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A preliminary investigation of endophytic fungal diversity at Hope, East Coast Demerara, Guyana

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Abstract

Foliar endophytic fungi spend a part of their life cycle on the leaves of plants. They may demonstrate no apparent symptoms but may also cause disease at a later time in the plant's life. Studies investigating foliar fungal endophytes of mangroves are limited. Therefore, the purpose of this study was to investigate the foliar fungal endophytes present on the leaves of three mangrove species: namely Red mangrove (*Rhizophora mangle*), Black mangrove (*Avicennia germinans*) and White mangrove (*Laguncularia racemosa*). The study site was an area located at Hope, East Coast Demerara, and South America, Guyana. Out of sixty (60) leaf samples that were prepared, fourteen (14) fungal isolates were identified. Most of the fungi isolated in the study were found to be Hyphomycetes (*Aspergillus, Fusarium, Penicillium, Alternaria, Cladosporium and Curvularia*) while the others were Zygomorphic (*Mucor* and *Rhizopus*). The ANOVA calculations for the isolates from the three mangrove species were found to not be statistically significant. *R mangle* was the preferred host out of the three (3) species. The findings of this study confirm that mangroves have rich endophytic diversity and demonstrate rich research and biochemical potential.

Keywords: Endophytic fungi; Foliar fungi; Leaf Endophytes; Mangroves; Mangrove endophytes; Host preference

1. Introduction

There are three (3) main types of mangroves that are found in Guyana namely Black mangrove (*Avicennia germinans*), White mangrove (*Laguncularia racemosa*) and Red Mangrove (*Rhizophora mangle*) [1]. Fungi affecting mangroves can be divided into three (3) broad groups: saprophytic, endophytic and pathogenic [2]. In particular, endophytic fungi are those fungi that invade plant tissues during their life cycle, but do not cause disease symptoms [3]. However, some are latent pathogens that can cause disease at a later time [3] [4].

The term endophyte has evolved over the years to refer not only to the location of the organism but the type of association that the fungi or bacteria have with the host [5]. Generally, the term is taken to mean a mycelial form in biological association with the living plant, at least for some period in aerial plant tissues [3] [5]. Fungi occur in tropical forests with a high diversity of plant species, and it is said that in over 300, 000 plant species, each is host to endophytes [5]. Studies on endophytic fungi in mangroves have been reported but studies on endophytic fungi in the tropics are scarce. In the published Checklist for Endophytic Fungi in the Tropical Regions, it was found that out of over one hundred species of fungi, eight (8) affected members of the Family Combretaceae, eight (8) also affected the Family Rhizophoraceae, and only three (3) affected the Family Verbenaceae [5]. The frequency of occurrence of fungi has been reported based on the percentage occurrence of fungi which are placed in groups according to artificially made frequency groupings. Additionally, the host species to which samples belong is largely responsible for the frequency of occurrence of fungi [6].

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There are several factors that influence the distribution of fungi on a plant. These can range from host species, the status of the host (example infected or disease free), the geographical location of the host, and additionally the amount of samples [5]. Leaf endophytic fungi display host specificity and host preference. However, it is not unusual for one endophyte to be present on more than one host [7]. Endophytes in plants are very diverse and include dominant taxa and rare taxa. The dominant taxa are usually found in only a single or a few host species and can be deemed as 'host specific.' On the contrary, many of them can be found and extracted from several hosts and are known to be 'accidental endophytes'. Usually, the term 'host preference' is used to refer to the endophytes that are not usually specific to a particular host plant [8] [9].

This study is scientifically significant since studies of this nature are not common. As such, the results would make a significant contribution to the endophytic fungal diversity of the Caribbean and subsequently the world. This research is important scientifically because it lends to the increasing knowledge of mangroves in Guyana. Mangroves have become a focal point locally since the intervention of the Guyana Mangrove Restoration Project (MRP) and the Low Carbon Development Strategy of Guyana. Currently, nothing is known about the fungal pathogens affecting mangroves in Guyana. Therefore, the results serve as a much needed catalyst in further research endeavors in the area of potential diseases that can affect mangroves in Guyana.

2. Material and methods

2.1. Site of work

This project was carried out in December, 2014, at a mangrove site at Hope, East Coast Demerara, Guyana, and growth of samples occurred in the Biology Laboratory of University of Guyana, Turkeyen Campus. The Hope location was selected because all three (3) of the mangrove species mentioned earlier occur sympatrically. Moreover, the area was one of the first areas replanted with mangroves by the Guyana Mangrove Restoration Project (GMRP) [1], and could possibly hold rich biological data.

2.2. Materials

Ziplock bags, Cooler, Ice, Field knife, Agar, Petri dish, Ruler, Petri dish, Sodium hypochlorite solution, Ampicillin, Deionized water, Incubator

2.3. Sampling method

Stratified random sampling was used to collect leaf samples. Sixty (60) mangrove leaf samples were collected from equal numbers of mangrove species plants (twenty of each of the three species). The trees selected were between fifteen (15) and seventy (70) cm in diameter and ten (10) to forty (40) metres in height.

