

Endophytic Microorganisms Isolated of Plants Grown in Colombia: A Short Review

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Abstract

Colombia is listed as the second largest country in plant diversity in the world, presenting more than 6000 species of endemic plants. The different genus and species of plants, as well as the various environments encountered in the country, are responsible for the countless amount of endophytic bacteria and fungi. So far, only a few endophytic microorganisms were isolated in Colombia, including the genera *Pseudomonas*, *Burkholderia*, *Chromobacterium*, *Curtobacterium*, *Acremonium*, *Alternaria*, *Aspergillus* and *Fusarium*, which have been isolated from rice, coffee, rose, grass and Espeletia plants. Fungi and bacteria isolated from these plants have great potential for use in biocontrol, bioremediation and in promoting plant growth. Colombia for its rich flora have become a promising country for finding new microorganisms associated with plants, especially those with potential for food industry, pharmaceuticals and agriculture.

Keywords: Endophytic microorganisms; Fungi; Bacteria and Colombia

Introduction

Colombia has the second largest biodiversity in the world, surpassing countries such as Indonesia, China, Mexico, USA and Australia. In the first place, the country with the largest biodiversity is Brazil, which has a considerable larger area than Colombia. According to the Royal Botanical Garden, Colombia is the country with the highest biological richness per m². From 2000 to 2009, 1,272 new biological species were described there, which represents 0.72% of new species in the Planet for this period [1-4]. In relation to its rich flora, Colombia is the second country with more diversity in plants, featuring 1,500 exclusive species. Moreover, it is the richest in ferns, mosses and lichens at neotropical level [2]. Plants establish relations with a range of microorganisms in different ways, some of them being endophytes. Unlike pathogens and opportunistic microorganisms, the endophytes live inside plant tissues without causing any damage [3,5]. Endophytes and plants live in symbiosis with mutual benefits. While the plant provides nutrients for the development of microorganisms, they help to promote plant growth by different mechanisms (nitrogen fixation, phosphate solubilization, iron chelation and hormone levels modulation) pathogen resistance herbivore defense and others [6-10].

There are about 300,000 species of plants and each can host one or even more species of endophytic microorganisms [11]. However, only a few have been thoroughly studied in relation to their endophytic microbiota. Therefore, finding new and beneficial endophytic microorganisms in this diversity of plants and ecosystems is highly likely [12]. These microorganisms showed a great potential for bioremediation, biocontrol, enzyme production, bioactive compounds, new secondary metabolites, plant growth and others [13-19].

In this way, the biological knowledge has the potential to stimulate public interest in biodiversity [20]. Investigation of endophytic microorganisms associated with different species of plants found in Colombia opens doors to new researches to better understand the Colombian microbial biodiversity, to identify new species or establish new genera of bacteria and fungi endophytes and consequently, to

explore future applications in different fields [21-23]. This short review summarizes the different genera and species of endophytic bacteria and fungi isolated from plants in Colombia, and their potential in many processes such as biocontrol, bioremediation, biocatalysis and development of new drugs.

Plant biodiversity in Colombia

With more than 56,000 species recorded in the Global Biodiversity Information Facility (GBIF), Colombia share with Brazil the first place worldwide in terms of biodiversity. The country is ranked by the United Nations Environment Programme as one of the 17 mega-diverse countries, hosting 70% of the world's biodiversity in only 10% of its territory [24]. Meanwhile, the Information System on Biodiversity (SIB) and the catalog of plants and lichens of Colombia establish that the country has 1,643 species of ferns and related, 262 species of palms, 4010 orchids, 45 species of gymnosperms, 1636 species of mosses and related, and 22840 species of angiosperms known [25,26]. Rangel-Ch conducted an inventory of Colombian flora, considering the geographical pattern of natural regions of Colombia, which led to the establishment of the plant component in the Pacific and Caribbean coast, and in Orinoco, Amazon and Andean regions [4] (Table 1). The Andean region has the largest concentration of biodiversity in Colombia and the Pacific coast has the highest concentration of wealth in flora of humid terrestrial biomes of the world. Meanwhile, the Colombian Paramo represents 60% of the wealth of flora among the high mountain

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Flora	Pacific coast	Caribbean coast	Orinoco region	Amazon region	Andean region
Species of flowering plants	4,525	4,274	4,347	11,500	7,600
Species of ferns	425	386	254	1050	510
Species of moss	132	230	86	800	174
Species of lichens	189	379	130	1,300	322
Species of liverworts	170	182	-	756	100

Table 1: Number of species of Colombian plants.

biome of Central and Northern South America.

