# Evaluation of endophytic microbiota of Calcutta 4 and Williams banana plants at *in vitro* and greenhouse level

**Isabel Adarve-Rengifo[1] • Javier C. Alvarez[1] • Luisa F. Posada[2*]**

**Abstract** Banana is the third agricultural product in Colombia, with the highest export value (852.527 USD) being preceded by coffee (2'330.255 USD) and flowers (1'501.064 USD) and positioning the country as the fifth worldwide banana exporter to countries like European Union, United Kingdom and United States. This crop, characteristic of the tropics, it's constantly threatened by pathologies like Black Sigatoka (caused by the fungus *Pseudocercospora fijiensis*), Moko (caused by the bacteria *Ralstonia solanacearum*) and one of the most aggressive the *Fusarium*'s wilt caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (also known as *Fusarium odoratissimum*). Stated pathologies compromise tissues, can produce productivity reduction and like *Fusarium oxysporum,* cause irreversible damages in this crop, sometimes leaving the soil infertile and unproductive. Wild variety Calcutta 4 banana plant, native of Southeast Asia, has shown resistance to different bacterial, fungal and nematode infections. Therefore, to identify differences in the composition of the endophytic microbiota of the wild variety of banana and the commercial cultivar Williams, this project was performed using culture-dependent methods, with isolation, morphologic characterization and taxonomic identification of endophytic microorganisms present among roots, pseudo-stem and leaves of these plants for greenhouse and *in vitro* level. For taxonomic identification, total DNA of isolated microorganisms were extracted, whole 16S gene region was amplified and then sequenced by Sanger method. Greenhouse assays using Calcutta 4 and Williams plants showed greater diversity through all tissues in genus and species of microorganisms, in comparison with Williams *in vitro* assays. Nonetheless, Calcutta 4 *in vitro* plants presented broader diversity in their tissues than Williams *in vitro* plants, suggesting that this variety possesses a richer native endophytic microbiota in contrast to the commercial cultivar. It would suggest that exists a correlation between the endophytic microbiota composition and the pathogen resistance.

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## Introduction

In Colombia, the production of commercial banana crops it's constantly threatened by diseases like black sigatoka disease caused by the fungus *Pseudocercospora fijiensis* [1], Moko caused by the bacteria *Ralstonia solanacearum* [2], and most recently *Fusarium* wilt caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (recently known as *Fusarium odoratissimum*) [3]. Thus, the evaluation of alternatives to reduce incidence of diseases or studies to determine some causes related to banana resistance in native cultivars are important topics for the development of Colombian banana industry, where this crop is important to the economic primary sector. In the view of above, the evaluation of native banana plants, as wild varieties like the native diploid Calcutta 4 originally from Southeast Asia [4], and their comparison with commercial banana microbiota is a possible research field to find differences that could be related to this resistance. Calcutta 4 variety has shown to be resistant to fungi *Pseudocercospora fijensis,* the nematode *Radopholus similis* and races 1 and 4 of fungi *Fusarium oxysporum* f. sp. *cubense*. Native from India, Calcutta 4 has been found in Australia and Honduras [5], in addition it has been found in Colombia nearby Palestina, Caldas. Commercial cultivar Williams is one of the most important in Colombia, hectares planted with banana in 2019 were 51.227, majority of this variety, obtaining a productivity of 1961 boxes per hectare, standing out notably the crops present in the areas of Urabá (Antioquia), Magdalena and La Guajira [6].

Today, commercial banana cultivars are seedless clones from genome A (species *Musa acuminata*) or the union of two genomes A and B (species *Musa acuminata* and *Musa balbisiana*) like "Grand Naine", "Williams" [7], "Valery" [8], among others cultivars. Their reproduction is asexual, using vegetative micropropagation or suckers (or pups); generating a reduction in the genetic base diversity and producing banana crops with a poor resistance to plagues and diseases [4].

Microorganisms that can be isolated from the tissues inside the plant without harming it, are called endophytes [9]; they can come directly from the seeds or mother plant, colonize plant tissues and act as a defense barrier against antagonistic activity or enhance plant development [10]. These endophytes get nutrients from the plants, establish in them and form colonies [11]. However, they are located in niches that are similar to those pathogens might harbor, and act as a defense barrier for the hosts. Some of these endophytes would promote plant growth due to the production of phytohormones like auxins as indoleacetic acid [11], nitrogen fixation and nutrients solubilization [12]. There is scant information about endophytes microorganisms for *in vitro* plants because studying the microorganisms-plant interactions is important and can contribute to the knowledge of plant development and health. For this reason, performing studies with culture-dependent and culture-independent techniques to determining microorganisms related to plant is a first step to elucidate diversity abundance and possible applications of plant microbiota, especially for commercial crops as banana that are grown using micro propagated meristems instead seeds [12].

The aim of this study was to identify and characterize the culturable endophytic microbiota of banana cultivar Williams and banana variety Calcutta 4 as well to determine the differences in presence and abundance of bacterial communities for both seedlings at *in vitro* and greenhouse level. This work would contribute in the future for the development of a biological supplement based on endophytic microorganisms from Calcutta 4 plants that are not found in Williams plants, in that way, that they can be able to provide resistance in susceptible plants.

## Materials and methods

### Plant material

Calcutta 4 plants from a natural system on early stages of development (two months), in field or ripe banana fruits, were collected from Calcutta banana plant from Universidad de Caldas' farm Montelindo in Palestina, Caldas (5.076280, -75.671235). These banana plants were transferred with soil to Universidad EAFIT, Medellín, Colombia and grown at greenhouse in their own soil in bags (2 kg). Calcutta 4 seeds were extracted from the fruit, collected, washed, cleaned, dried and storage to 22°C until sown at *in vitro* level. For growth, banana seeds were disinfected (ethanol 70% for 2 min, hypochlorite 2% for 10 min, ethanol 70% for 3 min and sterile water between washes) and pregerminated on Murashige and Skoog medium (4,33 g/L of M524 basal salt mixture with vitamins PhytotechLab and 5 g/L of agar BD 5) to 22°C and 12:12 h light and dark environment for 40 days. Obtained seedlings were transferred to fresh MS medium on glass flask and kept to previously conditions for 30 days until the assays.

Nine months developed banana Williams greenhouse plants were obtained from Meristemos de Colombia (UCO, Rionegro) and grown at Universidad EAFIT greenhouse using bags with 2 kg of soil (dark soil, rice husk and organic material in a ratio of 1:1:0.5, vivero Tierra Negra) and were kept to environmental conditions (23-30°C and mean relative humidity of 60%) for 3 months until this evaluation. Meristems of Williams banana plants were obtained from the named before provider and transferred to Bioprocess laboratory of Universidad EAFIT for these assays.

For greenhouse assays 15 plants were grown and five of them were randomly selected for bacterial isolation to obtain 10 g of plant material that were finally processed. For assays with meristems evaluations, *in vitro* banana plants inside three pots, with 45 meristems each one, was used and 10 g of meristems were randomly selected for bacterial isolation. For Calcutta 4 *in vitro* banana plants, we germinated 500 seeds plant seeds and 50 germinated plants were selected to obtain 10 g of banana tissues for bacterial isolation. These evaluations were done twice in different times.

**Culture of endophytic microbiota**

To determine the native endophytic microbiota that composes the different sections of banana plants Calcutta 4 and Williams, leaves, pseudo-stem and roots were disinfected and checked for non-presence of epiphyte organisms washing them with ethanol 70% (2 min), hypochlorite 2% (10 min), ethanol 70% (1 min) and between washes, rinsed with water. After that, tissues were macerated using a dilution 1:10 with $KH_2PO_4$ pH 7,4. with Ultraturrax (ULTRA-TURRAX® Tube Drive 2004IK3645000) to perform serial dilutions with resulting suspension of each banana plant section. These serial dilutions were sown on culture media **AIA** (0,1 g/L of L-asparagine; 0,5 g/L of $K_2HPO_4$; 0,001 g/L of $FeSO_4*7H_2O$; 2 g/L of sodium caseinate; 0,1 g/L of $MgSO_4*7H_2O$ and 15 g/L of agar) for semi selective isolation of actinomycetes [13], **PIA** (1,4 g/L of $MgCl_2$; 20 g/L of proteose peptone; 10 g/L of $K_2SO_4$; 0,025 g/L of triclosan y 13,6 g/L of Bactoagar 281210, Difco) pH 7,0) for isolating *Pseudomonas* [14], **YDC** (20 g/L of $CaCO_3$, 20 g/L of glucose, 10 g/L of yeast extract and 15 g/L of Bactoagar pH 7,0) to isolate Gram-negative bacteria [15], **TSA 50%** (9 g/L of Bactoagar BD and 20 g/L of TSA 105458, Merck) a broad spectrum rich medium [15] and **R2A** (0,5 g/L of proteose peptone, 0,5 g/L of casein acid hydrolysate, 0,5 g/L of yeast extract, 0,5 g/L of glucose, 0,5 g/L of soluble starch, 0,3 g/L of $K_2HPO_4$, 0,05 g/L of magnesium sulphate, 0,3 g/L of sodium pyruvate and 15 g/L of Bactoagar pH 7,0), a rich medium with broad spectrum [16]. Two repetitions for two biological replicates for three Petri dishes per dilutions and for each medium were performed. These culture media were selected with the purpose of increase the growth of the microorganisms previously mentioned due to their semi selective isolation character. Obtained morphotypes were purified and preserved in TSB + Glycerol 20% at -80°C.

**DNA isolation and taxonomic classification**

DNA extraction was carried out by heat shock at 96°C during 10 min or using DNA extraction kit (Ultraclean Microbial DNA, Mobio, USA). The DNA quality was determined using electrophoresis in agarose gel 1%, quantified by Nanodrop 2000 (Thermo Scientific™ NanoDrop™ 2000) and after that, amplified by PCR (Mix: 0,02 U/μL of Taq polymerase 5 U/mL; 0,2 μM of primers (8F y 1492R); 0,2 mM of dNTP's-Mix; 1,5 mM of $MgCl_2$; 1X of Taq buffer, 100 ng of DNA and PCR water for 50 μL). PCR conditions were initial denaturation at 94°C (3 min), 30 cycles of amplification, denaturation at 94°C (30 s), annealing at 60°C (20 sec), extension at 72°C (90 sec) and final extension at 72°C (5 min) in a T1000 ThemoCycler (Biorad, USA). The PCR products were evaluated using gel electrophoresis 1% agarose and NanoDrop 2000. Samples with positive band were sequenced by Sanger method using the primer 907R for the 16S [17] complete gene (1400 pb) and the results were processed and analyzed using our bioinformatic pipeline [18]. Additionally, another taxonomic allocation method was evaluated using Geneious bioinformatics Software (version 2020.0.5) trimming the ends, the primer and vectors from the sequence of the 16S rRNA gene. Then used for homology search by the Basic Local Alignment Search Tool (BLAST) software (http://blast.ncbi.nlm.nih. gov) algorithm at National Center for Biotechnology Information (NCBI), choosing the sequence with the highest % Pairwise Identity and Bit Score.

