



## DIVERSITY OF ENDOPHYTIC BACTERIA ASSOCIATED WITH ROOTS OF COMMERCIALY IMPORTANT PLANT SPECIES

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### ABSTRACT

Endophytic bacteria have become a native biological alternative associated with healthy plant tissues to improve crop production and replace agrochemicals that cause serious environmental damage. In this work, endophytic bacteria were isolated from different roots of colosoana grass, angletón, mombasa, rice, yam and the medicinal plant known as wild oregano in two departments of the Caribbean region, Colombia. The amount of endophytic bacteria in roots of the six plant species analyzed ranged between  $8.7 \times 10^7 \pm 2.3 \times 10^9$  CFU/g of roots. A total of 242 strains of endophytic bacteria were isolated, showing a high richness of these bacteria that can be biological alternatives for growth promotion, biological control and the production of secondary metabolites with antimicrobial activity and biotechnological potential for the six species analyzed.

**Keywords.** Plant species, bacteria, endophytes, roots, strains.

### INTRODUCTION

Endophytic bacteria are a group of symbiont microorganisms that inhabit plant tissues of healthy plants without causing disease symptoms to their host (Kandel et al., 2017). They contribute to plant health through the secretion of growth regulatory factors, production of phytohormones and microbial metabolites, which allows for the reduction of nutritional and pathogenic stress in the plant (Porras and Bayman, 2011; Kandel et al., 2017). Endophytic bacteria are considered a great biotechnological tool due to their ability to fix nitrogen, solubilize phosphate and produce siderophores, which has favored crop production and yield (Sahoo et al., 2017).

Among the traditional Colombian crops, yams (Dioscoreae), colosoana grass, angletón, mombasa, rice and medicinal and aromatic plants are of great socio-economic importance in the Caribbean region, especially in the departments of Córdoba and Sucre, and are part of the diet of the population and cattle populations. The crops are managed in an artisanal way with the use of family labor, constituting a product of peasant economy for the departments of the Caribbean region.

Based on the importance of endophytic bacteria for plant species, the aim was to isolate these bacteria from six plant species of agricultural and biotechnological interest for the region and to determine the presence and quantity of strains present in different conditions and crops.

## MATERIALS AND METHODS

**SAMPLING.** Sampling was carried out at different sites with different plant species of interest for the region. At each selected site, a representative sampling was carried out, taking 15-20 random subsamples at a depth of 0-20 cm, collecting soil and roots at the same time. The subsamples were homogenized to form one sample per site and per plant species with an approximate weight of 2,000 g, which were deposited in plastic bags labelled with the farm number, district, area sown with the grass and date of collection. For each sample collected, isolation and quantification of endophytic bacteria communities associated with the roots of each plant species sampled was carried out (Pérez et al., 2010; Pérez et al., 2015).

## ISOLATION, QUANTIFICATION AND PURIFICATION OF ENDOPHYTIC BACTERIA.

The collected samples were sieved to separate soil (stones, gravel) and roots. Once sieved, microbiological analyses of endophytic bacteria populations in the roots were carried out. Ten roots per sample were individually placed in 10 50 mL erlenmeyer flasks for surface disinfection by washing in distilled water and neutral detergent for one minute, followed by four rinses in sterile distilled water. The washed roots were transferred to new flasks containing sterile water for isolation of endophytic bacterial communities.

For the isolation of endophytic bacteria, a surface sterilization process was used for each root (Pérez et al., 2010), which consisted of the following steps: two washes of the root in sterilized distilled water, followed by shaking for 15 min in potassium phosphate buffer solution 0.05 mol L<sup>-1</sup>, pH 7.0; immersion for 1 min in 70% alcohol; shaking for 5 min in 5% sodium hypochlorite solution and Tween 80%; again immersion for 1 min in 70% alcohol followed by shaking for 15 min in potassium phosphate buffer 0.05 mol L<sup>-1</sup>, pH 7.0 and, finally, washing four times in sterile distilled water (Pérez et al., 2018).

The process was repeated twice. To confirm the sterilization of the root surface, an aliquot of the last wash was spread on a plate containing nutrient agar culture medium and incubated at 28 °C for 72 hours. The roots were then transferred to a tube containing nutrient broth and incubated at 28 °C for 72 hours to certify the absence of microorganisms on the surface of the roots to be used for the isolation of culturable endophytic bacteria (Pérez et al., 2010).

The density of bacteria per root, in CFU. g. of roots<sup>-1</sup>, was estimated by direct colony counting on plates. During counting, colonies that were distinguishable in shape, surface appearance, color and size were observed and selected. Selected morphotypes were purified and maintained on nutrient agar for further analysis and identification (Pérez et al., 2010, Pérez et al., 2018).

