Original Research Article

Molecular Characterization of Fungi Associated With Dump Site Soil

ABSTRACT

Aims: A study was conducted to identify fungal species associated with dumpsite soil in University of Port Harcourt using molecular techniques.
Methodology: Molecular methods for determining the species of a fungus based on the amplification and sequencing of the internal subscribed spacer (ITS) region of the fungal rRNA operon using molecular markers was applied. Soil sample was collected from a dumpsite in the University of Port Harcourt, Rivers State, Nigeria. Isolation of fungi associated with the dumpsite soil was carried out using spread plate method. Fungal genomic DNA was extracted using Quick-DNA Fungal/Bacterial Miniprep kit. The ITS1-2 gene of the isolates was amplified by Polymerase Chain Reaction (PCR) using the primer pair; ITS4 and ITS5.
Results: The sequences of the amplified ITS region were blasted against known sequences on the National Centre for Biotechnology Information (NCBI) database. Nucleotide sequence analysis revealed the species identity of the fungal isolates to be: *Aspergillus fumigatus, Trichoderma harzianum, Aspergillus felis, Aspergillus templicola, Aspergillus flavipes, Aspergillus fumigatus and Cunninghamella binariae*. Phylogenetic analysis was carried out to ascertain the relationship between the isolates and other closely-related isolates on GenBank.
Conclusion: The molecular techniques employed successfully identified the organisms to the species level as these techniques are based on the genetic constitution of organisms. There is need to adopt these these techniques for microbial biodiversity study of various soil samples. The result obtained from this study will complement the information on the fungal organisms associated with dumpsite soil.

Keywords: Dumpsite, Fungi, Phylogeny, Sequencing, Soil

1. INTRODUCTION

Soils are naturally occurring physical covering of the earth's surface and represent the interface of three material states: solids (geological and dead biological materials), liquid (water) and gases. There are four major categories of soil components: organic, minerals, liquid and gases; and there are three properties associated with the soil: chemical (eg: pH), physical (eg: texture) and biological (eg: microbial accumulation) [1]. Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates, as well as plants and algae. These soil dwellers provide food or nutrients that support organisms that live above and below ground.

Dumpsites act as inoculums of pathogens to the soil, which can lead to the emergence of infections [2]. Soil-borne pathogens cause diseases in plants with fungal pathogens being the most virulent, chiefly responsible for root rots, wilts, blight and other plant diseases that are usually difficult to control with conventional methods [3].

Identification of fungal species using morphological and microscopic techniques is laborious and prone to errors [4]. Molecular characterization is based on the genetic composition of organisms which reveals more information than the traditional cultural technique which is based on the morphological characteristics of organisms. The use of Polymerase Chain Reaction (PCR) to amplify the Internal Transcribed Spacer (ITS) region of fungi and subsequently sequencing the amplified region provides adequate information for identification of fungal species. The use of molecular methods of identification of microorganisms can lead to successful identification of novel species and should be employed in the study of microbial diversities [5]. Characterization of the microbial population of soil is essential for the understanding of biodiversity of soil ecosystem and for the development of control strategies against pathogenic organisms. This study was aimed at isolating and identifying the fungi associated with dumpsite soil using both traditional cultural techniques and basic molecular techniques in order to determine the most effective technique that will be able to classify the organisms to the species level.

2. MATERIAL AND METHODS

2.1 Study area and Sampling

The study was carried out at the Regional Center for Biotechnology and Bio-resources Research, University of Port Harcourt, Rivers State, Nigeria. Sequencing of the PCR products was done at the International Institute of Tropical Agriculture (IITA), Ibadan. The dumpsite is an open dumpsite situated in University of Port Harcourt and consisted of disposed papers, bottles, food remnants, plastics, tin cans, grasses, polyethene bags, leathers, faeces and urine. Soil samples were obtained from five different points at a distance of 6 feet apart. Samples were collected under the refuse pile at 0-15cm soil depth, using an auger borer. Fifteen grammes of soil was collected. The soil was transferred into a sterile zip bag and taken to the laboratory for fungal isolation.

