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Molecular Diversity And Phylogenetic Characterization Of Soil Fungal Communities Associated With Maize Cultivation Across Agricultural Ecosystems In A Southwestern State, Nigeria

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Abstract
Background: *Soil fungal communities play essential ecological roles in nutrient cycling, organic matter decomposition, plant health regulation, and agricultural productivity. Despite their importance, molecular data describing fungal diversity in agricultural soils remain limited in tropical ecosystems, particularly in understudied regions of sub-Saharan Africa. This study investigated the diversity and molecular characteristics of fungal communities associated with maize-cultivated soils collected from five agricultural locations in Ogun State, southwestern Nigeria.*
Materials and Methods: *Soil samples were collected from Obantoko, Ajebo, Ilaro, Irogun, and Oke Shida. Fungal isolation was performed using direct plating and serial dilution techniques on Potato Dextrose Agar. Morphologically distinct isolates were purified and subjected to genomic DNA extraction followed by amplification of the internal transcribed spacer (ITS) region using ITS1 and ITS4 universal primers. Polymerase chain reaction products were sequenced and species identification was performed through Basic Local Alignment Search Tool analysis using the National Center for Biotechnology Information database. Phylogenetic relationships were inferred using maximum likelihood analysis.*
Results: *A total of thirteen fungal isolates representing twelve species distributed across six genera were identified. The genus Aspergillus was the dominant taxonomic group, accounting for six identified species, while Aspergillus terreus showed the highest occurrence frequency. Sequence similarity analysis revealed high homology with reference sequences ranging from 98.34% to 99.83%. Phylogenetic analysis separated isolates into two major evolutionary clusters, indicating measurable genetic diversity independent of geographical sampling location.*
Conclusion: *These findings provide baseline molecular evidence of fungal diversity in maize-associated agricultural soils and contribute important insights for soil microbial ecology, agricultural disease surveillance, and sustainable crop management in tropical agroecosystems.*
Keyword: *Soil fungi, ITS sequencing, fungal diversity, molecular characterization, phylogenetic analysis, maize rhizosphere, agricultural microbiology*

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I. Introduction

Soil microbial communities perform critical ecological functions that sustain terrestrial ecosystems and agricultural productivity. Among these microorganisms, fungi constitute one of the most ecologically important groups because of their involvement in nutrient cycling, decomposition of organic matter, symbiotic plant interactions, and regulation of soil health[1]. Recent studies have demonstrated that soils harbor some of the highest levels of terrestrial biodiversity, with fungal communities contributing substantially to ecosystem stability and agricultural sustainability[2].

The rhizosphere, defined as the narrow soil zone influenced directly by plant roots, is among the most biologically active regions of terrestrial ecosystems. Fungal communities inhabiting this zone establish complex ecological relationships with plants, ranging from beneficial interactions that improve nutrient acquisition to pathogenic associations capable of causing economically important crop diseases[3]. In maize production systems,

soil-associated fungi significantly influence plant health, nutrient availability, disease suppression, and overall crop productivity.

Agricultural soils harbor fungal species with both beneficial and detrimental ecological roles. Beneficial fungi such as *Trichoderma* species are recognized for their biocontrol activity and ability to enhance plant growth, whereas genera such as *Aspergillus*, *Penicillium*, and *Fusarium* contain species capable of producing mycotoxins and causing plant diseases that threaten food safety and crop production[4]. Understanding fungal community composition in agricultural soils is therefore essential for disease monitoring and sustainable agricultural management.

Conventional fungal identification based on colony morphology and microscopic examination remains useful for preliminary taxonomic classification. However, morphological methods often present limitations in distinguishing closely related fungal species because phenotypic expression may vary considerably under environmental conditions. Molecular identification using DNA-based markers has significantly improved taxonomic resolution, with the internal transcribed spacer region recognized as the universal fungal DNA barcode because of its high discriminatory capacity[5,6].

Despite increasing interest in soil microbial ecology, molecular studies investigating fungal diversity in tropical agricultural soils remain limited in Nigeria. Most previous investigations have relied primarily on morphology-based identification approaches, leaving substantial knowledge gaps regarding fungal biodiversity and phylogenetic relationships in important agricultural ecosystems. Therefore, this study aimed to isolate, molecularly identify, and characterize fungal species associated with maize rhizosphere soils collected from selected agricultural locations in Ogun State, Nigeria, while evaluating their phylogenetic relationships through sequence-based molecular analysis.