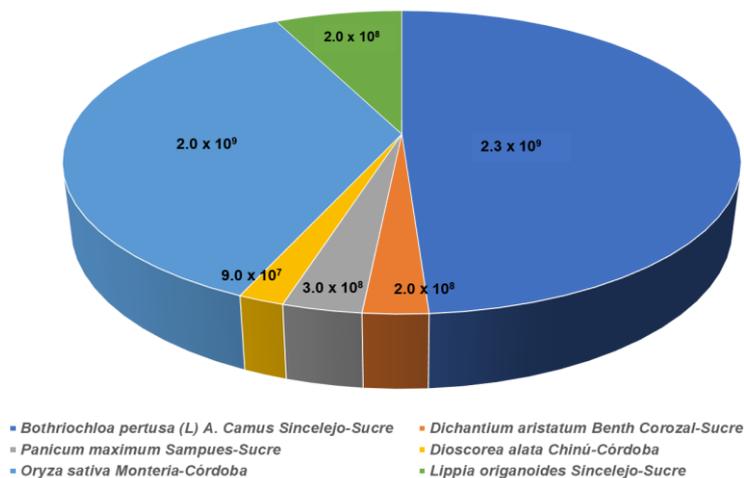
## RESULTS AND DISCUSSION

Sampling was carried out in two departments of the Colombian Caribbean region in which the following plant species were collected according to department and locality as described in table 1.

**Table 1.** Plant species collected, location and sampling site

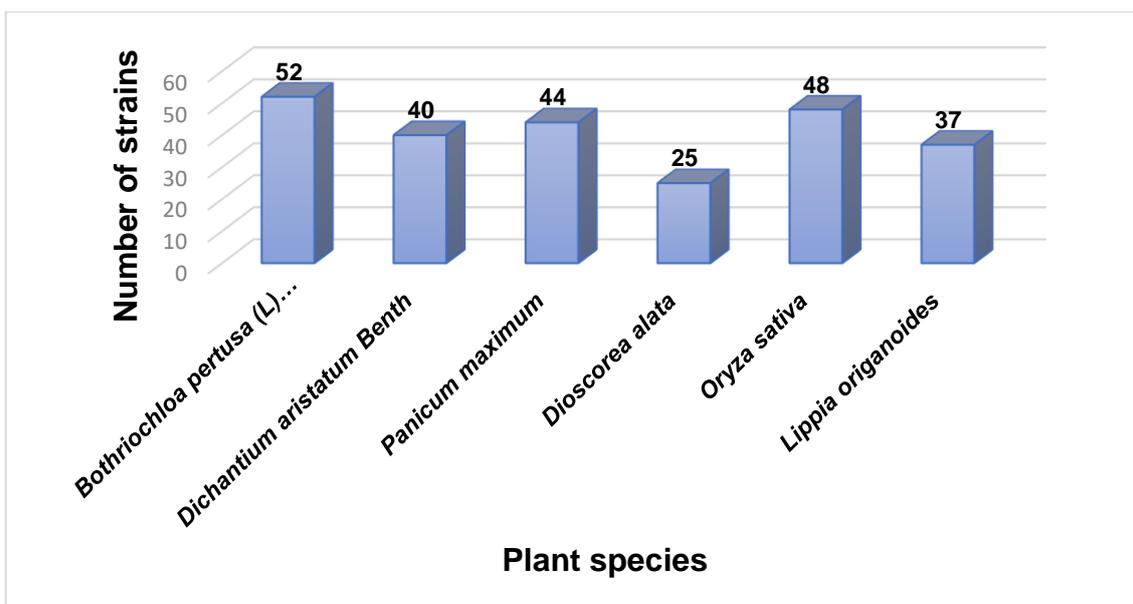
Plant species	Locality	Geo-referencing
<i>Bothriochloa pertusa</i> (L) A. Camus	Sincelejo-Sucre	northwest of the country at 9° 18' 17" north latitude, 75° 23" west latitude
<i>Dichantium aristatum</i> Benth	Corozal-Sucre	Latitude 9.31847 and longitude -75.2933. It is part of the continent of South America and is located in the northern hemisphere.
<i>Panicum maximum</i>	Sampués-Sucre	Latitude: 9.183, Longitude: -75.383 9° 10' 59" North, 75° 22' 59" West
<i>Dioscorea alata</i>	Chinú-Córdoba	Latitude: 9.117 ; Longitude: -75.4 ; Latitude: 9° 7' 1" North ; Longitude: 75° 24' 0" West.
<i>Oryza sativa</i>	Montería-Córdoba	Latitude: 84° 48' 03" N; Longitude: 75° 51' 20" W
<i>Lippia origanoides</i>	Sincelejo-Sucre	northwest of the country at 9° 18' 17" north latitude, 75° 23" west latitude

Figure 1 shows the amount of endophytic bacteria in roots of six plant species of economic interest for the region. As can be seen, the grass species *Bothriochloa pertusa* (L) A. Camus, is the one with the highest amount of bacteria in roots ( $2.3 \times 10^9$  CFU/g root), followed by *Oryza sativa* ( $1.7 \times 10^9$  CFU/g root) and the least amount was isolated in *Dioscorea alata* ( $8.7 \times 10^7$  CFU/g root).



**Figure 1.** Population density of endophytic bacteria associated with roots of six plant species of economic interest for the Colombian Caribbean region.