2.4. Culturing and identification of samples

Samples of collected mature leaves were transported to the laboratory located at University of Guyana Turkeyen Campus. The median portion of the leaf was cut into 5 mm squared segments, sterilized in 70 percent ethanol, 0.5 percent sodium hypochlorite solution and then rinsed in sterile water [7]. Two segments per leaf were selected and divided between petri plates with prepared Potato Dextrose Agar and incubated for fifteen (15) days. The fungi growing from different segments isolated at 3, 7 and 15 days portion of the plate from which it came was discarded after isolation to prevent overgrowth of cultures. Six weeks after isolation, the fungi were classified [4] [7].

3. Results and discussion

The aim of this research was to isolate and determine the endophytic fungal species present on three (3) different mangrove species.

From Table 1, it can be seen that fourteen (14) species of endophytes were isolated from sixty (60) leaf samples that were prepared. One hundred (100) percent of samples had endophytic growth. These results are similar to those of other studies which prove that a large sample size does not mean that there would be a large number of isolates. Therefore, the smaller the tissue fragment size, the greater the chance of species richness and genotype diversity, because there would be those species that are not widespread and those that are slow growing that would be recovered [8]. Endophytes are sensitive to growth on Agar and are better observed directly on host surfaces.

Table 1 Endophytic Fungal species found on leaves of mangrove species

Endophytic Fungal Species		Mangrove species isolated from
1	*Alternaria alternata*	*Laguncularia racemosa*
2	*Alternaria solanae*	*Rhizophora mangle*
3	*Aspergillus flavus*	*Avicennia germinans, Laguncularia racemosa, Rhizophora mangle*
4	*Aspergillus fumigatus*	*Rhizophora mangle*
5	*Aspergillus niger*	*Avicennia germinans, Laguncularia racemosa, Rhizophora mangle*
6	*Cladosporium*	*Avicennia germinans, Laguncularia racemosa*
7	*Curvularia*	*Rhizophora mangle*
8	*Fusarium proliferatum*	*Rhizophora mangle*
9	*Mucor*	*Rhizophora mangle*
10	*Penicillium*	*Avicennia germinans, Laguncularia racemosa, Rhizophora mangle*
11	*Rhizopus stolonifer*	*Rhizophora mangle*
12	*Mycelia sterilia dark I*	*Avicennia germinans, Rhizophora mangle*
13	*Mycelia sterilia dark II*	*Laguncularia racemosa, Rhizophora mangle*
14	*Mycelia sterilia dark III*	*Avicennia germinans, Laguncularia racemosa, Rhizophora mangle*

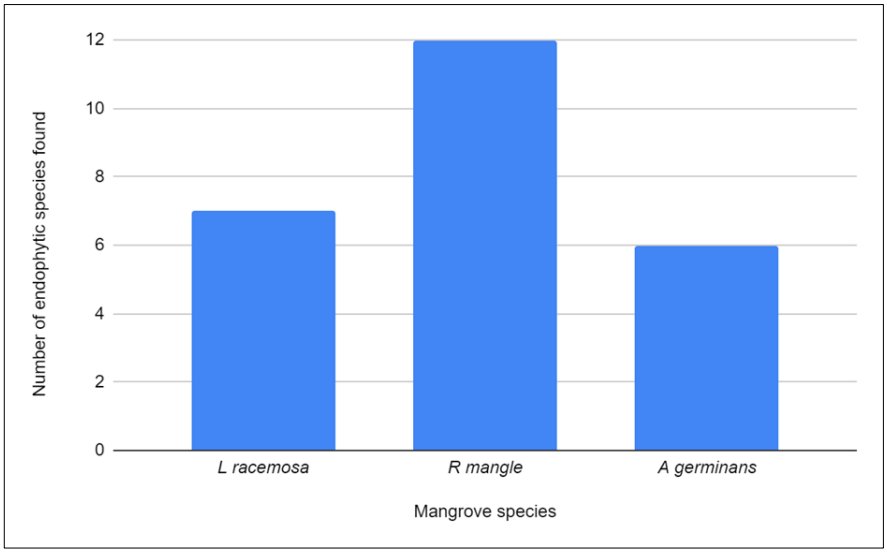

Figure 1 Number of endophytic species found per mangrove species

As such, some species actually resist in-vitro culture which includes the fragment plating method (as used in the study). It is likely that *the in vitro* method underestimated the true diversity and richness of endophytes [9].

Figure 1 above shows that *R mangle* had the highest number of endophytic species found while *A. germinans* had the lowest. In this study, it was found that the host specificity rate was very low. As such, the term 'host preference' is more

applicable since the endophytic species present on *Rhizophora mangle* are not known to be generally host specific. Rather, they preferred *Rhizophora mangle* because they facilitate endophytic growth [8].