II. Materials And Methods

Study Area and Sample Collection

Soil samples were collected from five agricultural locations in Ogun State, southwestern Nigeria: Obantoko, Ajebo, Ilaro, Irogun, and Oke Shida. Samples were obtained from the maize rhizosphere, where microbial activity is concentrated. Approximately 5 g of soil was aseptically collected using sterile spatulas and transferred into sterile polyethylene bags. Samples were transported to the Plant Pathology Laboratory, Department of Botany, University of Ibadan, Nigeria, and stored at 4–10°C before analysis.

Isolation of Fungi

Fungal isolation was carried out using serial dilution and direct plating techniques on Potato Dextrose Agar (PDA). For serial dilution, 1 g of soil sample was suspended in sterile distilled water and serially diluted to 10^{-4}. Aliquots from selected dilutions were inoculated onto PDA plates and incubated at room temperature for five days. Direct plating involved inoculating soil particles directly onto prepared PDA plates. All experiments were performed in triplicate.

Purification and Morphological Identification

Distinct fungal colonies were subcultured repeatedly on fresh PDA plates to obtain pure cultures. Preliminary identification was performed based on colony pigmentation, growth pattern, margin characteristics, texture, and microscopic morphology according to standard mycological procedures.

DNA Extraction and PCR Amplification

Genomic DNA was extracted using the Quick-DNA Fungal/Bacterial Kit according to manufacturer instructions. Molecular identification was performed by amplification of the internal transcribed spacer region using universal primers ITS1 and ITS4 following the protocol described by White et al[7]. Polymerase chain reaction amplification was carried out using OneTaq Quick-Load 2X Master Mix.

DNA Sequencing and Species Identification

Amplified PCR products were confirmed by agarose gel electrophoresis and purified enzymatically using ExoSAP-IT PCR Cleanup Reagent. DNA sequencing was performed in forward and reverse directions using BrilliantDye Terminator Cycle Sequencing Kit Version 3.1. Sequence purification was performed using Zymo DNA Sequencing Cleanup Kit and analyzed using ABI 3500XL Genetic Analyzer. Generated chromatograms were processed using DNASTAR software, and species identification was conducted through Basic Local Alignment Search Tool searches against the National Center for Biotechnology Information nucleotide database. Extracted DNA was sequenced in forward and reversed direction. A total of 13 pure isolates obtained from the soil samples were analysed. The (ITS) PCR reactions were conducted using the ITS1 and ITS4 primers. Figure 1 shows the amplification region of some of the isolates during PCR.

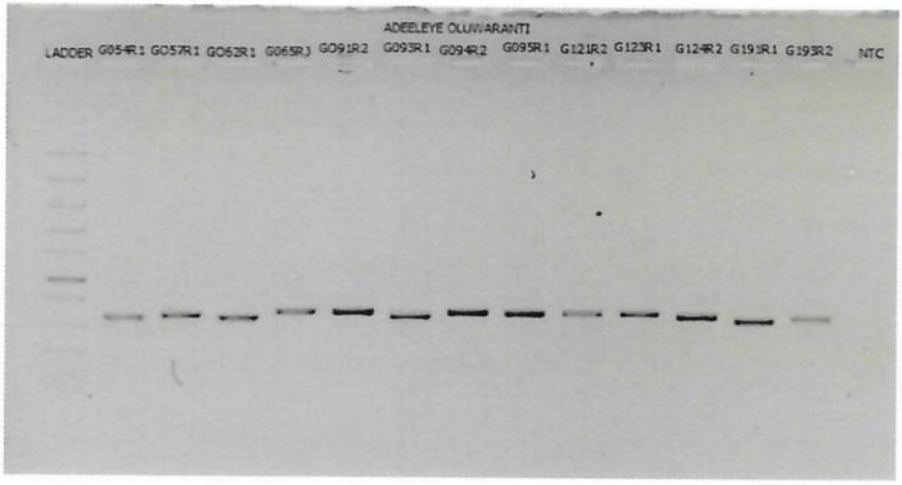

Figure 1: Photographic image showing amplification of ITS target region in agarose gel

Phylogenetic Analysis

Phylogenetic relationships among fungal isolates were inferred using maximum likelihood analysis implemented in MEGA11 software. Evolutionary relationships were evaluated based on aligned nucleotide sequences obtained from sequencing analysis.