Figure 2 shows the number of species of endophytic bacteria isolated from roots of different plant species. The figure shows a higher number of strains for the pasture species *Bothriochloa pertusa* (L) A. Camus established in cattle farms in the municipality of Sincelejo and a lower number of strains for cultivation of yam Creole variety (*Dioscorea alata*), established in the municipality of Chinú, belonging to the department of Córdoba. Pérez et al., (2010), in a study conducted on population density of endophytic bacteria on roots of *Bothriochloa pertusa* (L) A. Camus in three municipalities of the department of Sucre (Sincelejo, Corozal and Tolú) found a greater presence of bacterial morphotypes in the livestock farms of the municipality of Sincelejo, followed by Corozal and a lower quantity in the livestock farms of the municipality of Tolú.



**Figure 2.** Number of strains isolated from roots of various plant species of commercial interest.

In this study, a population density of  $1.7 \times 10^9$  CFU/g root and a number of 48 strains isolated from the roots of these rice plants was found for the rice crop (*Oryza sativa* (L)), cultivated in the locality

of Mocarí, department of Córdoba. In 2012, Perez and collaborators in a study on the diversity of endophytic bacteria associated with rice plants in the department of Cordoba, reported a greater colonization of endophytic bacteria in roots,  $3.2 \times 10^{10}$  CFU/g of tissue with respect to the stem, leaves, flag leaf and panicle.

Likewise, Barbosa et al. (2023), in a study of the diversity of endophytic bacteria isolated from four rice varieties in the department of Córdoba, found that the varieties with the highest densities were F2000 ( $1.37 \times 10^4$  CFU/g of tissue) followed by F473 ( $1.2 \times 10^4$  CFU/g of tissue); the lowest density of bacteria was for FTana and F67, which had densities of ( $1.0 \times 10^4$  and  $9.7 \times 10^3$  CFU/g of tissue), respectively.

According to the results of the amount of endophytic bacteria in roots of *Dioscorea alata* cultivated in the municipality of Chinú-Córdoba, which was  $8.7 \times 10^7$  CFU/g root and a total of 28 strains. From the total of samples analyzed, 92 morphotypes of endophytic bacteria were isolated. In a study conducted by Doncel in 2017 on endophytic bacteria isolated from yam (*Dioscorea* spp.) cultivation with production of metabolites with antifungal activity against *Colletotrichum gloeosporioides* Penz in the department of Sucre, they report a population density of endophytic bacteria, obtained by agar count analysis of  $5.30 \times 10^8$  and  $2.00 \times 10^9$  CFU/g of tissue.

According to the results of the amount of endophytic bacteria in roots of *Dioscorea alata* cultivated in the municipality of Chinú-Córdoba was  $8.7 \times 10^7$  CFU/g root and a total of 28 strains. 92 morphotypes of endophytic bacteria were isolated from the total number of samples analyzed. In a study carried out by Doncel in 2017 on endophytic bacteria isolated from yam (*Dioscorea* spp.) with production of metabolites with antifungal activity against *Colletotrichum gloeosporioides* Penz. in the department of Sucre, they report a population density of endophytic bacteria, obtained by agar count analysis of  $5.30 \times 10^8$  and  $2.00 \times 10^9$  CFU/g of tissue.

Finally, it was found that the amount of endophytic bacteria associated with roots of *Lippia organoides* was  $3.4 \times 10^8$  CFU/g root and a total of 37 species isolated.

Arrieta et al., (2017) reported the presence of endophytic bacteria associated with different tissues of *L. organoides* in greater presence of colonies in roots  $4.1 \times 10^8$ , in stems  $2.5 \times 10^8$  and to a lesser extent in leaves  $2.3 \times 10^6$  respectively. For *Panicum maximum*  $1.5 \times 10^8$  CFU/g root with a number of strains.

The presence of native or introduced strains of endophytic bacteria is normally observed in the root and lower part of the stem, with stem to leaf decline occurring (Lamb et al., 1996). Seasonal and geographical variations, type of plant tissue (Mocali et al., 2003), host species and cultivars, and interaction with other beneficial microorganisms (Araujo et al., 2002), can also influence the colonization pattern and presence of endophytic bacteria.

Figure 3 describes the cultural characteristics on the surface of the R2A culture medium of the different strains of endophytic bacteria isolated from roots of six plant species of commercial interest in the Colombian Caribbean region.



**Figure 3.** Cultural characteristics in R2A culture medium of endophytic bacterial strains isolated from roots of six plant species of commercial interest in the northern region of Colombia.

According to the cultural characteristics on R2A medium, a total of 242 strains of endophytic bacteria were isolated, distributed by plant species as shown in figure 2.

## CONCLUSION

In the present study, a total of 242 strains of endophytic bacteria isolated from the roots of six plant species of agricultural and medicinal interest in the Caribbean region of Colombia were isolated. Endophytic bacteria represent a great utility for diverse applications, such as biological control of different diseases, plant growth promotion and bioremediation of contaminated environments. The number of endophytic bacterial species varies significantly with respect to tissue type and sampling site. The morphotypes evaluated showed antifungal activity by confrontation assays and bacterial secondary metabolites.

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## AUTHORSHIP CONTRIBUTIONS

All authors have jointly and equally contributed to the argumentation and writing of the manuscript.

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## CONFLICT OF INTEREST

None.

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