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(RESEARCH ARTICLE)

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Fungal diversity in selected coastal mangrove ecosystems in East Berbice Corentyne, Guyana, South America

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Abstract

There is limited data on the fungal diversity in mangrove ecosystems in Guyana. This study investigated fungal diversity during the dry season in three selected coastal mangrove ecosystems along the Corentyne Coast of East Berbice, Guyana. Sampling was conducted within randomly established 50 m x 50 m plots containing 20 mini transects of length 12.5 m x 10 m in the overwash mangrove ecosystem at each of six study sites within the three study locations. Samples retrieved from the study plots included leaf, bark, soil and fruiting bodies. These were placed into separate bags and labelled appropriately. Environmental parameters were recorded at each study plot. Fruiting bodies were used to identify macrofungal species, and the leaf, bark and soil were used to prepare cultures from which microfungal samples were identified. The species that were identified were used to compile a checklist of fungal species, and diversity indices were calculated. A total of thirty (30) species were identified from macrofungal fruiting bodies retrieved at the study sites. The results further revealed that an increase in pH and salinity and a decrease in temperature resulted in an increase in species richness. Tidal activity also appeared to reduce species richness. *Aspergillus, Rhizopus* and *Trichoderma* were the dominant species at the three study sites with high relative species abundance. There also appeared to be some instances of substrate specificity.

Keywords: Fungal diversity; Mangroves; East Berbice; Guyana

1. Introduction

Mangroves are commonly found in the tropical or subtropical coastal regions across the world [1]. They have physically adapted their leaves, roots and reproductive methods to survive in the harsh, dynamic environments of soft, low oxygenated soils and varying salinity [2]. One of mangroves' most essential roles is protecting vulnerable coastlines from wave action, thereby preventing coastal erosion.

The coast of Guyana is populated in some areas with mangrove trees that serve as a habitat for terrestrial and aquatic organisms. Some of these organisms utilize the roots, leaves, stems or bark of these plants as growth media, as these are ideal for their growth and feeding habits [3] [4].

One group of organisms that utilize debris in the mangrove ecosystem is fungi. These eukaryotic organisms digest food externally and absorb nutrients directly through their cell walls. Fungi contribute to the balance of the overall ecosystem, play an important role in nutrient cycles and support the mangrove ecosystem by helping with the decomposition of fallen leaves, broken roots, dead bark, and any other organic material depleted of life [5][6]. Mangroves and fungi share a mutualistic relationship with the waste from fungi's external digestion providing nutrient

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for the mangroves and organisms. Although the mangrove forest is a habitat to many organisms, fungi inclusive, these organisms must be compatible enough to live in extreme conditions of high salinity, moisture and tidal activities.

There is limited information on fungi in mangrove ecosystems from Guyana. This research was conducted to undertake a preliminary study to investigate this gap and to provide some information about the fungal diversity in local mangrove ecosystems in East Berbice Corentyne, Guyana. The mangrove ecosystems that were investigated represented mixed stands of Black mangroves (*Avicennia germinans*) and White mangroves (*Laguncularia racemosa*) with a predominance of Black mangroves.

This study has the potential to add to the body of knowledge on fungi in Guyanese mangrove ecosystems and contribute information to assist the Guyana Mangrove Restoration Project (GMRP) in their efforts to understand the dynamics in local mangrove ecosystems. The results from this study can be a useful baseline for understanding fungal diversity in local mangrove ecosystems, contribute information to inform decisions to promote conservation of mangrove and reduce mangrove forest degradation in Guyana.

2. Material and methods

2.1. Study Location

This research was undertaken at three different locations along the Coast of East Berbice Corentyne (Region 6). The study locations were the mangrove forest at Palmyra, Bushlot Beach, and Wellington Park and two study sites were identified from each location.

2.2. Sampling Method, Collection and Storage of samples

A 50m x 50 m plot was randomly established in each study area and 20 mini transects of 12.5 m x 10 m were demarcated within each study plot. One sample of leaf, bark and soil was retrieved from each mini transect and placed in labelled Ziploc bags to prevent contamination and facilitate easy transportation to the laboratory. All samples were stored in a cooler at 22° C - 24° C to increase the survival rate of the hyphae and spores. Simultaneously, three parameters (temperature, salinity and pH) were recorded *in situ*.

