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Molecular Characterization of Soil-Derived Dermatophytes with Pathogenic Potentials

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Abstract

Dermatophytes are keratinophilic filamentous fungi capable of degrading keratinized tissues such as skin, hair, and nails. This study investigated the molecular identity and pathogenic characteristics of dermatophytes isolated from garden soils in Southeastern Nigeria. A total of 100 soil samples were randomly collected and processed using serial dilution and culture techniques. Isolates were characterized using macroscopic, microscopic, biochemical, and molecular methods. Pathogenicity was evaluated through haemolytic activity and Congo red dye uptake assays, while molecular identification was performed using PCR amplification and sequencing. Four dermatophyte species were identified: *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Paraphyton cookei*. Haemolysis testing showed that 42.31% of isolates exhibited β-haemolysis while 57.69% showed α-haemolysis. Congo red dye uptake assay revealed complete dye retention in 51.92% of isolates, indicating strong pathogenic potential. Molecular analysis demonstrated ≥95% similarity with reference strains in GenBank. The findings establish garden soil as an important environmental reservoir of pathogenic dermatophytes and highlight exposure risks among individuals engaged in soil-related activities.

Keywords: Molecular Characterization, Dermatophytes and Pathogenic Potentials

Introduction

Dermatophytes are keratinophilic filamentous fungi capable of degrading keratinized tissues such as the skin, hair, and nails. These fungi invade keratinized tissues and obtain nutrients through keratinase enzyme production (Arshah *et al.,* 2015; Kumar *et al.,* 2018). Dermatophytes are transmitted through direct contact with infected humans (anthropophilic transmission), animals (zoophilic transmission), contaminated soil (geophilic transmission), and indirectly through fomites. Dermatophytosis is primarily caused by fungi belonging to the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*. Soil serves as a major ecological reservoir of dermatophytes and other keratinophilic fungi. Loamy soils particularly harbor significant dermatophyte populations due to their saprophytic adaptability. Individuals such as farmers who frequently interact with soil are therefore predisposed to infection. Despite the rising global prevalence of dermatophytosis, environmental surveillance of soil-borne dermatophytes remains insufficient, particularly in developing countries. This study therefore evaluated garden soil as a potential reservoir of pathogenic dermatophytes.

Materials and Methods

Study Area: The study was conducted at Umuoma, Uli, Ihiala Local Government Area, Anambra State, Nigeria. Uli lies between latitudes 5.47°N and 5.783°N and longitudes 6.52°E and 6.87°E in Southeastern Nigeria. The area is characterized by rainforest vegetation and two major climatic seasons: rainy and dry seasons. Annual rainfall averages 1,600 mm with relative humidity of approximately 80% at dawn.

Isolation of Dermatophytes from Garden Soil

Sample Collection: A total of 100 soil samples were randomly collected from different soil types (loamy, clay, and sandy soils) within Umuoma community using a soil auger sterilized with 70% ethanol. Soil samples were collected at a depth of 10 cm and transferred into sterile polyethylene bags. Samples were transported to the Microbiology Laboratory for immediate analysis.

Culturing and Purification of Isolates: One gram of each soil sample was aseptically weighed and suspended in 100 mL sterile distilled water to obtain a stock suspension. Tenfold serial dilutions were prepared by transferring 1 mL aliquots into test tubes containing sterile distilled water. Aliquots (1 mL) from appropriate dilutions were inoculated onto Potato Dextrose Agar supplemented with cycloheximide using the pour plate technique. Plates were incubated in an inverted position at 32 ± 2°C for 7 days. Distinct colonies were subcultured to obtain pure isolates.

In Vitro Technique: Pathogenic potential was evaluated by assessing haemolytic activity and Congo red dye uptake as indicators of virulence.

Haemolysis on Blood Agar: Blood agar plates were inoculated with test isolates and incubated at 35 ± 2°C for 24 hours. Clear zones around colonies indicated β-haemolysis, greenish zones indicated α-haemolysis, while absence of zones indicated no haemolysis.

Reaction with Congo Red Dye: Sabouraud Dextrose Agar mixed with Congo red dye was inoculated with isolates and incubated at 35 ± 2°C for 24 hours. Wrinkled red colonies indicated positive dye uptake, whereas smooth pink colonies indicated negative reactions.