The geographical position, the influence of two seas, the climate variability and topography are reasons that could explain the rich flora of Colombia [2,4]. Although there are records of some biological groups as birds, other groups are still poorly documented. Among the taxonomic groups underrepresented in the data portal SIB there are mainly viruses, bacteria, protozoa and fungi.

Endophytic bacteria isolated from plants grown in Colombia

Rice is the third most important product in Colombian agriculture and plays an important role in the diet and feeding of Colombian households [27]. Pérez et al. determined the diversity of endophytic bacteria population associated to different tissues of rice plants and their antimicrobial activity against *Burkholderia glumae*, a gram negative bacillus responsible for causing grain rot, sheath and rice seedling [28]. The samples analyzed were taken from the genebank of the Experimental Station of Rice Victoria National Fund, located in the municipality of Mocarí, from the department of Córdoba. A total of 89 endophytic bacteria isolates from tissues of the rice plant. Four varieties described as Fedearroz 2000 (F2000), Fedearroz 473 (F473), Fedearroz Mocarí (Fmocari) and Fedearroz 733 (F733) were studied. The rice varieties with greater population density of endophytic bacteria were F733 and Fmocari with 1.77×10^{10} and 1.7×10^{10} CFU/g tissue, respectively, whilst the F273 and F2000 varieties had densities of 2.0×10^7 and 1.56×10^7 CFU/g tissue. Of the 89 isolated morphotypes, 28 showed antibacterial activity in vitro against *B. glumae*. Morphotypes isolated of stem showed higher inhibitory activity than morphotypes of roots. Rice varieties with higher density of associated bacteria have a higher tolerance to the disease of bacterial panicle blight, when compared with those with lower count of bacteria endophytes [29]. In this work, we comprise the importance of studying endophytic microorganisms and their potential applications, since one or more of these microorganisms may have secondary metabolites or develop strategies to combat pathogens of host plants, as exemplified above by the 28 endophytes, which presented activity against pathogenic *B. glumae*.

Pérez-Cordero et al studied the resistance to lead in endophytic bacteria isolated from commercial rice varieties. The samples analyzed were obtained from commercial varieties grown in the Experimental Station "La Victoria del Fondo Nacional del Arroz", located in the city of Montería, Córdoba. A total of 168 morphotypes of endophytic bacteria from roots, leaves and tillers were isolated from the varieties called Fedearroz 2000 (F2000), Fedearroz 473 (F473), Fedearroz Mocarí (Fmocari) and Fedearroz 733 (F733). In this study, the highest population density was observed in the root (3.045×10^{10} CFU/g tissue) compared to tillers (4.35×10^9 CFU/g of tissue) and leaves (7.34×10^8 CFU/g tissue). The highest bacteria density was observed in the varieties F733 (2.12×10^{10} CFU/g of tissue) and Fmocari (2.09×10^{10} CFU/g tissue) compared to the varieties F2000 (1.56×10^7 CFU/g of tissue) and F473 (2.07×10^7 CFU/g tissue). Among all the morphotypes isolated,

only two were able to grow in different concentrations of $Pb(NO_3)_2$; which were identified as *Pseudomonas putida* and *Burkholderia cepacia* by the gallery system API20E and compared to BioMerieux database, St Louis, MO, USA. The morphotype identified as *P. putida* showed higher growth in treatments with Pb than *B. cepacia* [30].

