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Exploring Fungi-mediated Biodegradation Pathway of Polymers in Environmental Systems(Dump Sites)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Context: The growing presence of environmental plastic has garnered escalating attention and worry in recent decades. While ease of production, durability, cost-effectiveness, and resistance to corrosion are valued attributes for consumer goods, they contribute to the persistent environmental presence, leading to a widespread surge in plastic pollution.

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Aim: To find out various microbial- mediated biodegradation pathway of polymers (black nylon and white nylon).
Settings and Design: The research was carried out by obtaining dump soil from a mechanic dump site and a hospital dump site in Ibadan, Oyo state.
Materials and Methodology: Dump soil were obtained, diluted(serial) and Cultered and morphological and microscopic observation were carried out while polythene bags which are black nylon and white nylon were also degraded at Day 5, 10 ,15 and 20.
Results: Biodegradation of polymers is influenced by both polymer characteristics and the presence of specific microorganisms in the environment. Fungi, with species like *A. niger, A. flavus, and C. fungus* were identified in a dump site. Weight analysis of polythene bags (black nylon and white nylon) over days revealed slight changes, indicating some degradation. PCR analysis confirmed genetic material from fungal isolates, highlighting the specificity and sensitivity of the chosen primers.
Conclusion: The presence of Aspergillus species in the dump site suggests their potential for bioremediation efforts in challenging environments

Keywords: Fungi; polymers; dump site; biodegradation; soil.

1. INTRODUCTION

The estimated quantity of improperly managed plastic waste in the environment was reported to range from 60 to 99 million metric tonnes in 2015 [1]. Plastic is an environmental contaminant. [2,3], and its persistence in the environment, and the potential harm that it may cause to organisms and ecosystems is an emotive modern day reality.

Quantifying the volume of plastic waste in the environment presents a challenge, leading to varying global estimates of plastic waste. For example, Lebreton, L. C., et al., [4] propose an annual global input of plastic waste from rivers to the marine environment of 1.15–2.41 million tons, while Schmidt, Krauth, and Wagner [5] put this figure at 0.41–4 million tons. There are many different plastic polymers that make up plastic pollution: polystyrene (PS), polyethylene (PE), and polypropylene can all be found as contributing environmental pollutants. Despite being a recyclable material and being environmentally toxic, plastics in the United States have a recycling rate of just 8.8% as of 2016 [6].

Unconstrained plastic debris can be transported through and between environments via natural processes like wind, water currents, and animal movement. It can also occur through human activities such as improper disposal or littering. Environmental modeling can estimate plastic concentrations and abundances in the environment. Geyer, Jambeck, and Law [7] estimate that 79% of the 6,300 metric tons of plastic waste generated up to 2015 are either in landfill or in the natural environment. However, quantifying the amount of plastic waste in the environment is challenging, and global estimates of plastic waste vary. plastics may also act as vectors for other pollutants. The ingestion of plastics to which chemicals are sorbed can occur through a variety of pollutants, including pesticides, industrial chemicals, and other environmental contaminants [8].

Biodegradable polymers are materials that can work for a limited time before degrading into readily discarded products through a regulated procedure [9]. They might be made from a variety of wastes or/and bioresources, such as wastes of food, animal, agro-waste as well as other sources such as starch, and cellulose. Bioplastics made from renewable resources are often less expensive than those made from microbial resources prompting producers to concentrate on making bioplastics from renewable resources [10]. The use of biodegradable polymers has environmental benefits such as regeneration of raw materials, biodegradation and reduction of carbon dioxide emissions that are led to global warming [11].

Microorganisms such as bacteria and fungus such as Specific types of bacteria, such as certain strains of Pseudomonas, Bacillus, and Clostridium, as well as fungi like Aspergillus and Penicillium may consume biodegradable polymers and convert them to water, Carbon dioxide, and methane. The biodegradation process depends on the material's composition [12]. The polymer morphology, polymer structure, chemical and radiation treatments, and polymer molecular weight are all parameters that

influence the biodegradation process [13]. Biodegradable polymers are also called biopolymers [14]. There are two reasons to use the polymers from renewable resources; (i) environmental concerns in terms of increasing plastic waste and global warming as a result of releasing of carbon dioxide when burning waste, and (ii) petroleum resources are limited and ended [13].