Characterization of Pathogenic Isolates

Macroscopic Characteristics: Colony morphology including colour, texture, pigmentation, growth rate, and surface features were examined using standard mycological procedures.

Slide Culture Technique: Slide culture technique was performed to preserve fungal structures for microscopic examination. Agar blocks were inoculated and incubated at 28°C for 4 days before staining and observation.

Molecular Characterization of Selected Fungal Isolates

DNA Extraction and Purification: Genomic DNA was extracted using Zymo Research DNA MiniPrep™ kit according to manufacturer's protocol.

Determination of DNA Quality: DNA purity was assessed using NanoDrop spectrophotometer at absorbance ratios of 260/280 nm.

PCR Amplification and Gel Electrophoresis: PCR amplification was carried out using a thermal cycler under standardized cycling conditions. Amplified products were separated on 1% agarose gel and visualized under UV illumination.

DNA Sequencing and Computational Analysis: PCR products were sequenced using ABI DNA sequencer. Generated sequences were cleaned and aligned .Consensus sequences were analyzed using BLAST algorithm. Isolates showing ≥95% similarity with reference strains were considered identified.

Statistical Analysis: Data were presented using tables and percentages. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) at 95% confidence level. Pairwise comparisons were conducted using Student's t-test

Results

The cultural characteristics of the fungal isolates revealed variations in growth rate, colony texture, pigmentation, conidial morphology, and urease production. Isolate M exhibited a moderate to rapid growth rate with a velvety and smooth texture. Its conidia appeared pyriform and round, consisting predominantly of numerous microconidia, and the isolate produced urease. Isolate N showed a moderate growth rate with a powdery texture. The colony pigmentation appeared mauve with a white edge. Numerous microconidia were observed, while urease production was not detected. Similarly, isolate P demonstrated a slow to moderate growth rate with a downy texture. The colony colour appeared white with grey margins. The conidia were predominantly club-shaped microconidia, and urease production was absent. Isolate Q exhibited a moderate to rapid growth rate with a felty colony texture and light brown to pale pigmentation. The conidia appeared predominantly as club-shaped to balloon-shaped microconidia (Table 1). The study revealed that the fungal isolates demonstrated pathogenic potential when exposed to human red blood cells and Congo red dye (Table 2). Out of the nine organisms isolated from the garden soil samples, only isolates M, N, P, and Q exhibited pathogenic characteristics. Isolates M and P displayed complete pathogenic features, whereas isolates N and Q showed partial pathogenic characteristics, as presented in Table 2. The study further highlighted the occurrence of pathogenic strains among the four pathogenic isolates (M, N, P, and Q). Out of the 52 isolates obtained from these groups, 42.31% exhibited β-haemolysis, while 57.69% showed α-haemolysis. Additionally, 51.92% demonstrated complete reaction with Congo red dye, producing tiny red colonies with wrinkled surfaces, whereas 48.18% showed partial reaction with Congo red, as presented in Table 3. The nature of nucleic acid extracted from the isolates is presented in Table 4. The results showed that the absorbance ratio at 260 nm and 280 nm ranged between 1.80 and 1.90, indicating high purity of the extracted nucleic acid and confirming that the genetic material was DNA. Molecular characterization based on polymerase chain reaction amplification and gene sequencing identified the isolates as *Trichophyton mentagrophytes* strain UKJ 594119 (TMU5), *Paraphyton cookei* strain IFM40904 (PCM4), *Trichophyton rubrum* strain CBS118892 (TR), and *Trichophyton tonsurans* strain DSM112177 (TTD1).

Table 1: Characteristic of the fungal isolates

Isolate Code	Growth Rate	Texture	Colour	Conidia	Urease
M	Moderate to rapid	Velvety and smooth	White	Pyriform, round, numerous mainly microconidia	Positive
N	Moderate	Powdery	Mouve with white edge	Numerous microconidia	Positive/Negative
P	Slow to moderate	Downy	White and gray at the edge	Club-shaped microconidia	Negative
Q	Moderate to fast	Felty	Light brown to pale	Club-shaped to balloon microconidia	Positive

Table 2: *In vitro* pathogenic potentials of the fungal isolates

Isolate	Haemolysis	Reaction with Congo red
M	β –hemolysis	Tiny red with wrinkled surface
N	α –hemolysis	Red with wrinkled surface
P	β –hemolysis	Tiny red with wrinkled surface
Q	α –hemolysis	Red with wrinkled surface

