Research & Reviews: Journal of Microbiology and Biotechnology

e-ISSN: 2320-3528
p-ISSN: 2347-2286

# Bioprospection of Plant Growth-Promoting Endophytic Bacteria from the Roots of the Medicinal Plant *Aloe vera*

**Cintia Faria da Silva[1,2], Luciana Cristina Vitorino[2*], Maria Andreia Correa Mendonca[2], Edson Luiz Souchie[2], Welington Luiz de Araujo[3], Manuella Nobrega Dourado Ribeiro[3], Leonardo Cunha de Albuquerque[4], Marcos Antonio Soares[5]**

[1]Institute of Tropical Pathology and Public Health, Federal University of Goias, Goiania, Goias, Brazil

[2]Agricultural Microbiology Laboratory, Federal Institute of Education, Science and Technology Goiano Campus, Rio Verde, Goias, Brazil

[3]Laboratory of Molecular Biology and Microbial Ecology, University of Sao Paulo, Sao Paulo, Brazil

[4]Federal Institute of Education, Science and Technology Goiano, Campus Morrinhos, Goias, Brazil

[5]Department of Botany and Ecology, Institute of Biosciences, Federal University of Mato Grosso, Cuiaba, Mato Grosso, Brazil

**Research Article**

Received date: 31/10/2018

Accepted date: 27/11/2018

Published date: 05/12/2018

***For Correspondence:**

**Edson Luiz Souchie,** Agricultural Microbiology Laboratory, Federal Institute of Education, Science and Technology Goiano Campus, Rio Verde, Goias, Brazil

**E-mail:** edson.souchie@ifgoiano.com.br

**Keywords:** Endophytes; Auxin; Phosphate solubilization; Antibiosis; Functional traits; Plant growth promotion; Xanthorrhoeaceae.

## ABSTRACT

Endophytic bacterial communities are dynamic, capable of spreading throughout plant tissues, and perform metabolic functions that may benefit the host plant. Despite the current high interest in symbiotic microorganisms of medicinal plants, little is known about the functional traits of the endophilic bacteria of *Aloe vera* (L.) *Burm. f.*, which has diverse chemical composition characterized by the synthesis of various bioactive substances. As this composition can be influenced by the endophytic microbiota, we tested the hypothesis that the root bacteria of *Aloe vera* present multifunctionality with potential in biotechnology for plant growth. A total of 129 endophytic bacteria from three environments, a field, garden, and nursery (n=3), were isolated, and phylogenetic analysis revealed the presence of four groups: *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. The functional trait for indole acetic acid (IAA) synthesis was observed in 32 of the isolates, with emphasis on the 135V *Enterobacter tabaci* strain, which revealed the highest production (225.2 μg $mL^{-1}$). The results found for Bayyvar phosphate solubilization were not expressive, with the highest values observed for the bacterium 149H *Paraburkholderia* sp. (45.7 mg $L^{-1}$). The 3V isolate *Brevibacillus agri* presented 45.6% antagonism against *S. sclerotiorum*. The greatest inhibition of the phytopathogens *Fusarium* sp. and *Rhizoctonia* sp. was observed with 149H *Paraburkholderia* sp. and 348V *E. tabaci*, respectively. This was

e-ISSN: 2320-3528
p-ISSN: 2347-2286

the first study to evaluate the potential of endophytic bacteria of *Aloe vera*. Our results indicate that the isolates 135V and 348V *E. tabaci* and 149H *Paraburkholderia* sp. have potential for *in vivo* testing in promoting plant growth.

## INTRODUCTION

Plants establish intrinsic relationships with a range of microorganisms, such as endophytic, rhizospheric, and mycorrhizal organisms, which have aroused the interest of the scientific community owing to their proven benefits [1-5]. These microorganisms are naturally associated with the host plant and can occur endogenously. This is true for endophytic microorganisms, which colonize internal plant tissues for at least a part of their life-cycle [6] without causing functional damage or disease symptoms [5,7]. In addition, they may be dependent on the host plant, through vertical transmission or vector insects [8].

Endophytic bacterial communities are dynamic and capable of spreading systemically throughout plant tissues [9]. Such colonization has several benefits, since this community can communicate and interact efficiently, regardless of the environmental conditions to which the plant is subjected [10]. This increases plant growth [11] and maximizes protection against plant pathogens [12], which can enhance resistance to stress [13,14] and may stimulate the synthesis or production of bioactive compounds of interest [15-17].

Several studies have confirmed that bacterial strains associated with roots can promote plant health and growth through mechanisms such as phytostimulation, biofertilization, and/or biocontrol [18-20]. Several genera, such as *Pseudomonas* [21-22], *Pantoea*, *Bacillus* [23], *Serratia*, *Enterobacter* [24], *Azospirillum* [25], and *Paraburkholderia* [26] are beneficial for plant growth.

The association between endophytic plants and microorganisms results in direct physiological effects on plant growth and development, such as: nitrogen fixation [27] and phosphate solubilization [23,28], as well as the production of ammonia [29], siderophores [18], phytohormones [30,31], and hydrolytic enzymes [27]. These benefits meet the needs of current plant cultivation through sustainable methods that reduce the use of chemical fertilizers and pesticides, replacing conventional mechanisms to preserve soil biological diversity. Therefore, a strategic alternative is the selection and use of microorganisms with biotechnological application, which influence plant growth and health, as well as soil quality and nutrient cycling.

Knowledge about plant-soil relationships and the deleterious effects that excessive or inadequate application of chemical fertilizers have on the environment have changed farmers' views regarding the use of biological products, such as microbial inoculants; their use has increased globally owing to their proven agronomic effects [32]. The production of P fertilizers is expensive because they are based on the chemical processing of insoluble rock phosphate, which makes access to farmers under-capitalized and typically family-based, onerous. Thus, biotechnology based on phosphate-soluble bioinoculants have been proposed as alternatives to increase P availability to crops and reduce fertilizer use [33].

Bacteria, fungi, and actinomycetes isolated from medicinal plants such as *Asclepias sinaica* [34], *Echinacea purpurea*, *Lonicera japonica* [35], *Rauwolfia serpentina*, *Gymnema sylvestre*, *Stevia crenata*, *Bacopa monnieri*, *Andrographis paniculata*, *Withania somnifera* [36], *Teucrium polium* [37], *Terminalia bohera*, and *Manihot esculenta* [38], have been shown to be efficient in agriculture, and have been applied to different crops owing to the multiple functions they perform.

Medicinal plants act in a specific manner when selecting endophytes, since the choice may be based on the secondary metabolites produced by the plant and the composition of the root exudates; therefore, these microbial communities diversify according to their nutritional needs, the type of soil, and the environment in which they are found [39]. Studies have shown that bioactive compounds synthesized by host medicinal plants are also produced by the endophytic microbiota [40-43], and there is evidence that metabolic synthesis pathways have evolved independently in plants and microorganisms [44,45], and for horizontal gene transfer [46]. Metabolites other than those produced by the medicinal plant can also be produced by the endophytic microbiota, contributing to the heterogeneity of the phytochemical profile and plant biofunctions [47-49]. Therefore, medicinal plants may represent readily available sources of microorganisms with biotechnological potential [34,50].

e-ISSN: 2320-3528
p-ISSN: 2347-2286

*Aloe vera L. Burm. f.* is a plant known worldwide for traditional medicine and is of significant commercial importance [51,52]. It is widely used for the production of cosmetics, tonics, and in the food industry [52], and has great medicinal potential, with approximately 75 active ingredients described [53]. Considering that this potential may be due in part to the endopathic community of *Aloe vera*, we tested the hypothesis that the root endophytic bacteria of this plant were multifunctional, which could contribute biotechnologically to agriculture.

Recently, Akinsanya et al. [54] isolated endophytic bacteria from *Aloe vera*, including *Pseudomonas hibiscicola*, *Macrococcus caseolyticus*, *Enterobacter ludwigii*, and *Bacillus anthracis*, which can produce bioactive compounds of medical importance with antimicrobial activity against bacterial pathogens. In another study, Akinsanya et al. [55] evaluated the diversity of the endophytic bacterial community of this plant by metagenomics, and demonstrated a greater colonization in the root system; however no studies have examined the functional traits expressed by this community. Therefore, to address this gap, we aimed to isolate cultivable bacterial isolates associated with the roots of *Aloe vera* obtained from heterogeneous environments, and to test the biotechnological potential of the strains in terms of plant growth.

## MATERIAL AND METHODS

### Study Area and Sample Collection

The root systems of three *Aloe vera* individuals were sampled in different environments in the city of Rio Verde, GO, central-western Brazil, in the following locations and geographical coordinates: (1) nursery (latitude 17°47.224'S, longitude 050°57.966'W, and altitude of 786 m), in interaction with diverse vegetation, between arboreal and herbaceous species; (2) garden (latitude 17°48.137'S, longitude 050°55.824'W, and altitude of 701 m) in contact with herbaceous plants, especially medicinal plants, receiving periodic cultural treatments; and (3) field (latitude 18°13.407'S, longitude 51°01.174'W, and altitude of 553 m), anthropic region, of little vegetation (**Figure 1**). Sampling was performed with the objective of recovering a wide diversity of cultivable bacteria, increasing the chances of isolating bacteria with functional traits for biotechnological application. To avoid that climatic factors could affect the dynamics of the microbial community, interfering with the results, all *Aloe vera* plants were sampled on the same day (02/05/2015), between 08 AM and 10 AM. This was a rainy day, and the temperature between the collection areas varied between 19 ˚C and 20˚C, while the relative air humidity remained at 80%.

The individuals selected had no apparent disease symptoms. The collected roots were stored in properly identified plastic bags and transported to the Agricultural Microbiology Laboratory of IF Goiano, Rio Verde Campus for analysis.