III. Results

Preliminary culture-dependent analysis revealed substantial morphological diversity among fungal isolates recovered from the five sampling locations. Colony morphology showed considerable variation in pigmentation, texture, growth pattern, and colony margin characteristics.

A total of thirteen fungal isolates were successfully recovered and subjected to molecular characterization. Molecular identification based on ITS sequencing identified twelve fungal species distributed among six genera. These included six species belonging to *Aspergillus* (*A. flavus, A. terreus, A. niger, A. udagawae, A. aculeatus,* and *A. pseudodeflectus*), two *Penicillium* species (*P. cataractarum* and *P. menonorum*), and one species each of *Trichoderma reesei, Fusarium chlamydosporum, Trametes polyzona,* and *Talaromyces flavus*.

Sequence similarity analysis revealed high homology with reference sequences deposited in GenBank, ranging between 98.34% and 99.83%, confirming reliable species-level identification.

The genus *Aspergillus* represented the dominant fungal group, accounting for six of the twelve identified species. *Aspergillus terreus* showed the highest distribution frequency and was detected in more than one sampling location.

Phylogenetic analysis grouped the fungal isolates into two major evolutionary clusters. Phylogenetic analysis between the Trichoderma isolates and some other ones which are already submitted on the NCBI is presented in Figure 2. The clustering pattern suggested measurable genetic diversity among isolates and indicated that evolutionary relatedness was not strongly associated with geographical sampling location.

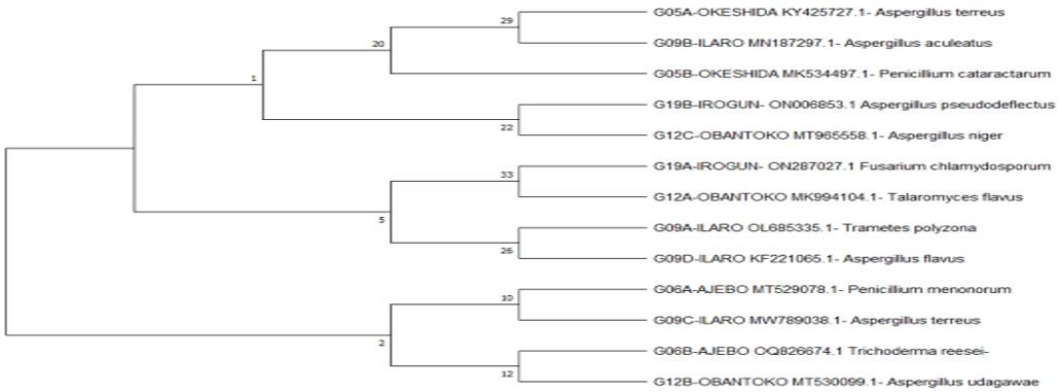

Figure 2 Phylogenetic relationship between the fungal isolates already documented in NCBI

IV. Discussion

The present study demonstrated substantial fungal diversity within maize-associated agricultural soils collected from five locations in Ogun State, Nigeria. Twelve fungal species belonging to six genera were identified, confirming the ecological complexity of fungal communities associated with tropical agricultural soils.

Preliminary identification based on morphological and cultural characteristics demonstrated substantial phenotypic diversity among fungal isolates.The morphological appearance of the isolated bioagents and pathogens are presented in Figures 3, 4 and 5 below. These findings agree with previous studies that have reported the usefulness of morphological approaches for preliminary fungal taxonomy, particularly for soil-borne fungi isolated from agricultural ecosystems[8,9]. However, morphology based classification alone may present limitations in distinguishing closely related fungal taxa.