2.2 Isolation of fungi from dumpsite soil

Isolation of fungi from the dumpsite soil was carried out using the serial dilution method. One gramme of dumpsite soil was transferred into a test tube containing 10 ml of sterile physiological saline. The soil was mixed well with the solution and 1ml was aseptically transferred to another test tube containing 9 ml of physiological saline. This was repeated for three times and 0.1ml aliquot from the 1,000x dilution was aseptically transferred onto the surface of freshly prepared Potato Dextrose Agar (PDA). A sterile glass rod was used to spread the inoculums all over the surface of the PDA for even distribution of microorganisms. Ten replicates were used and the inoculated plates were incubated at room temperature (25 ^{o}C ± 2 ^{o}C) for 7 days. Individual colonies distinguished by their discrete colours were picked from the plates using sterile forceps and sub cultured on freshly prepared PDA plates to obtain pure cultures.

2.3 Fungal DNA extraction, gene amplification and sequencing

Genomic DNA was extracted following the protocol of Quick-DNA^{TM} Fungal / Bacterial MiniPrep Kit (Zymo Research Group, California, U.S.A.) with slight modifications. The mycelium from each fungal isolate was scraped off from a 7-day fully grown culture using a sterile surgical blade and transferred into a sterilized mortar. Liquid nitrogen (-196 ^{o}C) was added to the isolate in the mortar in order to freeze the sample for easy homogenization. After the sample was completely frozen, 750 µl of bashing bead buffer (composed of Tris-hydrogen chloride, sodium chloride, sodium deoxychlorate and sodium dodecyl sulfate) was added, and the sample was immediately homogenized using pestle. The mixture was transferred into a 1.5 ml microcentrifuge tube, 200 µl of nuclease free water was added into the tube and centrifuged at 10,000 x *g* for 1 minute. The supernatant was discarded and the pellet was subsequently used for cell lysis, precipitation and DNA purification, following the manufacturer's protocol. Isolated DNA was stored at 4 ^{o}C for further analysis.

DNA quantity and purity were measured using NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware, U.S.A). DNA purity was determined as the ratio of UV absorbance by DNA at 260 nm to absorbance at 280 nm. DNA quality was determined by gel electrophoresis on a 1 % agarose gel.

The fungal ITS1-2 gene sequences were amplified using the primer pair, ITS4- TCCTCCGCTTATTGATATGS (forward) and ITS5- GGAAGTAAAAGTCGTAACAAGG (Reverse) through Polymerase Chain Reaction (PCR). PCR cocktail contained 3 µL of genomic DNA (10 ng/µL), 0.1 µL of 5 µ/µl Taq DNA polymerase, 2.5 µL of 10x PCR buffer, 1.0 µL of DMSO, 0.8 µL of 2.5 mM DNTPs, 1.0 µL of 25 mM $MgC1_2$ (Promega), and 1 µL each of forward primer and reverse primer (concentration of 5µM). The total reaction volume was made up to 25 µl using 13.4 µl Nuclease free water. Amplifications were performed in a thermal cycler (Eppendorf) using an initial denaturation step of 94 ^{o}C for 5 minutes, followed by 36 cycles of denaturation at 94 ^{o}C for 30 seconds, annealing at 54 ^{o}C for 30 seconds and elongation for 45 seconds at 72 ^{o}C with a final extension for 7 min at 72 ^{o}C. The amplicons (PCR products) from the above reaction were separated by gel electrophoresis on 1.5 % agarose gel.