Another example is the Colombian coffee, which is recognized worldwide for their superior quality product and one of the best in the world in terms of aroma and flavor. The Colombian coffee area corresponds to approximately 869,158 hectares and 566,000 families engaged in cultivation. Around one million people are economically dependent on the process-related activities, marketing and export of coffee. This agricultural and industrial activity always had a significant economic importance in the country, representing 4% of the total GDP in the last decade [31]. Vega and contributors isolated endophytic bacteria in stem, leaves and berries of *Coffea arabica* L. collected in Colombia, Hawaii and Mexico. Colombia samples were obtained from the National Coffee Research Center, CENICAFE, Chinchiná, city of Caldas department. Among the 87 endophytic bacteria isolated in this study, 67 were isolated from coffee plants grown in Colombia, which belong to the genera Bacillus, Burkholderia, Cedecea, Chromobacterium, Curtobacterium, Enterobacter, Escherichia, Klebsiella, Methylobacterium, Pseudomonas, Serratia, Stenotrophomonas and Variovorax. The different bacterial genera associated with coffee plants allow the development of new researches in the sense of to determine the interactions between endophytic bacteria, their host plants and others endophytes, production of metabolites, among others [32].

The grass "Colosoana" (*Bothriochloa pertusa*) has colonized most of Colombian grasslands, and despite its immature leaves have crude protein levels of 12% and digestibility of 60 to 70%, they are reconsidered by some farmers as an undergrowth vegetation [33]. Pérez et al analyzed the endophytic flora of grass grown on cattle farms located in the municipalities of Corozal, Sampués and Tolu in Sucre, Colombia. The population density for endophytic bacteria isolated from Colosua grass roots ranged from 3.1 to 6.7×10^5 CFU, 4.2 to 6.7×10^5 CFU and 3.2 to $5, 0 \times 10^5$ CFU for the Corozal, Sampués and Tolu samples, respectively. The study showed no significant differences between total CFU. Moreover, the highest density of isolated endophytic bacteria was found in Sampués [34].

Endophytic fungi isolated from plants grown in Colombia

The flower industry in Colombia emerged facing the external market, and it is the most important production of non-traditional exports in the country. Colombia ranks the second in the world in the export of flowers, only behind the Netherlands [35]. The flower production is mainly based on carnations and roses, which are the most extensive and diversified cultures [36]. Salgado Salazar et al isolated some endophytes from the rose (*Rosa hybrida*) leaves grown in urban Bogotá, and in the northeastern and northwestern areas. From the 560 sub-fragments of leaves inoculated in culture media, only 92 were colonized by endophytes. By conventional methods using taxonomic keys, 31 isolates were identified to genus or species. Among the isolated genera were described Acremonium, Alternaria, Aureobasidium, Cladosporium, Chaetomium, Gliocladium, Nigrospora, Nodulisporium, Phoma, Xylaria and Coelomycete. The lower number of isolates obtained, when compared to the other studies, could be related to the characteristics of the investigated plants, not being native and located in this particular case, in the city of Bogotá, a completely urbanized area with high levels of air pollution. Endophytes reported here may have a great potential

for the future tests of antagonism against plant pathogens [37].

Colombia has 4,270 registered species of orchids, of which 1,572 are unique to the country. Their great diversity coupled with the beauty of their flowers have made their cultivation and exportation commercially and economically interesting for the country [38,39]. Lizarazo-Medina et al. determined that the diversity and the composition of the endophytic mycobiota from leaves and roots of *Cattleya perivaliana* and *Cattleya trianaei* grown in the greenhouse El Cerro of the Colombian Orchid Society, located in Fredonia (Antioquia), Colombia. They were isolated a total of 323 fungi species from 1,200 fragments of leaves and roots. These species were classified taxonomically considering the macroscopic and microscopic characters in 14 genera and five morphotypes. Fungal isolates belong to the genera *Colletotrichum*, *Fusarium*, *Sclerotium*, *Botryotrichum*, *Aureobasidium*, *Chromelosporium*, *Gonatobotrys*, *Monilinia*, *Cladosporium*, *Curvularia*, *Gloeosporium*, *Trichoderma*, *Exophiala* and *Nodulisporium*. *Fusarium* was the most abundant in roots for all species, while in the leaves the most abundant were *Colletotrichum* and *Sclerotium* [39]. Ordóñez et al isolated and identified root endophytes in orchids of *Vanilla* genus in the wild, in order to determine their effect on the growth of *V. planifolia* plants when inoculated into the substrate. *Vanilla* sp. plants were collected in the Gulf of Morrosquillo and Montes de Maria (Sucre), Sierra Nevada de Santa Marta (Magdalena), San Pedro de Uraba, San Luis, San Jeronimo and Porce (Antioquia), Yopal (Casanare), Serranía de la Macarena (Caquetá) and Buenaventura (Valle del Cauca). By sequencing the ITS regions, the fungi were identified as *Ceratobasidium*, *Phomopsis*, *Hypoxylon*, *Xylariaceae*, *Phoma*, *Trichoderma* and *Bipolaris*. Biomass and growth of orchid plants inoculated with different isolated fungi showed significant differences for the variables height, root length, root mass and leaf mass, which highlights the importance of these fungi in the protection and improvement of nutrition in these plants [40].

