



Diversity of Endophytic Fungi of *Psidium guajava* (Myrtaceae) and Their Antagonistic Activity against Two Banana Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MAN and BLN initiated the project, guided the research work and revised the protocol. Author ENT managed the literature searches, wrote the protocol, managed the isolation and identification of endophytic fungi and phytopathogen and antagonistic activity of the study and wrote the first draft of the manuscript. Authors SAYF and FM managed molecular identification of fungi. All authors read, corrected and approved the final manuscript.

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ABSTRACT

Aims: The present study was carried out to determine the diversity of endophytic fungi that colonize the leaves of *Psidium guajava*, and to evaluate their antagonistic activity against *Fusarium oxysporum* f.sp. *cubense* and *Mycosphaerella fijiensis* which are the two main phytopathogens of banana plants.

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Place and Duration of Study: The research was carried out at Microbiology Laboratory, Faculty of Sciences, University of Yaoundé I and Microbiology Laboratory, Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine Bucharest, between April 2018 and February 2020.

Methodology: Fragments of surface sterilized leaves of *Psidium guajava* were inoculated on Potato Dextrose Agar supplemented with chloramphenicol. The isolated and purified endophytic fungi were identified based on their macroscopic and microscopic characters using a mycological atlas as guide. The non-sporulating isolates were identified by comparing the ITS regions of their DNA to those of known fungi registered in the GenBank database. The antagonistic activity of the endophytic fungi isolated against *Fusarium oxysporum* and *Mycosphaerella fijiensis* was screened using dual culture method.

Results: A total of 28 endophytic fungal were isolated from the leaves of *Psidium guajava* corresponding to a colonization frequency of 33.33%. These isolates were identified as: *Aspergillus* sp., *Botryosphaeria* sp., *Fusarium* sp., *Neoscytalidium* sp., *Xylaria* sp., *Phyllosticta capitalensis*, *Cercospora apii*, *Xylaria longipes*, *Phomopsis* sp., *Phomopsis asparagi*, *Aspergillus versicolor*, *Pallidocercospora thailandica*, and *Xylaria grammica* that belonged to the Deuteromycota and Ascomycota divisions. These endophytic fungi inhibited the growth of *Fusarium oxysporum* f.sp. *cubense* and *Mycosphaerella fijiensis* with the percentage inhibition varying respectively from 23.25% to 73.52% and from 21.36% to 100%. The species *Botryosphaeria* sp., *Phomopsis* sp., *Phomopsis asparagi*, and *Xylaria longipes* exhibited the greatest activity.

Conclusion: The leaves of *Psidium guajava* have a fairly varied diversity of endophytic fungi. These endophytic fungi can serve as potential biological control agents against Panama and Sigatoka diseases of banana and also would produce secondary metabolites with antifungal properties.

Keywords: Diversity; endophytic fungi; antagonistic activity; *Psidium guajava*; banana pathogens.

1. INTRODUCTION

The term endophyte is derived from the Greek words, “endon” meaning “within”, and “phyton” meaning plant. Originally, it was coined by Heinrich Anton de Bary in 1866 [1] to define any organism occurring within plant tissues [2]. Endophytes are an endosymbiotic group of microorganisms – often bacteria or fungi – that colonize the inter- and/or intracellular locations of plants [3,4]. Although bacteria and fungi are both able of living as endophytic microorganisms, the majority of the endophyte research have focused on endophytic fungi [5].