Amplified products were sequenced on ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Massachusetts, United States of America) using Sanger sequencing. Single-stranded DNA template, a DNA polymerase, a DNA primer, di-deoxynucleotide triphosphates (ddNTPs) and deoxynucleotide triphosphates (dNTPs) were used for sequencing. Each dideoxynucleotide (ddNTP) incorporated intermittently terminated DNA chain elongation as they lack 3'-OH group which is responsible for the formation of a phosphodiester bond between two nucleotides. This stopped the extension of DNA.

2.4 Statistical Analysis and Phylogeny

Frequency of occurrence of fungi was determined based on the Score Method recommended by Ataga and Akueshi [6]; where the numeral “1” represents absence of fungi and “2” represents absence of fungi; so that any value above “1” indicates presence of fungi. The identification of the isolated fungi by traditional cultural method was carried out with reference to Umechuruba and Elenwo [7] and Ataga *et al*. [8]. For identification using molecular technique, sequence editing which comprises trimming and sequence alignment using Clustal W were performed on MEGA X. Sequences were blasted on NCBI database for identification of species. The sequences of the ITS1-2 gene of the isolates were compared with the ITS sequences in GenBank. Best BLAST hits were used for the construction of neighbor-joining phylogenetic tree using the maximum composite likelihood method and evolutionary analysis was conducted on MEGA X software [9].

3. RESULTS AND DISCUSSION

3.1 Fungal isolates obtained from dumpsite soil

A total number of seven (7) fungi were isolated from the dumpsite soil. The pure cultures of the fungi are presented in Figure 1. The frequency of occurrence of each isolate was determined. Isolates 2 and 7 had the highest frequency of occurrence (1.8), followed by isolate 1 (1.7), isolates 3 and 4 (1.6), isolate 5 (1.5), and isolate 6 (1.4). Table 1 shows the morphological description of the isolates with their frequencies of occurrence. The Zymo Fungal / Bacteria Miniprep kit effectively extracted the fungal DNA with 260/280 nm ratio (DNA purity) between 1.28 and 1.97.