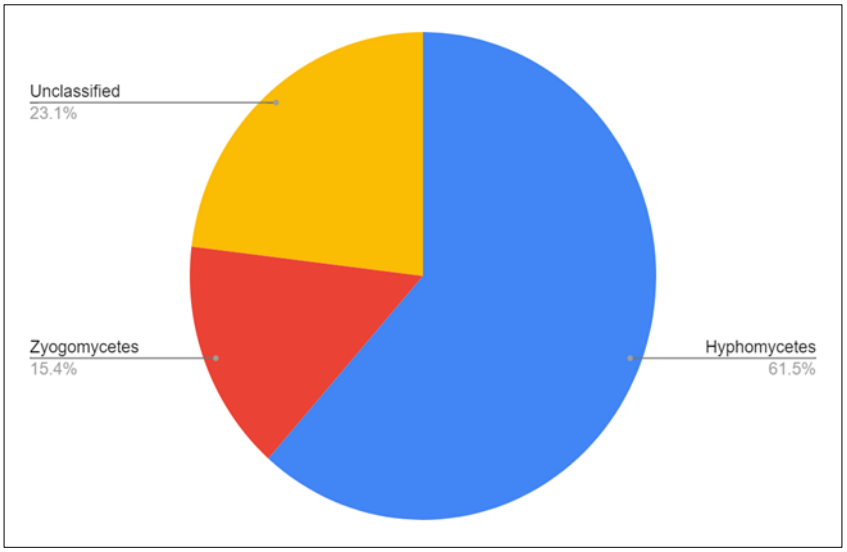

Figure 2 Distribution of Fungal Groups of isolates recovered in study

Most of the fungi isolated in the study were found to be Hyphomycetes (*Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Cladosporium* and *Curvularia*) while the others were Zygomorphic (*Mucor* and *Rhizopus*). Hyphomycetes are a group of fungi that are widespread and are known as primary pathogens. As primary pathogens, the spread of disease on their host is a part of the completion of their life cycle [10].

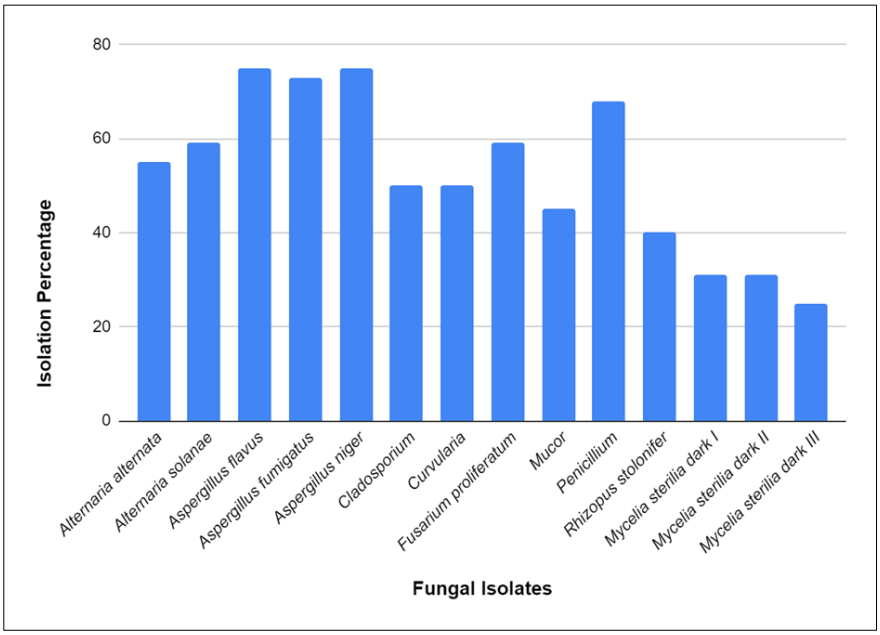

Figure 3 The isolation percentage for the foliar fungal endophytes

Our results here are similar to that of [5], [6], and [9], who found that in mangroves distributed in India, out of the 118 species isolated, *Aspergillus* was most common, followed by *Penicillium*, *Curvularia* and *Alternaria*. However, *Aspergillus* and *Penicillium* are considered as surface contaminants if the sterilization was not effective. Therefore, it is inconclusive if these large numbers of *Aspergillus* and *Penicillium* could be attributed to either improper sterilization or an actual reflection of endophytic species diversity [9].

4. ANOVA calculations for endophytic species

The ANOVA calculation for the number of endophytic species was found to not be statistically significant with a P value of 0.85.

5. Conclusion

This study investigated the preliminary results of the foliar fungal endophytes at an area of Hope, East Coast Demerara, and Guyana. Each sample examined was host to foliar fungal endophytes. There were fourteen (14) species that were isolated and these were: *Alternaria alternata*, *Alternaria solanae*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium*, *Curvularia*, *Fusarium proliferatum*, *Mucor*, *Penicillium*, *Rhizopus stolonifer*

Mycelia sterilia dark I, *Mycelia sterilia* dark II, and *Mycelia sterilia* dark III. Out of these endophytes, the endophytes most frequently isolated were *Aspergillus flavus*, *Aspergillus niger* and *Penicillium*. *R mangle* was most diverse in endophytic species while *Avicennia germinans* was lowest. *Aspergillus niger*, *Penicillium* and Mycelia sterilia Dark III were isolated from all three mangrove species.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Kimberly Craig and Abdullah Ansari hereby declare that there is no conflict of interest in this manuscript.

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