Visible macro-fungal fruiting bodies within each study plot were collected, placed in labelled bags, stored in a cooler at 22°C - 24°C and taken back to the laboratory, where they were identified using standard identification keys.

2.3. Laboratory Work

Standard protocols for undertaking microbiological work were adhered to before, during and after implementing the methodology. The methodology employed was adapted from [7]. Leaf and bark samples collected were washed in separate beakers using 10 ml of distilled water. Five (5) grams of each soil sample were diluted in a beaker using 10 ml of distilled water.

Nutrient Potato Dextrose Agar was used as the growth medium in this research. One (1) 500 mg Ampicillin (antibiotic) tablet was placed into the liquid agar and dissolved before pouring into the Petri plates. The antibiotic was used to reduce bacterial growth in the agar plates. By adhering to proper plating techniques, the samples were then plated on Potato Dextrose agar, labelled accordingly and placed under 25° C - 27° C for 72 hours. Microbial colonies that developed after incubation were sub-cultured on PDA slants to obtain pure cultures.

2.4. Identification of pure cultures

After sterilization, microscope slides were prepared for each pure culture obtained and prepared slides were examined under the microscope. Characteristics such as spore shape and hyphae structure were used to categorize the specimen and visible morphological characteristics were used for identification and verified by the use of taxonomical keys [8] [9] [10].

2.5. Identification of fruiting bodies found at each sampling site

The identity of fruiting bodies found at each sampling site was determined by their morphological characteristics using the keys [9] [11] [12] [13].

2.6. Data Analysis

All statistical analyses and calculations were done using the Statistical Package for Social Sciences Version 24 (SPSS v24).

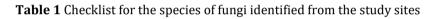
3. Results and discussion

The aim of this study was to conduct a preliminary investigation to identify and document fungal species and fungal diversity in mangrove ecosystems at three study locations along the coast of East Berbice Corentyne, Guyana. The mangrove forests at Palmyra, Bushlot Beach, and Wellington Park were sampled in the dry season during the months of July, August and September.

3.1. Species of fungi recorded from study sites

A total of thirty (30) species of fungi were identified (Table 1). Nine (9) species of microfungi belonging to eight (8) different genera were recorded from the cultured samples. Three of these fungi were Yeast based, but their specific identification was not possible and they were identified based on their colour as Cream-coloured yeast, White-coloured yeast and Pink-coloured Yeast.

The cultures prepared from the leaves, bark and soil obtained from the study sites yielded nine (9) fungal species (Table 2). *Aspergillus spp., Trichoderma spp.,* and *Rhizopus spp.* were found to be the dominant species identified from all of the substrates.



No.	Domain	Kingdom	Division	Class	Order	Family	Genus	Species
1								spp.
2						Amanitaceae	Amanita	
3						Amamateae	Amamita	spp.
4					Agaricales			spp.
5						Agaricaceae		spp.
6								spp.
7								spp.
8							Agaricus	spp.
9								spp.
10				es			Lycoperdon	spp.
11				lycet		Mycenaceae	Mycena	spp.
12				Agaricomycetes				spp.
13				Agar	Cantharellales	Cantharellaceae	Cantharellus	spp.
14					Polyporales	Polyporaceae	Hexagonia	spp.
15			æ	Basidiomycetes	Polyporales			spp.
16	ic		Basidiomycota		Polyporales			spp.
17	Eukaryotic	ji	diom		Polyporales			spp.
18	Euka	Fungi	Basic		Unknown A			spp.

19					Unknown B			
20					Unknown C	spp.		
21				Pezizomycetes	Pezizales	Pezizaceae	Peziza spp.	spp.
22				Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	spp.
23								spp.
24							Penicillin	spp.
25			Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	spp.
26						Nectriaceae	Fusarium	spp.
27		Asco		Saccharomycetes	Saccharomycetales	ist		
28					White Colored Yeas			
29					Cream Colored Yea			
30			Zygomycota	Mucormycotina	Mucorales	Mucoraceae	Rhizopus	spp.