Phosphorus (P) is an essential macronutrient for the growth and development of plants. Microorganisms play a key role in the cycle of P, in particular, on the release of its organic and inorganic forms through solubilization and mineralization [41,42]. Perez et al isolated fungi that were capable of solubilizing phosphates from Colosua grass root grown on cattle farms located in Sincé, Sucre, Colombia. A total of 43 morphotypes of endophytes were isolated and determined by taxonomic keys: 36 from Deuteromycetes genus, three from *Penicillium*, two *Aspergillus niger*, one *Zygomycetes* and one *Paecilomyces*. The genera of isolated fungi with phosphate solubilizing activity were identified as *Aspergillus*, *Penicillium* and *Paecilomyces* [43].

Vega et al studied the diversity of endophytic fungi of plants grown coffee in Colombia, Hawaii and Puerto Rico. The Colombia samples were taken at the National Coffee Research Center CENICAFE located in Chinchiná, Caldas. A total of 843 endophytic fungi were isolated, of which 267 were obtained from plants cultivated in Colombia (32%). Colombian isolates contained 113 genotypes, a plurality (Fisher's alpha) of 75.3 and the dominant genotype was *Colletotrichum* sp. In addition, they were isolated *Agaricomycetes* sp., *Ascomycota* sp., *Aspergillus* sp., *Beauveria* sp., *Botryosphaeria* sp., *Cercospora* sp., *Cladosporium* sp., *Clonostachys cf. rosea*, *Colletotrichum* sp., *Exobasidiomycetes* sp., *Fusarium* sp., *Hymenochaetaceae* sp., *Hypocreales* sp., *Neosartorya* sp., *Paecilomyces* sp., *Penicillium* sp., *Petriella* sp., *Pezizomycotina* sp., *Phomopsis* sp., *Pleosporaceae* sp., *Pleosporales* sp., *Pseudozyma* sp., *Sordariales* sp., *Sordariomycetes* sp., *Sporobolomyces* sp., *Stereum* sp., *Tilletia*, *Trametes* sp., *Trichoderma* sp., *Xylaria* sp., *Xylariaceae* sp. and *Xylariales* sp. Genotypes isolated from plants grown coffee in Colombia among of the exclusive fungi endophytes of only single tissue [44]

Fungi	Number of genotype	Tissue
<i>Aspergillus fumigatus</i>	1	Leaves
<i>Bauveria</i> sp.	1	
<i>Clonostachys cf. Rosea</i>	1	
<i>Colletotrichum</i> sp.	4	
<i>Aspergillus</i> sp.	1	Steam
<i>Colletotrichum</i> sp.	1	
<i>Trichoderma</i> sp.	1	
<i>Colletotrichum</i> sp.	1	Crown
<i>Homopsis</i> sp.	1	
<i>Tilletia</i> sp.	1	Berry

Table 2: Endophytics fungi from only single tissue of coffee.

(Table 2).