**Diversity estimation**

To estimate local and global diversity of endophytic bacteria in Calcutta 4 and Williams tissues, PAST 4.01 (Paleontological Statistics software) was used. An individual rarefaction analysis was performed for each treatment (1. Calcutta 4 greenhouse plants, 2. Williams greenhouse plants, 3. Calcutta 4 *in vitro* plants and 4. Williams *in vitro* plants), testing maximum cultivable taxa and then developing a matrix with the genus/species found across the four groups and their abundance in each one. The population abundance is obtained from the quantities of FCU/g per isolate, while the individuals' abundance is the number of identified microorganisms. The analysis of alpha diversity (locally) was done for tissues inner of the same variety/cultivar and the analysis of beta diversity (globally) was done across variety/cultivar. For alpha diversity, the indexes Shannon-Weiner, Simpson and Berger-Parker were determined and for beta diversity the Whittaker index [19].

### Statistical analysis

It was determined if there were significant differences between Calcutta 4 and Williams banana plants and their organs for *in vitro* and greenhouse levels using a diversity permutation analysis complemented with a T diversity analysis for Shannon and Simpson indexes, with a confidence of 95%. This statistical test determines if Calcutta 4 and Williams are not equal in abundance and diversity of the native endophyte microorganisms' present (PAST 4.01).

## Results

### Determination of native endophytic microbiota of roots, pseudo-stem and leaves from banana plants variety Calcutta 4 and cultivar Williams, at *in vitro* and greenhouse level

This project has as purpose to evaluate the microbiota present in Calcutta 4 and Williams banana plants and compare them to suggest that the presence of those microorganisms and/or their differential quantities would cause Calcutta 4 plants resistance to diseases. Endophytic microbiota from roots, pseudo-stem, and leaves of banana plants Calcutta 4 and Williams were isolated, cultivated and subsequently identified by sequencing of 16S rDNA, determining the abundance in each tissue. All microorganisms were deposited in the collection "Microorganisms isolated from banana Williams and Calcutta 4 crops" under permission ARG166 according to the plant origin and development stage (Table 1). The most abundant collection, in terms of numbers of genera and species isolated, was collection B from Calcutta 4 greenhouse banana plants; on the other hand, collection D isolated from Williams *in vitro* banana plants, only showed six different genera after the microorganisms' isolation. Also, this collection was the most homogeneous and less abundant when microorganisms' species were analyzed (Table 1).

**Collection B** was formed by 125 species isolated from Calcutta 4 banana plants with approximately two months of age, in their wild propagation zone and soil (see table S1 Online Resource 1). 116 isolates were identified and nine were not determined. The genus with more representation was *Bacillus* with 36% isolates followed by the genus *Agrobacterium* with 8%, those isolates that could not be identified with 7,2% and the genera *Stenotrophomonas* and *Pseudomonas* with a 6,4% and 5,6% (Figure 1). The specie with the highest proportion in this collection was *Bacillus cereus* with a 10,4% followed by those species that were not able to be identified and the species belonging to the genus *Bacillus sp*. (that were not able to be discriminated) both with 7,2%. The specie *Stenotrophomonas maltophilia* was found in 6,4% and the species *Bacillus subtilis* and *Raoultella planticola* were found each one in a proportion of 4,8% (Figure 2).

Endophytes were studied according to the tissue where were found. Population abundance of isolates in pseudo-stem was 64,38%, 23,81% in roots, and 11,35% in leaves (Figure 3-A). As for individuals' abundance, the proportions of isolates were 44,72% in pseudo-stem, 36,04% in leaves, and 19,24% in roots (Figure 3-A). Different culture media were evaluated seeking to have a broad spectrum of microorganisms, the population abundance of isolates was of 45,54% in AIA, 41,58% in TSA 50%, 6,84% in R2A, 5,94% in YDC and finally 0,07% in PIA (Figure 3-A). Individuals' abundance were 29,6% in TSA 50% media, 27,20% in AIA, 20,80% in YDC, 12,00% in R2A and 10,40% in PIA (Figure 3-A).

**Table 1.** Nomenclature and isolates quantity of the collections obtained, collection dates, and origin.

| Collection | Origin plant | Plant development stage | Date | Number of isolates |
|---|---|---|---|---|
| B | Calcutta 4 | Greenhouse | July 17th, 2018 | 125 |
| C | Williams | Greenhouse | August 18th, 2018 | 81 |
| D | Williams | *In vitro* | November 29th, 2018 | 75 |
| E | Calcutta 4 | *In vitro* | March 7th, 2019 | 48 |
| **Total isolates** | | | | 329 |

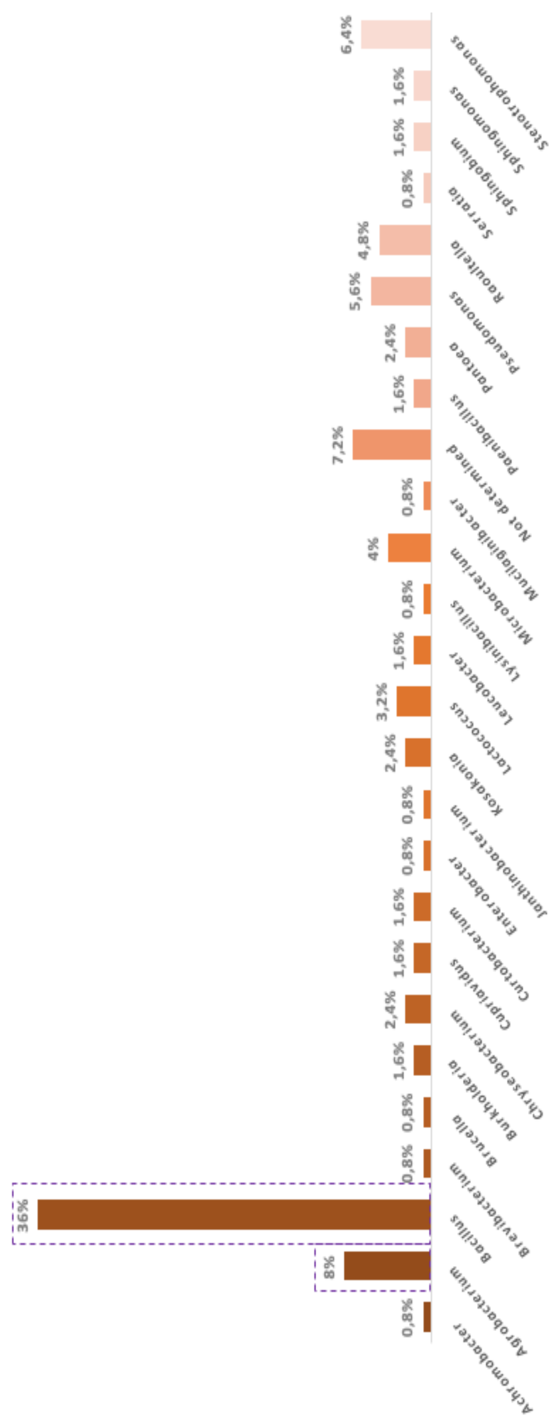



**Figure 1**. Relative abundance of culturable endophyte microorganisms found in greenhouse classified by genus Calcutta 4 banana plants.

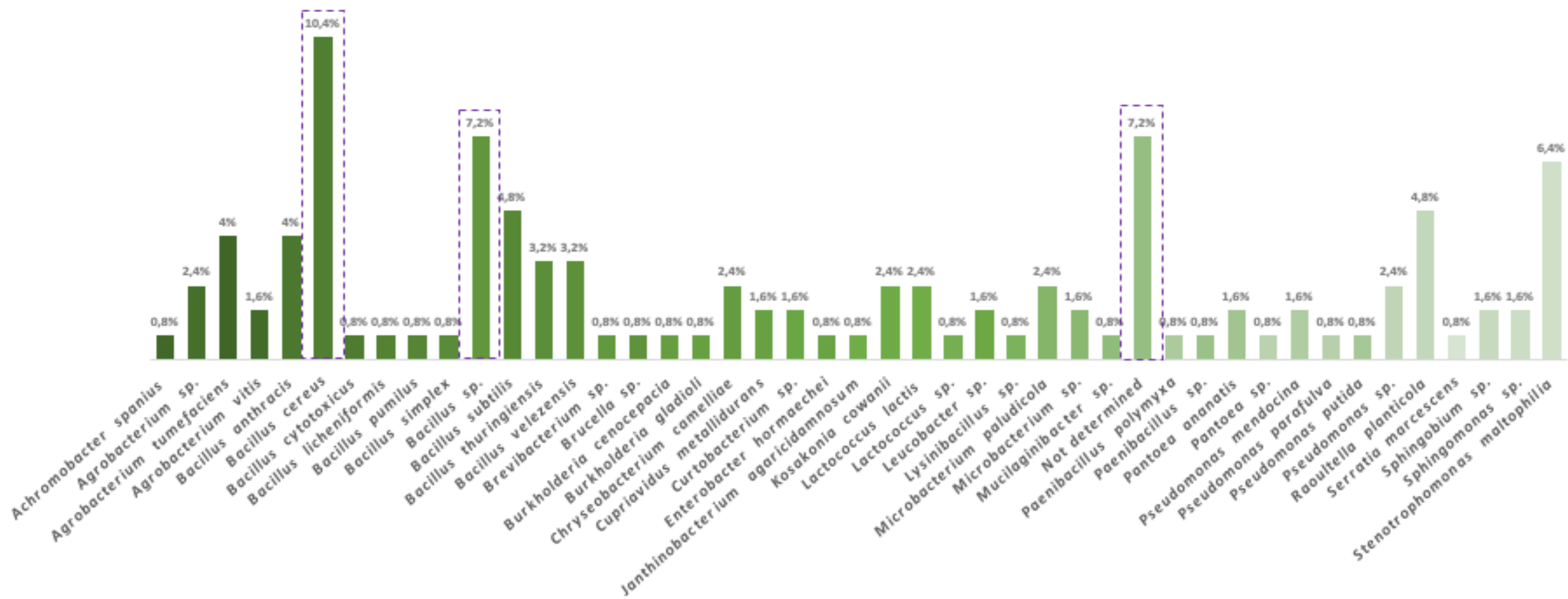



**Figure 2.** Relative abundance of culturable endophyte microorganisms found in greenhouse classified by species Calcutta 4 banana plants.