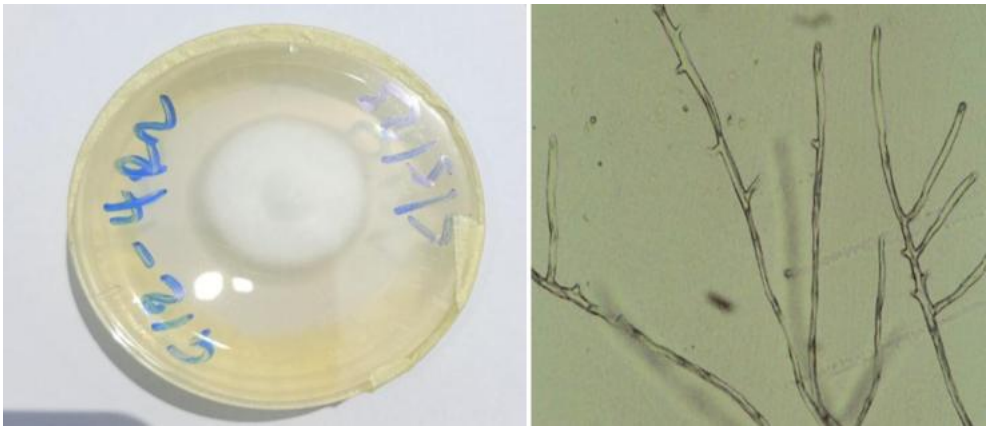

Figure 3. Morphological and microscopic (×40) characteristics of Talaromyces flavus*s*

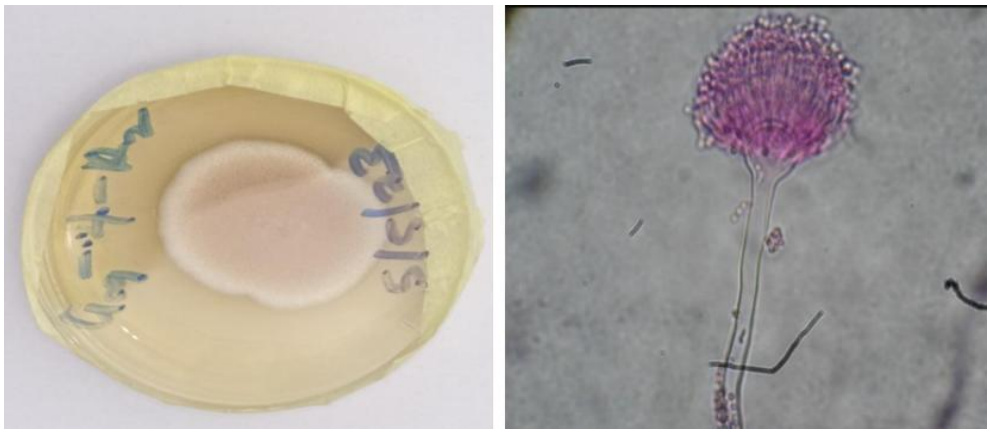

Figure 4. Morphological and microscopic (×40) characteristics of *Aspergillus terreus*

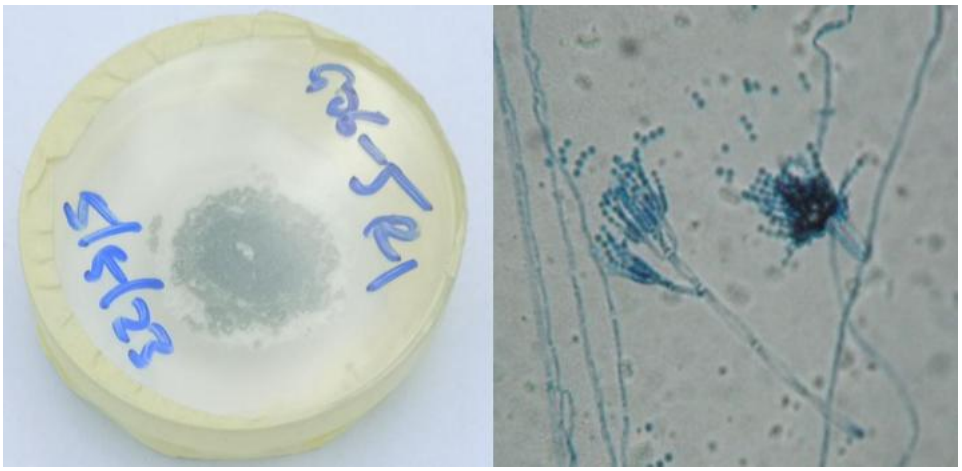

Figure 5. Morphological and microscopic (×40) characteristics of *Penicillium menonorum*

Table 1: Blast result showing the percentage identity of samples derived in NCBI website