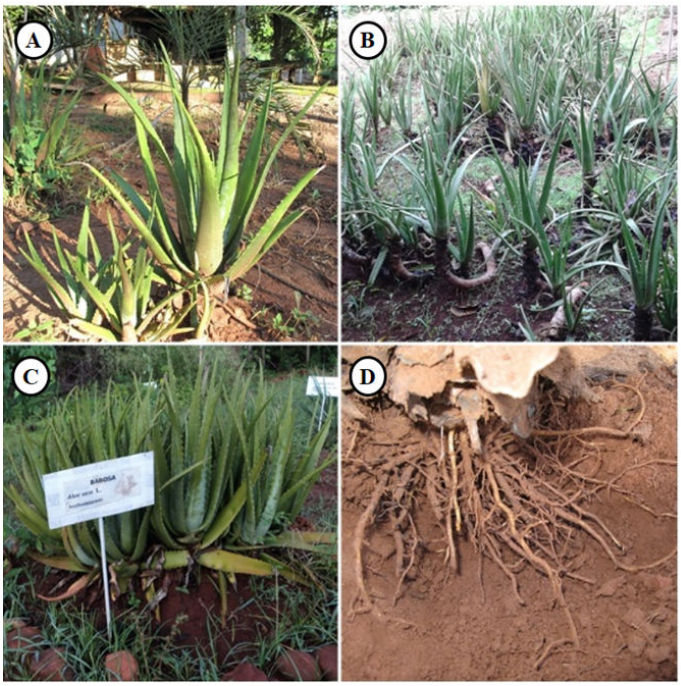


**Figure 1:** Environments from which *Aloe vera* roots were collected. A) Field: Farm; B) Garden of the Planta e Vida Cooperative; C) Nursery of the University of Rio Verde, GO; D) Extensive root-system of *Aloe vera*.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

### Endophytic Bacteria Isolation

Endophytic bacteria were isolated using root fragments. These were washed in running water to remove excess adhered soil and fragments were then shaken in water and neutral detergent (1%) at 70 rpm for 10 min to reduce the density of epiphytic microorganisms. The surface of the fragments was disinfected by successive washes in ethanol (70%), 2.5% sodium hypochlorite (active chlorine), and ethanol (70%) for 1 min, 5 min, and 30 sec, respectively. At the end of the process, four washes were performed using autoclaved distilled water. In addition, an aliquot of 100 μL was taken from the final wash for inoculation in a nutrient broth (3 g meat extract, 5 g peptone) at 28°C for 24 h to test the efficiency of the disinfestation process.

The disinfested fragments were cut to approximately 1 cm in length and placed on Petri dishes containing potato dextrose agar (Acumedia®; PDA). The growth of endophytic bacteria was monitored until day 10. The frequency of colonization was evaluated considering the percentage of fragments with at least one endophytic bacteria, in relation to the total fragments analyzed, according to the formula below.

Frequency of colonization = (No. fragments/ Total fragments) x 100

### Spore Extraction of Arbuscular Mycorrhizal Fungi (AMF)

Three samples of rhizospheric soil were collected from the same sampling sites at 0-20 cm depth. Spores were extracted by the wet-sieving method [56], whereby 50 g of dry soil was passed through 0.42 mm and 0.053 mm mesh sieves and centrifuged at 1,811x g in water and at 804x g in a solution of 50% sucrose, for 3 min and 2 min, respectively. The spore density was determined by counting under z Zeiss Discovery V8 (40x) stereomicroscope on a plate with canalettes.

### Identification and Phylogenetic Relationships of Isolates

Total genomic DNA was extracted from the purified bacteria as described by Cheng and Jiang [57]. Bacterial species were identified based on partial sequencing of the 16S rRNA, using the universal primers 27F (5'-AGA GTT TGA TCM TGG CTCAG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') [58].

PCR products were purified using the PureLink Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen®) as instructed by the manufacturer. Sequencing was performed by the Sanger method. Fragments with 16S gene sequences of at least 900 bp were analyzed by the software BLASTn, and each strain was identified through the National Center for Biotechnology Information Blast (www.ncbi.nlm.nih.gov/BLAST) [59].

The phylogenetic relationships of the isolates were inferred using 661 SNPs found in the sequences obtained from the 16S regions aligned with type sequences extracted from the database of the Ribosomal Database Project and the National Center for Biotechnology Information Blast using the software CLUSTAL OMEGA [60]. The reference sequences were chosen based on the degree of similarity to the sequences obtained from the isolates, given by the databases.

The evolutionary model of the TrN+G sequences was selected using Bayesian Information Criterion (BIC), implemented in software JMODELTEST 2 [61]. The phylogenetic tree was inferred through the software MR BAYES v.3.2.6. [62], using methods based on Bayesian inference. Four independent runs were performed, with 10 Ч $10^6$ generations assigned to each chain, with probability distribution a posteriori for every 500 generations. Before calculating the consensus tree, and to ensure the convergence of the chains, the first 2,500 trees sampled were discarded. Subsequently, the recovered phylogeny was tested by the bootstrap method, with 5,000 replications, through the MEGA 7 program [63]. The tree obtained was visualized and edited with the program FigTree v 1.4.2 [64]. A sequence from *Methylobacterium rhodinum* was used as an out-group.

### Selection of Endophytic Bacteria and Auxin Production

Indoleacetic acid (IAA) production was used to investigate the multifunctional potential of bacteria; only bacteria with this potential were tested for other traits. The use of this criterion reflects the fact that root endophytic bacteria are associated with the biosynthesis of this phytohormone, and can directly affect its homeostasis, while rhizosphere microorganisms tend to be more associated with nutrient supply [65].

IAA production was quantified using the colorimetric method developed by Gordon and Weber [66]. The bacterial isolates were cultured under constant stirring at 90 rpm in nutrient broth medium (3 g meat extract, 5 g peptone) at 28°C for 72 h. The optical density ($OD_{600}$) of all bacterial samples was adjusted to 0.3 by dilution with saline solution (0.85%).

IAA production was quantified in triplicate. Bacterial cultures were inoculated in nutrient broth medium supplemented with L-tryptophan (1%) and maintained for 72 h at 28°C in the dark at 90 rpm. As a control, the broth culture medium was used with tryptophan without bacterial inoculum. After incubation, the samples were centrifuged (12,000 rpm for 5 min

e-ISSN: 2320-3528
p-ISSN: 2347-2286

at 10°C), then 1 mL of the supernatant from each isolate was transferred to a test tube, and 1 mL of Salkowski reagent (1,875 g $FeCl_3.6H_2O$, 100 mL $H_2O$, and 150 mL $H_2SO_4$) was added.

The tubes were kept in the dark for 15 min at 28°C for further quantification of IAA in a spectrophotometer (530 nm). IAA concentrations were obtained using the equation of the calibration curve obtained with commercial IAA [31]. The 32 strains with the most satisfactory results for IAA production were then tested for other functional traits.

### Phosphate Solubilization

Bacterial cultures were standardized at an $OD_{600}$ of 0.3 and inoculated in triplicate, with 1 mL in each penicillin glass containing 9 mL of GY culture medium (10 g glucose, 2 g yeast extract), supplemented separately with four phosphate sources: 5g $L^{-1}$ of reactive natural phosphate from Bayyvar, Peru (12.8% P), 5g $L^{-1}$ from tricalcium phosphate [$Ca_3(PO_4)^2$], 2g $L^{-1}$ from iron phosphate ($FePO_4$), and 1g $L^{-1}$ of aluminum phosphate ($AlPO_4$). These remained under constant agitation at 90 rpm for 72 h at 28°C. As a control, the GY medium was used with each phosphate source. Subsequently, the pH was measured. The amount of inorganic P was determined by the colorimetric method described by Murphy and Riley [67]. Bacterial phosphate solubilization was estimated using the standard curve equation.

### Siderophore Production

Siderophore production was evaluated using the universal methodology adapted by Schwyn and Neilands [68]. The bacterial isolates were cultured in tryptophanin soybean culture medium, diluted 1/10, and incubated in an oven at 28°C for 72 h. The cell suspension was centrifuged at 12,000 rpm for 10 min, 1 mL of the supernatant was transferred into test tubes, and then 1 mL of chromoazide S (CAS) indicator solution was added. Conversion of the blue color of the CAS solution in the supernatant to orange-yellow over the 15 min period indicated that the isolate was capable of producing siderophores.

### Antibiosis to Phytopathogenic Fungi

The antagonism of bacteria against the phytopathogens *Sclerotinia sclerotiorum* (white mold), *Fusarium* sp., and *Rhizoctonia* sp. was tested using the double culture method [69]. Initially, four endophytic bacteria were inoculated at the edge of each plate containing PDA culture medium, 3 cm from the center of the plate, where a 5 mm diameter pathogen mycelium disc was deposited. For the control treatment, a plate containing the phytopathogen in the center was used. Evaluations were made relative to the control plates, where mycelial growth was observed up to 3 cm away from the center, by visual analysis.

Isolates presenting some degree of phytopathogen inhibition were individually tested in triplicate. In a Petri dish containing PDA medium, a 5 mm diameter culture dish containing endophytic bacterium and each pathogenic isolate was inoculated at equal distance. The control treatment consisted of a plate containing only the phytopathogen. The plates were incubated at 25°C until the mycelium of the pathogen developed, without the presence of the endophytic bacteria, on the whole culture medium. Subsequently, the zone of inhibition provoked by the different endophytic bacteria was evaluated.

The diameter of each fungus was measured with a pachymeter and the zone of fungal growth inhibition was confirmed by the production of suppressive compounds by the bacterium. The percentage suppression by each treatment was calculated using the relative index (RI), as follows:

RI (%) = (RC−RX)/ RC x 100, where:

RC = radius of the pathogen colony in the control treatment

RX = radius of the pathogen colony paired with the endophytic isolate

### Enzymatic Evaluation

The enzymatic activity (amylase, cellulase, pectinase, and protease) of the strains was evaluated. A pre-inoculum was performed in which bacteria were grown in nutrient broth culture medium for 48 h at 90 rpm at room temperature. For the tests, 5 μL of each isolate was inoculated by the micro-plating technique in the culture medium used for each test, and four strains were evaluated per Petri dish.

### Amylase Production

To determine amylolytic activity, the bacteria were inoculated by micro-plating in nutrient agar (NA) medium supplemented with 0.2% soluble starch [70] and incubated for 72 h at 28°C. The culture medium was then covered with a Lugol solution for 15 min. Following exposure to metallic iodine, a transparent halo was observed around colonies that produced amylase [71].

e-ISSN: 2320-3528
p-ISSN: 2347-2286

### Cellulase Production

To determine cellulase production, culture medium was used as described by Cattelan [72], whereby the bacteria were inoculated in trypticasein soy agar medium (TSA) containing 10 g/L cellulose and incubated for 72 h at 28°C. A Congo red solution (0.3%) was added to each plate for 15 min and incubated at room temperature. The excess was then removed and NaCl (1M) solution was added for discoloration and incubation for 15 min. Cellulase-producing bacteria presented a light-colored halo with orange borders, indicative of hydrolysis [73].

### Pectinase Production

To confirm the production of pectinases, the method proposed by Hankin et al. [74] was modified. Briefly, PDA medium plus 10 g of pectin/L was used. Bacteria were grown for 48 h at 28°C and the plate was covered for 15 min with hexadecyltrimethylammonium bromide solution (1%). This reagent precipitates intact pectin in the medium, and light zones around the colony indicate the degradation of pectin via the action of pectinase.

### Protease Production

YPD medium (5 g peptone, 3 g yeast extract, 10 g glucose, and 20 g agar containing 2% casein) was used, and solubilized in hot water followed by autoclaving for 10 min at 110°C. The plates were incubated for 72 h at 28°C. Bacteria were cultivated in this medium; protease-positive bacteria formed a light halo around their colonies, indicating the presence of proteases [71].