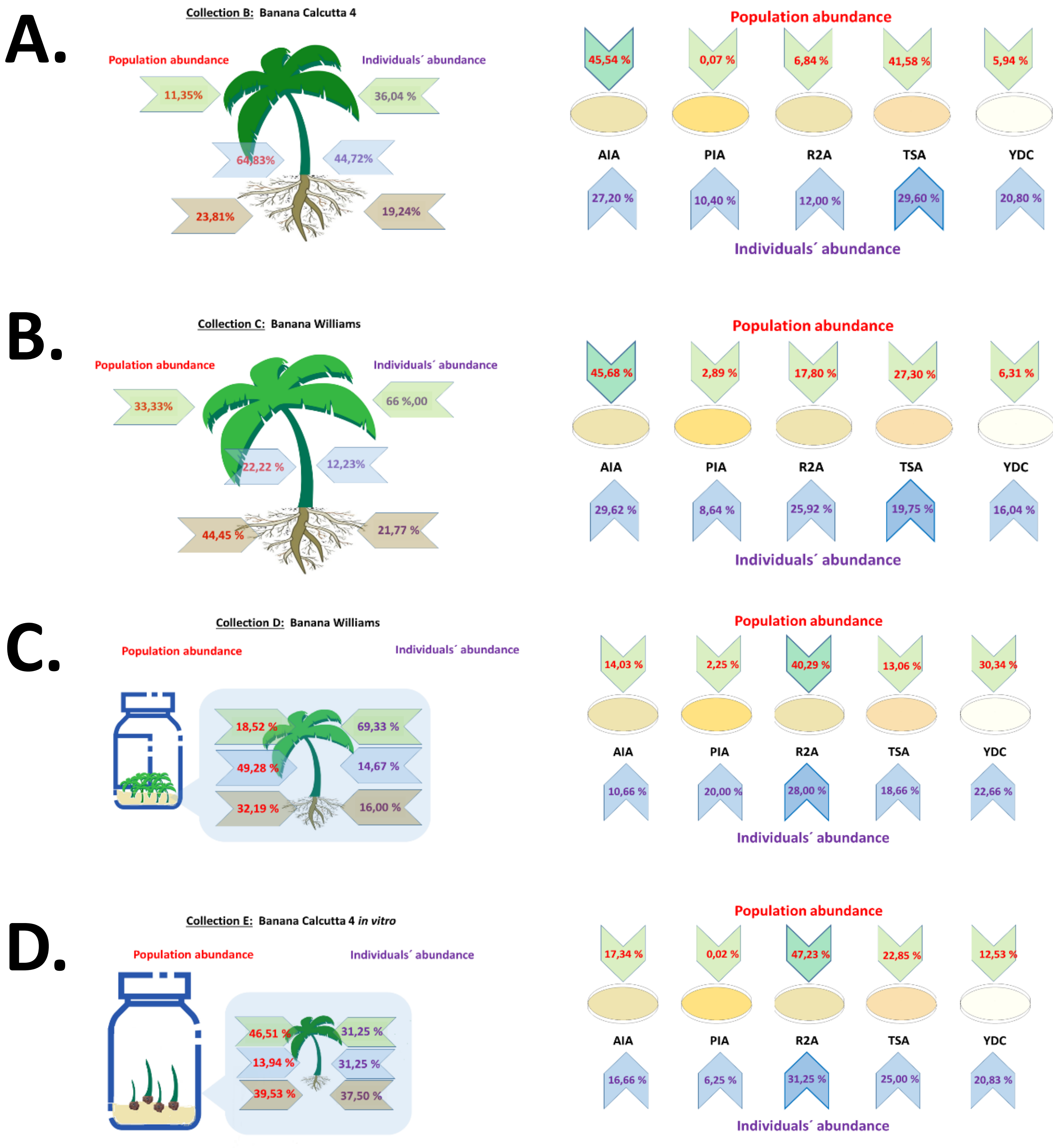



**Figure 3.** Proportion of isolates in population abundance and individuals' abundance for **a.** Collection B, **b.** Collection C, **c.** Collection D, **d.** Collection E; according to the tissue of isolation's origin and culture media.

**Collection C** was formed by 81 endophytes isolated from greenhouse Williams banana plants of four months of development (see table S2 Online Resource 1). 79 isolates were identified and two were not determined. *Bacillus* was the genus in the highest proportion (29,63%), followed by *Pseudomonas (*23,50%), *Paenibacillus* (6,20%) and the genera *Agrobacterium* and *Staphylococcus* were found in a 4,93% (Figure 4). The species found in the highest proportion were *Pseudomonas sp*. 12,34% (indiscriminate)*, Pseudomonas putida* 9,87% and *Bacillus pumilus* 8,64% followed by the species *Bacillus cereus, Bacillus subtilis* and *Staphylococcus epidermidis* in a 4,93% and the species *Agrobacterium tumefaciens* and *Paenibacillus sp*. (indiscriminate) in a 3,70% (Figure 5).

Population abundance according to the tissue was the 44,45% in roots, 33,33% in leaves and 22,22% in pseudo-stem (Figure 3-B). Nevertheless, individuals' abundance was 66,00% in leaves, 21,77% in roots, and 12,23% in pseudo-stem (Figure 3-B). For the different culture media, population abundance was of 45,68% in AIA, 27,30% in TSA 50%, 17,80% in R2A, 6,31% in YDC and 2,89% in PIA (Figure 3-B). And the individuals' abundance was 29,62% in AIA, 25,92% in R2A, 19,75% in TSA 50%, 16,04% in YDC and 8,64% in PIA (Figure 3-B).

In **collection D,** 75 isolates from nine months developed *in vitro* Williams banana plants were obtained (see table S3 Online Resource 1), 73 were identified and two were not determined. The genus found in highest proportion was *Stenotrophomonas* in a 45,33% followed by *Bacillus* in a 25,33% and *Pseudomonas* in a 21,33% (Figure 6).

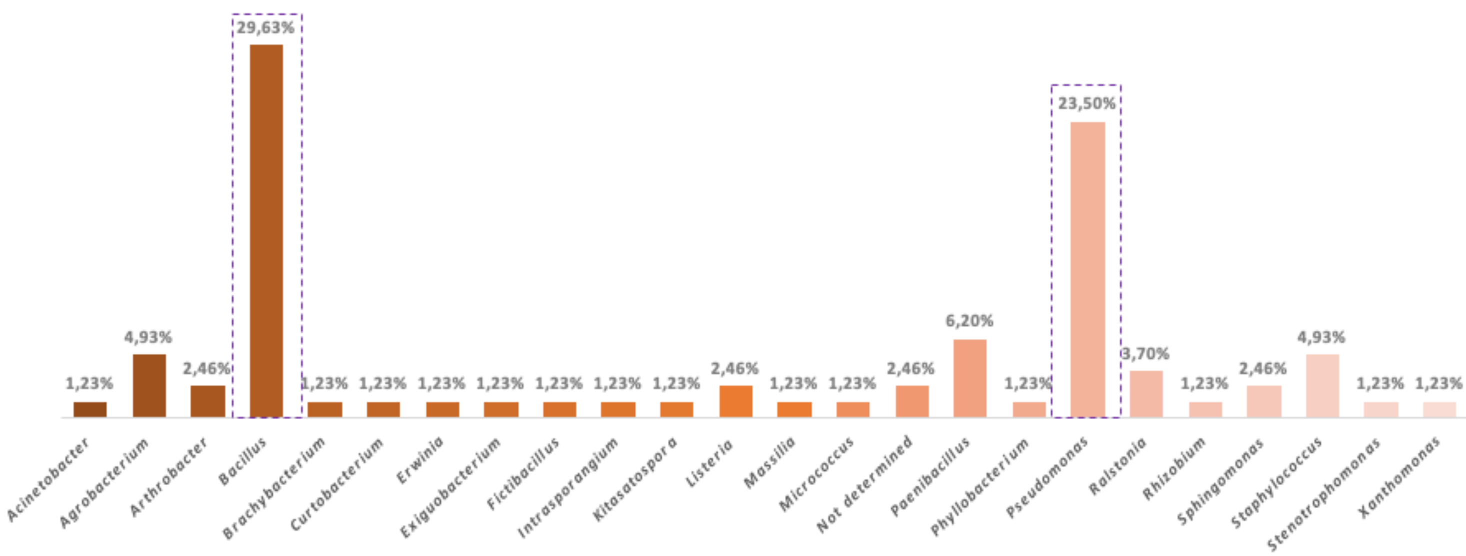



**Figure 4.** Relative abundance of culturable endophyte microorganisms found in greenhouse classified by genus Williams banana plants.

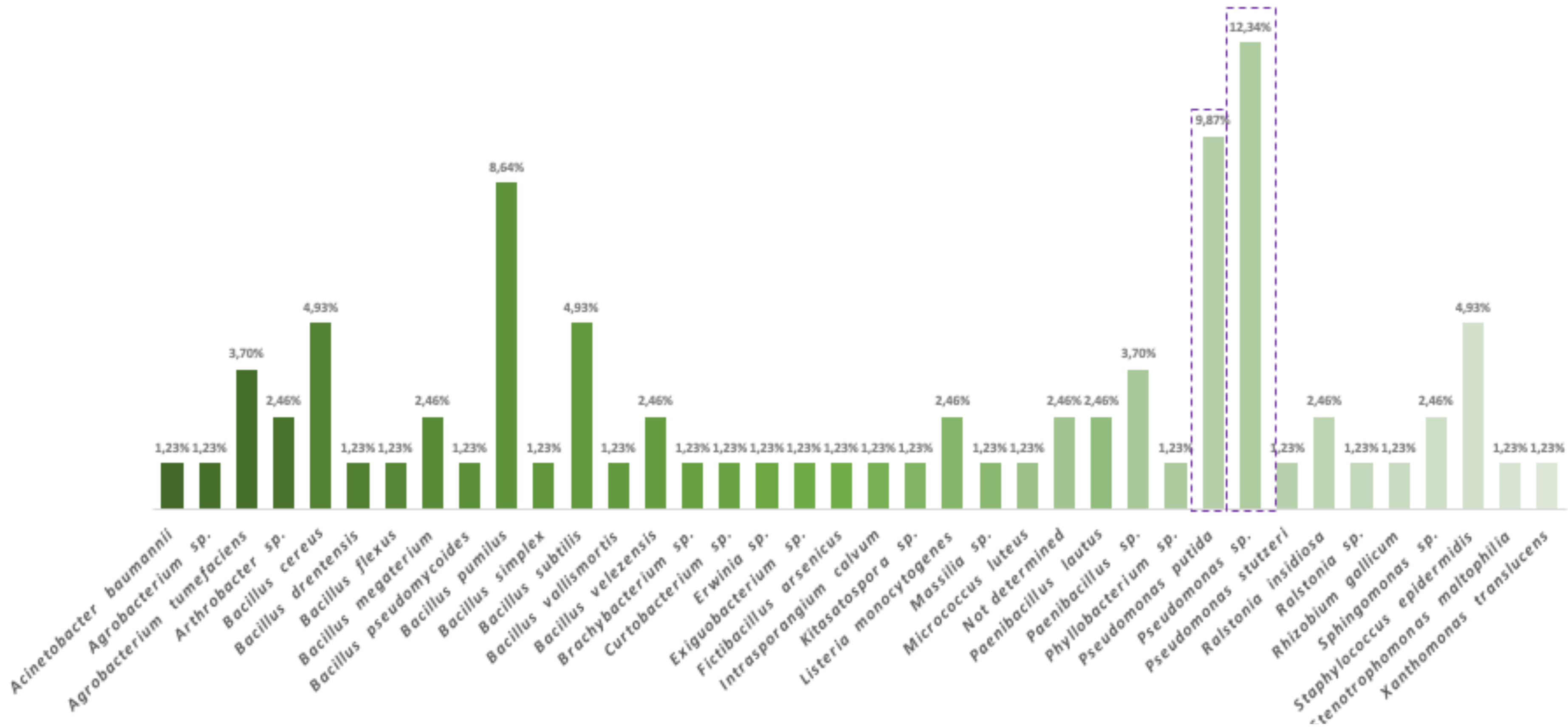



**Figure 5.** Relative abundance of culturable endophyte microorganisms found in greenhouse classified by species Williams banana plants.

