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Distribution and pathogenicity profile of dematiaceous fungi isolated at ULI community, Anambra state

Abstract

Superficial mycosis has been described as the major source of debilitating skin infection, especially in rural areas. This study examined the distribution and pathogenicity profile of dematiaceous fungi isolated at Uli community, Anambra State. Two hundred and ten (210) soil samples were randomly collected from three different soil types (loamy, clay, and sandy soil) at Uli community using a soil auger. The soil samples were analyzed using standard microbiological technique. The fungal isolates were characterized based on their morphology, slide culture technique, and atlas of clinical mycology. The pathogenicity potential of the isolates was evaluated using Wistar rats. *Exophiala jeanselmei* was mostly isolated (40%) from Umuoma community, followed by *Cladophialophora carrionii* (30%) from the same community, while the least was *Scedosporium apiospermum* (10%) from Aluoha community. Loamy soil yielded the highest fungal isolates 13(65%), followed by clay soil 4(20%) while sandy soil yielded the least fungal isolates 3(15%). Statistically, there was a significant difference ($P < 0.05$) in the distribution of the fungal isolates in the different soil types. Umuoma yielded the highest number of fungal isolates 10(50%), followed by Umuaku 6(30%) while the least was Aluoha 4(20%). The Wistar rats infected using the isolates developed erythematous lesions, which were confirmed by culturing scrapings from the infected site. The study showed that dematiaceous fungi are mostly found in loamy soil, of which *Exophiala jeanselmei* had the most frequent occurrence. Also, dematiaceous fungal isolates were able to cause erythematous lesions on the laboratory animals, which confirmed their pathogenicity potentials.

Keywords: Dematiaceous fungi, erythematous lesions, infection, pathogenicity

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Introduction

Dematiaceous fungi are group of organisms that are diverse in nature, having the ability to produce dark pigmentation known as melanin in their hyphae or spores.[1] Dematiaceous or dark fungi are highly saprophytic, and are capable of causing infections in humans, animals, and plants.[2] They can be grown on different microbiological media (Saboraud dextrose agar, Potato dextrose agar), where they produce dark grey colonies, white, brown, and black on agar plate surface while reverse pigmentation is dark coloured. The melanin pigment has been described as a virulent factor.[2] Dark fungi are abundant in nature, some common genera include; *Cladosporium, Curvularia*, *Exophiala*, *Bipolaris* etc.

Research had shown that distribution of dark fungi is localized due to their saprophytic nature.[2] The organisms had been reported to inhabit environment that provides opportunity for degradation, due to their saprophytic nature.[1] Most of the fungi had been isolated in the soil, rotten wood, air, and water.[1] However, it is worthy to note that soil harbors most of the organisms, and their distribution in the soil is influenced by several factors such as soil structure and texture.[3]

Dematiaceous fungi had been revealed to be responsible for subcutaneous infections in both humans and animals.[1] They are highly pathogenic to humans, both individuals that have vibrant immune defense mechanisms and immunodeficient are infected.[2] Individuals in rural areas that are engaged in farming activities, hunting, fetching of firewood are highly predisposed to the fungal agents, as they can enter the body through piercing or traumatic inoculation via broken wood or stick in the soil. The fungi usually attack the lower limbs with appearance of lesions.[2] These fungal species also disrupt the functioning of transplanted organ, resulting in morbidity as reported by Yew *et al.*[2]

Several researchers had worked on the distribution of dematiaceous fungi in soil and their pathogenicity potential such as Yew *et al.*,[2] Revanker and Suton[1] and Chukwuma *et al.*[3] but few research had been geared towards the distribution and pathogenicity profile of dematiaceous fungi isolated at Uli community. Hence, this research is aimed at evaluating the distribution and pathogenicity profile of dematiaceous fungi isolated at Uli community. The result obtained from this study would contribute immensely to checking the spread of fungal infection associated with dark fungi.

Materials and methods

Collection of Samples

Soil samples comprising of 70 loamy, 70 clay, and 70 sandy soil were collected from three communities (Aluoha, Umuaku, and Umuoma) at Uli, Ihiala L.G.A, Anambra State. The samples were collected using a sterile spoon at a depth of 10 cm. The samples were put in a sterile polythene bag and were conveyed to the Department of Microbiology Laboratory, COOU for analysis, which was carried out within 2 h.