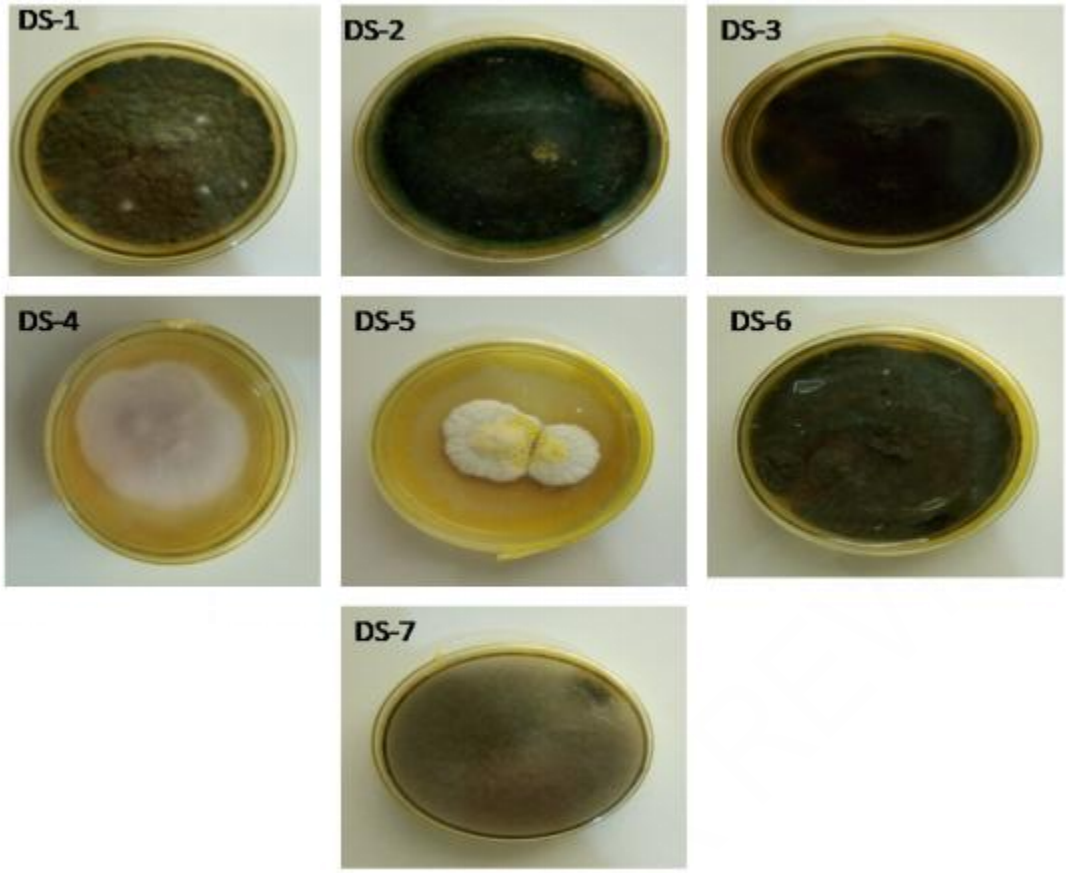

Figure.1: Fungal isolates obtained from University of Port Harcourt waste dumpsite soil and cultured on PDA.

Note: DS-1 to DS-7 represent isolate ID 1 to 7.

Table 1: Fungi isolated from dumpsite soil at room temperature (25^{o}C ± 2^{o}C).

Isolate ID	Morphology	Frequency of Occurrence	Suspected Organism
DS-1	Rough, dark green mycelia	1.7	*Aspergillus* sp.
DS-2	Smooth, dark green mycelia	1.8	*Trichoderma* sp.
DS-3	Smooth, dark and hard mycelia	1.6	*Aspergillus* sp.
DS-4	Smooth, white, cotton-like mycelia	1.6	*Aspergillus* sp.
DS-5	Hard, white mycelia with yellow spores in the middle	1.5	*Aspergillus* sp.
DS-6	Smooth, hard dark green mycelia	1.4	*Aspergillus* sp.
DS-7	Grey hairy mycelia with dark base	1.8	*Cunninghamella* sp.

3.2 Amplified products from ITS1-2 gene

Extracted genomic DNA from the seven fungal isolates were successfully amplified using the ITS4 and ITS5 primer pair. Amplified DNA showed bands on gel when viewed under UV light. The fragment produced by each species on 1.5 % agarose gel is presented in Figure 2.

L 1 2 3 4 5 6 7

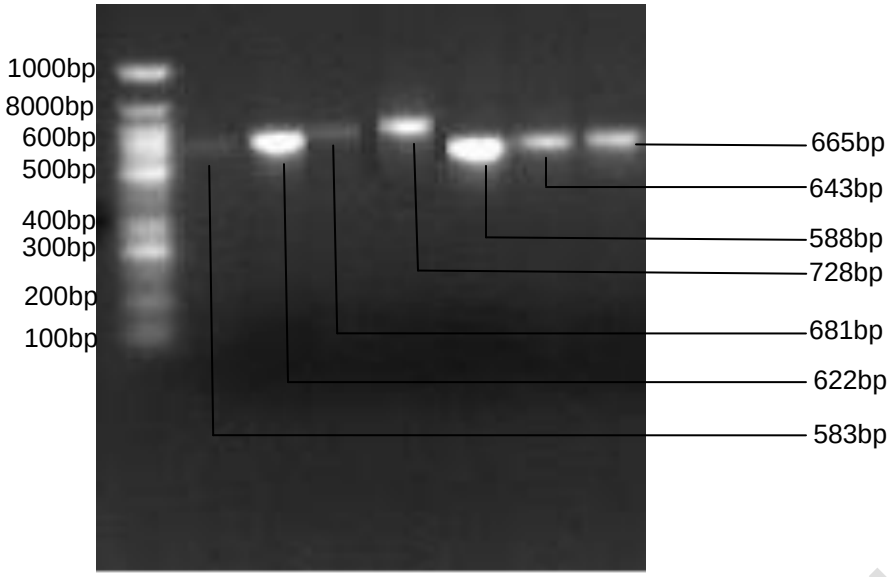

Figure 2: Gel electrophoresis profile of amplified ITS1-2 gene sequences of the fungal DNA from University of Port Harcourt waste dumpsite soil.
Note: L – 1 kb DNA ladder; Lane 1 to 7- Amplicons from fungal isolates.

