Original Research

Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

DOI : http://doi.org/10.22438/jeb/39/6/MRN-693

JEB™

p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP


# Endophytic bacteria from root nodules of *Ormosia macrocalyx* with potential as plant growth promoters and antifungal activity



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## Key words

Antagonism
Bioinoculum
Endophytes
Nitrogen fixation
Plant growth promoting bacteria

## Publication Info

Paper received : 13.07.2017
Revised received : 10.12.2017
Re-revised received : 23.01.2018
Accepted : 12.02.2018



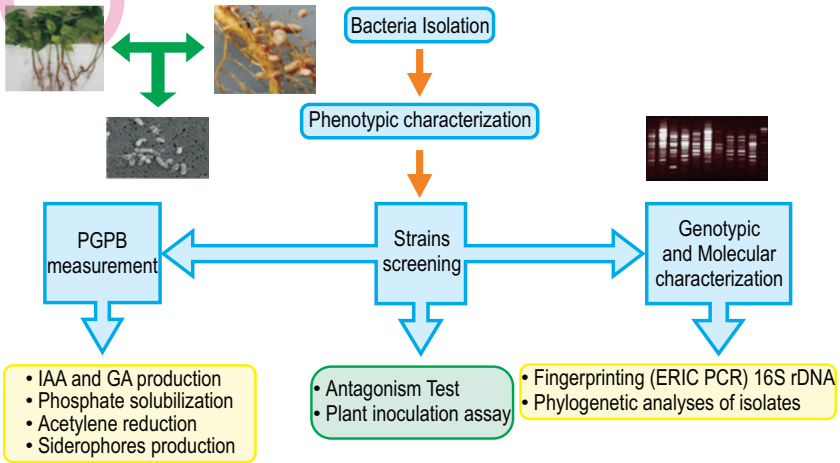
## Abstract

**Aim:** The present study was carried out to determine the diversity of cultivable endophytic bacteria associated to leguminous *Ormosia macrocalix*, and selected strains with potential as plant growth-promoting bacteria (PGPB) and antifungal activity.

**Methodology:** The isolated microorganisms were characterized using morphological, physiological and biochemical tests. The genetic diversity and phylogeny of isolated microorganisms were revealed by ERIC-PCR and sequencing of 16S rDNA. The plant growth promoting ability, plant inoculation assays and test for antagonism were evaluated to know the potential as plant growth-promoting bacteria.

**Results :** The amount of 105 endophytic bacteria was extracted from the root nodules of legume. According to the phenotypic characteristics and phylogenetic analysis based on 16S rDNA sequences, these strains were grouped within the bacterial genera *Bacillus*, *Citrobacter*, *Enterobacter*, *Novosphingobium, Paenibacillus*, *Pantoea* and *Ensifer.* Due to the capacity for nitrogen fixation, auxin production, phosphate solubilization and antagonism against certain pathogenic fungi were confirmed to be done by the plant growth promoting bacteria. The isolate of *Pantoea* sp. CA-02 produced the highest concentrations of IAA and *Ensifer* sp. CA-14 showed the maximum nitrogen fixation potency. The *Bacillus* sp. CA-11 and *Pantoea* sp. CA-02 isolates exhibited higher degree of phosphate solubilization. Isolate CA-02 showed ability to inhibit the pathogenic fungi *Fusarium oxyporum* and *F. solani*. The fungus *Fusarium verticilloides* was strongly inhibited by isolate of *Citrobacter* sp. CA-15.

**Interpretation:** Bacterial endophytes have potential use as bio-inoculants for the cultivation and propagation of leguminous tree *O. macrocalyx*. Use of endophytes may be particularly important in growing plants with high biological potential for reintroduction into their natural habitat.



## Introduction

The leguminous trees play an important ecological role in the functioning and productivity of terrestrial ecosystems in tropical forest, considering the capability of these plants to fix atmospheric nitrogen in association with a broad variety of rhizobacteria (Wang *et al.*, 2006).