Endophytic fungi inhabit a unique biological niche, because of their ability to asymptotically colonize plant tissues [6]. It can colonize host tissues in different organs, including leaves, stems, barks, roots, fruits, flowers, seeds, petioles, inflorescences of weeds, buds, and also dead and hollow hyaline cells of plants [7-11]. During their growth inside the living tissues of the plant, endophytic fungi establish complex relationship with their host plants, which involve mutualism, antagonism, and rarely parasitism [12,13]. Generally, in the symbiotic relationship fungal endophytes receive shelter and nutrients from the host, while the host plant might benefit from an array of attributes that

include: safe guarding against natural enemies such as pathogens and herbivores [14,15]; promoting plant growth [16]; and increasing the resistance of plants to abiotic stress factors such as drought, salinity, variations in temperature and heavy metal toxicity in soil [17]. It appears that fungal endophytes are ubiquitous in nature, as they have been isolated from almost every plant species examined [18]. Of the nearly 300,000 plant species forming the vegetal biodiversity of earth, each individual plant is host to one or more endophytes and can consequently constitutes an opportunity to find new and interesting endophytic microorganisms [19]. In fact, until now, only few of the existing plants have been completely studied in relation to their endophytic content [20]. According to Chowdhary et al. [21], the diversity of fungal endophyte is 7% out of total of 1.5 million fungi species on earth. Therefore, medicinal plants are a promising source for exploration of endophytic fungal diversity.

The endophytic fungi of medicinal plants are important because of their capacity to produce structurally and biologically unique natural bioactive compounds [21-24]. They are known as an important source of various bioactive secondary metabolites which, once isolated and characterized, may also have potential for use in

industry, medicine, and agriculture [19,22]. *Psidium guajava* (*P. guajava*) is a fructiferous tree belonging to the *Myrtaceae* family. It is an important food crop and medicinal plant in tropical and subtropical countries, widely used as food (due to its sweet and vitamin-containing fruits) and in folk medicine around the world [25]. In Cameroonian pharmacopeia, it is used to treat many diseases such as diarrhea, and dysentery. In Togo, a decoct of *P. guajava* leaves is used to treat abdominal pain, diarrhea and digestive mycosis [26-29]. This plant is a source of various phytochemicals such as saponins, triterpenic acids, and flavonoids [25]. Many studies have revealed its antimicrobial activity [29-32]. Additional pharmacological properties attributed to extracts of *P. guajava* include antioxidant, hepatoprotective, antiallergic, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, anticough, anti-inflammatory, antinociceptive, hypoglycemic, and antidiabetic activities, thus, supporting its uses in traditional medicine [25].

Banana constitutes the main fresh fruit subject to significant international trade around the world. Rich in mineral salts and vitamins, it is part of staple diet of millions of people around the world and particularly in Africa. It is cultivated in more than 120 countries in the tropical and subtropical regions across the five continents and especially by small producers for whom its cultivation constitutes a source of employment and income. With a global production of just over 145 million tons, it ranks first in fruit production and is the fourth most important crops in the world after rice, wheat and corn [33]. In Cameroon, the banana sector occupies a prominent place among the main food crops contributing to the country's food security. Its annual production estimated a 1,538,085 tons and contributes to about 6% of the country's agricultural gross domestic product. However, in spite of this capital importance, the cultivation of bananas in Cameroon, as in all the other producing countries of the world, comes up against numerous parasitic constraints like Panama and Sigatoka diseases. The latter two, also known as fusarium wilts and black leaf streak disease, respectively caused by the fungi *Fusarium oxysporum* f.sp. *cubense* (*F. oxysporum*) and *Mycosphaerella fijiensis* (*M. fijiensis*) represent the greatest threats to banana production [34-36].

The present study was carried out to determine the diversity of endophytic fungi from leaves of *P. guajava* and to evaluate the antagonistic

activity of endophytic fungi isolated against *F. oxysporum* and *M. fijiensis* the two main phytopathogens of bananas.

2. MATERIALS AND METHODS

2.1 Collection of Host Plants

Healthy and mature *P. guajava* plant was carefully chosen for sampling. Symptoms-less and apparently healthy leaves were collected at the University of Yaoundé I (Cameroon). The plant parts were brought to the laboratory in sterilized bags and processed within a few hours after sampling (24 hours after collection).