3.3 DNA Sequences and Blasting

The numbers of base pairs of the sequenced ITS1-2 region of the fungal isolates are: 583bp, 622 bp, 681 bp, 728 bp, 588 bp, 643 bp and 665 bp for samples 1 to 7 respectively. The sequence alignment of each isolate was more than 200base pairs which is ideal for the identification of microorganisms.

BLAST results revealed the species identity of the fungal isolates to be: DS 1- *Aspergillus fumigatus*; DS 2- *Trichoderma harzianum*; DS 3- *Aspergillus felis*; DS 4- *Aspergillus templicola*; DS 5- *Aspergillus flavipes*; DS 6- *Aspergillus fumigatus*; DS 7- *Cunninghamella binariae*. The taxonomic affinities of the ITS1-2 gene sequences with the most closely related sequences on GenBank are shown in Table 2. The sequences of the fungal isolates were submitted to the GenBank and accession numbers were assigned to each isolate as shown in Table 3.

Table 2: Putative Taxonomic affinities of sequence types inferred from BLAST search of ITS sequences

Sample ID	Taxonomic affinity (Gene bank no)	Percentage Similarity
DS-1	*Aspergillus fumigatus* (MK732091.1)	90.04
DS-2	*Trichoderma harzianum* (KR868343.1)	100
DS-3	*Aspergillus felis* (MH865509.1)	84.56
DS-4	*Aspergillus templicola* (KP987081.1)	99.20
DS-5	*Aspergillus flavipes* (MH865240.1)	99.81
DS-6	*Aspergillus fumigatus* (MH141301.1)	90.17
DS-7	*Cunninghamella binariae* (MH858865.1)	99.00

Table 3: Accession numbers and strain numbers of fungal isolates

Sample	Accession numbers	Strain numbers
DS-1	MN602899	RCBBR_AEA1
DS-2	MN602970	RCBBR_AEA2
DS-3	MN603056	RCBBR_AEA3
DS-4	MN602898	RCBBR_AEA4
DS-5	MN602941	RCBBR_AEA5
DS-6	MN603087	RCBBR_AEA6
DS-7	MN603006	RCBBR_AEA7

3.4 Phylogenetic Analysis

The sequence analysis showed genetic diversity among the isolates with five isolates belonging to the genus *Aspergillus.* The phylogenetic tree constructed revealed the organisms (on GenBank) that are most closely related to the isolates obtained from the dumpsite soil, as presented in Figure 3. The vertical lines on the trees indicate the difference between the branches. The greater the length of the vertical line, the more the difference between the branches. The isolates were found to be closely-related to*Aspergillus fumigates*, *Trichoderma harzianum*, *Aspergillus felis*, *Aspergillus templicola*, *Aspergillus flavipes*, *Aspergillus fumigates* and *Cunninghamella binariae*. These organisms are in turn closely related to *Curvularia affinis, Aspergillus micronesiensis, Aspergillus parafelis, Botryosphaeria obtusa, Gongronella guangdongensis* and*Trichoderma aureoviride*.