*Ormosia macrocalyx* Ducke (Fabaceae), commonly known as *caracolillo*, is a nodulating leguminous tree (Cernusak *et al.,* 2011) occurring in tropical forests ranging from southern Mexico to Panama. This legume is highly appreciated by the Mexican Mayan communities due to its wood quality, foliage and seeds, which are used in crafts (Pérez-Hernández *et al.*, 2011) and to the restoration of degraded areas because of its leafy canopy (Gonçalves *et al*., 2011). Deforestation, excessive logging and fires have reduced populations of *O. macrocalyx*. According to the Mexican official standard NOM-059-ECOL-2001, this tree is under special protection as it is a species legume threatened with extinction (DOF, 2011). Therefore, programs aimed at reforestation of *O. macrocalyx* have been undertaken. However, when the seedlings are grown under greenhouse conditions, these are often attacked by pathogenic fungi, mainly by *Fusarium* species, which cause severe root rot and leaf wilt (Ozbay and Newman, 2004; Culebro-Ricaldi *et al.,* 2017), making plants have slow growth and high mortality rate (Pérez-Hernández *et al.*, 2011). To solve this problem, farmers have commonly used systemic fungicides, but to the limited extent. On the contrary, these treatments have contributed to environmental pollution, as these chemicals are persistent and highly toxic (Bawa, 2016). The PGPB microorganisms were used for the biological control of phytopathogenic fungi this has led to a promising potential strategy to reduce the use of agrochemicals (Martínez-Rodríguez *et al*., 2014). The PGPBs may be isolated from different plant parts: roots, nodules, leaves, flowers and sprouts of legumes and contribute to plant growth (Rosenblueth and Martínez-Romero, 2006). Some PGPBs can enter the interior of root nodules of the legume and establish endophytic populations. Endophytic microbes promote plant growth by helping plants acquire nutrients, e.g. by way of nitrogen fixation, phosphate solubilizing mechanism or iron chelation, by preventing pathogen infections via antifungal or antibacterial agents, by outcompeting pathogens for nutrients by siderophore production, or by imparting systemic resistance in plants (Dudeja *et al.,* 2012).

In addition to rhizobia, a wide range of cultivable endophytic bacteria have been isolated, mainly from different herbaceous legume such as alfalfa, clover, soybean and peanut (Stajkovic, 2009, Ibáñez *et al.,* 2009). Also, endophytic bacteria have been isolated from leguminous trees. For instance, *Citrobacter*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Pantoea* and *Salmonella*, and were isolated from nodules and bark tissues of *Conzattia multi flora* (Wang *et al.,* 2006). Some of these bacteria significantly promoted the growth of *Conzattia* seedlings. In another study, thirty-nine endophytic bacterial strains were isolated from the nodules of *Lespedeza* sp. and 16S rDNA sequences grouped the bacteria into the following nine different genera: *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Dyella*, *Methylobacterium*, *Microbacterium*, *Rhizobium* and *Staphylococcus*. Most of the isolates showed various plant growth promoting activity (Palaniappan *et al.,* 2010). These studies on endophytic bacterial diversity have revealed the existence of many species that can establish an association with leguminous trees. On the other hand, the Leguminosae family includes more than 18000 known species and nodulation has only been investigated in a small fraction of these plants (Sprent, 2001). It is important to trace endophytes in other species of leguminous tree in order to determine their potential use as PGPBs.

The present study aimed to characterize the diversity of cultivable endophytic bacteria associated with the legume *O. macrocalyx*, and to determine their beneficial capability in promoting growth, as well as their antifungal activity against pathogenic fungi.

## Materials and Methods

**Isolation of bacteria from nodules:** The isolates were obtained from the root nodules of *O. macrocalix*, a native legume species that grows in the rainforest of an ecological reserve of Nacajuca, Tabasco, Mexico. The root nodules were collected from young seedlings. Nodules were carefully washed under sterilized water by immersing them in 95% ethanol for 5 min, followed by immersing them 5% is sodium hypochlorite solution for 10 min and finally rinsing eight times with sterile water. The surface-sterilized root nodules were crushed in a sterile plate, and bacteria were isolated by streaking the nodule extraction using yeast extract-mannitol (YEMA) medium. The petri dishes were incubated aerobically at 28°C for 5 days. The developed bacterial colonies were purified by streaking single colonies on the same media and then these were examined microscopically. The pure cultures were preserved at 4°C temporarily in 65% glycerol-YM broth at 80°C for long term storage.

**Phenotypic characterization:** The characterization of isolates were carried out on YEMA medium. The tolerance of salt was evaluated at 28°C with 0.5, 1.0, 2.0, 3.0 and 5.0% (w/v) NaCl concentrations, while the pH levels were determined at 4.0, 5.0, 9.0 and 11.0. The production of acid or alkali was evaluated on the same medium by adding with 25 mg $ml^{-1}$ bromothymol blue as a pH indicator. Antibiotic resistance was tested following the process recommended by Martínez-Romero *et al.* (1991). In addition, aluminum and copper tolerance of the isolates were analyzed in solid YEMA medium.

**DNA extraction and fingerprinting (ERIC-PCR):** The DNA genomic was isolated according to the manufacturer´s

specifications during the DNA Isolation Kit (ROCHE®, Basel, Switzerland). PCR primers ERIC 1R and ERIC2 were used to study the genomic fingerprinting (Versalovic *et al*., 1994).