The biodegradable plastics sector shows great promise. However, they need to be developed in tandem thorough examination of end-of-life processes of treatment and a worldwide integration with organic management of waste as selective biowaste collection expands. Biodegradable plastics have the benefit of being able to be handled biologically at the end of their lives (composting or anaerobic digestion).

2. MATERIALS AND METHODOLOGY

2.1 Materials

Apparatus: Test tubes and test tube rack, Conical flasks, Beakers, Weighing balance, Forcep, Measuring cylinder, Masking tape, Microscope, Slide and slide cover, Marker, Wireloop and Flames, Soil sample, Polythene bags, Pipette.

Reagents: Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose broth, Ethanol, Mercury and Lactophenol.

2.1.1 Sample collection from different sites for microbial degradation

The dry soil samples and Polythene bags were collected from two different dump sites and a mechanic workshop in Ibadan, Oyo state which are 54km apart. The samples were separated and labeled according to their location. The soil samples were collected from the plow layer (0–15 cm in depth) of the soil at different places. About 100 g of the soil were taken and packed into labeled sterilized bottles.

2.1.2 Surface sterilization of polythene bag

The collected polythene bags from the mechanic dump site and the hospital dump site were cut into small pieces and cleaned with tap water and surface sterilized with ethanol. Then washed with distilled water, 0.1% mercuric chloride and again washed with distilled water.

2.2 Methodology

2.2.1 Isolation of soil fungi

One gram of the soil sample from both mechanic and hospital dump sites was suspended in 10 ml of sterile distilled water to make serial dilutions (10^{-1} to 10^{-5}). One mL of each dilution was placed on Potato Dextrose Agar (PDA) containing 1 % streptomycin. The plates were incubated at 28˚C in the dark. The plates were observed for one week. The cultures were incubated at room temperature in an incubator for three to five days.

2.2.2 Media preparation

The soil fungi were isolated by the soil dilution method. 4g of Sabouraud Dextrose Agar (SDA) was weighed and dissolved in 60ml of distilled water and autoclave at 121°C for 15 minutes. The media was allowed to cool. The media was then poured aseptically into the petri-dishes and allowed to solidify

2.2.3 Serial dilution

9 test tubes were arranged in a test tube rack, 3 test tubes for each of the 3 samples. 9ml of distilled water was pipette into each of the test tubes. 1g of the soil sample was added into the first test tube containing the distilled water and shakes vigorously to have a homogeneous mixture. 1ml of the solution in the first test tube was pipette into the second test tube and the second test tube to the third test tube. This procedure is subsequently replicated for the remaining samples. 1ml of the solution was spread on the freshly prepared media for culture and stored at room temperature for one week.

2.2.4 Subculture and pure culture

After the incubation, the fungal colonies were observed, and the pure cultures were maintained. Subculture of each of the different colonies was done on to a Sabouraud Dextrose Agar (SDA) plate and stored at room temperature for 7 days.

2.2.5 Microscopic observation

Fungal morphology was studied macroscopically by observing the colony features (color, shape,

size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium.

2.2.6 Degradation of polythene bag

200mL of nutrient broth was prepared and autoclave at 121°C for 15 minutes. 200mL of cooled nutrient broth was poured into eight 250ml sterile conical flasks. The sterile pre weighed polythene bag pieces was aseptically transferred into the nutrient broth. A loop full of fungal culture was inoculated into nutrient broth. The flasks were incubated at 37°C for 5, 10, 15 and 20 days. The polythene bags were carefully removed from the culture by using forceps after different days of incubation. The collected pieces was washed thoroughly with tap water, ethanol and then distilled water. The pieces were shade dried and weighed for final weight. The data were recorded. The same procedure was repeated for all the treated samples.