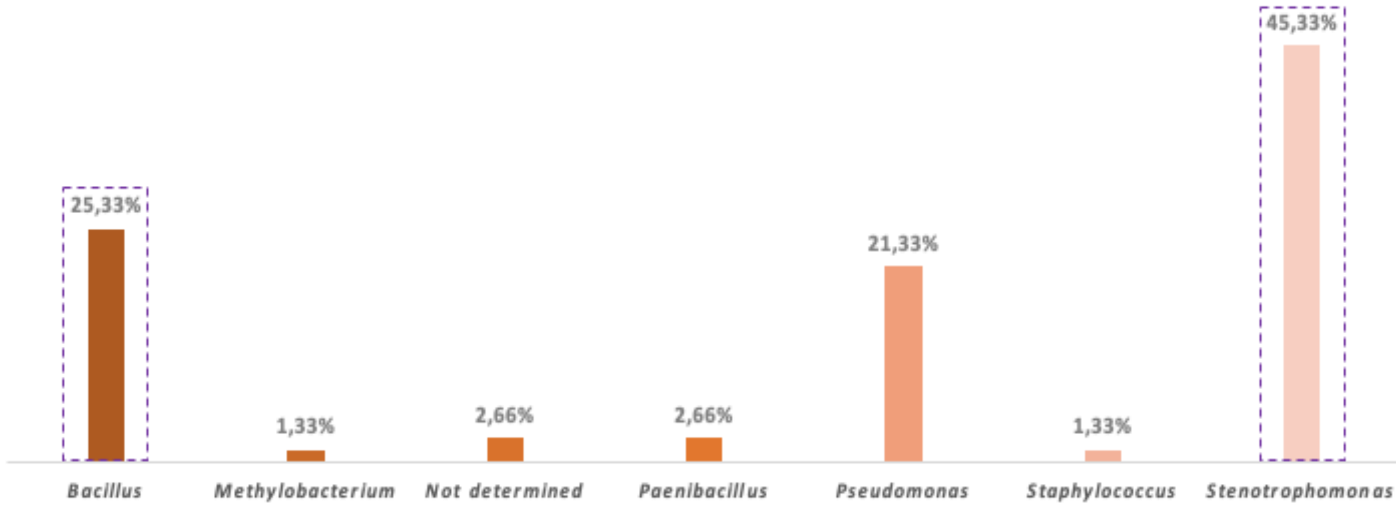



**Figure 6.** Relative abundance of culturable endophyte microorganisms found in *in vitro* classified by genus Williams banana plants.

The species found in higher proportions were *Stenotrophomonas maltophilia* 45,33%, *Pseudomonas azotroformans* 8,00% and *Pseudomonas enthomophilia* 6,70% (Figure 7). Population abundance according to the tissue of isolations was 49,28% in pseudo-stem, 32,19% in roots, and 18,52% in leaves (Figure 3-C). And the individuals' abundance was 69,33% in leaves, 16,00% in roots, and 14,67% in pseudo-stem (Figure 3-C). As for the population abundance according to the culture media it was 40,29% in R2A, 30,34% in YDC, 14,03% in AIA, 13,06% in TSA 50% and 2,25% in media PIA (Figure 3-C). And the individuals' abundance was 28,00% in R2A, 22,66% in YDC, 20,00% in PIA, 18,66% in TSA 50% and 10,66% in AIA (Figure 3-C).

For **collection E** were obtained 48 isolates from *in vitro* Calcutta 4 banana plants (see table S4 Online Resource 1) and identify 42 of them, six remained un-identified (not determined). The genera found in the highest proportions were *Bacillus* 18,75%, un-identified isolates, and *Chryseobacterium* 12,50%, *Herbaspirillum, Ralstonia,* and *Xanthomonas* 8,33% (Figure 8).

The most predominant species were *Chryseobacterium sp*. (indiscriminate) and un-identified isolates 12,50%, *Bacillus megaterium* 10,42%, *Herbaspirillum seropedicae, Rasltonia sp*. and *Xanthomonas translucens* 8,33% (Figure 9). The proportion of population abundance isolates according to tissue of isolation was 46,51% in leaves, 39,53% in roots, and 13,94% in pseudo-stem (Figure 3-D); and for individuals' abundance, it was 37,50% in roots, and 31,25% in leaves and pseudo-stem (Figure 3-D).

With regard to population abundance according culture media it was determined that 47,23% of the microorganisms were isolated from R2A media, 22,85% from TSA 50%, 17,34% from AIA, 12,53% from YDC and 0,02% from PIA culture media (Figure 3-D). Individuals abundance was 31,25% in R2A, 25,00% in TSA 50%, 20,83% in YDC, 16,66% in AIA and 6,25% in PIA (Figure 3-D).

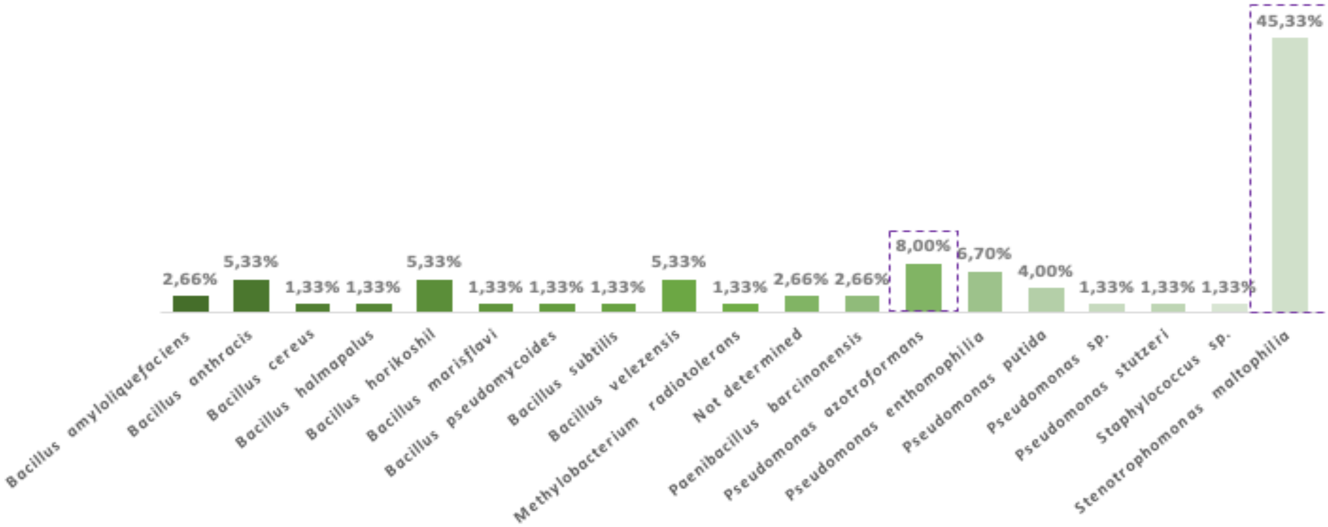



**Figure 7.** Relative abundance of culturable endophyte microorganisms found in *in vitro* classified by species Williams banana plants.

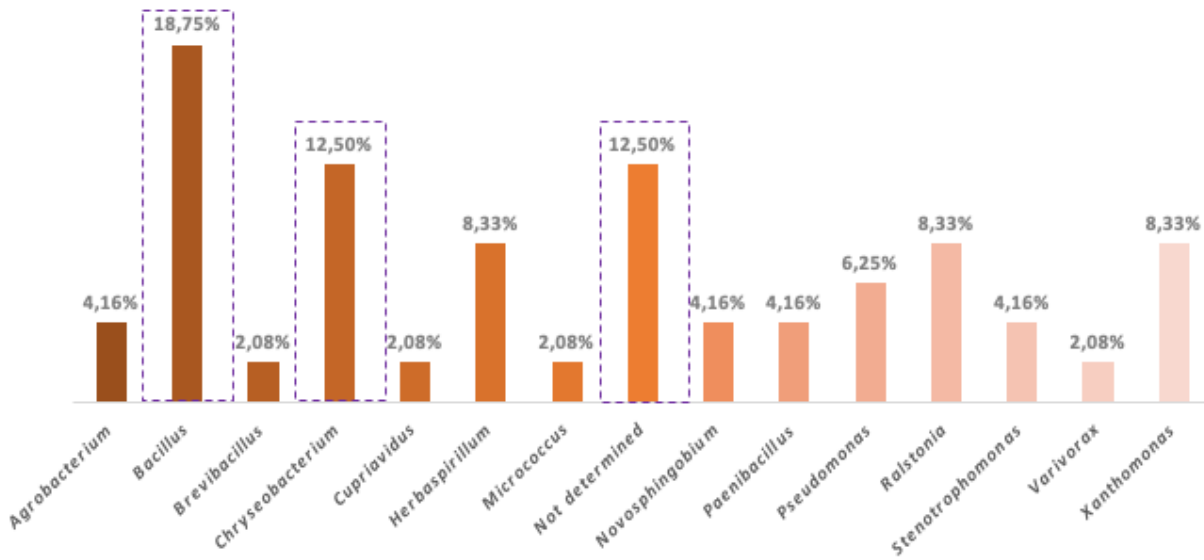



**Figure 8.** Relative abundance of culturable endophyte microorganisms found in *in vitro* classified by genus Calcutta 4 banana plants.