**16S rRNA gene sequencing and phylogenetic analysis:** PCR was performed with the bacterial universal 16S rDNA primers f D1-5´AGAGTTTGATCCTGGCTCAG 3´ and rD1-5´AAGGAGGTGATCCAGCC 3´ (Weisburg *et al.,* 1991). Before sequencing, the amplification mixture was purified using the Roche® PCR product purification system. All sequences were compared using BLAST (Altschul *et al*., 1990) and were aligned by the CLUSTAL X (2.0) software (Larkin *et al*., 2007). Phylogenetic and molecular evolutionary analyses were done with MEGA v5.2 software. The phylogenetic tree was constructed by Neighbour-Joining model (Saitou and Nei, 1987; Tamura *et al*., 2011). Finally, the 16S rRNA gene sequences were deposited in the GenBank database.

**Plant growth promoting ability of the isolates:** Solubilization of phosphate was analyzed as described by Nautiyal (1999). The IAA concentration from each isolate were determined by using HPLC equipment. The gibberellins ($GA_3$) produced by the isolates were determined by a colorimetric method as described by Holbrook *et al*. (1961). All isolates were tested for siderophore production by the Chrome Azurol S (CAS) plate assay. The fixing nitrogen of the isolates was determined by acetylene reduction activity (ARA) assay.

**Plant inoculation assays:** The selected isolates from each bacterial groups, identified previously by 16S rDNA gene sequence analysis, were used as inoculants. *Ormosia macrocalyx* seeds were scarified with $H_2SO_4$ for 15 min and surface sterilized with 1% (v/v) sodium hypochlorite for 10 min. Treated seeds were subjected to germination on 0.8% agar Petri dishes. The germinated seedlings were planted in pots filled with peat moss and enriched with free N medium and placed in a plant growth chamber. The plants were inoculated with 2 ml of bacterial suspension with a concentration of $1x10^6$ CFU $ml^{-1}$ (Bashan, 1986). Some plants were fertilized with 60 mg $KNO_3$-N per plant, and uninoculated plants were used as a control. Experimental units were distributed under a completely randomized design, while the evaluations were repeated four times. After 60 days, the plants were harvested and estimated for the plant height, plant fresh weight and root fresh weight. The total plant nitrogen content was determined by Kjeldahl method (Bremner and Mulvaney, 1982).

**Antifungal activity:** *Fusarium oxysporum* (Genbank accession number KX232463) was obtained from CIAD-Culiacán fungal collection, *F. solani* (Genbank accession number HQ530551) was obtained from CIAD-Culiacán fungal collection and *F. verticillioides* (Genbank accession number G1982311.1) was obtained as a monoconidial culture isolate from maize were used for antifungal activity tests. The bacteria were grown on YEM medium and incubated at 28°C for 24 hr. The fungus were grown on a potato dextrose agar medium on a plate at 28°C for 7 days in dark condition. A mycelial fragment (0.8 cm diameter) of fungus was placed at the center of the Petri dish with PDA medium, then each bacterial isolate was inoculated to confront at a distance of 3 cm in the same Petri dish. The plates were then incubated at 28°C in dark conditions for 5 days and the inhibition percentage was determined following the method of Martínez-Rodriguez *et al*. (2014).

**Statistical analysis:** The significance of the effect of the isolates on inoculation and antifungal activity, P-solubilizing, IAA and gibberellins production, as well as ARA activity was determined by ANOVA, and the treatment mean was tested for significance with the Tukey test ($P<0.05$).

## Results and Discussion

Legumes play an important role in the functionality and productivity of these natural systems in tropical forests, when considering the capacity of these legumes to capture essential nutrients, such as nitrogen and phosphorus, symbiotically with different bacterial species (Pajares and Bohannan, 2016). In this study, a total of 105 isolates were obtained from *O. macrocalyx* root nodules. The isolates were grouped by ERIC-PCR into 16 genomic fingerprints. The isolates showed different patterns which were considered for sequencing and phylogenetic analysis.