### Tolerance to Salinity

Four bacteria, which presented positive characteristics for plant growth in previous tests, were selected. The tolerance of these bacteria was evaluated in nutrient agar medium containing different concentrations of NaCl (0, 1, 2, 4, and 6%) and incubated for 48 h at 28°C, as described by Cardoso et al. [75].

### Statistical Analysis

Data were submitted to analysis of variance and the average AMF spore densities were compared by Tukey's test (5%). Average values for phosphate solubilization, IAA synthesis, and antibiosis were compared by the Scott-Knott test (5%). All analyses were performed using the software SISVAR [76].

## RESULTS

### Isolation and Density of Endophytic Bacteria and AMF Spores

The colonization frequency of endophytic bacteria in the root fragments was 100% for the nursery and garden samples and 98.6% for the field sample. In total, 129 endophytic bacteria were obtained, including 36 from the field, 20 from the garden and 73 from the nursery. Only 32 isolates were selected for subsequent studies based on auxin production as follows: one from the field, six from the garden, and 25 from the nursery (Figure 2).

The density of soil spores differed significantly between the environments evaluated; the density was highest in the nursery samples at 1,375 (50 $g^{-1}$) compared with the garden samples at 865 (50 $g^{-1}$) and field samples at 93 (50 $g^{-1}$).

e-ISSN: 2320-3528
p-ISSN: 2347-2286

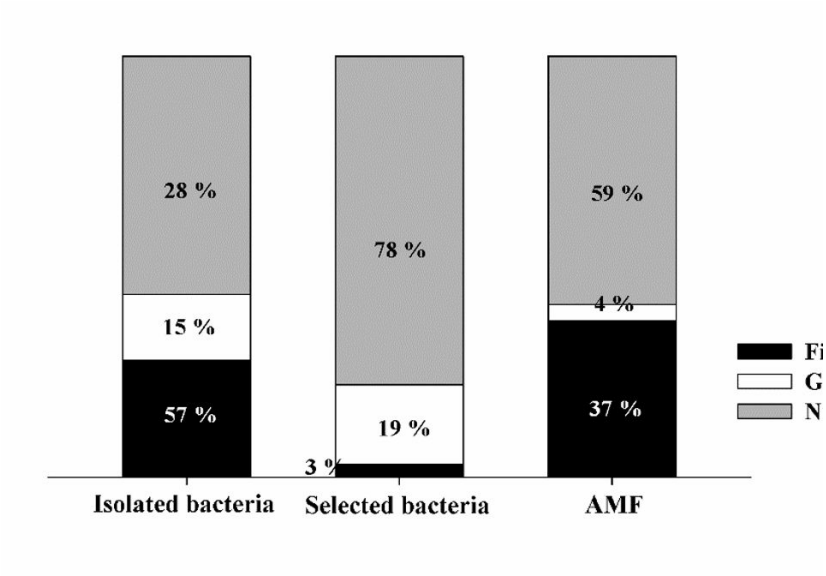


**Figure 2:** Percentage of endophytic bacteria isolated from the roots of *Aloe vera*. Bacteria were selected based on the production of indoleacetic acid and arbuscular microrrheic fungi (AMF) spores on the rhizospheric soil of the samples field, garden and nursery.

## Identification and Phylogenetic Analysis of Isolates

The identified bacteria represented 10 genera, distributed in four groups. Proteobacteria was the most abundant and was represented by the classes *Gammaproteobacteria* and *Betaproteobacteria*; *Firmicutes*, class *Bacilli*; *Bacteroidetes*, class *Flavobacteriia*; and *Actinobacteria*, class *Actinobacteria*. The 32 isolates were identified by the degree of genetic similarity in: *Enterobacter* sp. (4), *Enterobacter asburiae* (1), *Enterobacter tabaci* (8), *E. ludwigii* (4), *Pantoea agglomerans* (1), *P. cypripedii* (1), *Lelliottia nimipressuralis* (1), *Paraburkholderia* sp. (1), *Bacillus megaterium* (3), *B. agri* (1), *Lysinibacillus xylanilyticus* (1), *L. macroides* (2), *Microbacterium aerolatum* (1) and *Chryseobacterium taiwanense* (3) (Figure 3).

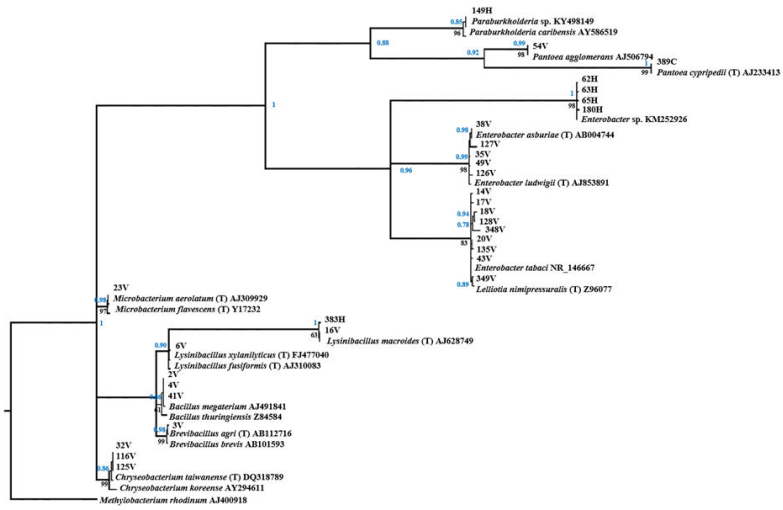


**Figure 3:** Similarity tree based on 16S gene sequences from endophytic bacteria isolated from the roots of *Aloe vera* in three different sites with relationships between the nearest strains. (T) Type strain. Letters following the isolate numbers indicate the sampling site (V =nursery, H=garden, and C=field). Black numbers below the nodes indicate the posterior probability, and the blue values above the nodes represent the bootstrap values based on 10,000 replicates.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

### IAA Production and Selection of Endophytic Bacteria

The ability to synthesize IAA was evaluated quantitatively in 129 endophytic bacteria; 24.8% (32 bacteria) presented positive results, with concentrations varying between 0.3 and 225.2 μg $mL^{-1}$. Nineteen bacterial isolates produced significant amounts of this phytohormone (greater than 80 μg $mL^{-1}$) and only seven isolates presented less than 20 μg $mL^{-1}$ of IAA. The 135V *E. tabaci* isolate produced the highest concentrations of IAA (Table 1).

**Table 1:** *In vitro* production of indoleacetic acid (IAA) by bacteria obtained from the roots of *Aloe vera*.

| Isolate | IAA (μg $mL^{-1}$) | $\sigma_M^{\pm}$ |
|---|---|---|
| 389C *Pantoea cypripedii* | 42.4 d | 1.5 |
| 62H *Enterobacter* sp. | 113.7 b | 10.3 |
| 63H *Enterobacter* sp. | 116.3 b | 6.7 |
| 65H *Enterobacter* sp. | 119.4 b | 8.8 |
| 149H *Paraburkholderia* sp. | 132.6 b | 1.9 |
| 180H *Enterobacter* sp. | 97.9 c | 4.5 |
| 383H *Lysinibacillus macrolides* | 5.7 d | 1.6 |
| 2V *Bacillus megaterium* | 6.4 d | 1.9 |
| 3V *Brevibacillus agri* | 0.3 d | 0.2 |
| 4V *Bacillus megaterium* | 5.3 d | 2.6 |
| 6V *Lysinibacillus xylanilyticus* | 7.9 d | 1.2 |
| 14V *Enterobacter tabaci* | 119.2 b | 1.4 |
| 16V *Lysinibacillus macrolides* | 29.9 d | 1.9 |
| 17V *Enterobacter tabaci* | 83.0 c | 8.6 |
| 18V *Enterobacter tabaci* | 113.3 b | 6.5 |
| 20V *Enterobacter tabaci* | 88.6 c | 10.8 |
| 23V *Microbacterium aerolatum* | 11.6 d | 6.7 |
| 32V *Chryseobacterium taiwanense* | 24.3 d | 1.2 |
| 35V *Enterobacter ludwigii* | 122.8 b | 3.7 |
| 38V *Enterobacter asburiae* | 152.1 b | 0.7 |
| 41V *Bacillus megaterium* | 8.3 d | 1.2 |
| 43V *Enterobacter tabaci* | 111.6 b | 12 |
| 49V *Enterobacter ludwigii* | 83.2 c | 11.1 |
| 54V *Pantoea agglomerans* | 20.7 d | 1 |
| 116V *Chryseobacterium taiwanense* | 22.1 d | 0.5 |
| 125V *Chryseobacterium taiwanense* | 24.3 d | 2.9 |
| 126V *Enterobacter ludwigii* | 101.0 c | 8.7 |
| 127V *Enterobacter ludwigii* | 115.9 b | 3.1 |
| 128V *Enterobacter tabaci* | 131.0 b | 6.8 |
| 135V *Enterobacter tabaci* | 225.2 a | 2.9 |

e-ISSN: 2320-3528
p-ISSN: 2347-2286

| | | |
|---|---|---|
| 348V *Enterobacter tabaci* | 103.4 c | 1.6 |
| 349V *Lelliottia nimipressuralis* | 104.0 c | 1.8 |

Average values followed by the same letter do not differ from one another by the Scott-Knott test (5%); σM = standard error of the mean.

## Phosphate Solubilization

Maximum Bayyvar phosphate solubilization was detected by the bacterium 149H *Paraburkholderia* sp. (45.7 $mgL^{-1}$), which reduced the pH to 3.3. The most effective solubilization of $Ca_3(PO_4)_2$ was observed with the isolate 389C *Pantoea cypripedii* (36.5 mg L-1), with pH 3.9. For $FePO_4$, only four bacteria demonstrated solubilization, although the levels observed were low, at ≤ 3.4. When using AlPO4 as a phosphate source, no solubilization was observed for any of the bacteria tested. Isolates 383H *Lysinibacillus macroides*, 6V and 16V *L. xylanilyticus*, and 116V and 125V *C. taiwanense* did not solubilize any of the phosphate sources tested. As expected, lower final pH values were related to higher solubilization levels (**Table 2**).