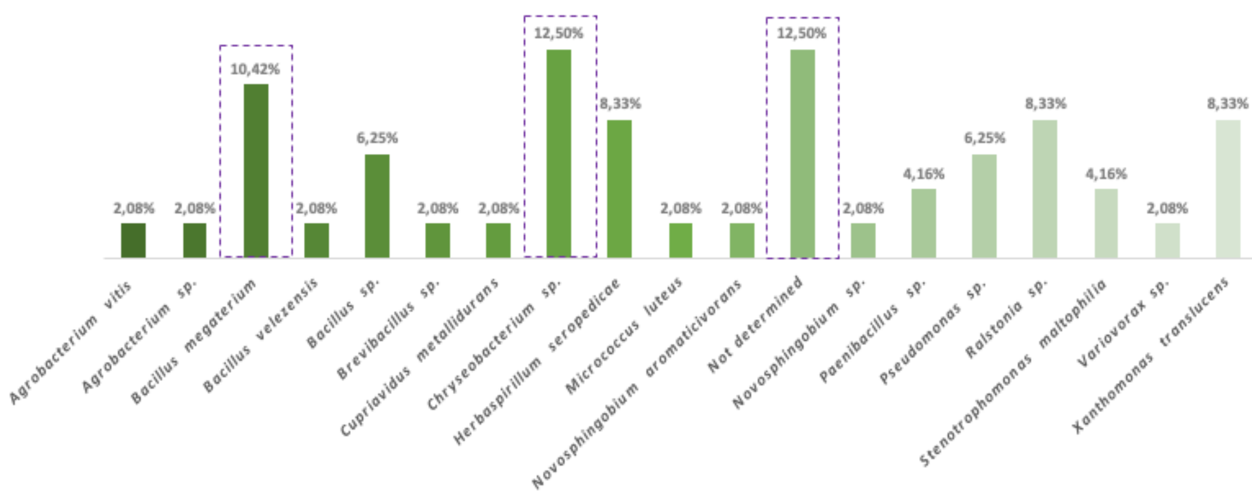



**Figure 9.** Relative abundance of culturable endophyte microorganisms found in *in vitro* classified by species Calcutta 4 banana plants.

To compare the results obtained using the Python script and the Basic Local Alignment Search Tool (BLAST) software at National Center for Biotechnology Information (NCBI) for the taxonomic characterization, the Geneious Prime version 2020.0.5 software was used (Table 2). Using Geneious software, it was possible to assign a taxonomic classification to 304 isolates from 326 total isolates; and 49 that were only identified up to genus level using the Python script, using the Geneious software were identified up to specie level. Four isolates that hadn't been identified with the Python script, accomplished their taxonomic assignation with Geneious software. 15 isolates remained un-identified and 10 that had been identified using the script, were not identified by the Geneious software, because their trimmed sequence was too short or because search results were not generated in BLAST. As well, 36 sequences that using the script identified a determined specie could not be determined using Geneious, only their genera.

Additionally, 19 isolates changed their genus, the most common of the changes occurred in five isolates that after being identified using the script belonged to the genus *Agrobacterium,* but after being identified using the Geneious software were part of the *Rhizobium* genus. Two isolates were identified as from the *Listeria* genus using the Python script but using the Geneious software those two isolates were identified as from the *Bacillus* genus.

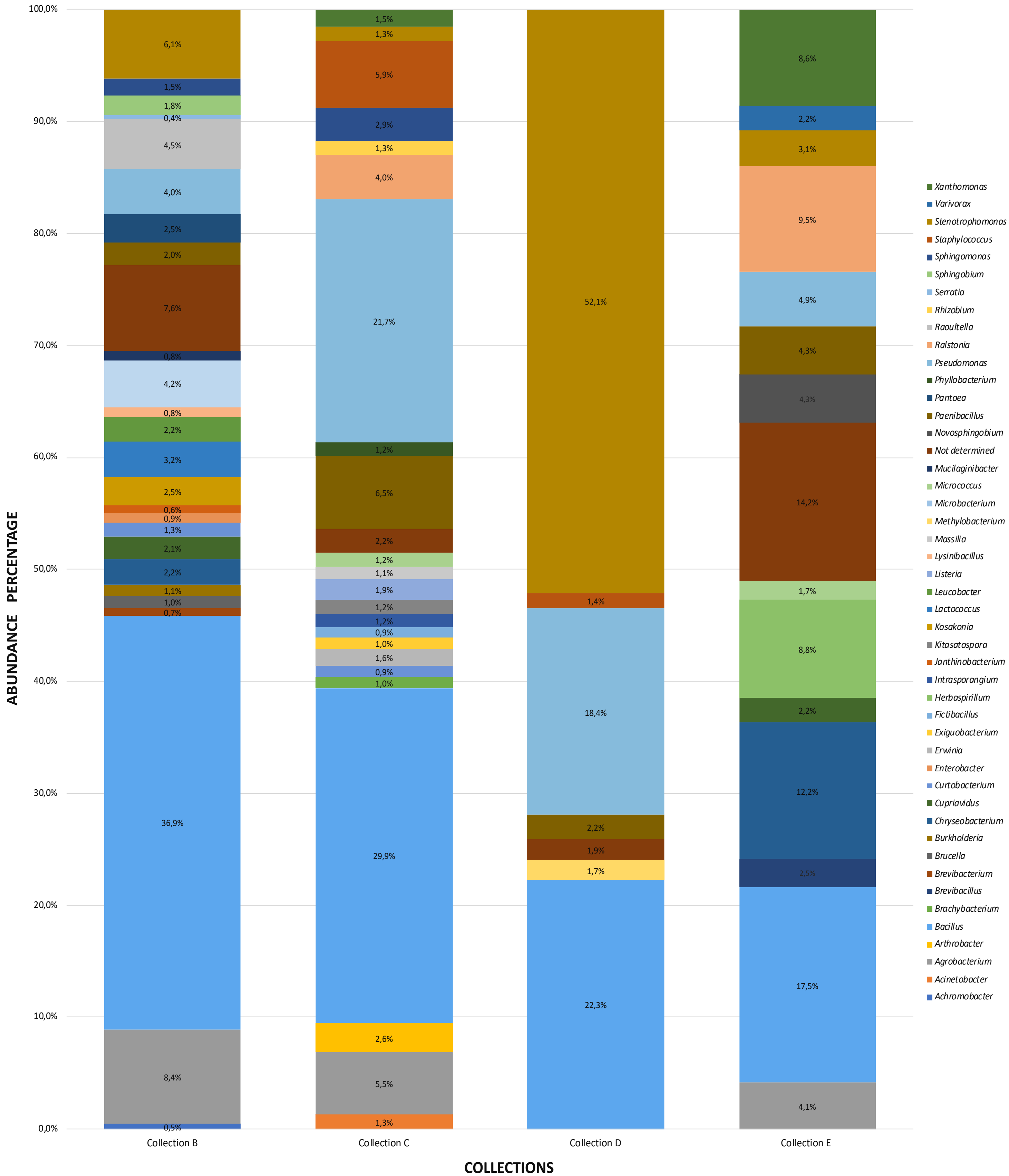
Other genus changes in some isolates were from *Bacillus* to *Staphylococcus, Bacillus* to *Paenibacillus, Achromobacter* to *Bordetella, Enterobacter* to *Kosakonia, Janthinobacterium* to *Herbaspirillum, Intrasporangium* to *Terrabacter, Erwinia* to *Pantoea*, *Paenibacillus* to *Microbacterium*, *Arthrobacter* to *Paenarthrobacter, Stenotrophomonas* to *Romboutsia, Staphylococcus* to *Bacillus,* and *Stenotrophomonas* to *Bacillus*.

To contrast, the abundance graphs of the strains are presented below. The genera identified in the four collections with Python script (Graphic 1) and the Geneious software (Graphic 2).

**Table 2.** Nomenclature and quantity of collection's isolates, collection data, origin plant and identification method

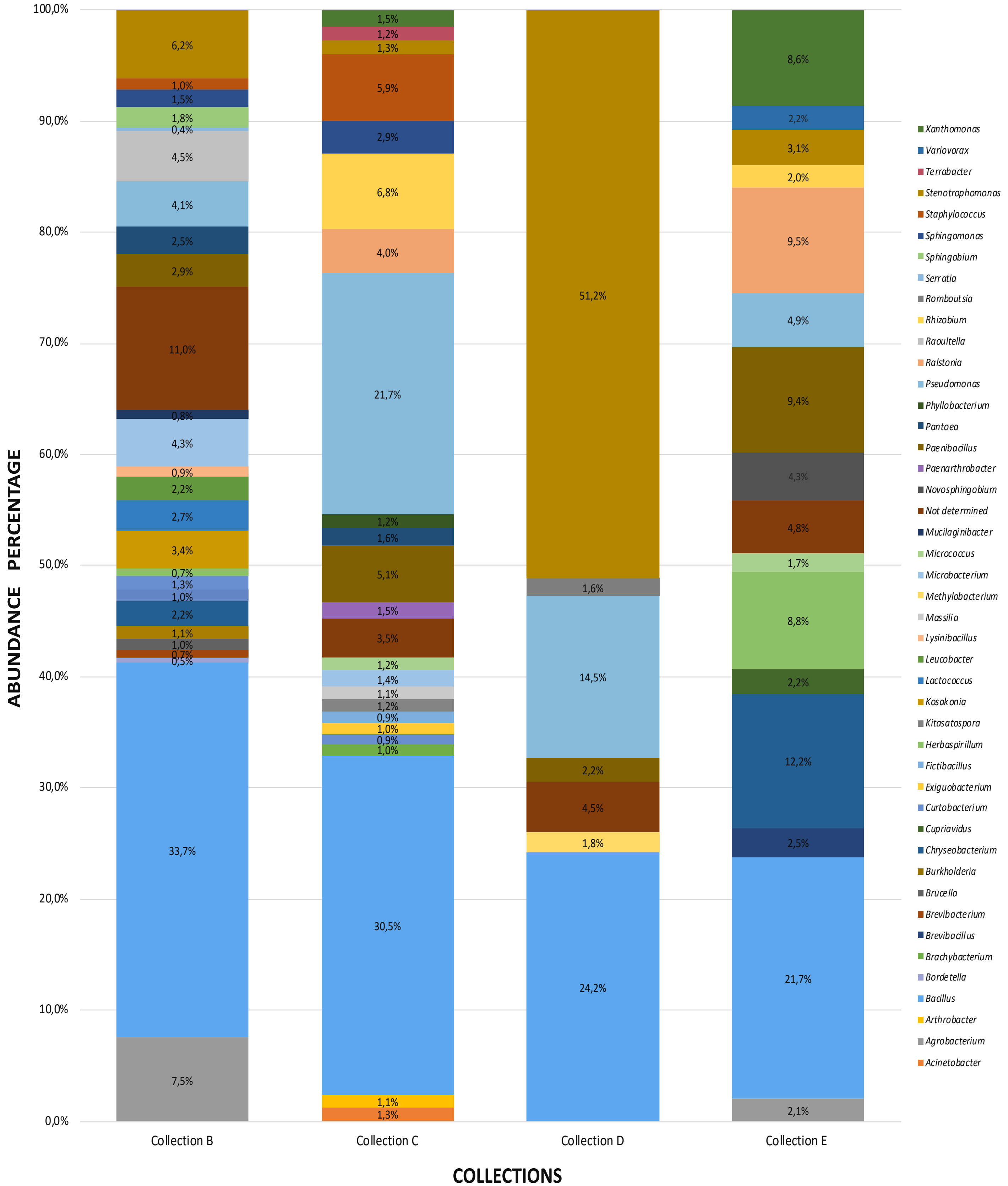
| Collection | Total isolates | Number of identified with Python script | Number of identified with Geneious |
|---|---|---|---|
| B | 125 | 116 | 112[a] |
| C | 81 | 79 | 78[b] |
| D | 75 | 73 | 68[c] |
| E | 48 | 42 | 46[d] |
| **Total isolates** | **329** | **310** | **304** |

(**a.** See table S1, figure S1, Online Resource 1; **b.** See table S2, figure S2, Online Resource 1; **c.** See table S3, figure S3, Online Resource 1; **d.** See table S4, figure S4, Online Resource 1).