2.2.7 Determination of degradation percentage of polythene bag

The percentage of degradation of polythene bag pieces by *Aspergillus niger and Candida fungus* was determined by calculating the percentage of weight loss of plastics. The percentage of weight loss was calculated by the following formula.

Percentage of weight loss= initial weight - final weight ÷ initial weight × 100.

2.2.8 Molecular identification of fungal species dna extraction and pcr amplification

The DNA Extraction of genomic DNA from the fungi was conducted from a one-week-old SDA culture using Zr Fungal/Bacterial Dna Miniprep (Manufactured By Zymo Research). PCR products were purified using the QIA quick PCR purification kit.

2.2.9 Sequencing and analysis

The PCR products were sent for sequencing to Bioformatics services, Mokola Ibadan, Nigeria. The obtained sequences were compared with the other related sequences.

3. RESULTS

3.1 Morphological and Microscopic Identification of Fungi

Table 1 shows the microscopic and morphological characteristics of three (3) fungal species isolated from dump site soils. The macroscopic and microscopic characteristics of the fungal species showed the organisms to be *A. niger, A. flavus, and C. fungus* respectively. Fungi demonstrated the ability to thrive in the soil of the dump site.

Table 1. Morphological and Microscopic identification of fungi isolated from 2 different dump sites (hospital and refuse) and mechanic workshop in Ibadan, Oyo state.

S/NO	Morphological	Microscopic characteristics	Organism
Isolate A1	White yellow colony covered by a dark brown layer.	The conidial heads are radial and they split into columns, the conidiophores are smooth and coloured	*Aspergillus niger*
Isolate A2	White yellow colony covered by a dark brown layer.	The conidial heads are radial and they split into columns, the conidiophores are smooth and coloured	*Aspergillus niger*
Isolate B1	A greenish, flat dry colony	Short, slender tube like structure, the canidiophores are short and inflated	*Candida fungus*
Isolate B2	Whitish colony then turns to yellow-green.	The conidial are characteristically green, the conidiophores are colorless and rough textured	*Aspergillus flavus*
Isolate C1	White yellow colony covered by a dark brown layer to black	The conidial heads are radial and they split into columns, the conidiophores are smooth and colored.	*Aspergillus niger*
Isolate C2	A greenish, flat dry colony.	Short, slender tubelike structure, the canidiophores are short and inflated	*Candida fungus*

3.2 Biodegradation of Polythene Bag by *Candida fungus* (Black Nylon)

Table 2 indicates to have uniform initial weight of the polythene bag (Black nylon) which is 0.06g for all the days of the treatment. There was slight changes in the final weight of the polythene bag (black nylon) with Day 5 and 10 having 0.05g as the final weight of the polythene bag (black nylon) and Day 15 and 20 had 0.06g as the final weight of the polythene bag (Black nylon).

Table 2. Biodegradation of polythene bag by *Candida fungus* (black nylon)

S.NO	Days of Treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1	5	0.06	0.05	1.67%
2	10	0.06	0.05	1.67%
3	15	0.06	0.06	0%
4	20	0.06	0.06	0%

3.3 Biodegradation of Polythene Bag by *Aspergillus Niger* (White Nylon)

Table 3 indicates to have uniform initial weight of the polythene bag (white nylon) which is 0.06g for all the days of the treatment. There was slight changes in the final weight of the polythene bag (white nylon) with Day 5 having 0.06g as the final weight of the polythene bag (white nylon) while Day 10 and 15 had 0.04g as the final weight of the polythene bag (white nylon) and Day 20 had 0.05g as the final weight of the polythene bag (white nylon).

Table 3. Biodegradation of polythene bag by *Aspergillus Niger* (white nylon)

S.NO	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1	5	0.06	0.06	0%
2	10	0.06	0.04	33.3%
3	15	0.06	0.04	33.3%
4	20	0.06	0.05	1.67%

PCR (Polymerase Chain Reaction) to amplify specific DNA regions from fungi isolates which are *Candida fungus, Aspergillus flavus and Aspergillus niger from both dump site*. In the gel image, Lane M contains molecular weight markers for reference, Lane C is the negative control, and Lanes A and B show PCR products from isolates A and B.