**Table 2:** pH and *in vitro* solubilization of Bayovar natural phosphate (P), tricalcium phosphate ($Ca_3(PO_4)_2$), and iron phosphate ($FePO_4$) by *Aloe vera* root endophytic bacteria

| Isolate | Bayovar phosphate | | | $Ca_{342}$ | | | $FePO_4$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | P soluble (mg $L^{-1}$ | $M^{\pm}$ | pH | P soluble (mg $L^{-1}$ | $M^{\pm}$ | pH | P soluble (mg $L^{-1}$ | $\sigma_M^{\pm}$ |
| 389C *Pantoea cypripedii* | 6.3 c | - | - | 3.9 a | 36.5 a | 0.18 | 3.4 a | 2.8 a | 0.02 |
| 62H *Enterobacter* sp. | 5.2 b | 7 c | 0.09 | 6.7 e | 4.9 g | 0.03 | 7 f | - | - |
| 63H *Enterobacter* sp. | 5.3 b | 5.7 c | 0.11 | 6.8 e | 2.1 h | 0.02 | 7.2 f | - | - |
| 65H *Enterobacter* sp. | 4.4 a | 10.3 b | 0.09 | 6.8 e | 2 h | 0.01 | 6.9 f | - | - |
| 149H *Paraburkholderia* sp. | 3.3 a | 45.7 a | 0.12 | 6.1 d | 29.6 b | 0.29 | 7 f | - | - |
| 180H *Enterobacter* sp. | 5 b | 12.9 b | 0.03 | 6.8 e | 2.1 h | 0.01 | 8 f | - | - |
| 383H *Lysinibacillus macroides* | 8.1 d | - | - | 8 f | - | - | 7 d | - | - |
| 2V *Bacillus megaterium* | 4.1 c | 2.6 d | 0.04 | 5.5 c | 8.7 f | 0.03 | 5.6 d | - | - |
| 3V *Brevibacillus agri* | 7.5 d | - | - | 7.1 e | - | - | 4.2 b | 2.1 a | 0.03 |
| 4V *Bacillus megaterium* | 4.5 a | - | - | 4.8 b | 23.8 c | 0.06 | 4.8 c | - | - |
| 6V *Lysinibacillus xylanilyticus* | 7.5 d | - | - | 8 f | - | - | 7.8 f | - | - |
| 14V *Enterobacter tabaci* | 6.8 c | - | - | 6.6 e | 8.4 f | 0.02 | 7.1 f | - | - |
| 16V *Lysinibacillus macroides* | 6.4 c | - | - | 8.2 f | - | - | 5.8 d | - | - |
| 17V *Enterobacter tabaci* | 6.9 c | - | - | 6.7 e | 9.1 f | 0.06 | 7.1 f | - | - |
| 18V *Enterobacter tabaci* | 4.8 b | 3 d | 0.03 | 4.9 b | 21.5 d | 0.03 | 5.3 d | 0.7 b | 0.02 |
| 20V *Enterobacter tabaci* | 6.7 c | - | - | 6.7 e | 5.9 g | 0.03 | 6.7 e | - | - |
| 23V *Microbacterium aerolatum* | 6 c | - | - | 5.3 c | 18.2 d | 0.07 | 5.7 d | - | - |
| 32V *Chryseobacterium taiwanense* | 6.9 c | - | - | 6 d | 6.1 g | 0.02 | 5.6 d | - | - |
| 35V *Enterobacter ludwigii* | 5.3 b | 12.3 b | 0.09 | 6.6 e | 12.6 e | 0.05 | 7.1 f | - | - |
| 38V *Enterobacter asburiae* | 6.7 c | - | - | 6.6 e | 4.1 g | 0.04 | 7 f | - | - |
| 41V *Bacillus megaterium* | 4.1 a | 2.1 d | 0.01 | 4.9 b | 25.6 c | 0.12 | 5.4 d | - | - |
| 43V *Enterobacter tabaci* | 6.7 c | - | - | 6.5 e | 6.7 g | 0.05 | 7.2 f | - | - |

e-ISSN: 2320-3528
p-ISSN: 2347-2286

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 49V *Enterobacter ludwigii* | 4.3 a | 16.9 b | 0.09 | 6 d | 3.3 h | 0.02 | 6 d | - | - |
| 54V *Pantoea agglomerans* | 5.1 b | 7.1 c | 0.08 | 6,1 d | 24.6 c | 0.05 | 4.8 c | 3.4 a | 0.01 |
| 116V *Chryseobacterium taiwanense* | 6.6 c | - | - | 6.2 d | - | - | 5.5 d | - | - |
| 125V *Chryseobacterium taiwanense* | 6.9 c | - | - | 6.2 d | - | - | 5.5 d | - | - |
| 126V *Enterobacter ludwigii* | 4.5 a | 9 b | 0.01 | 6.5 e | 14.5 e | 0.18 | 6.6 e | - | - |
| 127V *Enterobacter ludwigii* | 5.2 b | 11.5 b | 0.16 | 6.7 e | 3.8 h | 0.01 | 6.8 e | - | - |
| 128V *Enterobacter tabaci* | 6.6 c | - | - | 6.7 e | 2.4 h | 0.03 | 7 f | - | - |
| 135V *Enterobacter tabaci* | 4.4 a | 13 b | 0.01 | 6.7 e | 9 f | 0.13 | 5.6 d | - | - |
| 348V *Enterobacter tabaci* | 4.4 a | 14 b | 0.05 | 6.9 e | 2.4 h | 0.07 | 5.7 d | - | - |
| 349V *Lelliottia nimipressuralis* | 4.3 a | 5 c | 0.04 | 6.6 e | 5.5 g | 0.18 | 6.3 e | - | - |

Averages followed by the same letter do not differ from one another by the Scott-Knott test (5%). (-) indicates no solubilization; σM = standard error of the mean.

## Antibiosis and Siderophore Production

Initial screening with 32 bacteria revealed that 40.6% (13) of isolates exerted some degree of mycelial growth inhibition of *S. sclerotiorum*, 34.4% (11) on *Fusarium* sp., and 21.9% (7) against *Rhizoctonia* sp. (**Figure 4**). Quantitative assessment of antagonistic activity against *S. sclerotiorum* revealed a variation of 11.4 to 45.6% in terms of relative inhibition. Among the isolates, 383H *L. macroides*, 3V *Brevibacillus agri*, and 14V and 17V *Enterobacter tabaci* presented inhibition rates between 35.6 and 45.6%. For the phytopathogens *Fusarium* sp. and *Rhizoctonia* sp., the relative inhibition was lower, reaching no more than 34%, with the highest levels presented by the bacteria 149H *Paraburkholderia* sp. and 348V *Enterobacter tabaci* (**Table 3**). Siderophore production was negative for all bacterial isolates evaluated.

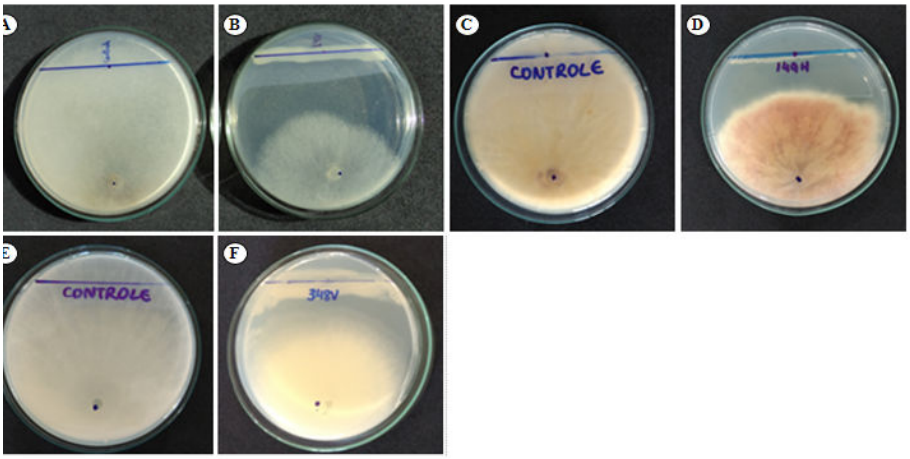


**Figure 4:** Double culture test between root endophytic bacteria of A. vera and phytopathogens *Sclerotinia sclerotiorum*, *Fusarium* sp., and *Rhizoctonia* sp. (A) *S. sclerotiorum* (control); (B) Antagonism between the phytopathogenic fungus *S. sclerotiorum* and the bacterium 14V *Enterobacter tabaci*. (C) *Fusarium* sp. (control); (D) Antagonism between *Fusarium* sp. and the bacterium 149H *Paraburkholderia* sp. (E) *Rhizoctonia* sp. (control); (F) Antagonism between *Rhizoctonia* sp. and the bacterium 348V *Enterobacter tabaci*.

**Table 3:** Inhibition of mycelial growth of phytopathogenic fungi *Sclerotinia sclerotiorum*, *Fusarium* sp., and *Rhizoctonia* sp. by endophytic bacteria in antagonism tests *in vitro*.

| Isolate | Sclerotinia sclerotiorum | | | Fusarium sp. | | Rhizoctonia sp. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | RC[1] (cm) | $\sigma_{M\,(RC)}^{\pm}$ | RI[2] (%) | RC[1] (cm) | $\sigma_{M\,(RC)}^{\pm}$ | RI[2] (%) | RC[1] (cm) | $\sigma_{M\,(RC)}^{\pm}$ | RI[2] (%) |