***Collection B:** greenhouse Calcutta 4 banana plants. **Collection C:** greenhouse Williams banana plants. **Collection D:** *in vitro* Williams banana plants. **Collection E:** *in vitro* Calcutta 4 banana plants.

**Graphic 1.** Relative genera abundance identified with Python script for collections B, C, D and E.




*__Collection B:__ greenhouse Calcutta 4 banana plants. **Collection C:** greenhouse Williams banana plants. **Collection D:** *in vitro* Williams banana plants. **Collection E:** *in vitro* Calcutta 4 banana plants.

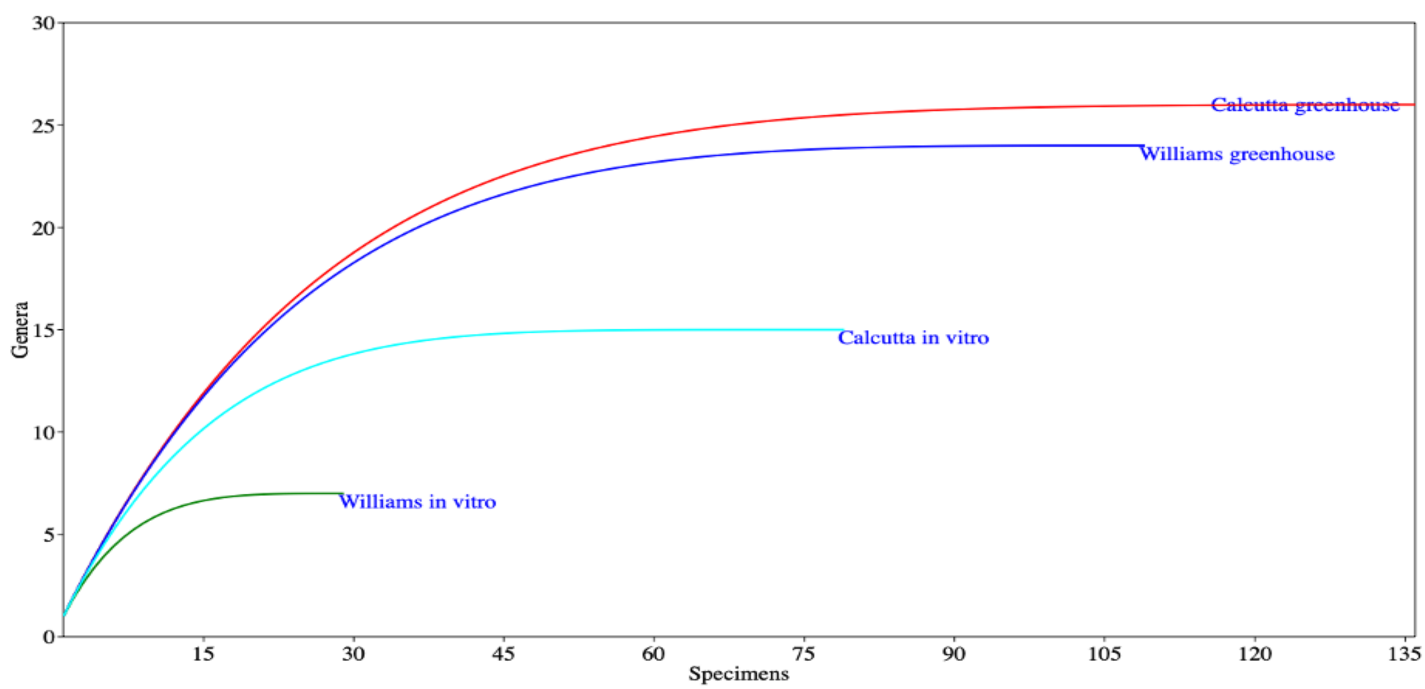
**Graphic 2.** Relative genera abundance identified with Geneious software for collections B, C, D and E.

**Estimation of diversity of native endophyte microorganisms present among the different banana plants, variety Calcutta 4 and cultivar Williams, at *in vitro* and greenhouse level.**

To be able to determine if the sampling processes and culturable microorganisms' isolation present in each plant were successfully performed, rarefaction curves were graphed. The behavior of the stationary phase in Graphic 3 shows that a highest number of genera present in each group was isolated, nevertheless, it varies according to the microorganisms' quantity present in each of them. Greenhouse Calcutta 4 plants are those with the highest number of genera of endophytic microorganisms in their tissues. In contrast, *in vitro* Williams plants are those that have least number of genera of endophytic microorganisms in their tissues.

Alpha (α) and beta (β) diversity analysis for greenhouse Calcutta 4, greenhouse Williams, *in vitro* Williams, and *in vitro* Calcutta 4 plants are presented regarding the genera (Table 3) and species found (Table 4), using the Python script with which more isolates were identified (310). It is important to mention that α diversity analyzes for a particular group's species richness while β diversity indicates the change of species that occurs between one group and another. Alpha diversity analysis shows a high number of different genera 26, 24 and 15 in greenhouse Calcutta 4, greenhouse Williams, and *in vitro* Calcutta 4 plants respectively; however, *in vitro* Williams plants presented a low number of genera, only seven different were found. These results can be compared with the values of the Simpson index obtained for each group. This indicates how diverse a community is. A value of 1 reflects high diversity and a value of 0 low diversity. Greenhouse Calcutta 4, greenhouse Williams, and *in vitro* Calcutta 4 plants present values of 0,81; 0,86 and 0,83 for this index, showing that communities are very diverse; unlike *in vitro* Williams plants where this index has a value of 0,38. This result means that *in vitro* Williams banana population is not diverse and there is a higher probability to select isolates of the same genus when they are randomly assigned (Table 3).

In three of the four groups it is possible to find that all genera are present in similar proportions because it was determined a dominance of 0,18 for greenhouse Calcutta 4; 0,13 for greenhouse Williams and 0,16 for *in vitro* Calcutta 4 plants. Instead, the greatest dominance found at *in vitro* Williams plants (0,61) where the *Stenotrophomonas* genus prevails over the others isolates.




**Graphic 3.** Rarefaction curves for greenhouse Calcutta 4, greenhouse Williams, *in vitro* Williams, and *in vitro* Calcutta 4 banana plants showing the number of genera as a function of sample size.

**Table 3.** Alpha diversity and Beta diversity indices according to genera found in Calcutta 4 greenhouse, Williams greenhouse, Williams *in vitro* and Calcutta 4 *in vitro* plants.

| Parameters | Calcutta 4 greenhouse (Collection B) | Williams greenhouse (Collection C) | Williams *in vitro* (Collection D) | Calcutta 4 *in vitro* (Collection E) |
|---|---|---|---|---|
| **α Diversity** | | | | |
| **Taxa_S** | 26 | 24 | 7 | 15 |
| **Individuals** | 85’470.250 | 13’418.300 | 2’070.800 | 15’716.000 |
| **Dominance_D** | 0,1847 | 0,1384 | 0,6184 | 0,163 |
| **Simpson_1-D** | 0,8153 | 0,8616 | 0,3816 | 0,837 |
| **Shannon_H** | 2 | 2 | 1 | 2 |
| **Berger-Parker** | 0,3627 | 0,231 | 0,7726 | 0,3309 |
| **Chao-1** | 26 | 24 | 7 | 15 |
| **β Diversity** | | | | |
| **Whittaker index** | 1,6111 | | | |

Shannon index indicates that in greenhouse Calcutta 4, greenhouse Williams, and *in vitro* Calcutta 4 plants there are greater uncertainty when choosing a specific genus randomly, because for the three groups a value of 2 was obtained, which doesn’t happen with the group of *in vitro* Williams plants, where the value of 1 for this index indicates that if an element from the group is randomly chosen it will belong to the *Stenotrophomonas* genus (Table 3).

Finally, low equity presented inside the *in vitro* Williams plants group is corroborated with Berger-Parker index value’s (0,77), because it represents the proportion of the dominant genus (*Stenotrophomonas*) compared to the others that were also found (the higher the value, the lower the equity will be among the genera found). For the Beta diversity analysis Whittaker index was evaluated and a value of 1,61 was obtained. This suggest that there are differences between the genera in each of the four evaluated groups, but some similarity and homogeneity is maintained between them. Also, part of the isolates are not considered as different communities (equality between genera is maintained, not increase in diversity); but there’s another portion that is considered as different (Table 3). Alpha diversity analysis was performed for the identified species, these were found in similar proportions in plants tissues: greenhouse Calcutta 4 (dominance 0,07), greenhouse Williams (dominance 0,09) and *in vitro* Calcutta 4 (dominance 0,16) which doesn’t happen in *in vitro* Williams plants where more than half of the isolates (dominance 0,61) belong to *Stenotrophomonas maltophilia* specie. This was corroborated using Simpson’s and Berger-Parker’s index. Greenhouse Calcutta 4, greenhouse Williams and *in vitro* Calcutta 4 plants obtained values of 0,92; 0,90 and 0,83 for Simpson index and 0,17; 0,21 and 0,33 for Berger-Parker index respectively. Diversity for species found at *in vitro* Williams plants showed lower diversity with a value of 0,38 for Simpson index and lower equity with value of 0,77 for Berger-Parker index. This is related to the high presence of *Stenotrophomonas maltophilia* in this assay (Table 4).

Shannon index (value of 3) suggest that endophytic microbiota of greenhouse Calcutta 4 and Williams plants is made up of various species of bacteria unlike the microbiota from *in vitro* Williams plants with a value of 1 and *in vitro* Calcutta 4 plants with a value of 2. By last, the Whittaker index for Beta diversity accounts for the different species among the four groups, and in this particular case, can’t let us assure that there is an existent total difference between the species found. In a certain way there is similarity between groups (Table 4).