Table 3: Occurrences of the pathogenic strains

Isolate	Number	Haemolysis		Congo Red	
		β –Reaction (%)	α –Reaction (%)	Complete (%)	Partial (%)
M	14	12(85.71)	2(14.29)	14(100.00)	0(0.00)
N	19	2(10.53)	17(89.47)	4(21.05)	17(78.95
P	8	7(87.50%)	1(12.50)	8(100.00)	0(0.00)
Q	11	1(9.09)	10(90.01)	3(27.27)	8(72.73)
Total	52	22(42.31)	30(57.69)	27(51.92)	25(48.18)

Table 4: Nature of nucleic acid extracted from the isolates

Isolate Code	Conc (mg/mL)	A_{280}	A_{260}	260/280
M	86.40	0.5294	0.9741	1.84
N	78.80	0.5160	0.9391	1.82
P	87.20	0.5271	0.9646	1.83
Q	72.30	0.4540	0.8399	1.85

Table 5: Molecular characteristics of the isolates

Code	Max Score	Total Score	Query Cover	E-Value	Percent Identity (%)	Accession Number	Description
M	1423	1423	100	0.0	100	MN064822.1	*Trichophyton mentagrophytes* strain UKJ 594119 (TMU5)
N	1339	1339	100	0.0	100	AF150738.1	*Paraphyton cookie* strain IFM40904 (PCM4)
P	1681	2340	100	0.0	100	JX431933.1	*Trichophyton rubrum* strain CBS118892 (TR)
Q	1552	1552	100	0.0	100	OM326334.1	*Trichophyton tonsurans* strain DSM112177 (TTD1)

Discussion

Soil is an important habitat for microorganisms, including pathogenic fungi such as dermatophytes, which are responsible for superficial mycoses. Fungal infections were previously neglected to some extent, as they were considered common conditions due to their effects being largely limited to superficial tissues such as the skin, nails, and hair. However, in recent times, these infections have increased in severity, leading to pronounced abnormalities and significant tissue damage in affected individuals. The characteristic features of dermatophytes isolated from the sampled garden soil in this study correspond to those documented by several researchers (Sharma, 2010; Jain and Sharma, 2011; Rizwana *et al.,* 2012; Shrivastav *et al.,* 2013; Yadav *et al.,* 2021). The ability of the fungal isolates to haemolyze red blood cells and retain Congo red stain may be attributed to their high pathogenicity profile. These virulence indicators suggest enhanced invasive and enzymatic capabilities of the isolates. This observation is consistent with findings reported by several researchers (Hiruma *et al.,* 2015; de Hoog *et al.,* 2017; Wang and Li, 2021; Klinger *et al.,* 2021; Wang *et al.,* 2021; Faway *et al.,* 2018; Iorizzo *et al.,* 2019; Gupta *et al.,* 2023), who studied the epidemiology and pathogenic mechanisms of dermatophytes such as *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, and *Trichophyton interdigitale*. Molecular characterization of the selected fungal isolates through in vitro analysis revealed the presence of *Trichophyton mentagrophytes* strain UKJ 594119 (TMU5), *Paraphyton cookei* strain IFM40904 (PCI4), *Trichophyton rubrum* strain CBS118892 (TR), and *Trichophyton tonsurans* strain DSM112177 (TTD1). Similar dermatophyte species have been isolated and characterized by other researchers (Hiruma *et al.,* 2015; de Hoog *et al.,* 2017; Wang and Li, 2021; Klinger *et al.,* 2021; Wang *et al.,* 2021; Faway *et al.,* 2018; Iorizzo *et al.,* 2019; Gupta *et al.,* 2023). However, the variations observed in the genera and species distribution may be attributed to differences in sample sources. In the present study, all isolates were obtained from garden soil, whereas some researchers (Klinger *et al.,* 2021; Wang *et al.,* 2021; Faway *et al.,* 2018) isolated dermatophytes from clinical samples such as skin scrapings, hair, and nails.

Conclusion

This study clearly demonstrates that dermatophytes are present in garden soil. The dermatophyte strains identified include

Paraphyton cookei strain IFM40904, *Trichophyton mentagrophytes* strain UKJ 594119, *Trichophyton rubrum* strain CBS118892, and *Trichophyton tonsurans* strain DSM112177. These strains exhibited substantial pathogenicity as demonstrated through in vitro analyses

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