e-ISSN: 2320-3528
p-ISSN: 2347-2286

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 389C *Pantoea cypripedii* | 8.0 c | 0.07 | 11.4 | 8.0 b | 0.07 | 11.5 | - | - | - |
| 62H *Enterobacter* sp. | - | - | - | - | - | - | - | - | - |
| 63H *Enterobacter* sp. | - | - | - | 8.4 b | 0.08 | 6.3 | 7.1 b | 0.04 | 21.5 |
| 65H *Enterobacter* sp. | - | - | - | 8.8 b | 0.05 | 1.9 | 8.8 c | 0.11 | 2.2 |
| 149H *Paraburkholderia* sp. | 6.5 b | 0.13 | 28.1 | 6.1 a | 0.05 | 32.2 | - | - | - |
| 180H *Enterobacter* sp. | 7.0 c | 0.05 | 21.9 | - | - | - | - | - | - |
| 383H *Lysinibacillus macroides* | 5.3 a | 0.5 | 41.4 | - | - | - | - | - | - |
| 2V *Bacillus megaterium* | - | - | - | - | - | - | - | - | - |
| 3V *Brevibacillus agri* | 4.9 a | 0.06 | 45.6 | - | - | - | - | - | - |
| 4V *Bacillus megaterium* | - | - | - | - | - | - | - | - | - |
| 6V *Lysinibacillus xylanilyticus* | - | - | - | - | - | - | - | - | - |
| 14V *Enterobacter tabaci* | 5.1 a | 0.17 | 43 | 8.1 b | 0.04 | 10.4 | - | - | - |
| 16V *Lysinibacillus macroides* | - | - | - | - | - | - | - | - | - |
| 17V *Enterobacter tabaci* | 5.8 a | 0.23 | 35.6 | - | - | - | - | - | - |
| 18V *Enterobacter tabaci* | 7.3 c | 0.11 | 19.2 | - | - | - | - | - | - |
| 20V Enterobacter *tabaci* | - | - | - | - | - | - | - | - | - |
| 23V *Microbacterium aerolatum* | 7.8 c | 0.23 | 13.7 | - | - | - | - | - | - |
| 32V *Chryseobacterium taiwanense* | - | - | - | - | - | - | - | - | - |
| 35V *Enterobacter ludwigii* | 6.2 b | 0.12 | 31.4 | - | - | - | - | - | - |
| 38V *Enterobacter asburiae* | - | - | - | 8.7 b | 0 | 3.3 | 7.7 b | 0.37 | 14.1 |
| 41V *Bacillus megaterium* | - | - | - | - | - | - | - | - | - |
| 43V *Enterobacter tabaci* | - | - | - | - | - | - | - | - | - |
| 49V *Enterobacter ludwigii* | - | - | - | 8.1 b | 0.23 | 10 | 7.1 b | 0.36 | 21.5 |
| 54V *Pantoea agglomerans* | 7.2 c | 0.07 | 19.7 | 7.9 b | 0.35 | 11.9 | - | - | - |
| 116V *Chryseobacterium taiwanense* | - | - | - | - | - | - | 7.1 b | 0.04 | 21.5 |
| 125V *Chryseobacterium taiwanense* | - | - | - | - | - | - | - | - | - |
| 126V *Enterobacter ludwigii* | - | - | - | - | - | - | - | - | - |
| 127V *Enterobacter ludwigii* | - | - | - | 8.9 b | 0.06 | 1.1 | - | - | - |
| 128V *Enterobacter tabaci* | - | - | - | 8.4 b | 0.06 | 6.7 | 8.9 c | 0.04 | 0.7 |
| 135V *Enterobacter tabaci* | - | - | - | - | - | - | - | - | - |
| 348V *Enterobacter tabaci* | 6.6 b | 0.17 | 27 | 6.8 b | 0 | 24.4 | 6.0 a | 0.28 | 33.7 |
| 349V *Lelliottia nimipressuralis* | 6.8 b | 0.03 | 24.8 | - | - | - | - | - | - |
| Control | 9.0 d | 0 | 0 | 9.0 b | 0 | 0 | 9.0 c | 0 | 0 |

Averages followed by the same letter do not differ by the Scott-Knott test (5%); [1]Radius of phytopathogen colony. [2]Relative inhibition. (-): indicates no inhibitory activity; $\sigma_M$ = standard error of the mean

Microscopic images showed relevant morphological changes after 4 days of *S. sclerotiorum* phytopathogen cultivation with endophytic bacteria. Some hyphae became swollen, twisted, and dehydrated, and others were collapsed (**Figure 5**).

e-ISSN: 2320-3528
p-ISSN: 2347-2286

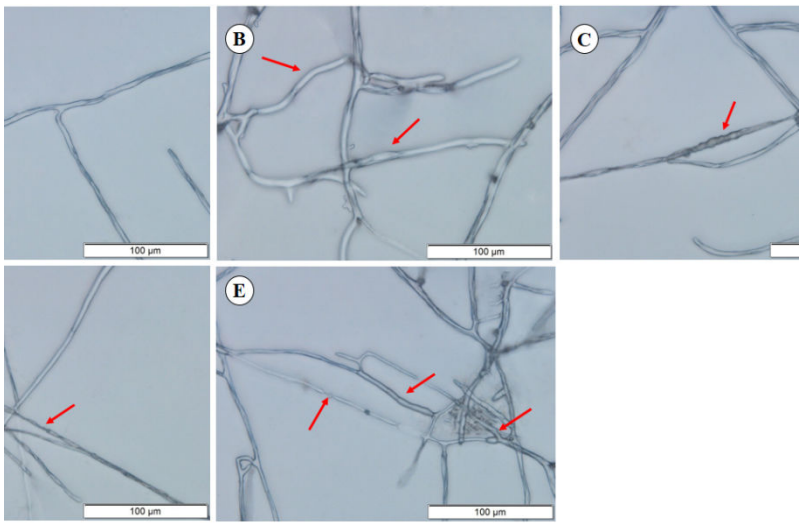


**Figure 5:** Morphological alterations of the mycelium of the phytopathogenic fungus *S. sclerotiorum* are indicated by red arrows following interaction with root endophytic bacteria of *Aloe vera*. (A) Control - hyphae of *S. sclerotiorum*; (B) interaction with the 3V *B. agri* bacterium – hyphae swollen; (C) 14V *Enterobacter tabaci* bacterium - hyphae twisted; (D) bacterium 17V *E. tabaci* - degenerative changes in the morphology of hyphae; (E) 383H *L. macroides* bacteria – hyphae collapsed or swollen.

## Enzymatic Evaluation and Salinity Tolerance

There was evidence of amylolytic activity in 34.4% (11) of the bacteria by bacterial isolates 2V, 4V, and 41V (*B. megaterium*), 3V (*B. agri*), 18V and 135V (*E. tabaci*), 23V (*M. aerolatum*), 32V, 116V, and 125V (*C. taiwanense*), and 349V (*L. nimipressuralis*). No cellulase, protease, or pectinase activity was observed for any the bacteria tested.

With respect to salinity, among the selected bacteria, 35V *E. ludwigii*, 135V *E. tabaci*, and 389C *P. cypripedii* presented good growth under all NaCl concentrations, while 149H *Paraburkholderia* sp. did not tolerate the highest concentrations of NaCl (4% and 6%).

## Multifunctional Potential of Isolates

None of the 32 isolates tested have all the 13 functional characteristics evaluated. Only three isolates were able to produce IAA, and six of the functional characteristics evaluated were observed in three bacteria (**Figure 6A**). The predominant features were IAA production and calcium phosphate solubilization (**Figure 6B**).

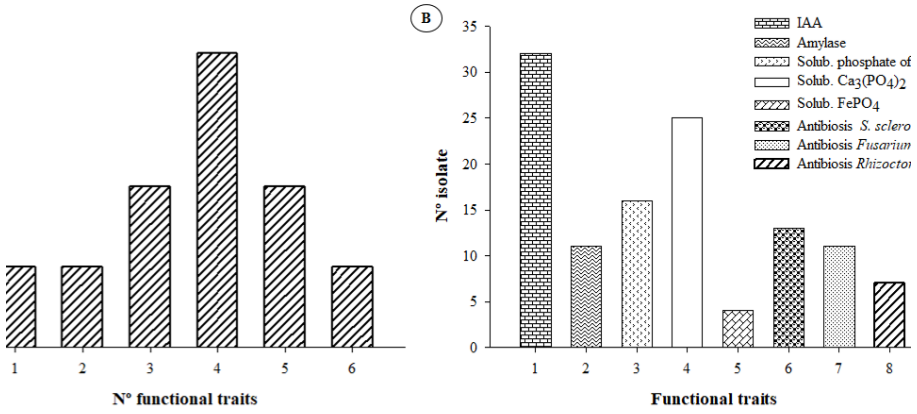


**Figure 6:** Multifunctional potential of endophytic bacteria associated with the roots of A. vera from different sites. A) Number of bacterial isolates expressing potential functional traits for biotechnology; B) Number of isolates presenting each functional trait evaluated (IAA biosynthesis, amylase production, Bayóvar phosphate solubilization, calcium and iron, and antibiosis to phytopathogens *S. sclerotiorum*, *Fusarium* sp., and *Rhizoctonia* sp.)

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Isolates 149H *Paraburkholderia* sp., and 135V and 348V *E. tabaci* were notable for their expression of the functional traits evaluated (**Figure 7**). The 135V isolate produced the highest levels of IAA detected, and showed potential for Bayyvar phosphate solubilization, amylase production, and salinity tolerance, while the 149H isolate produced the second highest level of IAA, and the highest values for Bayyvar phosphate solubilization, and relative inhibition of *Fusarium* sp. The 348V isolate showed good potential for IAA synthesis, Bayyvar phosphate solubilization, and phytopathogen inhibition, especially *Rhizoctonia* sp.

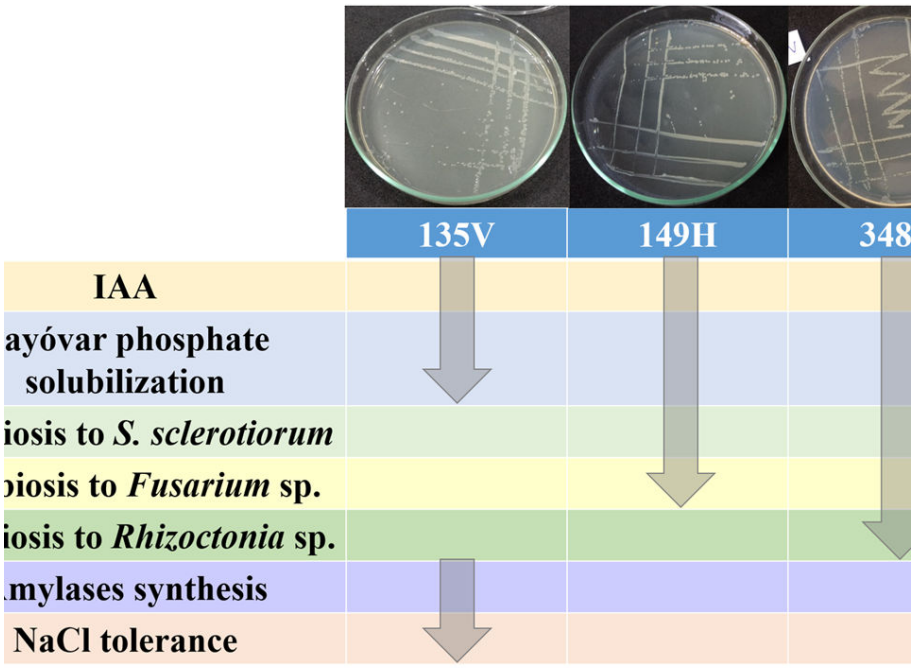


**Figure 7:** Endophytic bacteria 149H *Paraburkolderia* sp., and 135V and 348V *Enterobacter tabaci* isolates from the roots of *Aloe vera* and functional traits expressed by these isolates. Arrows indicate that the isolate expresses the indicated trait.

## Discussion

Despite the high interest in plants used for traditional medicine, little is known about the symbiotic associations of these plants with endophytic microorganisms [23,17,77]. The present study was the first to evaluate the multifunctional potential of bacterial strains isolated from the roots of *Aloe vera*.

High bacterial colonization and a high number of AMF spores were observed in the nursery. In this environment, *Aloe vera* plants were associated with a wide diversity of plants. According to Silva et al. [78], AMF density can be explained by the wide variety of vegetable species; thus, systems with high vegetation diversity present high values for this index.