Processing of Samples

The soil samples were serially diluted using normal saline. Ten millimeter of normal saline was put into test tubes containing 1 g of soil samples and tenfold serial was done to obtain the following dilutions: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Test tubes containing 10^{-4} and 10^{-5} dilutions were plated on Potato Dextrose Agar (PDA)

using spread plate technique. All the inoculated plates in triplicates were incubated in inverted position at 30±2°C for 7 days. The isolated colonies were then purified using a freshly prepared PDA.

Identification of the Isolates

The fungal growths were thoroughly scrutinized morphologically and microscopically. In morphological characterization, the colors, texture, size, margin, and reverse pigmentation were examined. Further identification was done using slide culture technique which enabled the structure of the melanized fungi to be clearly visualized as elucidated by Umedum and Iheukwumere.[4] The shape, color, and size of the conidia and hyphae were examined with the aid of a digital microscope and the overall identification was made using color atlas of clinical mycology.[4,5]

Pathogenicity Test

Two months Wistar rats numbering 18 with average weight of 150g were purchased, and acclimatized for 7days. During this period, the rats were provided with water and growers' feed inside a metallic cage that had optimum ventilation. The inoculum of the isolates was prepared and standardized using 0.5 MacFarland solution. The Wistar rats were divided into four groups. Each of the isolates (0.1mL) was inoculated into the rats subcutaneously using 1 mL syringe after disinfection and depilation using 70% ethanol and sterile blade, respectively. Dimethyl Sulfoxide (0.1mL) was inoculated into the control rats. The infected rats were fed and observed for 21 days. Erythematous lesions that developed on the infected site on the 14th day were scrapped and cultured on SDA and incubation followed at 30±2°C for 7 days.

Statistical Analysis

The distribution of the fungal isolates in the three communities was compared using ANOVA. P values lower than 0.05 ($P < 0.05$) were considered to reflect significant differences.

Results

The total number of four dark fungal species were isolated and identified from the sampled communities and their microscopic features are shown in Figure 1 & Table 1. *Exophiala jeanselmei* was isolated most frequently 8(40%), followed by *Cladophialophora carrionii* 6(30%), *Ochroconis mirabilis* 4(20%) while the least was *Scedosporium apiospermum* 2(10%) (Table2). In Table 3, most of the isolates were isolated from loamy soil 13(65%), followed by clay soil 4(20%) while the least was sandy soil 3(15%). Table 4 revealed that samples collected from Umuoma produced most of the dark fungi 10(50%), followed by Umuaku 6(30%) while the least was Aluoha 4(20%). Statistically, the distribution of the isolates in the three communities was significant ($P < 0.05$). The result of pathogenicity test revealed that all the Wistar rats infected using the fungal isolates survived, though erythematous lesions were seen at the site of infection. The scrapings collected from the infected skin yielded colonies of the inoculated fungi after incubation on SDA at 30±2°C for 7 days, which confirmed that the organisms were responsible for the lesions (Figure 2).

Table 1 Morphological and microscopical features of the fungal isolates

Isolate	Colony appearance on PDA	Texture and Elevation	Microscopic appearance	Type of Conidia	Reverse pigmentation	Suspected Fungal species
DME01	Dark brown	Mucoid, velvety, flat, rough surface and smooth edge	Brown septate hyphae	Brownish annelloconidia	Dark brown	*Exophiala jeanselmei*
DME02	Brown to olivaceous	Velvety, rough surface and smooth edge	Smooth septate hyphae	Brown Blastoconiddia	Brown	*Ochroconis mirabilis*
DME03	Olive brown	Velvety, downy, rough surface and smooth edge	Dark septate hyphae	Dark Blastoconiddia with branching chains	Black	*Cladosporium carrionii*
DME04	White	Velvety, rough surface and edge	Dark septate hyphae	Dark annelloconidia	Black	*Scedosporium prolificans*

Table 2 Frequency occurrence of fungal isolates

Fungal isolate	Frequency	% occurrence
Exophiala jeanselmei	8	40
Cladophialphora carrionii	6	30
Ochroconis mirabilis	4	20
Scedosporium apiospermum	2	10
Total	20	100

Table 3 Distribution of the fungal isolates in different soil types

Fungal isolate	Loamy soil	Clay soil	Sandy soil
Exophiala jeanselmei	5 (25%)	2(10%)	1(5%)
Cladophialphora carrionii	3 (15%)	2 (10%)	1 (5%)
Ochroconis mirabilis	3 (15%)	0 (0)	1 (5%)
Scedosporium apiospermum	2(10%)	0 (0)	0 (0)
Total	13 (65%)	4(20%)	3(15%)