2.2 Isolation and Identification of Endophytic Fungi

2.2.1 Isolation of endophytic fungi

The leaf samples were surface sterilized by the method described by Petrini et al. [37]. Briefly, the samples were washed under running tap water, then the washed leaf samples were treated by the following immersion sequence: 70% ethanol for 2 min followed by 2.5% sodium hypochlorite (NaOCl) solution for 3 min and 70% ethanol for 30 seconds. Then, samples were rinsed in double distilled, sterilized water for a couple of minutes before being dried on a blotting sheet. The surface-sterilized samples were cut, aseptically, into 1 cm × 1 cm length and were placed (5 segments on each plate) in Petri dishes containing Potato Dextrose Agar (PDA) (GMH096-500G HIMEDIA, India) medium supplemented with chloramphenicol (100 mg/L). The Petri dishes were sealed properly with parafilm to avoid desiccation of the medium and any contamination during this period and incubated at 27 ± 2°C for up to three weeks. The Petri dishes were monitored every day to check the growth of fungal colonies from the leaf segments. Individual hyphal tips that emerged from the edges of each treated plant bits were transferred separately onto fresh PDA medium and coded with a unique number until identification. The fungal strains in the pure culture were preserved on PDA slant at 4 to 5°C and were sub-cultured from time to time. The colonization frequency (CF %) of the endophytic fungi was calculated as described below [38].

$$CF = \frac{\text{Number of leaf segments colonized by endophytes}}{\text{Total number of leaf segments analysed}} \times 100$$

2.2.2 Identification of endophytic fungal isolates

Fungal identification was based on the morphology of the cultures and characteristics of the spores. The fungal isolates were characterized macroscopically by observing the top and reverse mycelia characteristic of 7 days old fungal culture on PDA. Fungal hyphae were mounted on microscopic slides and stained with lactophenol cotton blue and examined in 40X light microscopy. The fungal isolates were further identified on the basis of microscopic characters, for spore shape and phenotypic characteristics, for spore type, growth color, rate using standard manual [39]. Those from non-sporulated isolates will undergo molecular identification.

◆ **DNA preparation:** All the selected fungal strains were cultivated in Potato Dextrose Broth (PDB) at 28°C for 5 days. The cultures were centrifuged at 5,000 rpm for 5 min to collect the mycelium which was frozen in liquid nitrogen and ground into a fine powder. About 150-200 mg of this frozen and ground mycelium was used for DNA extraction using the commercial EZNA Fungal DNA kit according to the manufacturer's instructions.

◆ **PCR reaction and DNA digests:** The 5.8S-ITS region of each fungus was amplified by Polymerase Chain Reaction (PCR) using universal fungal primers ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' [40]. The PCR reactions were carried out in 50 µL of mix containing: 1.5 mM MgCl₂, 0.2 mM dNTP, 0.5 mM of each primer, 0.025 U of Taq polymerase and 10 to 30 ng of fungal DNA. The reactions were carried out in a thermal cycler using the program described by Phalip et al. [41]. The PCR products were allowed to migrate (electrophoresis) in 2% agarose gels. After electrophoresis, the gels were stained with ethidium bromide and the DNA bands were visualized under UV light. Sizes were estimated by comparison with DNA size markers.

◆ **DNA sequencing and sequence analysis:** The sequencing was carried out on DNA fragments 5.8-ITS generated by PCR and was done in the two orientations. The primers ITS1 / ITS4 were used. The sequences obtained were analysed by comparison with the sequences available in the National Center for Biotechnology Information (NCBI) Genbank

using the basic local alignment search tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). These sequences were then recorded in the NCBI's online database and accession numbers assigned.

2.3 Plant Pathogens

The banana plant pathogens: *F. oxysporum* and *M. fijiensis* were used in this study to evaluate antagonistic activity of endophytic fungi isolated. *F. oxysporum* was donated by the phytopathology laboratory of African Center for Research on Bananas and Plantains (CARBAP), located in Njombé (Cameroon) while *M. fijiensis* was isolated from banana plants affected by Sigatoka disease (black leaf streak disease).