Locations	Description	Accession lens	Max score	Total score	Query cover	E value	Per. Identity of Pathogen	Accession number
Oke shida	*Penicillium cataractarum*	588	976	976	99%	0.0	98.92%	MK534497.1
Oke shida	*Aspergillus terreus*	611	1019	1019	99%	0.0	99.48%	KY425727.1
Ajebo	*Trichoderma reesei*	602	1055	1055	98%	0.0	98.98%	OQ826674.1
Ilaro	*Trametes polyzona*	1730	1051	1566	86%	0.0	99.83%	OL685335.1
Ilaro	*Aspergillus aculeatus*	577	950	1324	95%	0.0	99.44%	MN187297.1
Ilaro	*Aspergillus terreus*	761	1135	1592	84%	0.0	96.41%	MW789038.1
Ilaro	*Aspergillus flavus*	939	989	1375	99%	0.0	99.64%	KF221065.1
Obantoko	*Aspergillus udagawae*	605	997	997	99%	0.0	99.82%	MT530099.1
Obantoko	*Aspergillus niger*	575	990	990	98%	0.0	98.41%	MT965558.1
Obantoko	*Talaromyces flavus*	521	933	933	95%	0.0	99.62%	MK994104.1
Irogun	*Fusarium chlamydosporum*	506	887	887	100%	0.0	99.01%	ON287027.1
Irogun	*Aspergillus pseudodeflectus*	864	949	949	99%	0.0	99.62%	ON006853.1
Ajebo	*Penicillium menonorum*	592	946	946	99%	0.0	98.34%	MT529078.1

Molecular characterization using ITS sequencing successfully identified all fungal isolates with sequence similarity values ranging between approximately 98% and 99%, confirming high-confidence species-level identification. The successful amplification of the ITS region supports previous studies identifying ITS as the most reliable universal DNA barcode marker for fungal identification because of its high interspecific variability and discriminatory power[5,6]. Blast result showing the percentage identity of samples derived in National Center for Biotechnology Information (NCBI) was shown in Table 1 above. The sequences obtained were compared with sequences deposited in the Genebank nucleotide database (NCBI), http//www.ncbi.nlm.gov/) for similarity using the BLAST program Basic Local Alignment Search Tool).

The predominance of *Aspergillus* species observed in this study is ecologically significant. Similar dominance patterns have been reported in agricultural soils where *Aspergillus* species demonstrate high ecological fitness under fluctuating environmental conditions and nutrient variability[10]. The ability of *Aspergillus* species to tolerate environmental stress and rapidly colonize organic substrates likely contributes to their widespread distribution in tropical agricultural ecosystems.

The identification of other genera including *Penicillium, Trichoderma, Fusarium, Trametes,* and *Talaromyces* further demonstrates the ecological complexity of the studied soils. Several of these fungi perform important ecological roles including nutrient mineralization, organic matter decomposition, biological control activity, and plant-pathogen interactions. Species belonging to *Fusarium* and *Aspergillus* may function as economically important plant pathogens capable of causing substantial crop losses.

Phylogenetic analysis revealed measurable genetic diversity among isolates and showed that clustering patterns were not strongly influenced by geographical sampling location. Similar observations have been reported in fungal ecology studies where environmental selection pressures exert stronger influence on microbial community composition than geographic separation alone[11].

Overall, the integration of conventional morphology with ITS-based molecular identification significantly improved species-level resolution and provides valuable baseline data on fungal biodiversity in agricultural soils from understudied tropical ecosystems.

V. Conclusion

This study demonstrated that maize-associated agricultural soils from selected locations in Ogun State, Nigeria, harbor diverse fungal communities with important ecological and agricultural significance. Through culture-dependent isolation, morphological characterization, ITS-based molecular identification, and phylogenetic analysis, twelve fungal species belonging to six fungal genera were successfully identified.

The predominance of *Aspergillus* species suggests strong ecological adaptation of this genus to tropical agricultural soil environments. The detection of fungal genera including *Aspergillus, Penicillium, Fusarium, Trichoderma, Trametes,* and *Talaromyces* highlights the functional complexity of soil fungal communities and

their potential implications for plant health, nutrient cycling, biodegradation processes, and agricultural disease ecology.

These findings provide valuable baseline molecular data on fungal biodiversity in maize-associated soils and contribute important evidence for soil microbial ecology, agricultural disease monitoring, and sustainable crop management. Future studies incorporating ecological diversity indices and metagenomic approaches are recommended to provide deeper understanding of fungal community dynamics in tropical agricultural ecosystems.

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