Figure 3: Phylogenetic tree showing the relationship between the fungal isolates obtained from University of Port Harcourt waste dumpsite and other fungal species on GenBank

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4. DISCUSSION

Dumpsite accommodates numerous and diverse microorganisms, some of which are pathogenic to other environmental inhabitants such as plants, animals and humans. PCR amplification of the ITS1-2 gene sequences of the fungal isolates yielded amplicons between 580 to 800 base pairs. Internal Transcribed Spacer (ITS) region has been reported for its importance in biodiversity study and is proven to be more reliable in distinguishing fungal species [10] than traditional cultural methods. The molecular techniques used in this study proved successful as they led to the identification of seven fungal species viz: *Aspergillus fumigatus, Trichoderma harzianum, Aspergillus felis, Aspergillus templicola, Aspergillus flavipes, Aspergillus fumigatus* and *Cunninghamella binariae*.

The genus *Aspergillus* belongs to the phylum Ascomycota, classEuromycetes, order Eurotiales and family Trichomaceae. *Aspergillus fumigatus* has been isolated from milled rice [11]) and semi-desert soils [12]. It is an opportunistic filamentous fungus, one of the most common *Aspergillus* species that cause diseases in individuals with immunodeficiency which include patients receiving immunosuppressive therapy for autoimmune or neoplastic disease, organ transplant recipients, and AIDS patients [13].*A. fumigatus* is a saprotroph, widespread in nature and is typically found in soil and decaying organic matter such as compost and heaps, where it plays an essential role in carbon and nitrogen recycling [14]. Colonies of the fungus produce thousands of minute grey-green conidia (2-3 µm) from their conidiophores and these

conidia readily become airborne. *A. fumigatus* is capable of growth at 37 ^{o}C and can grow at temperatures up to 50 ^{o}C, with conidia surviving at 70 ^{o}C, the typical temperature of self-heating compost heaps. Its spores are ubiquitous in the atmosphere.

Aspergillus felis is a thermo-tolerant fungus, with optimum growing temperatures of ≥45 ^{o}C and <20 ^{o}C. Species of the genus *Aspergillus* can cause aspergillosis, in a diverse range of human and animal hosts. *A. felis* can be easily misidentified as the closely related fungus *A. fumigatus*, which is a well-studied cause of disease in humans. However, *A. felis* is intrinsically more resistant to anti-fungal drugs than *A. fumigatus*[15].

Aspergillus templicola belongs to the section *Flavipedes*. Members *Flavipedes* are found in soils, rhizospheres, caves and indoor environments usually as endophytes in plants, contaminants in food and as human pathogens [16]. They have been isolated from hypersaline soils [17]. *Aspergillus flavipes* is another fungus belonging to the *Flavipedes* section in the genus *Aspergillus* andis one of the most common and widely distributed species of this section. The species was first described in 2014 [18]. *A. flavipes* has been reported to produce extrolite, a wide array of bioactive compounds such as sterigmatocystin, citrinin, and lovastatin [16]. *A. flavipes* is also an endophytic fungi capable of producing secondary metabolites such as flavipesin A, flavipesin B, guignardic acid , and dibutyl phthalate that exert antibiosis against plant and human pathogens [19].

Trichoderma harzianum, a filamentous soil fungus, is an effective bio-control agent of several economic important soil-borne plant pathogens and viral vectors [20]. They are present in nearly all soils, where they occur as opportunistic plant symbionts and antagonists to many phytopathogenic fungi, thereby protecting plants from diseases. They are used for foliar application, seed treatment and soil treatment for suppression of various disease-causing fungal pathogens. *Trichoderma* strains are naturally resistant to many toxic compounds including herbicides, fungicides and pesticides such as phenolic compounds.

Cunninghamella binariae is a filamentous fungus found in soil and plant materials, particularly at Mediterranean and subtropical zones. It has also been isolated from animal feces [21]. *Cunninghamella* is a genus in the order Mucorales covering species with distinctive morphological features. In addition to being common contaminants, *Cunninghamella* species cause infections such as Mucormycosis in immune-compromised patients with high mortality [22].