The results of this study demonstrated that the characteristics of the environment affect the functional diversity of bacterial communities. For example, 36 endophytic bacteria were isolated from the field, which has scarce vegetation, but only one of those species (*Pantoea cypripedii*) was able to produce IAA and present other functional traits. From the garden, where the specimen was closely associated with a variety of medicinal plants, 20 bacteria were isolated, of which six strains of *Enterobacter* sp., *Paraburkholderia* sp. and *Lysinibacillus macroide* presented both the initial trait for IAA production, as well as other traces evaluated. From the nursery, which is rich in arboreous and herbaceous species, a higher number of isolates (73) were obtained, and 25 of those had functional traits, represented by the species *P. agglomerans*, *Enterobacter* sp., *E. tabaci*, *E. ludwigii*, *E. asburiae*, *L. nimipressuralis*, *B. megaterium*, *B. agri*, *L. xylanilyticus*, *L. macroides*, *M. aerolatum*, and *C. taiwanense*.

We identified a high number of isolates from the groups *Proteobacteria* and *Firmicutis*, followed by *Actinobacteria* and *Bacteroids*. The predominance of these groups was also observed by Akinsanya et al. [54], who performed a metagenomic study to identify endophytic bacteria in tissues such as the leaves, stem, and roots of *Aloe vera*. In our samples, we identified bacteria that have previously been reported as growth promoters, with potential for biostimulation, biocontrol, and biofertilization [12,26,79,80]. These bacteria belong to the genera *Bacillus* [81,82], *Paraburkholderia* [26,83], *Enterobacter* [12,84], *Lysinibacillus* [85], *Microbacterium* [80] and *Pantoea* [86].

Some genera of the family *Enterobacteriaceae* are polyphyletic, including *Enterobacter* and *Paraburkholderia*, which are currently under revision, and thus hindered the allocation of new related species using only the 16S rRNA gene sequence. One example is the bacterium *L. nimipressuralis*, which was formerly classified as *Enterobacter* [87]. The genus

e-ISSN: 2320-3528
p-ISSN: 2347-2286

*Paraburkholderia*, containing growth-promoting species, was also recently renamed. This previously belonged to the genus *Burkholderia*, a group that contains opportunistic pathogens that are harmful to human and animal health [88]. The α- and β-Proteobacteria classes are related to nitrogen fixation [1,89]. The presence of the genera *Enterobacter*, *Pantoea*, and *Paraburkholderia* in the roots of *Aloe vera* may play an important role in nitrogen absorption in this plant.

In this study, the genus *Enterobacter* represented 54% of the isolates, with species demonstrating capacity for IAA production, Bayyvar phosphate, calcium and iron solubilization, and antibiotic activity against the phytopathogens *S. sclerotiorum*, *Fusarium* sp., and *Rhizoctonia* sp. Similar results were observed by Nutaratat et al. [90] with the strain *Enterobacter* sp. DMKU-RP206, which showed great potential for IAA production, phosphate solubilization, ammonia and siderophores production, and antagonism against the pathogenic rice fungi *Curvularia lunata*, *Rhizoctonia solani*, and *Fusarium moniliforme*.

Members of the Firmicutes phylum produce a range of antimicrobial metabolites, enzymes, and surfactants that promote growth and induce systemic resistance in plants [91]. Bacteria of the genus *Bacillus* have demonstrated resistance to metals and can be used in phytoremediation. In addition, they produce enzymes such as cellulases, amylases, and xylanases. Bacteria of the genus *Microbacterium*, belonging to the phylum *Actinobacteria*, have also shown potential for the synthesis of these enzymes, and for the solubilization of phosphates [92].

*Bacteriodetes*, represented here by the bacterium *Chryseobacterium taiwanense*, have been reported to have potential for biotechnological applications, including the production of enzymes and pigments [93-96]. The strains evaluated in this study were able to produce amylases and the phytohormone IAA.

Auxin was produced by 32 of 129 bacteria tested, with emphasis on 135V *E. tabaci*, a species for which there is limited literature. This was recently described as a new member of the genus, and isolated from the stem of a tobacco plant [97]. The levels of IAA synthesized varied between 0.3 μg $mL^{-1}$ and 225.2 μg $mL^{-1}$, whereby the maximum values were significantly higher than those observed for endophytic bacteria obtained from other medicinal species such as *Hyptis marrubioides* [98], *Phyllanthus amarus* [27], and *T. polium* [37].

Variations in the levels of IAA can be explained by the location of genes responsible for biosynthesis, and the locations of these genes modulate the levels of this phytohormone. When the genes are located on chromosomal DNA, they result in lower production; however, when they are located on the plasmid, they result in greater auxin production, as several copies of the gene are present [99].

The relationship between phosphate solubilization and culture medium acidification was confirmed, since the microbial solubilization of P in organic and inorganic soils is generally associated with the release of low-weight molecular organic acids. Through hydroxyl and carboxyl groups, these chelate the cations bound to phosphate, thereby converting it to the soluble form [100]. These organic acids may include acetate, lactate, malate, oxalate, succinate, citrate, and gluconate [101]. Other mechanisms of P solubilization include H+ excretion and acid phosphatase biosynthesis [102].

Gupta et al. [34] evaluated rhizospheric bacteria of *Aloe barbadensis* using *Pseudomonas synxantha*, *Burkholderia gladioli*, *Enterobacter hormaechei*, and *Serratia marcescens* and observed tricalcium phosphate solubilization between 340 mg $L^{-1}$ and 150 mg $L^{-1}$. These levels are high, when compared with those obtained in the present study using A. vera endophytic bacteria. We observed solubilization levels ranging from 45.7 $mgL^{-1}$ to 2.1 $mgL^{-1}$ for Bayyvar phosphate, 36.5 $mgL^{-1}$ to 2.1 $mgL^{-1}$ for $Ca_3(PO_4)_2$, and 3.4 $mgL^{-1}$ to 0.7 $mgL^{-1}$ for $FePO_4$. These levels indicate that solubilization is not the main functional trait of the analyzed strains.

Another important functional trait evaluated in the present study was antibiosis to phytopathogens. The genus *Brevibacillus* showed potential for the inhibition of *S. sclerotiorum*. This phytopathogen is a cosmopolitan necrotrophic that attacks a range of hosts, including more than 400 plant species [103], such as *Phaseolus vulgaris* [104], *Glycine max* [105], and *Brassica napus* [106]. In the present study, 3V *B. agri* induced 45.6% inhibition, which induced morphological changes in phytopathogenic hyphae (**Figure 5**).

Mohanty et al. [107] isolated endophytic bacteria from *Jatropha curcase* and observed a predominance of the genus *Brevibacillus*, with positive characteristics for IAA production and phosphatases, which induced the growth of maize seedlings. In the present study, the *B. agri* species showed low potential for IAA synthesis and phosphate solubilization, but significantly suppressed the phytopathogen *S. sclerotiorum*. In a study by Yue et al. [108], *Brevibacillus* inhibited the growth of *S. sclerotiorum*, and the formation of sclerotia, and Joo et al. [109] confirmed the effect of rhizospheric *Brevibacillus* on several species of *Fusarium*. Strains of this genus are considered potential candidates for biocontrol agents, owing to antibiotics production and biofilm formation (eg, Arrigoni et al. [110]; Shaf et al. [111]; Panda et al. [112]. *Brevibacillus brevi* strain FJAT-0809-GLX, as evaluated by Jianmei et al. [113], inhibits the proliferation of pathogens through the production of the antimicrobial substance ethylparaben, in addition to chitinase, with the suppression of seven types of pathogens: *Ralstonia solanacearum*, *Salmonella typhimurium*, *Escherichia coli*, *Fusarium oxysporum*, *Aspergillus niger*, *Fusarium solani*, and *Fusarium moniliforme*.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

In this context, the class Bacilli is the most representative among the bacteria capable of inhibiting the growth of phytopathogenic fungi, consistent with many antagonist strains [23,114-117]. This is because bacteria of this class synthesize antimicrobial substances, such as ethylparaben, and enzymes that attack the cell walls of phytopathogenic fungi, such as chitinases [113], and lipopeptides with antifungal properties [118-120]. Thus, several studies confirmed the potential of Bacilli as biocontrol agents and encourage their use in agriculture [113].

Conversely, the phytopathogen *Fusarium* sp. was inhibited to a greater extent by the bacterium 149H *Paraburkholderia* sp. with 32.2% RI. Huo et al. [121] also observed the inhibitory activity of a *Paraburkholderia* rhizosphere isolate on root rot fungus. However, the phytopathogen *Rhizoctonia* sp. was mainly inhibited by the endophytic 348V *E. tabaci*, with 33.7% RI (**Figure 4**). As it is a recently described species, additional data is not available in the literature confirming the ability of this bacterium to inhibit phytopathogens.

In addition to IAA synthesis, most of the strains tested (91%) expressed other functional traits of biotechnological importance. Therefore, our fundings support the hypothesis that root endophytic bacteria from *Aloe vera* display multifunctionality, and some have biotechnological potential. Some of the functional traits can act in synergism, favoring the growth and development of the vegetable and improving the health and performance of the crop. Among the functional traits evaluated, the indices obtained by the multifunctional 135V and 348V isolates, which are both *Enterobacter tabaci*, were notable. Although this is a recently described species, Salkar et al. [122] isolated a strain of this species with great biotechnological potential, suggesting its use in sustainable agriculture systems. Another notable isolate in relation to the tested indices was 149H *Paraburkholderia* sp.; this genus contains environmental bacteria, including promising candidates for biotechnological applications [26,123]. *In vivo* mechanisms for the use of this genus as inoculants have already been evaluated and positive effects on growth and productivity have been observed (e.g. Bernabeu et al. [26]; Rahman et al. [124]). Therefore, in this study, we have confirmed the *in vitro* biotechnological potential of several endophytic strains of *Aloe vera*, especially 135V, 348V, and 149H. This was the first study to evaluate the root endophytic bacterial community of *Aloe vera*, providing opportunities for the future use of endophytic bacteria of this species as inoculants for the promotion of vegetal growth.

## CONCLUSION

This study demonstrated the multifunctionality of endophytic root-endophytic bacteria of *Aloe vera* especially among the isolates 135V and 348V *Enterobacter tabaci* and 149H *Paraburkholderia* sp., which expressed a set of functional traits with potential application for plant growth. Future *in vivo* assessments should aim to confirm the potential of these strains for use as inoculants, biofertilizers, or biological control agents of phytopathogens in order to replace chemicals and increase crop growth, health, and productivity.