Table 2 Species of microfungi recorded from the cultured samples

Comme	Species	Palmyra			Wellington Park			Bushlot		
Genus		Soil	Leaf	Bark	Soil	Leaf	Bark	Soil	Leaf	Bark
Acrestillus ann	Green	+	+	+	+	+	+	+	+	+
Aspergillus spp.	Black	+	+	+	+	+	+	+	+	+
Trichoderma spp.		+	+	+	+	+	+	+	+	+
Rhizopus spp.		+	+	+	+	+	+	+	+	+
Fusarium spp.					+			+		+
Penicillin spp.							+			
Pink coloured Yeast			+			+			+	
White coloured yeast					+	+		+	+	+
Cream coloured yeast					+				+	+

Of the thirty species identified from all sites, twenty-one (21) species of macrofungi were identified from the fruiting bodies collected on-site during sampling (Table 3). Eleven (11) of these belonged to four (4) Genera, four (4) species belonged to four (4) Genera, three (3) species were identified to the order Polyporales and three (3) species remained unidentified and were recorded as Unknown A, B & C (Table 1).

Fungi	# of species	Palmyra	Wellington Park	Bushlot
Amanita spp.	4	✓		
Lepiota spp.	3	~		
Order: Polyporales 1	1	✓		
Order: Polyporales 2	1	~		
Order: Polyporales 3	1		\checkmark	~
Agaricus spp.	2	✓		
Unknown A	1	~		
Unknown B	1	✓		
Unknown C	1	\checkmark		
Peziza spp.	1	~		
Mycena spp.	2	~		
Lycoperdon spp.	1	\checkmark		
Cantharellus spp.	1	~		
Hexagonia hydnoides	1	\checkmark		

Table 3 Macrofungal species identified from the fruiting bodies collected during sampling

3.2. Diversity indices

Diversity indices give a measure of the species diversity in a given ecological community. They are either based on the number of species present (species richness) or the number of individuals per species (species abundance). The Shannon index assumes all species are represented in a sample and randomly sampled, whereas the Simpson index, a dominance index, gives more weight to common or dominant species. Table 4 compares the species index of diversity for the cultured samples for the different study sites using Simpson's Index of Diversity (D).

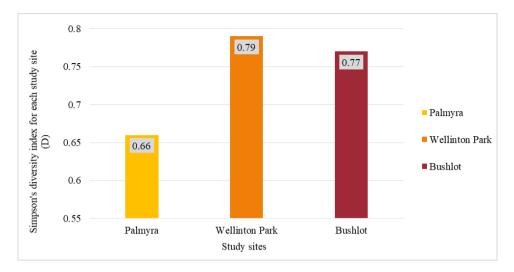
Table 4 Species index of diversity for cultured samples from the different study sites using Simpson's Index of Diversity(D)

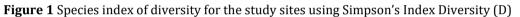
Simpson's Index of Diversity (D)	Sample type			
Sample Site	Leaf	Bark	Soil	
Palmyra	0.67	0.66	0.64	
Bushlot	0.71	0.77	0.64	
Wellington Park	0.75	0.59	0.79	

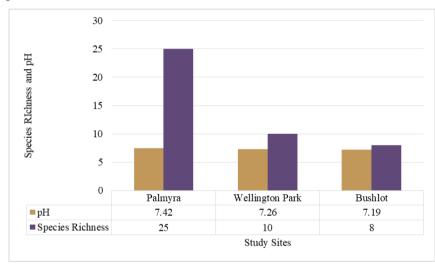
Figure 1 depicts the overall variation of the species diversity index for the individual study sites using Simpson's Index Diversity (D). The values show Palmyra having the lowest species index of diversity (0.66) while Wellington Park obtained the highest at 0.79, followed closely by Bushlot at 0.77.

Gilna *et. al.*, [7], Bessey [8] and Jennings *et. al.*, [13] reported differences in species composition of fungi from different parts of mangrove plants and noted that there might also be seasonal variation in fungal species composition and abundance. In addition, Gilna *et. al.*, [7] and Sridhar [14] alluded to the fact that mangrove ecosystems are very good environments for diverse fungal species. Like those mentioned above, this current study also demonstrated that the mangrove ecosystems investigated were good for fungi, but also had differences in species composition and diversity as shown by the results of the calculated Simpson's Diversity Index for each study site. This may be supported by Sridhar [14] and Saravanan *et. al.*, [15], who reported that because there is a diverse mix of vegetation in mangrove ecosystems,

this presents a major niche for fungal diversity. Further, the observed fungal diversity may also have been influenced by the age, diversity and physicochemical characteristics of the specific mangrove habitat [14].