Fresh and dry leaf samples from banana plants on different stages of infection with *M. fijiensis* were collected. The leaf samples were allowed to discharge ascospores on water-agar plates to produce monosporic cultures according to the method described by Carlier et al., [42]. Single germinated ascospores were transferred to potato dextrose agar (PDA) and incubated at 27°C for 14 days. The pure culture was inoculated on V8 medium for sporulation. The macroscopic characteristics of the cultures on the PDA and the microscopic characteristics of the cultures on the V8 medium allowed the identification of the isolates obtained. The isolate used was confirmed as *M. fijiensis* by molecular identification as described above.

2.4 Evaluation of Antagonistic Activity of Endophytic Fungi

The antagonistic activity of endophytic fungi isolated against the phytopathogens was screened using dual culture method described by Orole and Adejumo [43]. In Petri dishes containing the PDA medium, an explant of a pure culture of endophytic fungus (7 days old) was inoculated at a distance of 4 cm from an explant of pure culture of the phytopathogen (7 or 14 days old). The controls were represented by Petri dishes inoculated only with the phytopathogen. The dishes were incubated at 28°C for 7 to 14 days. The radial growth of phytopathogens was measured and the percentage inhibition calculated by the following formula [43].

$$\text{Percentage inhibition} = \frac{R1 - R2}{R1} \times 100$$

R1= radial growth of the phytopathogen in the control (alone)

R2= radial growth of the phytopathogen in double culture (in the presence of the endophytic fungus)

3. RESULTS

3.1 Identification of Plant Pathogen Isolated: *M. fijiensis*

M. fijiensis isolate appeared olive-white on PDA medium, with a black reverse. The shape of colonies was irregular with a rounded appearance. On V8 medium, *M. fijiensis* produces cylindrical conidia, most often curved and hyaline with their well-marked insertion hilum (Fig. 1).

The identity of *M. fijiensis* was confirmed by amplification and sequencing of its DNA followed by a comparison of the sequence obtained with those contained in the NCBI reference database. The sequence obtained of 488 base pairs has been registered in this database under the accession number MN744740.

3.2 Isolation and Identification of Endophytic Fungi

A total of 28 isolates of endophytic fungi were obtained with colonization frequency of 33.33%. Based on the macroscopic and microscopic characteristics, the 28 isolates obtained were morphologically grouped (Table 1).

The 4 groups (G1 to G4) isolates that produced spores were identified as: *Aspergillus* sp., *Botryosphaeria* sp., *Fusarium* sp., and *Neoscytalidium* sp., All nine groups (G5 to G13) of isolates with sterile mycelia were molecularly identified. The 5.8S-ITS region of their DNA was amplified, sequenced and compared to the sequences recorded in the NCBI reference database. These isolates were identified as

Aspergillus versicolor, *Cercospora apii*, *Pallidocercospora thailandica*, *Phomopsis asparagi*, *Phomopsis* sp., *Phyllosticta capitalensis*, *Xylaria grammica*, *Xylaria longipes*, and *Xylaria* sp. The sequences used to identify these sterile endophytic fungi were then recorded in the NCBI database and their accession numbers assigned (Table 2).

It was noted that 13 groups were identified as belonging to nine genera namely *Aspergillus*, *Botryosphaeria*, *Fusarium*, *Neoscytalidium*, *Cercospora*, *Pallidocercospora*, *Phomopsis*, *Phyllosticta*, and *Xylaria*. The genus *Xylaria* was the most represented with 3 species (23.1%), followed by the genera *Aspergillus* and *Phomopsis* with two species (15.3%) each (Fig. 2). These endophytic fungi isolated and identified belonged to the division of Deuteromycota (23.1%) and Ascomycota (76.9%).