## ACKNOWLEDGEMENT

The authors thank the Goiano Federal Institute - Rio Verde Campus for providing the infrastructure and materials to carry out this work; the Pro-Centro-Oeste Network and the Foundation for Research Support of the State of Goias (FAPEG) for financial assistance; and the Coordination for the Improvement of Higher Education Personnel (CAPES) for the doctoral scholarship granted to the first author.

## CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

## REFERENCES

1. Liotti RG, et al. Diversity of cultivable bacterial endophytes in Paullinia cupana and their potential for plant growth promotion and phytopathogen control. Microbiol Res. 2018;207:8-18.
2. Cordero I, et al. Rhizospheric microbial community of Caesalpinia spinosa (Mol.) Kuntze in conserved and deforested zones of the Atiquipa fog forest in Peru. Appl Soil Ecol. 2017;114:132-141.
3. Card SD, et al. Beneficial endophytic microorganisms of Brassica – A review. Biol Control 2015;90:102-112.
4. Ludwig-Mьller J. Bacteria and fungi controlling plant growth by manipulating auxin: Balance between development and defense. J Plant Physiol. 2015;172:4-12.
5. Reinhold-Hurek B and Hurek T. Living inside plants: bacterial endophytes. Curr Opin Plant Biol. 2011;14:435-443.
6. Hardoim PR, et al. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 2015;79:293-320.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

7. Schulz B and Boyle C. The endophytic continuum. Mycol Res. 2005;109:661-686.
8. Hardoim PR, et al. Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology. 2008;16:463-471.
9. Bacon CW and White Jr JF. Functions, mechanisms and regulation of endophytic and epiphytic microbial communities of plants. Symbiosis 2015;68:87-98.
10. Santoyo G, et al. Plant growth-promoting bacterial endophytes. Microbiol Res. 2016;183:92-99.
11. Khamwan S, et al. Characterization of endophytic bacteria and their response to plant growth promotion in Helianthus tuberosus L. Biocatal Agric Biotechnol. 2018;13:153-159.
12. Zhao L, et al. Antagonistic endophytic bacteria associated with nodules of soybean (Glycine max L.) and plant growth-promoting properties. Braz J Microbiol. 2018;49:269-278.
13. Kumar A and Verma JP. Does plant - Microbe interaction confer stress tolerance in plants: A review? Microbiol Res. 2018;207:41-52.
14. Sharma T, et al. Diversity of culturable bacterial endophytes of saffron in Kashmir, India. Springerplus. 2015;4:661.
15. McMullin DR, et al. Detection of foliar endophytes and their metabolites in Picea and Pinus seedling needles. Fungal Ecol. 2018;31:1-8.
16. Venugopalan A and Srivastava S. Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol Adv. 2015;33:873-887.
17. Alvin A, et al. Exploring the potential of endophytes from medicinal plants assources of antimycobacterial compounds. Microbiol Res. 2014;169:483-495.
18. Abbamondi GR, et al. Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. Chem Biol Technol Agric. 2016;3:1-10.
19. Gaiero JR, et al. Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot. 2013;100:1738-1750.
20. Verma VC, et al. Bio-control and plant growth promotion potential of siderophore producing endophytic Streptomyces from Azadirachta indica A. Juss. J Basic Microbiol. 2011;51:550-556.
21. Liffourrena AS and Lucchesi GI. Alginate-perlite encapsulated Pseudomonas putida A (ATCC 12633) cells: Preparation, characterization and potential use as plant inoculants. J Biotechnol. 2018;278:28-33.
22. Ali S, et al. Amelioration of high salinity stress damage by plant growth promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem. 2014;80:160-167.
23. Andreolli M et al. Diversity of bacterial endophytes in 3 and 15 year-old grapevines of Vitis vinifera cv. Corvina and their potential for plant growth promotion and phytopathogen control. Microbiol Res. 2016;183:42-52.
24. Otieno N, et al. Screening of large collections of plant associated bacteria for effective plant growth promotion and colonization. Asp Appl Biol. 2013;120:22-28.
25. Hungria M, et al. Draft Genome Sequences of Azospirillum brasilense Strains Ab-V5 and Ab-V6, Commercially Used in Inoculants for Grasses and Legumes in Brazil. Genome Announc. 2018;6:e00393-18.
26. Bernabeu PR, et al. Assessment of bacterial inoculant formulated with Paraburkholderia tropica to enhance wheat productivity. World J Microbiol Biotechnol. 2018;34:8
27. Joe MM, et al. Isolation of phosphate solubilizing endophytic bacteria from Phyllanthus amarus Schum & Thonn: Evaluation of plant growthpromotion and antioxidant activity under salt stress. J Appl Res Med Aromat Plants. 2016;3:71-77.
28. Pande A, et al. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. J Genet Eng Biotechnol. 2017;15:379-391.
29. Yaish MW, et al. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (Phoenix dactylifera L.) and their potential role in salinity tolerance. Antonie van Leeuwenhoek. 2015;107:1519-1532.
30. Lin L and Xu X. Indole-3-acetic acid production by endophytic Streptomyces sp. En-1 isolated from medicinal plants. Curr Microbiol. 2013;67:209-217.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

31. De Melo Pereira GV, Magalhres KT, Lorenzetii ER, Souza TP, Schwan RF. 2012. A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. Microb Ecol 63: 405-417.

32. Malusa E and Vassilev N. A contribution to set a legal framework for biofertilisers. Appl Microbiol Biotechnol. 2014;98:6599-6607.

33. Busato JG, et al. Compost biofortification with diazotrophic and P-solubilizing bacteria improves maturation process and P availability. J Sci Food Agric. 2017;97:949-955.

34. Fouda AH, et al. Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.). Ann Agric Sci. 2015;60:95-104.

35. Gupta H, et al. Analysis of plant growth promoting potential of endophytes isolated from Echinacea purpurea and Lonicera japфnica. J Soil Sci Plant Nutr. 2016;16:558-577.

36. Gupta M, et al. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of Aloe barbadensis Miller. Microbiol Res. 2012;167:358-363

37. Singh SP and Gaur R. Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against Sclerotium rolfsii in chickpea. J Appl Microbiol. 2016;121:506-518.

38. Hassan SE-D. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of Teucrium polium L. J Adv Res. 2017;8:687-695.

39. Ansary WR, et al. Endophytic Bacillus spp. from medicinal plants inhibit mycelial growth of Sclerotinia sclerotiorum and promote plant growth. Z Naturforsch C. 2018;73:247-256.

40. Maggini V, et al. Antagonism and antibiotic resistance drive a species-specific plant microbiota differentiation in Echinacea spp. FEMS Microbiol Ecol. 2018;94:118.

41. Danagoudar A, et al. Antioxidant and cytotoxic potential of endophytic fungi isolated from medicinal plant Tragia involucrate L. Pharmacognosy Res. 2018;10:188-194.

42. Tan X, et al. Diversity and bioactive potential of culturable fungal endophytes of Dysosma versipellis, a rare medicinal plant endemic to China. Sci Rep. 2018;8:5929.

43. Venieraki A, et al. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hellenic Plant Protection Journal. 2017;10:51-66.

44. Abdou R, et al. Botryorhodines A–D, antifungal and cytotoxic depsidones from Botryosphaeria rhodina, an endophyte of the medicinal plant Bidens pilosa. Phytochemistry. 2010;71:110-116.

45. Hamayun M, et al. Gibberellins producing endophytic fungus Porostereum spadiceum AGH786 rescues growth of salt affected soybean. Front Microbiol. 2017;8:686.

46. Jensen NB, et al. Convergent evolution in biosynthesis of cyanogenic defence compounds in plants and insects. Nat Commun. 2011;2:273.

47. Richards TA, et al. Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi. Plant Cell. 2009;21:1897-1911.

48. Nakaew N and Sungthong R. Seed phytochemicals shape the community structures of cultivable actinobacteria-inhabiting plant interiors of Thai pigmented rice. Microbiologyopen. 2018;7:e591.

49. Nisa H, et al. Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: A review. Microb Pathogen. 2015;82:50-59.

50. Aly AH, et al. Bioactive metabolites from the endophytic fungus Ampelomyces sp. isolated from the medicinal plant Urospermum picroides. Phytochemistry. 2008;69:1716-1725.

51. Passari AK, et al. In vitro and in vivo plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. PLoS One. 2015;10:e0139468.

52. Radha MH and Laxmipriya NP. Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. J Tradit Complement Med. 2015;5:21-26.

53. Zapata PJ, et al. Characterization of gels from different Aloe spp. as antifungal treatment: Potential crops for industrial applications. Ind Crops Prod. 2013;42:223-230.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

54. Bjшrklund G, et al. Brief Challenges on Medicinal Plants: An Eye-Opening Look at Ageing-Related Disorders. Basic Clin Pharmacol Toxicol. 2018;122:539-558.

55. Akinsanya MA, et al. Diversity, antimicrobial and antioxidant activities of culturable bacterial endophyte communities in Aloe vera. FEMS Microbiol Lett. 2015;362:184.

56. Akinsanya MA, et al. Metagenomics study of endophytic bacteria in Aloe vera using next-generation technology. Genom Data. 2015;6:159-163.

57. Gerdemann JW and Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol Soc. 1963;46:235-244.

58. Cheng HR and Jiang N. Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett. 2006;28:55-59.

59. Weisburg WG, et al. 16S Ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173:697-703.

60. Altschul SF, et al. Basic local alignment search tool. J Mol Biol. 1990;215:403-410.

61. Sievers F, et al. Fast, scalable generation of highquality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7:539.

62. Darriba, D, et al. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012;9:772.

63. Ronquist F, et al. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539-542.

64. Kumar S, et al. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870-1874.

65. Rambaut A. FigTree version 1.4.2. 2014 [computer program] http://tree.bio.ed.ac.uk/software/figtree/

66. Marasco R, et al. A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS One. 2012;7:e48479.

67. Gordon SA and Weber RP. Colorimetric estimation of indole acetic acid. Plant Physiol. 1951;26:192-195.

68. Murphy J and Riley JP. A modified single solution method for determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31-36.

69. Schwyn B and Neilands JB. Universal assay for the detection and determination of siderophores. Anal Biochem. 1987;160:47-56.

70. Mew TW and Rosales AM. Bacterization of rice plants for control of sheath blight caused by Rhizoctonia solani. Phytopathology. 1986;76:1260-1264.

71. Hankin L and Anagnostakis SL. The use of media for detection of enzymes production by fungi. Mycologia. 1975;67:597-607.

72. Strauss MLA, et al. Screening for the production of extracellular hydrolytic enzymes by non-Saccharomyces wine yeasts. J Appl Microbiol. 2001;91:182-190.

73. Cattelan AJ. Mйtodos quantitativos para determinaзго de caracterнsticas bioquнmicas e fisiolygicas associadas com bactйrias promotoras do crescimento vegetal. Londrina: Embrapa Soja. 1999.