Based on 16S rDNA gene sequences, *O. macrocalyx* nodule isolates were classified into seven bacterial genera, as *Bacillus, Citrobacter, Enterobacter, Paenibacillus, Pantoea, Ensifer* and *Novosphingobium* (Table 1). Isolates CA-10, CA-11 and CA-13 were grouped into the genus *Bacillus.* Isolate CA-15 was affiliated within the genus *Citrobacter* and had 86.4% 16S rDNA gene sequence similarity with *Citrobacter farmeri* CDC 2991-81. Isolate CA-03 was grouped within the genus *Enterobacter* and showed 95.1% similarity with *E. asburiae* JCM6051. Isolates CA-01, CA-04, CA-09 and CA-12 were clustered within of the genus *Paenibacillus*. CA-01 had 99.9 % similarity with *P. lupini* RLAHU15, while isolate CA-04 and CA-12 showed 96.9% similarity with *P. cineris* LMG 18439. Isolates CA-02, CA-05, CA-06, CA-07 and CA-08 were grouped into the genus *Pantoea*. Up to 99% of the CA-02 and CA-08 sequences were identical to *P. agglomerans*, while CA-05, CA-06 and CA-07 showed similarity with sequences of *P. dispersa.* Finding an isolate related to the genus *Ensifer*, isolate CA-14 had a 98.0% genetic similarity with *E. mexicanus* ITTG R7. This was also an important outcome, since these species has been reported as a bacterium with high potential to fix nitrogen (Lloret *et al*., 2007). The isolate CA-16 was grouped with members of the genus *Novosphingobium*. This taxonomic status of all the identified isolates was confirmed using phylogenetic analysis of the complete sequences of gene 16S rDNA (Fig 1). Likewise, the

**Table 1 :** Molecular identification of endophytic isolates of *O. macrocalyx* Ducke

| Isolate name | Accession No. | 16S rRNA sequence (bp) | Closely NCBI match | Species identity BLAST(%) | Class |
|---|---|---|---|---|---|
| CA-01 | KX389680 | 1169 | *Paenibacillus lupini* RLAHU15 (NR_134115) | 99.0 | Bacilli |
| CA-02 | KX389676 | 1240 | *Pantoea agglomerans* 76 (AM184097) | 94.0 | γ-proteobacteria |
| CA-03 | KX389685 | 1329 | *Enterobacter asburiae* JCM6051 (AB004744) | 95.1 | γ-proteobacteria |
| CA-04 | KX389681 | 1248 | *Paenibacillus cineris* LMG 18439 (AJ575658) | 96.6 | Bacilli |
| CA-05 | KX389678 | 1271 | *Pantoea dispersa* R7-534 (JQ659886) | 99.0 | γ-proteobacteria |
| CA-06 | KX389678 | 1202 | *Pantoea dispersa* R7-534 (JQ659886) | 97.5 | γ-proteobacteria |
| CA-07 | KX389679 | 1313 | *Pantoea dispersa* LMG 2603 (DQ504305) | 85.5 | γ-proteobacteria |
| CA-08 | KX389677 | 1244 | *Pantoea agglomerans* PB17 (EU360112) | 99.0 | γ-proteobacteria |
| CA-09 | KX389682 | 1334 | *Paenibacillus catalpa* D75(HQ657320) | 95.2 | Bacilli |
| CA-10 | KX389693 | 1113 | *Bacillus subtilis*LD170 (KJ534453) | 80.0 | Bacilli |
| CA-11 | KX389690 | 1211 | *Bacillus niacini*IFO15566 (AB021194) | 99.0 | Bacilli |
| CA-12 | KX389683 | 1220 | *Paenibacilluscineris*LMG 18439 (AJ575658) | 96.9 | Bacilli |
| CA-13 | KX389691 | 1322 | *Bacillus megaterium*IAM 13418 (D16273) | 97.1 | Bacilli |
| CA-14 | KX389671 | 1254 | *Ensifer mexicanus* ITTG R7 (DQ411930) | 98.0 | α-proteobacteria |
| CA-15 | KX389688 | 1266 | *Citrobacter farmeri* CDC2991-81 (AF025371) | 86.4 | γ-proteobacteria |
| CA-16 | KX389684 | 1312 | *Novosphingobiumresinovorum*NCIB87 (EF029110) | 95.5 | α-proteobacteria |

**Table 2:** Representative groups of endophytic isolates of *O. macrocalyx*, according to ERIC_PCR profiles

| Group No. | Bacterial genus | Isolates | Number of isolates | Representative isolate |
|---|---|---|---|---|
| I | *Bacillus* | CA-10CA-11CA-13 | 3 | CA-11 |
| II | *Citrobacter* | CA-15 | 1 | CA-15 |
| III | *Enterobacter* | CA-03 | 1 | CA-03 |
| IV | *Novosphingobium* | CA-16 | 1 | CA-16 |
| V | *Paenibacillus* | CA-01CA-04CA-09CA-12 | 4 | CA-12 |
| VI | *Pantoea* | CA-02CA-05CA-06CA-07CA-08 | 5 | CA-02 |
| VII | *Ensifer* | CA-14 | 1 | CA-14 |



study of genomic fingerprints, as well as phylogenetic analysis allowed to determine that there is a wide diversity and abundance of bacterial species associated with *O. macrocalyx*. Several of these bacteria could be considered as plant growth-promoting bacteria (PGPB).

