016. p. 117-143. Doi: https://doi.org/10.1007/978-81-322-6247-5_7

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D. Horizontal gene transfer to endogenous endophytic bacteria from poplar improved phytoremediation of toluene. Appl Environ Microbiol. 2005; 71(12): 8500-8505. Doi: https://doi.org/10.1128/AEM.71.12.8500-8505.2005

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013; 30(12): 2725-2729. Doi: https://doi.org/10.1093/molbev/mst197

Verma SK, Kingsley K, Irizarry I, Bergen M, Kharwar RN, White JF. Seed-vectored endophytic bacteria modulate development of rice seedlings. J Appl Microbiol. 2017;122(6): 1680-1691. Doi: https://doi.org/10.1111/jam.13463

Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991; 173(2): 697-703. Doi: https://doi.org/10.1128/JB.173.2.697-703.1991

White JF, Kingsley KL, Kowalski KP, Irizarry I, Micci A, Soares MA, et al. Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive reed grass (Phragmites australis). Plant Soil. 2018; 422:195-208. Doi: https://doi.org/10.1007/s11104-016-3169-6

Wong VA, Kante LF, Reyes RA, Sánchez CR, De Leon MA, Yañez OG, et al. Identification of bacteria from the rhizosphere of Jatropha curcas with characteristics of biotechnological interest. J Pure Appl Microbiol. 2015; 9: 2025-203

Xu M, Sheng J, Chen L, Men Y, Gan L, Guo S, et al. Bacterial community compositions of tomato (Lycopersicum esculentum Mill.) seeds and plant growth promoting activity of ACC deaminase producing Bacillus subtilis (HYT-12-1) on tomato seedlings. 2014. World J Microbiol Biotechnol. 30; 835–845. Doi: https://doi.org/10.1007/s11274-013-1486-y

Zhu X, Wang W, Crowley DE, Sun K, Hao S, Waigi MG, et al. The endophytic bacterium Serratia sp. PW7 degrades pyrene in wheat. Environ Sci Pollut Res Int. 2017;24(7):6648-6656. Doi: https://doi.org/10.1007/s11356-016-8345-y