**Table 4.** Diversity indices and Beta diversity according to species found in Calcutta 4 greenhouse, Williams greenhouse, Williams *in vitro* and Calcutta 4 *in vitro* plants.

| Parameters | Calcutta 4 greenhouse (Collection B) | Williams greenhouse (Collection C) | Williams *in vitro* (Collection D) | Calcutta 4 *in vitro* (Collection E) |
|---|---|---|---|---|
| **α Diversity** | | | | |
| **Taxa_S** | 45 | 38 | 19 | 19 |
| **Individuals** | 86’047.900 | 13’307.000 | 2’063.101 | 15’738.000 |
| **Dominance_D** | 0,07787 | 0,09286 | 0,6196 | 0,1604 |
| **Simpson_1-D** | 0,9221 | 0,9071 | 0,3804 | 0,8396 |
| **Shannon_H** | 3 | 3 | 1 | 2 |
| **Berger-Parker** | 0,1743 | 0,2179 | 0,7755 | 0,3304 |
| **Chao-1** | 45 | 38 | 19 | 19 |
| **β Diversity** | | | | |
| **Whittaker index** | 1,7769 | | | |

**Comparison of native endophyte microorganisms’ diversity associated with banana plants from variety Calcutta 4 and cultivar Williams and find differences between the communities present in their roots, pseudo-stem and leaves.**

To compare the evaluated plants’ endophytic microbiota, T and permutation tests were performed. T-test indicates whether two groups are significantly different and permutation test helps to corroborate the T-test results, because it indicates how similar two treatments are. With previous results, it is possible to see that from the six couples created with two of the four studied groups (Calcutta 4 group 1, greenhouse Williams group 2, *in vitro* Williams group 3 and, *in vitro* Calcutta 4 group 4) only one couple (Calcutta 4 and Williams greenhouse plants) doesn’t show statistically significant differences respecting the abundance and genera diversity that composes greenhouse Calcutta 4 and greenhouse Williams groups (Table 5).

With a diversity T-test it was compared between groups the genera abundance of each tissue from the different evaluated banana plants: greenhouse Calcutta 4, greenhouse Williams, *in vitro* Williams and, *in vitro* Calcutta 4 (Table 6). From the 18 evaluated couples, only three didn’t present statistically significant differences, which indicates that quantity and genera abundance of the microorganisms that were found in the evaluated tissues are similar (Table 6). Thus, leaves from greenhouse Calcutta 4 banana plants present similar endophyte microbiota to the endophyte microbiota from greenhouse Williams banana plants’ leaves, the endophytic microbiota from leaves of greenhouse Williams banana plants is similar to the *in vitro* Calcutta 4 banana plants’ leaves, and the endophytic microbiota from greenhouse Calcutta 4 banana plants’ roots is similar to greenhouse Williams banana plants’ roots (Table 6). These similarities do not show a total homology between the plants to be compared, only between the tissues that are being evaluated in each couple made up of two groups. For example, after doing the diversity T-test, the couple 1 – 2 formed by greenhouse Calcutta 4 and greenhouse Williams banana plants, didn’t show significant differences, as regards, endophytic microbiota doesn’t vary in terms of diversity and quantity (Table 5). However, if only the isolates from pseudo-stem of each plant are compared, a significant diversity and quantity difference will be found (Table 6).

The number of couples that presented statistically significant differences was 13 (Table 6); this implies that if the same tissue is compared between a couple, the endophytic microbiota of each plant's tissue, is different. For example, the couple 1 – 3 formed by roots of greenhouse Calcutta 4 and *in vitro* Williams banana plants is one of the couples with statistically significant differences, because from the total of microorganisms in roots, leaves and pseudo-stem of greenhouse Calcutta 4 plants, only 24% were isolated from its roots and the most abundant genus was *Bacillus* a 29% from the total roots isolates; conversely, 32% of the isolates from *in vitro* Williams banana plants were isolated from the roots and 66% of those roots isolates belonged to the *Stenotrophomonas* genus. In general, it is demonstrated that the P value for Shannon and Simpson indices in the T test are the same when couples of groups are compared.

Summarizing, it is apparent that every kind of banana plant evaluated in this project has a characteristic endophytic microbiota and it is related with its growth conditions, environment (greenhouse or *in vitro*) and organs of the plant. It could be related to the agronomic management for each groups of plants (origin, type of soil, weather conditions, disinfection process for growth, etc.), the transference of microorganisms from the source plant (source of seeds or meristem) and their genetic heritage.

**Table 5.** P values for Shannon and Simpson indices from T-test and permutation test for pairs of evaluated groups: (1) Calcutta 4 greenhouse. (2) Williams greenhouse. (3) Williams *in vitro*. (4) Calcutta *4 in vitro*.

| Couples | Test | Shannon index | Simpson index |
|---|---|---|---|
| 1 - 2 | T test | 1,E-01 | 5,E-01 |
| | Permutation test | 9,E-01 | 8,E-02 |
| 1 - 3* | T test | 7,E-24 | 1,E-06 |
| | Permutation test | 1,E-04 | 1,E-04 |
| 1 - 4* | T test | 5,E-24 | 4,E-07 |
| | Permutation test | 1,E-04 | 1,E-04 |
| 2 - 3* | T test | 3,E-23 | 2,E-06 |
| | Permutation test | 1,E-04 | 1,E-04 |
| 2 - 4* | T test | 2,E-18 | 8,E-06 |
| | Permutation test | 1,E-04 | 1,E-04 |
| 3 - 4* | T test | 6,E-15 | 1,E-04 |
| | Permutation test | 1,E-04 | 1,E-04 |

*There are statistically significant differences between the evaluated groups regarding the genera diversity found among them.

**Table 6.** P value for Shannon index from T-test for pairs of evaluated groups: (1) Calcutta 4 greenhouse. (2) Williams greenhouse. (3) Williams *in vitro*. (4) Calcutta *4 in vitro*.

| Couples | T-test P-value for Shannon index |
|---|---|
| Leaves 1 - 2 | 2,00E-01 |
| Leaves 1 – 3* | 3,00E-04 |
| Leaves 1 - 4* | 2,00E-07 |
| Leaves 2 - 3* | 6,00E-07 |
| Leaves 2 - 4 | 2,00E-01 |
| Leaves 3 - 4* | 1,00E-05 |
| Pseudosteam 1 - 2* | 3,00E-07 |
| Pseudosteam 1 - 3* | 2,00E-09 |
| Pseudosteam 1 - 4* | 9,00E-12 |
| Pseudosteam 2 - 3* | 6,00E-09 |
| Pseudosteam 2 – 4* | 2,00E-03 |
| Pseudosteam 3 - 4* | 3,00E-08 |
| Roots 1 - 2 | 2,00E-01 |
| Roots 1 - 3* | 5,00E-17 |
| Roots 1 - 4* | 3,00E-09 |
| Roots 2 - 3* | 4,00E-17 |
| Roots 2 - 4* | 2,00E-12 |
| Roots 3 - 4* | 1,00E-13 |

*There are statistically significant differences between the evaluated groups regarding the genera found in each tissue.

## Discussion

As previously mentioned for Collection B, originally from greenhouse Calcutta 4 banana plants, it was found that the *Bacillus* genus is the most abundant, so as the *Bacillus cereus* specie. The population abundance was higher in pseudo-stem and the culture media AIA, but the individuals' abundance was higher in roots and culture media TSA 50%. It is important to highlight that many of endophytes are taken up from the roots, but they can colonize the rest of the plant's tissues, establish and feed of the nutrients in the plant. It could be the reason why the major number of microorganisms are present in the pseudo-stem (population abundance), but it does not indicate diversity (individuals' abundance), which is higher in roots.

The collection C it's made from the isolated microorganisms of greenhouse Williams banana plants. Again, the genus *Bacillus* was the most abundant and in this case the specie with the highest proportion was *Pseudomonas putida*. Proportion of isolates in population abundance was higher in roots and the abundance of individuals was higher in leaves, both higher in culture media AIA, meaning that these tissues house the largest number and variety of isolates. Even culture media AIA promotes isolation of actinomycetes, sodium caseinate acts as nitrogen source and *Bacillus* genus is well known by its nitrogen-fixing bacteria [20] and the specie *Pseudomonas putida* has demonstrated to stimulate nitrogen fixation by plants' rhizosphere [21].

Collection D is the collection with the lowest genera variety found, of which most of them belong to *Stenotrophomonas* genus, standing out the specie *Stenotrophomonas maltophilia*. The population abundance was higher in pseudo-stem and higher in leaves regarding individuals' abundance. It was found that both abundances were higher in R2A when isolates from different culture media were evaluated. These results agree with what has been reported by other authors where *Stenotrophomonas* was found at metagenomic level as a predominant genus in banana plants' endophytic microbiota [22] and Williams banana plants at field level (David-Gonzales, 2014, Unpublished) and given their broad resistance to antibiotics, ability to establish endophytic associations with plants and the capacity of some species to promote plant growth and serve as a biocontrol and bioremediation agents [23] [24], this genus can be benefit for the plants.

And finally, the most abundant genus for Collection E was *Bacillus*, the specie found in higher proportion was *Chryseobacterium sp,* and population and individuals' abundance were higher in leaves and roots and culture media R2A. Because of the size of these plantlets and its short developmental stage, it can be possible to hypothesize that majority of microorganisms are present in leaves due to the lack of developed roots and pseudo-stem. Also, MS culture medium, where plants grew, is poor related to complex nutrients content, but plant can convert those ions and salts that compound it to more complex nutrients and transport them across *in vitro* banana plant. It could be a reason why microorganisms are located in other organs instead

roots, perhaps looking for areas with more nutrients to establish it.
It is worth mentioning that these culture media are not selective, they are semi-selective, indicating that the characteristic microorganism that tend to be isolated in these media are the most related to metabolize nutrients sources present there, but are not necessarily the only ones. Other species can proliferate there too, and it might be because they can use the C source from the media for their survival and growth.

Related to the disinfection protocols that were followed during this experiment, it might be the reason why *Bacillus* genus is found as the most abundant in three of the four groups of plants that were evaluated: bacteria belonging to *Bacillus* genus form aerobic endospores that are resistant to conditions of high temperatures, acidulated water and chemical agents [25] then when tissues and meristems from mother plants go through disinfection processes *Bacillus* are the bacteria transferred in higher proportion to the offspring [26]. As it has been previously mentioned in other studies, the *Bacillus* genus is one of the most abundant genera among the banana plants [27], [28] and has a broad potential for primary and secondary metabolites production, beneficial interaction with the host plant and antagonistic activity with phytopathogens, that is why it is used to produce bioproducts [29]. It is also found that bacteria from the genus *Stenotrophomonas* are abundant in the endophytic microbiota of banana plants [22], [24] as they are health indicators and can prevent osmotic stress by protecting the roots [30] as it has been found in this project, where the genus *Stenotrophomonas* is the main core of endophytes from Williams *in vitro* banana plants.