The works of other researchers, who employed the traditional cultural method of characterization, revealed similar fungi species with the ones isolated in this study, with species of the *Aspergillus* genus being predominant in the different waste dumpsites studied. Simon-Oke and Alade [23] carried out a study on two dumpsites in Akure metropolis in Nigeria and isolated *Aspergillus flavus, A. fumigatus, A.* niger and *Mucor mucedo* among other fungal and bacterial species. The authors suggested that the low bacteria and fungi count in one of the dumpsites may be as a result of smaller size of the dumpsite and the low human activities in the area. Eleanya *et al*. [24] reported the isolation of *Aspergillus niger, Fusarium* spp., *Rhizopus* spp. and *Mucor* spp. from dumpsites in Bwari, Federal Capital Territory, Nigeria. Williams and Hakam [25] isolated *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Saccharomyces* species from dumpsites in Port Harcourt. While the traditional cultural method they employed revealed similar fungi species, the molecular method of characterization employed in this study revealed that more fungi species such as, *Aspergillus flavipes*, *Aspergillus templicola, Trichoderma harzianum* and *Cunninghamella binariae* can also be found in dumpsite soil. Microorganisms tend to be more abundant in the soil when there is increased moisture for instance, during the rainy season [23].

The molecular method used in this study effectively characterized all the fungi isolates at the species level. This study has outlined some of the fungal organisms associated with dumpsite soil and the economic importance of the isolates have also been highlighted to give readers the insight into the beneficial or detrimental effects of the isolates. Some of the organisms obtained from this study are plant pathogens while others are of public health concern.

4. CONCLUSION

This study has shown that dumpsite soil holds diverse fungal species of public health significance. Though most of the isolates obtained from this study belong to the genus *Aspergillus*, other fungi genera isolated are equally of economic importance. The traditional cultural method employed in this study only aided in suggesting the suspected fungi isolated based on the morphological characteristics of the organisms. This method cannot be successfully used to identify microorganisms to the species level because some organisms look similar at the morphological and microscopic level and this may lead to misidentification. Molecular techniques have been revealed to be highly effective in the identification and characterization of microorganisms as they are based on the genetic constitution of organisms; and no two organisms are the same at the genetic level. These techniques also allow for the comparison of DNA sequences between known and unknown species, hence the need to adopt these methods for microbial biodiversity study of various soil samples. This study will complement the existing knowledge on the fungal organisms associated with dumpsite soil and also educate

individuals on the dangers of living close to dumpsites due to the adverse effects of health implications that can be caused by some of these dumpsite fungi.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Dominati E, Patterson M and Mackay A. A framework for classifying and quantifying natural capital and ecosystem services of soils. Ecol Econ. 2010;69:1958-1968.

2. Nyandjou JM, Yakubu SE, Abdullahi IO and Machido DA. Enteric bacteria of public health significance isolated from Zaria metropolis dumpsite soil. Sci World J. 2018;13(4):30-34.

3. Baysal-Gurel F, Gardener BM and Miller SA. (2012). Soil borne diseases management in organic vegetable production. eOrganic. Accessed 14 October 2021. Available: https://articles.extension.org/pages/64951/soilborne-diseases-management-in-organic-vegetable-production.

4. Adeniyi M, Titilawo Y, Oluduro A. Molecular identification of wild Nigerian mushrooms using internal transcribed spacer: polymerase chain reaction. AMB Express. 2018;8:148-156.

5. Elijah AI, Atanda OO, Popoola AR and Uzochukwu SV. Molecular characterization and potential of bacterial species associated with cassava waste. Niger Food J. 2014;32(2):56-65.

6. Ataga AE and Akueshi CO. Fungi associated with sunflower seeds in Nigeria. Seed Res. 1986;1(24):64-65.

7. Umechuruba CI and Elenwo EN. Diagnostic techniques in mycology with practical exercise. Belk Publishers. 1997;30-42.

8. Ataga AE, Elenwo EN and Nwachukwu EO. Laboratory exercises and series in mycology. ACOTEC Technologies, Port Harcourt, Nigeria. 2010;58-64.