On a previous study, endophytic fungi present in plants of *Coffea arabica*, *Coffea congensis*, *Coffea dewevrei* and *Coffea liberica* collected in Colombia, Hawaii and Maryland were isolated by Vega et al. [45]. The samples analyzed consisted of leaves, roots, stem and various parts of the coffee berry (crown, peduncle, pulp and seeds). Thirteen *Penicillium* endophytic species were isolated from *Coffea arabica* during the study. In the Colombian samples, the isolated species found were *Penicillium brevicompactum*, *P. brocae*, *P. oxalicum*, all from leaves of plants grown in CENICAFE located in Chinchiná, Caldas. The fungus *P. oxalicum* produces ochratoxin A, however, does not pose a risk to human health because of the amount produced is very low (0.037 ppb). The fact that none of the *Penicillium* species are reported as pathogens of *Coffea* spp. implies that these endophytes are not latent pathogens, suggesting commensal relationships or mutuals with coffee plants [45].

Seeds can benefit from microorganisms associated with it, which can play a key role in their preservation and preparation of the medium for germination [46-48]. Seed endophytes are transmitted from generation to generation, thereby ensuring the benefits related to the promotion of growth and biocontrol for future plants [48]. Endophytes of green coffee beans from Guatemala, Colombia, India, Kenya, Papua New Guinea, Puerto Rico and Vietnam were isolated by Vega et al. A total of 19 isolates were obtained during the study; three were isolated from seeds from Colombia and were identified as *Aspergillus tubingiensis*, *Penicillium* sp. and *Gibberella olsonii* [49].

Miles et al isolated endophytic fungi from two species of *Espeletia* (Asteraceae): *Espeletia grandiflora* and *Espeletia corymbosa*, endemic plants of Cruz Verde Paramo, Colombia. The biocontrol ability of the isolated fungi against phytopathogenic fungi was evaluated. A total of 60 endophytic fungi were isolated and identified morphologically in 13 genera: *Aureobasidium*, *Beauveria*, *Chaetomium*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Leptosphaerulina*, *Nigrospora*, *Paecilomyces*, *Penicillium*, *Scopulariopsis*, *Stemphylium* and *Trichoderma*. In addition, isolates were molecularly identified to species level in the following genera (Table 3).

All isolated fungi were tested in biocontrol and many species showed biological activity against several bacterial, fungal and oomycete plant pathogens. In addition, this work demonstrates that the bioactive metabolites are not only produced in the presence of the plant pathogen. This work showed that the two plant species may be sources of numerous microorganisms, which in turn may be sources of a range of bioactive compounds [50].

The plants studied in Colombia related to endophytic microorganisms are illustrated (Figure 1).