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Table 4 Distribution of the fungal isolates in the sampled communities

Fungal isolate	Aluoha	Umuaku	Umuoma
Exophiala jeanselmei	2 (10%)	3(15%)	6 (30%)
Cladophialophora carrionii	1 (5%)	1 (5%)	2(10%)
Ochroconis mirabilis	0 (0)	2 (10%)	1 (5%)
Scedosporium apiospermum	1 (0)	0 (0)	1 (5%)
Total	4(20%)	6(30%)	10(50%)

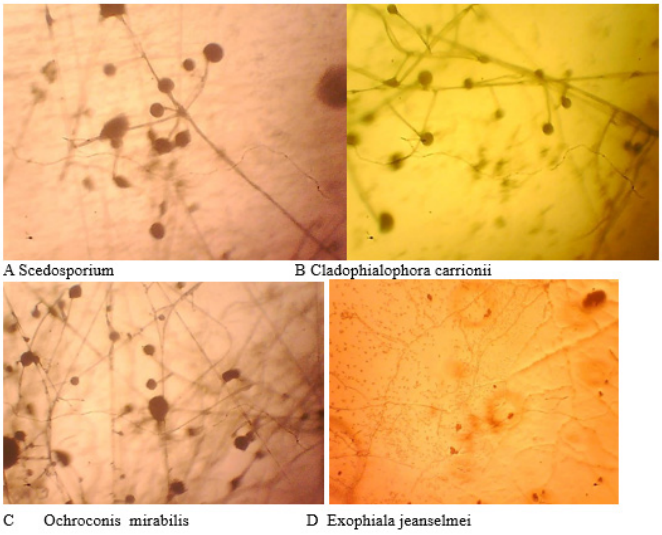

Figure 1 Mcroscopic features of dematiaceous fungi in slide culture and lactophenol cotton blue staining.

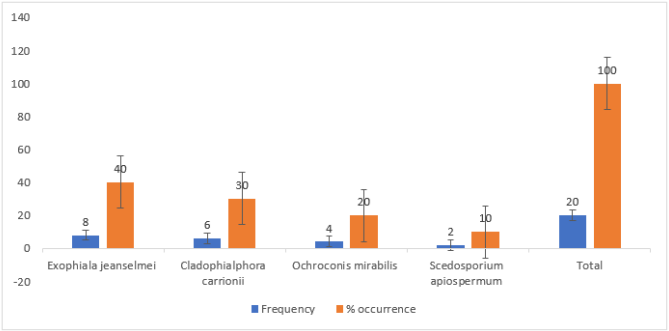

Figure 2 Frequency occurrence of fungal isolates.

Figure 3 Erythematous lesions on the infected Wistar rats.

Discussion

The need to discover sources of pathogenic fungi has become paramount in clinical mycology due to high rate of fungal infections in the society. This research has revealed that dark fungi are found in different soil types with characteristic reverse dark pigmentation and prominent conidia. This observation agrees with the finding of several researchers.[1-3] The highest number of dark fungi isolated from loamy soil could be ascribed to high humus which emanated from high biodegradation as the organisms are well-known saprophytes. This conforms to the observation made by Chukwuma *et al*.,[3] Osuji *et al,*[6] who investigated the distribution and pathogenicity of dematiaceous fungi and obtained highest number of dark fungi from loamy soil. The presence of the four isolates at Umuoma community could be ascribed to variation in the habitat of the fungi. The fungi were able to adapt to environmental conditions that scared other fungal species. Similar conclusion was drawn by Chukwuma *et al*.[3] The ability of the isolates to produce lesions on the Wistar rats portrayed their pathogenicity potential. This confirms the previous literatures that documented that dark fungi are responsible for subcutaneous infections.[3-10] However, there was variation in the fungal isolates which could be ascribed to regional differences. Chukwuma *et al*.[3] isolated from samples obtained from Awka North while samples in this study were obtained from Anambra South.

Conclusion

This study has shown that dark fungi are mostly found in loamy soil due to high humus. Also, soil samples collected from Umuoma community yielded the highest number of dark fungi due to high availability of degradable organic materials. These dark fungi are pathogenic due to their ability to produce lesions on the skin of Wistar rats.

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Conflict of interest

The authors hereby declare that there was no conflict of interest throughout the period of this study.

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