9. Kumar S, Stecher G, Li M, Knyaz C and Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547-1549.

10. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA *et al*. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. PNAS USA. 2012;09(16):1-6.

11. Yazdani D, Zainal-Abidin MA, Tan YH and Kamaruzaman S. Molecular identification of *Aspergillus* and *Eurotium* species isolated from rice and their toxin-producing ability. Microbiology 2011;80(50):720–727.

12. Giusiano GE, Piontelli E, Fernández M S, Mangiaterra ML, Cattana ME, Kocsublé S *et al*. Biodiversity of species of *Aspergillus* section fumigatiin semi-desert soils in Argentina. Rev Argent Microbiol. 2017;49(3):247-254.

13. Ben-Ami R, Lewis RE, Kontoyiannis DP. Enemy of the immunosuppressed state: an update on the pathogenesis of *Aspergillus fumigatus* infection. Br J Haematol. 2010;150(4):406-417.

14. Fang W and Latge JP. Micobe profile: *Aspergillus fumigatus*: a saprophytic fungi and opportunistic fungal pathogen. Microbiology 2018;164(8):1009-1011.

15. Barrs VR, Van-Doorn TM, Houbraken J, Kidd SE, Martin P, Pinheiro MD *et al*. *Aspergillus felis* sp. nov., an emerging agent of invasive aspergillosis in humans, cats and dogs. PLoS One 2013;8(6):1-11.

16 Hubka V, Nováková A, Kolarik M, Jurjevic Z and Peterson SW. Revision of *Aspergillus* section Flavipedes: seven new species and proposal of section Jani sect. nov. Mycologia 2015;107(1):169-208.

17. Arzanlou M, Samadi R, Frisvad JC, Houbraken J and Ghosta Y. Two novel *Aspergillus* species from hypersaline soils of the National Park of Lake Urmia, Iran. Mycol Prog. 2016;15(10-11):1081-1092.

18. Visagie CM, Hirook, Y, Tanney T, Whitfield E, Mwange K, Meijer M *et al*. *Aspergillus, Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. Stud Mycol. 2014;78:63-139.

19. Zhang H, Tang Y, Ruan C and Bai X. Bioctive secondary metabolites from the endophytic *Aspergillus* genus. Rec Nat Prod. 2016;10(1):1-16.

20. Brunner K, Zeilinger S, Ciliento R, Woo SL, Lorito M, Kubicek CP and Malc RL. Improvement of the fungal biocontrol agent *Trichoderma atroviridae* to enhance both antangonism and induction of plant systemic disease resisance. Appl Environ Microbiol. 2005;71:3959-3965.

21. Nguyen TT, Hyde KD and Lee HB. *Cunninghamella binariae*, *Mucor ardhlaengiktus,Mucor gigasporus* and *Umbelopsis changbaiensis*, Newly recorded species from amphibian feces and soil in Korea. Phytotaxa 2019;425:19-34.

22. Yu J, Walther G, Van-Diepeningen A, Van-Den-Ende A, Li RY, Moussa T*et al*. DNA barcoding of clinically relevant *Cunninghamella* species. Med Mycol J.. 2014;53(2):99-106.

23. Simon-Oke IA and Alade T. Survey andIdentification of bacteria and fungi associated with two dumpsites in Akure metropolis. Microbiol Res Int. 2018;24(5):1-8.

24. Eleanya EU, Oyegue TO, Rwang PG, Ozoude TO and Okey-Ndeche NF. Microbiological analysis of dumpsites in Bwari, Federal Capital Territory, Nigeria. Microbiol Res Int. 2017;22(3):1-6.

25. Williams JO and Hakam K. Microorganisms associated with dumpsite in Port Harcourt Metropolis, Nigeria. J Ecol Nat Environ. 2016; 8(2):9-12.