Regarding the results of the current investigation, some similarities and differences were found with other studies where genera such as *Bacillus, Paenibacillus, Pseudomonas,* and *Methylobacterium* were found in higher abundance in the endophytic microbiota from plant tissues like roots, pseudo-stem, and leaves of *in vitro* Williams banana plants [22]. These results are quite similar to those obtained in the present study because genera *Bacillus* and *Pseudomonas* were also two of the three genera found in higher abundance and although in lower proportions, microorganisms belonging to the genera *Paenibacillus* (3%) and *Methylobacterium* (1%) were found. It is important to highlight that in this study the genus obtained in highest proportions of *in vitro* Williams banana plants by culture-dependent methods was *Stenotrophomonas*. This genus was almost 50% of the total genera found. For the study of García Giraldo, 2017, this genus comprised more than the 80% of total genera, results that were obtained by culture-independent methods (metataxonomic evaluations of the regions V3-V4 of 16S rDNA gene) and similarly happened with *Staphylococcus* genus found by culture-dependent methods in this study and by culture-independent methods in García Giraldo, 2017 study, but in both studies found in lower proportions.

Posada Uribe, 2017 presented pioneering study for banana *in vitro* endophytes where similar results are reported for endophytic microbiota of *in vitro* Williams banana plants' roots. Microorganisms of genus *Bacillus* were the most predominant, and genera as *Pseudomonas, Methylobacterium* and *Paenibacillus* were found in higher proportions like in the present study. However, in our project we evaluated differential isolation for pseudo-stem, roots and leaves tissues, and the reported source did not discriminate banana sections [27]. Besides, in the present study 58,3% of the microorganisms from the endophytic microbiota of *in vitro* Williams banana plants' roots that were able to be identified, belong to the *Stenotrophomonas* genus (which is not found in Posada Uribe, 2017, investigations) and the 33,33% and 8,33% to the genera *Pseudomonas* and *Bacillus* respectively. Genera like *Micrococcus, Microbacterium, Staphylococcus, Rhodopseudomonas, Acinetobacter, Moraxella,* and *Athrobacter*, although found in lower proportion in Posada, 2017 were not found among the roots' isolates of the present study, for example *Staphylococcus* genus was isolated but from leaves and in a very low proportion, around 1%.

To date and per the reviewed literature, there are no works reported about endophytic microbiota of greenhouse Williams banana plants. Nonetheless, there are studies about microbiota's diversity associated to banana plants in other cultivars (*Musa paradisiaca*) coming from smallholder cropping system due to 16S rRNA metagenome sequencing, where genera like *Ralstonia, Sphingomonas, Methylobacterium, Flavobacterium,* and

*Pseudomonas* are found [31] as in our study (except for the *Flavobacterium* genus and *Methylobacterium* in Williams greenhouse plants).

Like wilt pathogens, endophytes can colonize plants' vascular tissue and their presence can be beneficial to the plant and can even suppress disease when a pathogenic microorganism is present [32], [33]. Some reports have evaluated banana endophytes inoculation for plant *Fusarium* wilt infections, finding high survival rates for plants with bacterial inoculation even when infected, increasing tolerance to wilt disease and growth when compared to the control [34]. Endophytes act as biocontrol agents because can produce defense metabolites or have a direct antagonistic activity against pathogens [32]. Also, some of them can increase size and vigor's plant, and induce resistance [32], [34], [35] without causing possible disease or interfering with its normal development [32]. Wild bananas have a higher number of endophytes genera perhaps due to their coevolution with soil's microbiota [34], having an enormous potential and their introduction is suggested to be previous field transplantation in a nursery stage so they can be properly established [34] and induce plants to produce host defense enzymes [36].

In Jie et al., 2009 the effects of artificial inoculation of greenhouse Cavendish banana plants with a mixture of endophytic microorganisms (culturable and nonculturable) from healthy Cavendish plants were evaluated. These plants were evaluated to reduce *Fusarium oxysporum* f. sp. *cubense* race 4 infection. It was found that this mixture contained bacteria from *Bacillus, Bradyrhizobium, Sphingomonas, Herbaspirillum, Burkholderia, Ralstonia, Variovorax, Pseudomonas, Enterobacter, Pantoea, Shigella, Escherichia,* and *Nevskia* genus, and some of them reduced up to a 67% the wilt disease. Some of these genera were found at the present study in greenhouse Williams banana plants *Bacillus, Sphingomonas, Ralstonia* and *Pseudomonas*.

There are genotypic plant studies that aim to improve domesticated banana plants' (Giant Cavendish cv. Baxi) resistance to diverse pathogens by engineering bacteria that can express certain enzymes or hormones that promote plant growth and wilt resistance [37] or that look up for defence-related expresed genes among resistant plants [38]. Regarding to variety Calcutta 4, some studies, aim to determine the relationship between its phenotypic characters and their response to the inoculation with *M. fijiensis* and the interaction with the pathogen at a molecular level [39] [40] but there haven't been found studies that characterize their native endophytic microbiota.

Although the changes of genus by using the Python script or the Geneious software occurred in low proportions, they can be attributed to the precision with which the trimmed sequence is obtained in the Geneious software. It is because not only the primer and the fragments where there is more noise during the sequencing are eliminated, possible contaminating vectors are eliminated too; or can also be because of the phylogenetic relationships that exists between some genera. For example, the most common change was from *Agrobacterium* genus to *Rhizobium* genus and these two are genera phylogenetically related but still, there are no conclusions if they are considered as one [41]–[43]. Other changes like *Bacillus – Listeria* and *Bacillus – Staphylococcus* might be by the fact that these genera are paraphyletic and belong to the Bacillales order [44] like the genera *Bacillus – Paenibacillus* that also belong to this order. Few genus changes are related to microorganisms of the same families like *Achromobacter – Bordetella* from Alcaligenaceae family [45], *Enterobacter – Kosakonia* from Enterobacteriaceae family [46], *Janthinobacterium – Herbaspirillum* from Oxalobacteraceae family [47], *Intrasporangium –Terrabacter* from Intrasporangiaceae family [48] and *Arthrobacter – Paenarthrobacter* from Micrococcaceae family [49].

Respecting the results obtained from the diversity's estimation of native endophyte microorganisms present among the different banana plants, it is verified that endophytic microbiota's plants that have grown in greenhouses are more diverse than the ones that have grown in aseptic conditions (*in vitro*); inferring that the environmental exposure can influence their richness. Also, it is possible to contrast the endophytic microbiota from a native plant with one that has been throughout the process of domestication which in time leads to a decrease of the own plant's tissues bacterial variety [50], [51]. The above indicates that naturally and under sterile conditions, Calcutta 4 and Williams banana plants present a distinctive and unique native endophyte microbiota because at *in vitro* conditions it is notorious that both are significant different, which leads that when compared with another variety/cultivar and even with their homologous from a different environment, the endophytic microbiota changes and still presents significant differences between one and another (greenhouse Calcutta vs. *in vitro* Calcutta 4 and greenhouse Williams vs. *in vitro* Williams). Nonetheless, greenhouse conditions might suit endophytic microbiota from two different plants to the point where there are no significant differences between them (greenhouse Calcutta 4, greenhouse Williams). See table 5.

There are several studies about banana plants' endophytic microbiota where they rely on culturable and non-culturable methods and results are variable among them. It has been found that when molecular approaches are employed the main core of endophytes is composed by bacteria from the *Stenotrophomonas* genus in *in vitro* Williams banana plants [22] and *Serratia* genus from shoot-tip tissue of Grand Naine banana plants [52] and from the Enterobacteriaceae family mostly present in banana plants' pseudo-stem [53]. Thus, it is important to perform culturable-dependent isolations because they can help to elucidate the microbiota's composition and offer scope towards new analysis and tools development for the agricultural sector [52].

Finally, the findings of this work allow suggesting that there are significant differences among banana plants' microbiota when compared to different development stages and varieties/cultivars. This information is important to begin researches related to differential microbiota among varieties and stages for bioproducts development based on beneficial microorganisms. The reference for this investigation could be microbiota found in banana Calcutta 4 at different stages that is absent in commercial banana, as the genera that are only found in greenhouse or *in vitro* Calcutta 4 banana plants in higher proportions like *Raoultella* (4,5%), *Microbacterium* (4,2%) and *Lactococcus* (3,2%) from greenhouse banana Calcutta 4; *Herbaspirillum* (8,8%), *Novosphingobium* (4,3%) and *Brevibacillus* (2,5%) from *in vitro* banana Calcutta 4; and the genera *Chryseobacterium* (2,2% - 12,2%) and *Cupriavidus* (2,1% - 2,2%) found in both greenhouse and *in vitro* Calcutta 4 banana plants. It might be one of the reasons that confers to the plants, resistance to different types of plagues.

## Conclusions

The endophytic microbiota of roots, pseudo-stem, and leaves from greenhouse Calcutta 4, greenhouse Williams, *in vitro* Williams, and *in vitro* Calcutta 4 banana plants was determined due to the identification of isolates obtained by culture-dependent methods. The most diverse microbiota was from greenhouse plants of variety Calcutta 4, mainly constituted in a 36% by bacteria of *Bacillus* genus and to a lesser extent by the genera *Agrobacterium*, unidentified isolates, *Stenotrophomonas,* and *Pseudomonas*; and by the species *Bacillus cereus*, *Bacillus sp*., unidentified isolates, and *Stenotrophomonas maltophilia.* Greenhouse Williams plants have an endophytic microbiota composed mostly of bacteria from the genera *Bacillus* 29,63% and *Pseudomonas* 23,50% and the species *Pseudomonas sp*., *Pseudomonas putida,* and *Bacillus pumilus*. The less diverse microbiota belongs to the *in vitro* Williams banana plants, where approximately 45,33% of the identified isolates belong to the *Stenotrophomonas* genus and the specie *Stenotrophomonas maltophilia*. At least the genera *Bacillus*, *Chryseobacterium*, unidentified isolates, and species *Chryseobacterium sp*., unidentified isolates, and *Bacillus megaterium* composed mostly the native endophytic microbiota of *in vitro* Calcutta 4 banana plants.
Diversity indices were estimated for the evaluated groups and it was found that *in vitro* Williams banana plants present fewer genera and species variety in their tissues where a specific genus and specie predominates among the other isolates; in the other groups we found a higher microbiological richness. By estimating beta diversity, it was possible to conclude that both the difference of genera and species between one group or another is higher and there is not so much global similarity.

The endophytic microbiota was compared between groups and not significant differences were obtained for greenhouse Calcutta 4 and Williams banana plants but there were differences for other reported treatments pairs. The same occurred when compared the same organ between two different groups, all presented significant differences regarding the population of endophytes present in the evaluated organs except for the leaves of greenhouse Calcutta 4 and Williams plants, the leaves of greenhouse Williams and *in vitro* Calcutta 4 plants and roots of greenhouse Calcutta 4 and Williams banana plants. A good isolation of morphotypes was performed, since rarefaction curves were obtained in the stationary phase, which verifies the adequate sampling effort for the process.

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## Supplementary material

**Online Resource 1:** **Tables S1-S4** and **Figures S1-S4**. Lists of collections' isolates identified by python script and Geneious software. Genera and species figures of collections' isolates identified by Geneious software. (1,1 MB)

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