74. Teather RM and Wood PJ. Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol. 1982;43:777-780.

75. Hankin L, et al. Improved solid medium for the detection and enumeration of pectolytic bacteria. Appl Microbiol. 1971;22:205-209.

76. Cardoso AS, et al. Characterization of rhizobia isolates obtained from nodules of wild genotypes of common bean. Braz J Microbiol. 2017;48:43-50.

77. Ferreira DF. Sisvar: a computer statistical analysis system. Ciкnc Agrotec. 2011;35:1039-1042.

78. Pereira SIA, et al. Endophytic culturable bacteria colonizing Lavandula dentata L. plants: Isolation, characterization and evaluation of their plant growth-promoting activities. Ecol Eng. 2016;87:91-97.

79. Silva CF, et al. Comunidade de fungos micorrнzicos arbusculares: diversidade, composiзго e glomalina em area revegetada com sesbвnia. R Bras Ci Solo. 2014;38:423-431.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

80. Numan M, et al. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: A review. Microbiol Res. 2018;209:21-32.

81. Sun W, et al. Bacterial communities of three plant species from Pb-Zn contaminated sites and plant-growth promotional benefits of endophytic Microbacterium sp. (strain BXGe71). J Hazard Mater. 2018.

82. Haque MD A, et al. Diversity of indigenous endophytic bacteria associated with the roots of Chinese cabbage (Brassica campestris L.) cultivars and their antagonism towards pathogens. J Microbiol. 2016;54:353-363.

83. Munjal V, et al. Genotyping and identification of broad spectrum antimicrobial volatiles in black pepper root endophytic biocontrol agent, Bacillus megaterium BP17. Biol Control. 2016;92:66-76.

84. Bolнvar-Anillo HJ, et al. Burkholderia tropica una bacteria con gran potencial para su uso en la agricultura. TIP Rev Esp Cienc Quнm Biol. 2016;19:102-108.

85. Dolkar D, et al. Stress tolerance and plant growth promotion potential of Enterobacter ludwigii PS1 isolated from Seabuckthorn rhizosphere. Biocatal Agric Biotechnol. 2018;14:438-443.

86. Kumar K, et al. Alleviation of the adverse effect of salinity stress by inoculation of plant growth promoting rhizobacteria isolated from hot humid tropical climate. Ecol Eng. 2017;102:361-366.

87. Trifi H, et al. Effectiveness of the plant growth-promoting rhizobacterium Pantoea sp. BRM17 in enhancing Brassica napus growth in phosphogypsum-amended soil. Pedosphere. 2017.

88. Brady C, et al. Taxonomic evaluation of the genus Enterobacter based on multilocus sequence analysis (MLSA): proposal to reclassify E. nimipressuralis and E. amnigenus into Lelliottia gen. nov. as Lelliottia nimipressuralis comb. nov. and Lelliottia amnigena comb. nov., respectively, E. gergoviae and E. pyrinus into Pluralibacter gen. nov. as Pluralibacter gergoviae comb. nov. and Pluralibacter pyrinus comb. nov., respectively, E. cowanii, E. radicincitans, E. oryzae and E. arachidis into Kosakonia gen. nov. as Kosakonia cowanii comb. nov., Kosakonia radicincitans comb. nov., Kosakonia oryzae comb. nov. and Kosakonia arachidis comb. nov., respectively, and E. turicensis, E. helveticus and E. pulveris into Cronobacter as Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov., respectively, and emended description of the genera Enterobacter and Cronobacter. Syst Appl Microbiol. 2013;36:309-319.

89. Dobritsa AP and Samadpour M. Transfer of eleven species of the genus Burkholderia to the genus Paraburkholderia and proposal of Caballeronia gen. nov. to accommodate twelve species of the genera Burkholderia and Paraburkholderia. Int J Syst Evol Microbiol. 2016;66:2836-2846.

90. Estrada-De Los Santos P, et al. To split or not to split: an opinion on dividing the genus Burkholderia. Ann Microbiol. 2016;66:1303-1314.

91. Nutaratat P, et al. High-yield production of indole-3-acetic acid by Enterobacter sp. DMKU-RP206, a rice phyllosphere bacterium that possesses plant growth-promoting traits. 3 Biotech. 2017;7:1-15.

92. Bibi F. Diversity of antagonistic bacteria isolated from medicinal plant Peganum harmala L. Saudi J Biol Sci. 2017;24:1288-1293.

93. Roman-Ponce B, et al. Plant growth-promoting traits in rhizobacteria of heavy metal resistant plants and their effects on Brassica nigra seed germination. Pedosphere. 2017;27:511-526.

94. Puentes-Tйllez PE and Salles JF. Construction of Effective Minimal Active Microbial Consortia for Lignocellulose Degradation. Microb Ecol. 2018;76:419-429.

95. Zhao R, et al. Chryseobacterium takakiae sp. nov., a member of the phylum Bacteroidetes isolated from Takakia lepidozioides. Int J Syst Evol Microbiol. 2015;65:71–76.

96. Wu YF, et al. Chryseobacterium taihuense sp. nov., isolated from a eutrophic lake, and emended descriptions of the genus Chryseobacterium, Chryseobacterium taiwanense, Chryseobacterium jejuense and Chryseobacterium indoltheticum. Int J Syst Evol Microbiol. 2013;63:913-919.

97. Tai CJ, et al. Chryseobacterium taiwanense sp. nov., isolated from soil in Taiwan. Int J Syst Evol Microbiol. 2006;56:1771-1776.

98. Duan YQ, et al. Enterobacter tabaci sp. nov., a novel member of the genus Enterobacter isolated from a tobacco stem. Antonie Van Leeuwenhoek. 2015;108:1161-1169.

99. Vitorino LV, et al. Solubilization of calcium and iron phosphate and in vitro production of indoleacetic acid by endophytic isolates of Hyptis marrubioides Epling (Lamiaceae). IRJOB. 2012;3:47-54.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

100.Spaepen S and Vanderleyden J. Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol. 2011;3:1-13.

101.Behera BC, et al. Diversity, mechanism and biotechnology of phosphate solubilizing microorganism in mangrove - A review. Biocatal Agric Biotechnol. 2014;3:97-110.

102.Vassilev N, et al. Stress-tolerant P-solubilizing microorganisms. Appl Microbiol Biotechnol. 2012;95:851-859.

103.Satyaprakash M, et al. Phosphorous and phosphate solubilising bacteria and their role in plant nutrition. Int J Curr Microbiol Appl Sci. 2017;6:2133-2144.

104.Kabbage M, et al. Pathogenic attributes of Sclerotinia sclerotiorum: Switching from a biotrophic to necrotrophic lifestyle. Plant Sci. 2015;233:53-60.

105.Rocha R, et al. Selection of endophytic fungi from comfrey (Symphytum officinale L.) for in vitro biological control of the Phytopathogen sclerotinia sclerotiorum (Lib.). Braz J Microbiol. 2009;40:73-78.

106.Zhang F, et al. Biocontrol potential of Trichoderma harzianum isolate T-aloe against Sclerotinia sclerotiorum in soybean. Plant Physiol Biochem. 2016;100:64-74.

107.Chen Y, et al. Inhibitory efficacy of endophytic Bacillus subtilis EDR4 against Sclerotinia sclerotiorum on rapeseed. Biol Control. 2014;78:67-76.

108.Mohanty SS, et al. Endophytes of Jatropha curcas promote growth of maize. Rhizosphere. 2017;3:20-28.

109.Yue L, et al. Preliminary study on antagonistic mechanism of Brevibacillus laterosporus B8 against two plant pathogenic fungi. China Plant Protection. 2015;3:004.

110.Joo HJ, et al. A Brevibacillus sp. antagonistic to mycotoxigenic Fusarium spp. Biol Control. 2015;87:64-70.

111.Arrigoni E, et al. Tissue age and plant genotype affect the microbiota of apple and pear bark. Microbiol Res. 2018;211:57-68.

112.Shaf S, et al. Dynamics of bacterial class Bacilli in the deepest valley lake of Kashmir-the Manasbal Lake. Microb Pathogen. 2017;104:78-83.

113.Panda AK, et al. Brevibacillus as a biological tool: a short review. Antonie van Leeuwenhoek. 2014;105:623-639.

114.Jianmei C, et al. Identification of ethylparaben as the antimicrobial substanceproduced by Brevibacillus brevis FJAT-0809-GLX. Microbiol Res. 2015;172:48-56.

115.Douriet-Gamez NR, et al. Genomic analysis of Bacillus sp. strain B25, a biocontrol agent of maize pathogen Fusarium verticillioides. Curr Microbiol. 2018;75:247-255.

116.Francisco NF, et al. In vitro antagonism of Bacillus strains against Fusarium species. Mycopath. 2016;14:15-19.

117.Zouari I, et al. Biocontrol activity of the endophytic Bacillus amyloliquefaciens 1 strain 2 CEIZ-11 against Pythium aphanidermatum and purification of its bioactive 3 compounds. Biol Control. 2016;100:54-62.

118.Tumbarski Y, et al. Antimicrobial activity against some saprophytic and pathogenic microorganisms of Bacillus species strains isolated from natural spring waters in Bulgaria. Br Microbiol Res J. 2014;4:1353-1369.

119.Toral L, et al. Antifungal activity of lipopeptides from Bacillus XT1 CECT 8661 against Botrytis cinerea. Front Microbiol. 2018;9:1315.

120.Villegas-Escobar V, et al. Lipopeptides from Bacillus sp. EA-CB0959: Active metabolites responsible for in vitro and in vivo control of Ralstonia solanacearum. Biol Control. 2018;125:20-28.

121.Torres MJ, et al. Biological activity of the lipopeptide-producing Bacillus amyloliquefaciens PGPBacCA1 on common bean Phaseolus vulgaris L. pathogens. Biol Control. 2017;105:93-99.

122.Huo Y, et al. Paraburkholderia panacihumi sp. nov., an isolate from ginseng-cultivated soil, is antagonistic against root rot fungal pathogen. Arch Microbiol.2018;200:1151

123.Salkar K, et al. Largest freshwater lake 'Loktak' in Manipur needs urgent conservation. Curr Sci. 2018;114:2436-2437.

124.Silva PRA, et al. Draft genome sequence of Paraburkholderia tropica Ppe8 strain, a sugarcane endophytic diazotrophic bacterium. Braz J Microbiol. 2018;49:210-211.

125.Rahman M, et al. Plant probiotic bacteria Bacillus and Paraburkholderia improve growth, yield and content of antioxidants in strawberry fruit. Sci Rep. 2018;8:2504.

Research & Reviews: Journal of Microbiology and Biotechnology

e-ISSN: 2320-3528
p-ISSN: 2347-2286