3.3 Antagonistic Activity of Endophytic Fungi

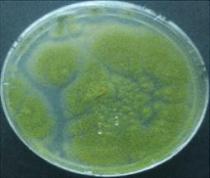
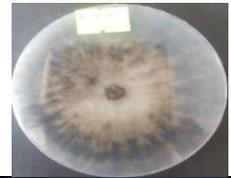
The antagonistic activity of endophytic fungi isolated against *F. oxysporum* and *M. fijiensis* was screened by dual culture method. The results obtained were recorded in the Table 3.

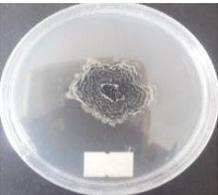
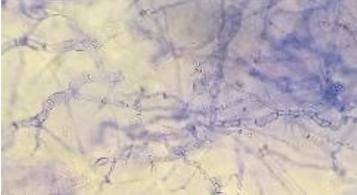
Table 3. shows that all the endophytic fungi exert an antagonistic activity against the phytopathogens tested. The antagonistic activity of these endophytic fungi expressed by the percentages of inhibition varied from 23.25% to 73.52% and from 21.36% to 100% respectively for *F. oxysporum* and *M. fijiensis*. The endophytic fungi *Botryosphaeria* sp., *Phomopsis* sp., *Phomopsis asparagi* and *Xylaria longipes* inhibited the growth of the two phytopathogens used by more than 50%. Fig. 3 below presents the antagonistic activity exerted by some endophytic fungi against the phytopathogen tested.



Fig. 1. Macroscopic (a: front; b: reverse) and microscopic (c) characteristics of *M. fijiensis* after 14 days of incubation

Table 1. Macroscopic and microscopic characteristics of the groups of isolates of endophytic fungi obtained

Group code	Macroscopic features		Microscopic features	Description and identity
G1				Downy and powdery mycelial colonies of green colors, fast growing, septate mycelium, unbranched conidiophore bearing an aspergillus head, presence of many round spores Inference: <i>Aspergillus</i> sp.
G2				Cotton-like colonies, initially white in color, turning to black with age. Fast growing. Septate mycelium, initially hyaline, turning to black with age Inference: <i>Botryosphaeria</i> sp.
G3				White fluffy colonies, septate mycelium, presence of some microconidia, spindle-shaped macroconidia, curved and quite pointed at the ends Inference: <i>Fusarium</i> sp.
G4				Woolly colonies varying in color from white to brown, septate and hyaline hyphae, presence of arthroconidia with a central septum Inference: <i>Neoscytalidium</i> sp.

Group code	Macroscopic features		Microscopic features	Description and identity
G5				<p>Flat colonies with undulate margin, initially white, gradually become greenish to dark green. Septate and sterile mycelium</p> <p>Inference: unidentified</p>
G6				<p>White erumpent colonies with smooth, even margins and moderate aerial mycelium. Slow growing. Septate and sterile mycelium</p> <p>Inference: unidentified</p>
G7				<p>Round and velvety colonies, of white colors. Fast growing. Mycelium formed stomata at prolonged incubation time. Septate and sterile mycelium</p> <p>Inference: unidentified</p>
G8				<p>White and fast-growing colonies, forming growth rings, with sparse and slightly raised mycelium. Branched, septate, hyaline and sterile mycelium.</p> <p>Inference: unidentified</p>
G9				<p>Flat, moderate growing and white colonies becoming dark brown with age. Branched, septate, hyaline and sterile mycelium.</p> <p>Inference: unidentified</p>