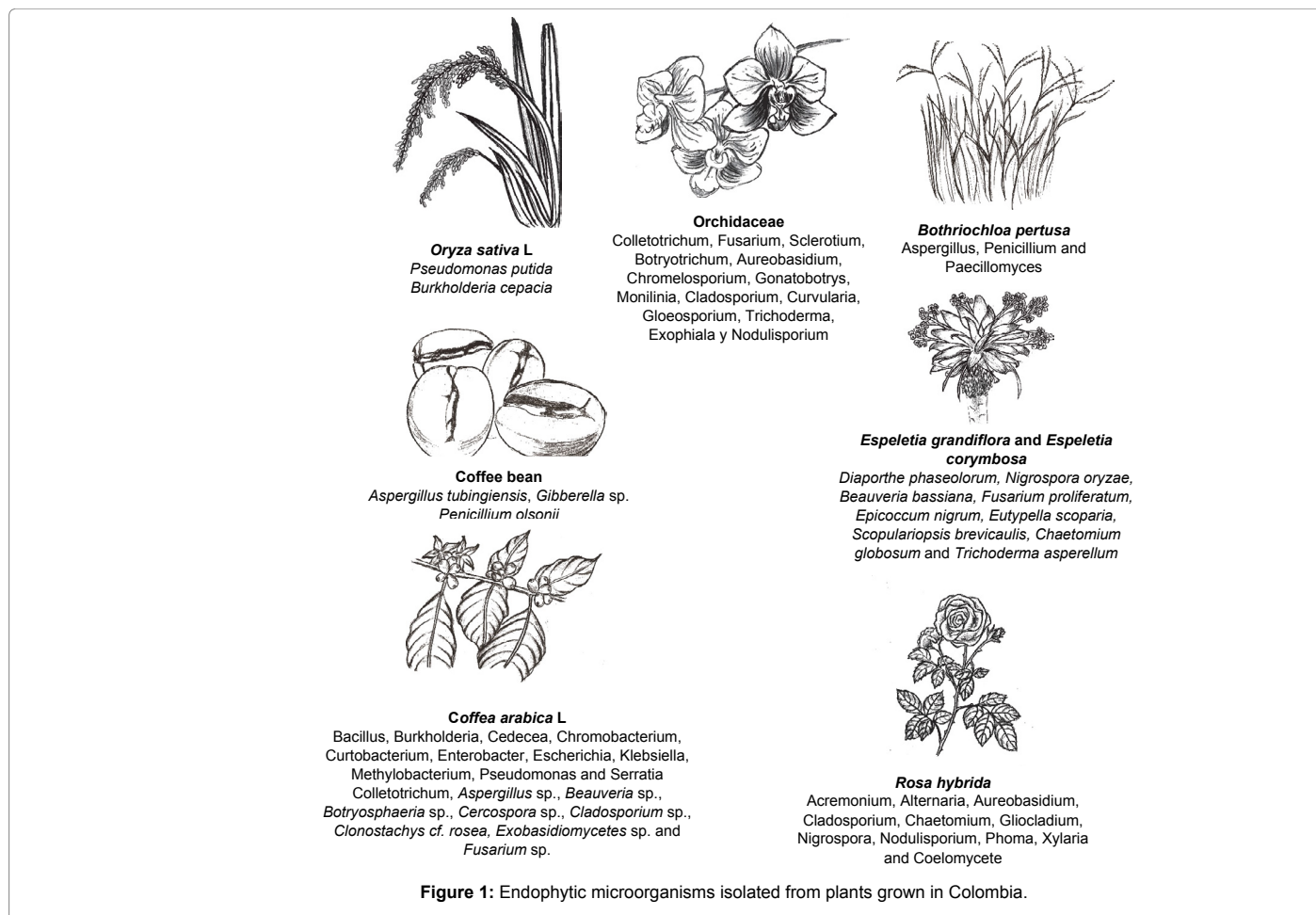


Figure 1: Endophytic microorganisms isolated from plants grown in Colombia.

Fungi	Number of isolates
<i>Diaporthe phaseolorum</i>	22
<i>Nigrospora oryzae</i>	15
<i>Beauveria bassiana</i>	12
<i>Fusarium proliferatum</i>	9
<i>Epicoccum nigrum</i> <i>Eutypella scoparia</i>	4
<i>Scopulariopsis brevicaulis</i> <i>Chaetomium globosum</i> <i>Trichoderma asperellum</i>	3
<i>Aporospora terricola</i> <i>Cladosporium tunuissimum</i> <i>Hypoxyton stygium</i> <i>Leptodontidium orchidicola</i> <i>Leptosphaerulina chartarum</i>	2
<i>Aureobasidium pullulans</i> <i>Bipolaris sorghicola</i> <i>Botrytis fabae</i> <i>Cladosporium cladosporioides</i> <i>Coprinellus micaceus</i> <i>Curvularia oryzae</i> <i>Eucasphaeria capensis</i> <i>Paecilomyces sinensis</i> <i>Paraconiothyrium sporulosum</i> <i>Pestalotiopsis disseminata</i> <i>Phoma glomerata</i> <i>Penicillium commune</i> <i>Stemphylium vesicarium</i> <i>Trichoderma atroviride</i> <i>Xylaria polymorpha</i>	1

Table 3: Endophytic fungi isolated from two species of Espeletia (Asteraceae).

Conclusion

In this short review, we could relate the great biodiversity of plants and endophytic microorganisms available in Colombia to be studied and mainly the few existing studies on these endophytes. Colombia has a wide variety of climates and environments, being bathed by the Pacific Ocean coast and the Caribbean coast, with countless native plants. This review presents its vast unexplored biodiversity and encourages a deeper look at these unstudied microorganisms, which can hide solutions for various diseases, pathogens and industrial processes.

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