3.3. Environmental parameters

Figure 2 Variation in pH and influence on total fungal species richness for study sites

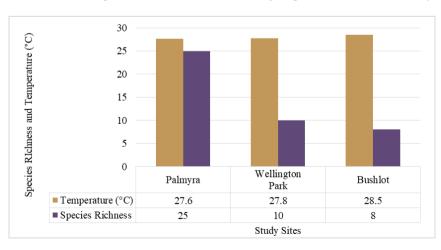


Figure 3 Variation in temperature and influence on total fungal species richness

Figure 2, Figure 3 and Figure 4, show how the fungal species richness for the study sites varied with pH, salinity and temperature. It was observed that as salinity and pH increase, there is an increase in the total species richness of fungi for individual study sites. However, a decrease in temperature results in an increase in the total species richness of fungi for individual study sites. This increase in species richness is supported by [14] and later explained by Jennings [16], Wichern *et. al.*, [17] and Rath *et. al.*, [18], as fugal species are adapted to saline environments and can fully complete their life cycles in coastal and marine environments. Also, the environmental conditions (temperature, pH, salinity) may have determined which fungal species was present because fungal spores or hyphae must be compatible enough to withstand environmental conditions.

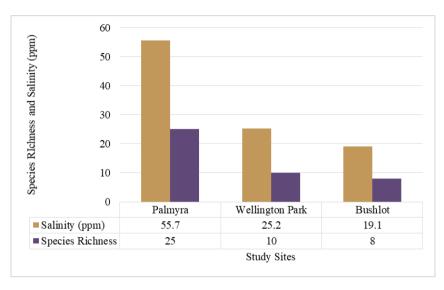


Figure 4 Variation in salinity (ppm) and influence on total fungal species richness

It was reported by [18] that high levels of salinity tend to increase fungal activity, thus contributing to an increase in fungal species richness and relative species abundance. Also, Vanegas *et. al.*, [19] reported that soil salinity acts as a factor to determine fungal community composition in mangroves. In addition, Lee *et. al.*, [20] reported that terrestrial fungi tend to dominate areas that are not regularly inundated by tidal regimes.

The current study areas experience periodic tidal inundation. Ravikumar *et. al.*, [21] notes that although decaying wood, seedlings, leaves and other substrata of mangroves serve as the appropriate hosts for fungi, periodic inundation of mangrove ecosystems does not allow for many fungi to associate with mangrove trees [22]. This may account for the low total species richness observed at Wellington Park and Bushlot and the high species richness at Palmyra. It was observed during sampling that the incoming tides would flood the study area, resulting in the debris being submerged. When the tide subsided, it tended to wash away a large amount of debris, thus reducing the substrate on which fungi may become established.

4. Conclusion

This study yielded thirty species of fungi over all of the study sites. *Aspergillus spp., Rhizopus spp., Trichoderma spp.* and a pink-coloured yeast were the dominant microfungi when their relative species abundance was considered. *Amanita spp.* and *Lepiota spp* were the dominant macrofungal species when their relative abundance across study sites was considered. Species belonging to the Order Polyporales were found to be common among the study sites.

There was a high species richness (25) at Palmyra, followed by Wellington Park (10) and Bushlot, which had the lowest species richness (8). With respect to the cultured samples, the study site at Wellington Park yielded the highest species index of diversity at 0.79, followed by Bushlot at 0.77 and Palmyra with the lowest at 0.66.

The functional diversity of an ecosystem is highly influenced by environmental conditions (temperature, pH, and salinity). In this study, the tidal activities appeared to affect fungal diversity since the incoming and outgoing tides washed away an extensive amount of debris, thus reducing available substrate material, which ultimately reduced fungal diversity.

This study was a preliminary one to investigate the presence and diversity of fungi in mangrove ecosystems along the Corentyne Coast in Guyana, South America. Previous studies have reported on this aspect in other parts of the world, however, not much was done in Guyana. The information derived from this study can therefore serve as a useful base for the planning and execution of other similar and detailed investigative studies on fungal diversity in mangrove ecosystems in Guyana.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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