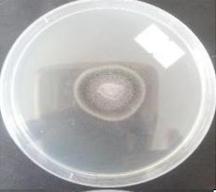
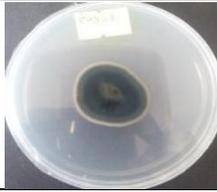
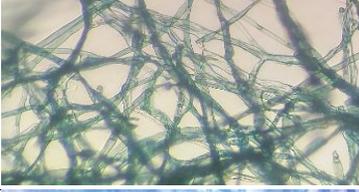
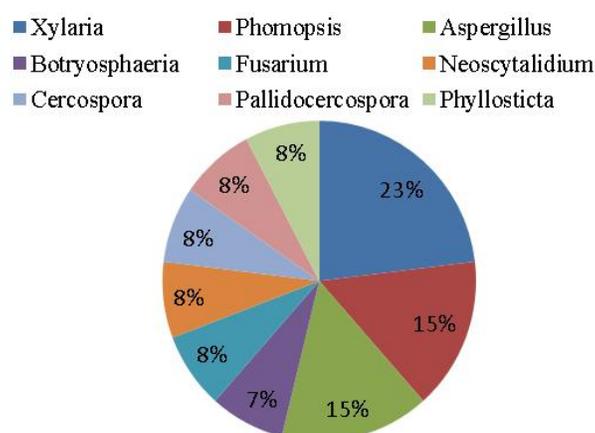
Group code	Macroscopic features		Microscopic features	Description and identity
G10				<p>Slow growing colony varying in color from White to green with a brown underside. Septate and sterile mycelium</p> <p>Inference: unidentified</p>
G11				<p>Colonies erumpent, circular with sparse aerial mycelium and even lobate, of dirty white colors. Slow growing. Septate and sterile mycelium.</p> <p>Inference: unidentified</p>
G12				<p>White, cottony with a yellowish underside, abundant aerial mycelium with regular margin. Moderate growing. Branched, septate and sterile mycelium.</p> <p>Inference: unidentified</p>
G13				<p>White, cottony, abundant aerial mycelium with irregular margin. Moderate growing. Septate and sterile mycelium.</p> <p>Inference: unidentified</p>

Table 2. Identification of groups of sterile isolates of *P. guajava* and their accession numbers

Group	DNA fragments length (bp)	Identity percentage (%)	Identification	Accession number
G5	561	99.67	<i>Phyllosticta capitalensis</i>	MN744737
G6	476	100	<i>Cercospora apii</i>	MN744735
G7	499	98.94	<i>Xylaria longipes</i>	MN744731
G8	494	98.37	<i>Phomopsis</i> sp.	MN744732
G9	528	98.64	<i>Phomopsis asparagi</i>	MN744736
G10	465	99.57	<i>Aspergillus versicolor</i>	MN744734
G11	493	99.40	<i>Pallidocercospora thailandica</i>	MN744739
G12	501	99.32	<i>Xylaria grammica</i>	MN744733
G13	459	97.77	<i>Xylaria</i> sp.	MN744738

**Fig. 2. Different genera of endophytic fungi isolated from *P. guajava* leaves****Table 3. Inhibition percentage of the growth of phytopathogen by endophytic fungi isolated and identified**

Group code	Name	Inhibition percentage (%) against	
		<i>F. oxysporum</i>	<i>M. fijiensis</i>
G1	<i>Aspergillus</i> sp.	39.85	93.09
G2	<i>Botryosphaeria</i> sp.	54.89	100
G3	<i>Fusarium</i> sp.	42.11	100
G4	<i>Neoscytalidium</i> sp.	37.05	100
G5	<i>Phyllosticta capitalensis</i>	23.25	21.36
G6	<i>Cercospora apii</i>	25.41	32.29
G7	<i>Xylaria longipes</i>	73.52	100
G8	<i>Phomopsis</i> sp.	59.88	100
G9	<i>Phomopsis asparagi</i>	57.14	100
G10	<i>Aspergillus versicolor</i>	47.30	40.45
G11	<i>Pallidocercospora thailandica</i>	35.81	36.72
G12	<i>Xylaria grammica</i>	48.37	100
G13	<i>Xylaria</i> sp.	40.63	100