Later, a representative of each one of the bacterial entophytes (Table 2) was selected to perform several studies related to phenotypic characterization and to the biological potential of strains as PGPB. The highest number of endophyte isolates were Gram-negative, aerobic, non-spore, forming rods, and they grew faster in YEMA medium. Bacterial cells formed colonies with various sizes and colors. About 85% of isolates had the capacity to form pigments and abundant exopolysaccharides (EPS). The EPS forms a protective layer for the bacteria, which allows tolerance to abiotic stress and contributes to the colonization of root surface (Sandhya and Ali, 2015). All isolates can grow well at 37°C and 44°C, with the exception of *Ensifer* sp. CA-14 and *Novosphingobium* sp. CA-16, which showed little growth at higher temperatures (Table 3). Most of the isolates have capacity to grow in the range from pH 5.0 to 9.0. The *Pantoea* sp. CA-02 stood out for its capacity to grow in the range from pH 4.0 to 11.0. For tolerance to NaCl, endophytic isolates have the ability to grow in the range from 0.5 to 3.0%, except *Paenibacillus* sp. CA-12. This result is important due to salinity because it is one of the most severe environmental problems affecting the crop yield and it has been shown that most cultivations are sensitive to higher salinity of soil. Therefore, PGPB microorganisms can be used as an alternative to improve soil fertility. In relation to the antibiotic test, *Citrobacter* sp. CA-15 showed more ability to tolerate different antibiotics tested, as compared to other isolates (Table 3). Furthermore, it was found that all isolates had the capacity for tolerance of higher concentrations of $Al^{+3}$ and $Cu^{+2}$. Also, this study shows that all isolates were able to produce siderophores. Siderophore-producing rhizobacteria are also known to convey induced systemic resistance to plants and suppressiveness to the soil, and have been implicated in the biocontrol of several plant diseases (Saha *et al*., 2016).

The potential of endophytic isolates from *O. macrocalyx* root nodule as PGPB was evaluated based on the ability to solubilize phosphate, fix nitrogen, and to biosynthesize auxins (IAA) and gibberellins ($GA_3$). The *Pantoea* sp. CA-02 isolate produced a higher concentration of IAA (6.19 mg $l^{-1}$) and of $GA_3$

**Table 3 :** Characteristics of endophytic isolates of *O. macrocalyx* Ducke

| Characteristics | Isolate | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| Gram stain | (+) | (-) | (-) | (-) | (-) | (-) | (-) |
| Colony aspect on YEMA medium: | Light-beige cream | Bright-yellow mucoid | Cream-white mucoid | Bright-yellow mucoid | Yellowish dried | Light-white cream | Pearly-white mucoid |
| Growth on YEMA agar at | | | | | | | |
| 37ºC | + | + | + | + | + | + | + |
| 44 ºC | + | + | + | - | + | + | - |
| Growth on YEMA medium to different pH at 28º C | | | | | | | |
| 4.0 | - | - | - | - | - | - | - |
| 5.0 | - | - | + | + | + | + | + |
| 7.0 | + | + | + | + | + | + | + |
| 9.0 | + | + | + | - | + | + | + |
| 11.0 | + | + | - | - | + | + | - |
| Tolerance to NaCl | | | | | | | |
| 0.5 % | + | + | + | + | + | + | + |
| 1.0 % | + | + | + | + | + | + | + |
| 2.0 % | + | + | + | + | + | + | + |
| 3.0 % | + | + | + | + | + | + | + |
| 5.0 % | - | - | - | - | + | - | - |
| Tolerance to antibiotics (ug $ml^{-1}$): | | | | | | | |
| Ampicillin (100) | - | + | - | - | - | - | - |
| Amikacin (10 ) | + | + | + | - | + | + | + |
| Cephalothin (30) | - | + | - | + | - | + | + |
| Cefotaxime (30) | - | + | - | - | - | + | + |
| Chloramphenicol (100) | + | + | + | + | - | + | + |
| Gentamicin (10 ) | + | + | + | - | + | + | + |
| Netilmicin (20 ) | + | + | + | - | + | + | + |
| Tolerance to heavy metals (ug $ml^{-1}$): | | | | | | | |
| $Al^{+3}$ (500) | + | + | + | + | + | + | -+ |
| $Cu^{+2}$ (100) | + | + | + | + | + | + | + |
| Production of siderophore | + | + | + | + | + | + | + |

Taxon **1**, *Bacillus* sp. CA-11; **2**, *Citrobacter* sp. CA-15; **3**, *Enterobacter* sp. CA-03; **4**, *Novosphingobium* sp. CA-16; **5**, *Paenibacillus* sp. CA-12; **6**, *Pantoea* sp. CA-02, and **7**, *Ensifer* sp. CA-14. **+**, Positive; -, Negative