4. DISCUSSION

In recent years, endophytic fungi have captured the attention of researchers because of their ability to produce many natural compounds with

broad spectrum of antimicrobial activities. Living in the internal tissues of plants without harming them, they have been isolated from all plants studied to date [2,44] and it is estimated that each plant would host on average of 50 species

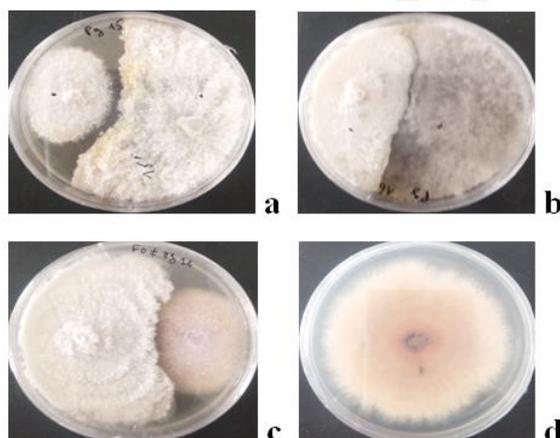


Fig. 3. Antagonistic activity of endophytic fungi against *F. oxysporum* : (a), (b) and (c) : *F. oxysporum* and endophytic fungi in dual culture ; (d): *F. oxysporum* only.

of endophytes [45,46]. In order to increase the probability of discovering new natural compounds produced by these fungi, the selection of plant material for their isolation is governed by assumptions based on: the habitat of the plant, its unusual longevity and its use in traditional medicine [22,47]. Thus, several studies have shown that endophytic fungi isolated from medicinal plants produce important bioactive secondary metabolites that can be used in industry, medicine and agriculture [4,22,48,49]. This justifies the choice of *P. guajava* used in this study. Additionally, *P. guajava* is a medicinal plant that grows in the tropics and subtropics [50,51]. Several studies have shown that plant tissues, in particular leaves, are excellent reservoirs of endophytic microorganisms [52,53]. Healthy leaves of *P. guajava* have been selected for the isolation of endophytic fungi. This is because plant leaves exhibit a rich diversity of endophytes and are easy to handle compared to other parts [54].

Isolation of endophytic fungi from leaves of *P. guajava* showed a percentage colonization rate of 33.33%. The macroscopic and microscopic characteristics of the endophytic fungi was used to group the isolates into 13 morphologically distinct groups. Four spore producing genera were identified: *Aspergillus* sp., *Botryosphaeria* sp., *Fusarium* sp., and *Neoscytalidium* sp.. Lack of sporulation is a common problem in the identification of endophytic fungi, because their identification by traditional methods is difficult [55]. Hence, the use of barcodes of their DNA seems to be an effective means for a consistent and precise

identification [56,57]. Indeed, the ITS1-ITS2 intergenic regions of DNA, combined, have been proposed as a 'barcode of life' for animal species, plants and fungi [58]. Thus, PCR followed by sequencing make it possible to compare the informative sequences contained in the ITS regions of an unknown fungal sample with the sequences identified and listed in a database [59]. This molecular method allowed the identification of the non-sporulating nine groups of endophytic fungi isolates of *P. guajava* leaves as *Phomopsis* sp., *Xylaria longipes*, *Pallidocercospora thailandica*, *Xylaria grammica*, *Aspergillus versicolor*, *Cercospora apii*, *Phomopsis asparagi*, *Phyllosticta capitalensis*, and *Xylaria* sp.. Our results differ from those obtained by Enyi et al. [60] and Susilawati et al. [61] who in Nigeria and Indonesia respectively isolated 2 and 4 endophytic fungi from the leaves of *P. guajava*. This difference shows that the colonization of plants by endophytic fungi varies from one locality to another. Similarly, several studies have shown that the endophytic colonization of plants is a function of environmental factors such as temperature, precipitation and atmospheric humidity as well as the age of the plant [45,62-66].