**Table 4 :** IAA and GA production, phosphate solubilization and acetylene reduction activity (ARA) of endophytic isolates of *O. macrocalyx* Ducke

| Isolate | IAA (mg $l^{-1}$) | GA (mg $l^{-1}$) | P-solubilization (mg $l^{-1}$) | ARA[¶] |
|---|---|---|---|---|
| *Bacillus* sp. CA-11 | 15.2 B[§] | 3.97 BC | 37.8 A | 242.3 BC |
| *Citrobacter* sp. CA-15 | 5.7 D | 2.97 C | 13.3 D | 106.1 C |
| *Enterobacter* sp. CA-03 | 10.3 C | 3.05 C | 25.5 BC | 627.4 AB |
| *Novosphingobium* sp. CA-16 | 10.4 C | 2.82 C | 24.2 C | 804.3 A |
| *Paenibacillus* sp. CA-12 | 12.0 C | 3.95 BC | 31.2 AB | 740.7 A |
| *Pantoea* sp. CA-02 | 19.6 A | 6.22 A | 36.9 A | 751.5 A |
| *Ensifer* sp. CA-14 | 16.7 B | 4.47 B | 32.0 AB | 821.3 A |
| HSD[¥] (P<0.05) | 2.16547 | 1.39203 | 6.95898 | 471.36 |

[§]Mean values of three replicates. Means followed by same letter are non significant (Tukey test, P<0.05); [¶] ARA: acetylene reduction assay (µmol $C_2H_4$ per culture fresh weigh $h^{-1}$); [¥]HSD: Honest Significant Difference

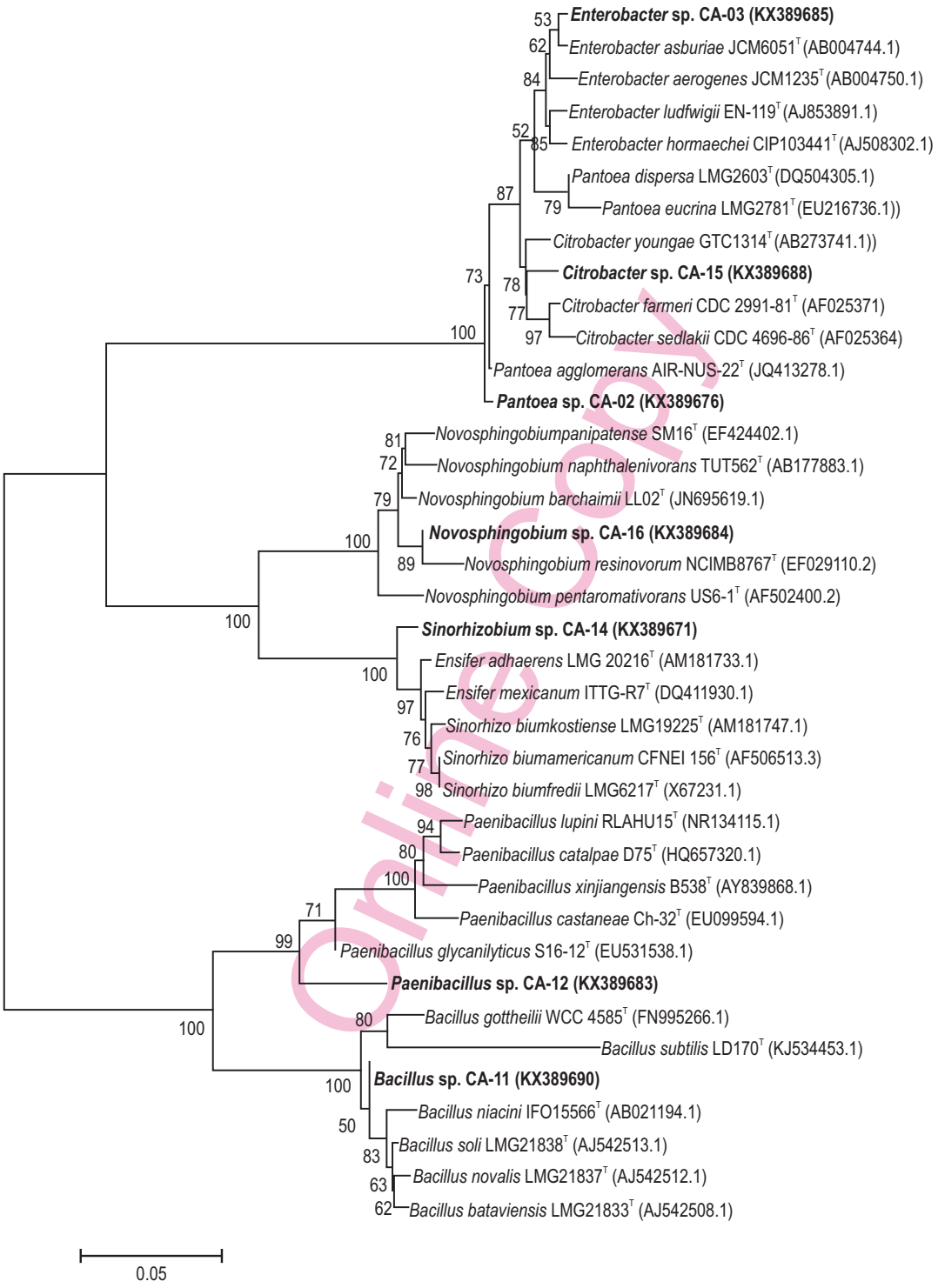





**Fig. 1 :** Phylogenetic tree based on 16S rDNA gene sequences of representative endophytic strains isolated from *Ormosia macrocalyx*. The accession numbers for the sequences are indicated within parentheses. Those generated in this study are shown in bold.

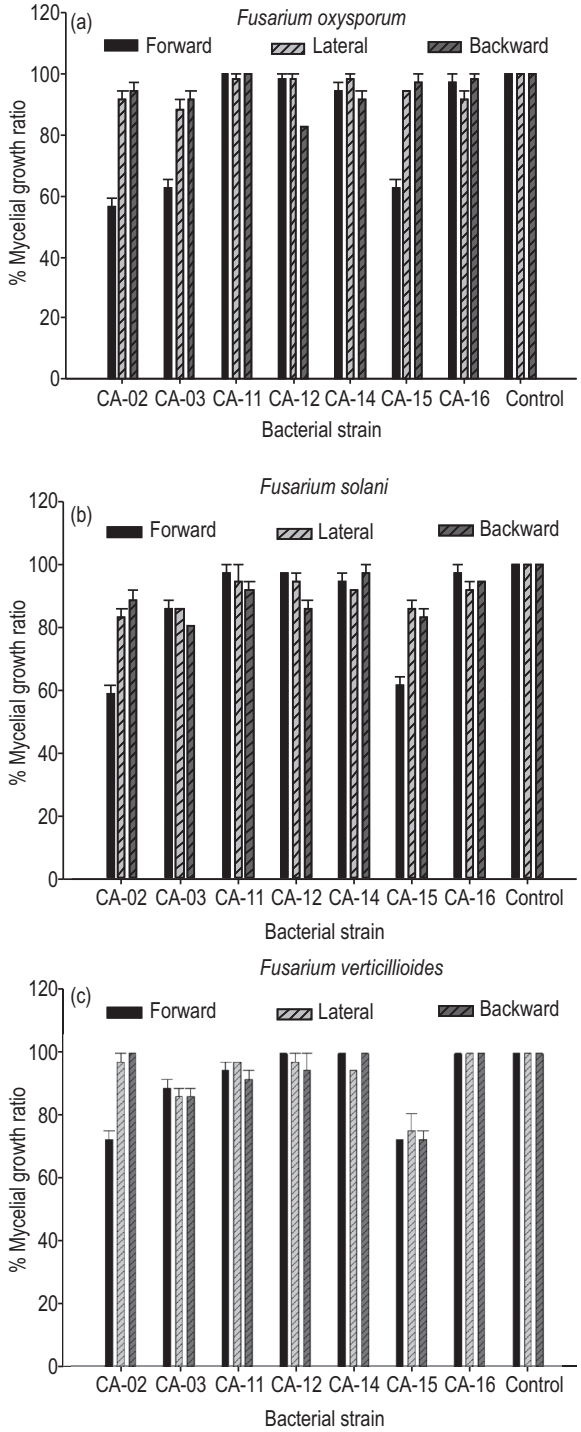



**Fig. 2:** Quantification of phytophatogenic fungi *Fusarium oxysporum*, *Fusariu solani* and *Fusarium oxysporum* forward, lateral and backward mycelial growth ratio in the CA-02, CA-03, CA-11, CA-12 CA-14, CA-15 and CA-16 strains. Vertical bars represent SD of mean of three replications.

(6.22 mg $l^{-1}$) as compared to the other isolates (Table 4). IAA and $GA_3$ are important plant hormones and play a significant role in plant growth (Singha *et al*., 2017). *Bacillus* sp. CA-11 showed a higher capacity to solubilize phosphate (37.8 mg $l^{-1}$), followed by *Pantoea* sp. isolate CA-02 (29.8 mg $l^{-1}$). Additionally, the *Ensifer* sp. CA-14 exhibited maximum capacity for nitrogen-fixation in comparison to other isolates (Table 4). Therefore, in this study all endophytic isolates have potential to biosynthesize IAA, $GA_3$ and solubilize phosphate, as well as the ability for nitrogen fixation. These results suggest that nitrogen and phosphorus are important nutrients for the development and metabolism of *O. macrocalyx* legume.