These fungi identified in this study have also been reported as endophytes of several other medicinal plants by other researchers [67-74]. The endophytic fungi isolated in this study belong to 9 genera: *Aspergillus*, *Botryosphaeria*, *Fusarium*, *Neoscytalidium*, *Cercospora*, *Pallidocercospora*, *Phomopsis*, *Phyllosticta*, and *Xylaria* whose species have already been isolated from different parts of *P. guajava*

[62,75,76]. The genus *Xylaria* is the most isolated followed by the genera *Aspergillus* and *Phomopsis*. These genera are among the most dominant genera among endophytes, and their species generally adapt to different plant tissues [55,77]. Fungi of the genera *Xylaria* and *Phomopsis* have been reported to be dominant components of tropical plants [78,79]. All these isolated and identified fungi belong to the divisions of *Deuteromycota* and *Ascomycota*. Previous studies have shown that these divisions contain more than 85% of endophytic fungi [55,80-82].

Endophytic fungi are studied for their multiple antimicrobial properties, including their antifungal activity. This has been demonstrated in several studies [83-88]. The *in vitro* screening of this antifungal activity is largely carried out by the double culture method (direct confrontation test). The results obtained from this direct confrontation test showed that all the endophytic fungi isolated inhibited the mycelial growth of *F. oxysporum* and *M. fijiensis*, thus reflecting their antifungal activity against these phytopathogens. This antifungal activity was marked either by the presence of a fine clear halo between the two fungi in double culture (endophytic fungus-*F. oxysporum*), or by the total invasion of the phytopathogen (*M. fijiensis*) by the endophytic fungus. This suggested that production of bioactive compounds as a result of competition for space and substrate as well as mycoparasitism are the mechanisms by which these isolated endophytic fungi exert their antifungal activity against the phytopathogens tested. Indeed, the antagonistic activity of endophytic fungi is based on several mechanisms such as competition for space and substrate, mycoparasitism, or the production of bioactive metabolites [89]. Percentages of inhibition of mycelial growth of phytopathogens greater than 50% was obtained with *Botryosphaeria* sp., *Phomopsis* sp., *Phomopsis asparagi*, and *Xylaria longipes*. These percentages of inhibition are comparable to those obtained by Taribuka et al. [84] with endophytic fungi of the genus *Trichoderma* against *F. oxysporum* sp. *cubense*. Furthermore, in a similar study conducted by Villamizar-Gallardo et al. [85], the results showed that the growth of *Phytophthora palmivora* and *Moniliophora roreri*, two cocoa pathogens, was respectively inhibited by 82.3 and 80.7% by an endophytic fungus of the genus *Botryosphaeria* (*B. quercum*). Likewise, the antagonistic activity of the endophyte *Xylaria* sp. against *Fusarium solani*

was demonstrated in the study by Hamzah et al. [83].

5. CONCLUSION

The results obtained showed that the leaves of this plant are colonized by *Aspergillus* sp., *Botryosphaeria* sp., *Fusarium* sp., *Neoscytalidium* sp., *Phomopsis* sp., *Aspergillus versicolor*, *Cercospora apii*, *Pallidocercospora thailandica*, *Phomopsis asparagi*, *Phyllosticta capitalensis*, *Xylaria grammica*, *Xylaria longipes* and *Xylaria* sp. that belonged to the *Deuteromycota* and *Ascomycota* divisions. These endophytic fungi inhibited the growth of *F. oxysporum* and *M. fijiensis*. The species *Botryosphaeria* sp., *Phomopsis* sp., *Phomopsis asparagi* and *Xylaria longipes* exhibited the greatest activity. This antagonistic activity observed in this work shows that endophytic fungi isolated could serve as a potential biological control agent against Panama and Sigatoka diseases of banana and also could produce secondary metabolites with antifungal properties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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