Inoculation of selected isolates had significant effect on the growth of *O. macrocalyx* plants (Table 5). The inoculated plants with endophyte isolates increased significantly the total height and fresh weight, while the root weight and total nitrogen content for inoculated plants with *Ensifer* sp. CA-14 were significantly higher in comparison to the control plants ($P < 0.05$).

**Antifungal activity:** The results revealed that *Pantoea*sp. CA-02, *Enterobacter*sp.CA-03, *Citrobacter*sp. CA-15 and *Paenibacillus* sp. CA-12 exhibited inhibition against *F. oxysporum* (Fig 2). Isolate CA-02 showed the strongest inhibition against mycelial growth (40%), CA-03 and CA-15 reduced growth (35%), while CA-12 inhibited mycelial growth (18%). The isolates that exhibited inhibition against *F. solani* were CA-02, CA-03, CA-15, CA-12 and *Ensifer sp*. CA-14. Isolates CA-02 and CA-15 showed the strongest inhibition against mycelial growth 38.5% and 35.5%, respectively. The inhibition of lateral mycelial growth was significant with CA-02 and CA-03 reducing 13.7%, while CA-03 and CA-15 inhibited 19.2% and 15.7% mycelial growth, respectively. The growth of *F. verticillioides* was inhibited with the isolates CA- 02,CA-03, CA-11 and CA-15. The latter significantly inhibited forward (28.4%), lateral (19.2%) and backward (21.9%) mycelia growth, compared to control. CA-03 showed inhibition against mycelial forward growth at 24.7%. Mousa *et al*. (2015) reported that a species of *Citrobacter* and three isolates of *Paenibacillus* had antifungal activity against pathogenic *Fusarium* isolates of maize. Choi *et al*. (2008) indicated that *Paenibacillus* produces fusaricidin compounds, polyketides and non-ribosomal peptides that hold back fungal pathogens. The results of this study is similar to the study of Martínez–Rodríguez *et al*. (2014) who reported *Bacillus sp*.inhibited the growth of *F. oxysporum*, which indicated that inhibition of lateral and backward growth was related to the volatile compounds released.

In conclusion, the present study provides valuable information about the phenotypic and genotypic diversity of endophytic bacteria associated with *Ormosia macrocalyx*. It was found that bacterial isolates belong to the genera *Bacillus*, *Citrobacter*, *Enterobacter*, *Novosphingobium, Paenibacillus*, *Pantoea* and *Ensifer.* All endophytic isolates had the capacity for nitrogen fixation, production of IAA and $GA_3$, solubilization of

**Table 5 :** Growth parameters and total nitrogen content of *O. macrocalyx* plants inoculated with endophytic isolates

| Treatment | Plant height (cm) | Plant fresh weight (g) | Root fresh weight (g) | Total shoot nitrogen (mg per plant) |
|---|---|---|---|---|
| Uninoculated | 30.42 B[§] | 2.73 AB | 1.18 C | 73.32 D |
| *Bacillus* sp. CA-11 | 32.02 B | 2.97 AB | 1.57 BC | 100.02 BCD |
| *Citrobacter* sp. CA-15 | 31.35 B | 2.75 AB | 1.49 C | 98.97 BCD |
| *Enterobacter* sp. CA-03 | 30.57 B | 2.32 B | 1.12 C | 77.4 D |
| *Novosphingobium* sp. CA-16 | 38.82 A | 3.20 A | 1.45 C | 85.2 CD |
| *Paenibacillus* sp. CA-12 | 38.70 A | 3.15 A | 2.03 AB | 96.67 BCD |
| *Pantoea*sp. CA-02 | 40.32 A | 3.12 A | 1.97 AB | 146.47 AB |
| *Ensifer* sp. CA-14 | 41.80 A | 3.45 A | 2.07 A | 175.92 A |
| $KNO_3$-N (60 mg per plant) | 41.52 A | 3.13 A | 1.96 AB | 135.25 ABC |
| HSD[¥] (P<0.05) | 4.70362 | 0.8053 | 0.46687 | 53.3467 |

[§]Mean values of four replicates. Means followed by same letter are non-significant (Tukey test, P<0.05); [¥]HSD: Honest Significant Difference

phosphate and showed antifungal activity against pathogenic fungi. Therefore, these bacterial endophytes could be promising bioinoculants to enhance cultivation and reforestation of *O. macrocalyx* legume.

## Acknowledgment

Thank to the '*Tecnologico Nacional de Mexico*', projects No. 6211.17-P and 6212.17-P' for the financial support of this work.

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