In Panel A, the PCR of the fungal universal region was performed. Lane M in this panel represents a molecular weight marker. The bands labeled A, B, and C represent different isolates of *Candida guilliermondii.* It's noteworthy that Isolate 19, identified as *Candida guilliermondii,* exhibits a high pairwise similarity of 98.97% with *Candida intermedia* strain JYC551. The PCR-RFLP (Restriction Fragment Length Polymorphism) technique was employed to characterize and differentiate various fungal isolates, providing valuable insights into their genetic similarities and differences.

Fig. 1a. Examining a Fungal Isolate

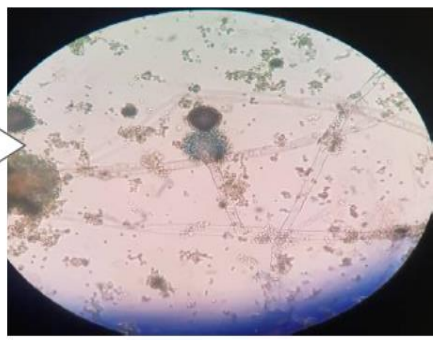

Fig. 1b. Examining a Fungal Isolate at a Microscopic Level

Fig. 2a. Isolated Organism from mechanic dump site

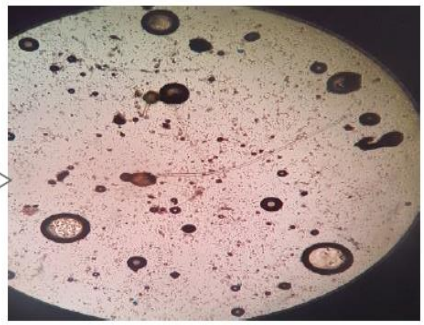

Fig. 2b. Isolated Organism from mechanic dump site Under microscope)

Fig. 3a. Isolated Organism from hospital dump site

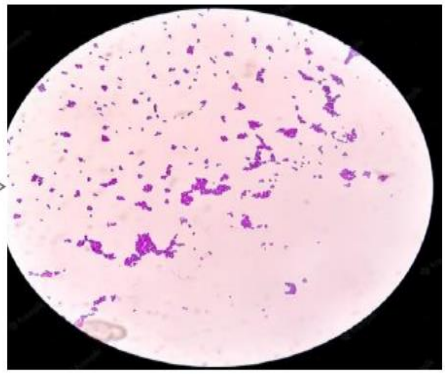

Fig. 3b. Isolated Organism from hospital dump site (Under microscope)

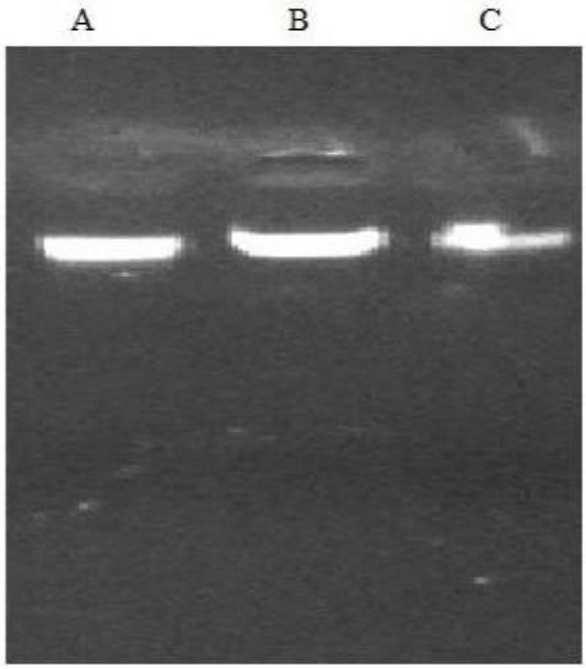

Fig. 4. Gel image showing high molecular weight DNA extracted from the isolates

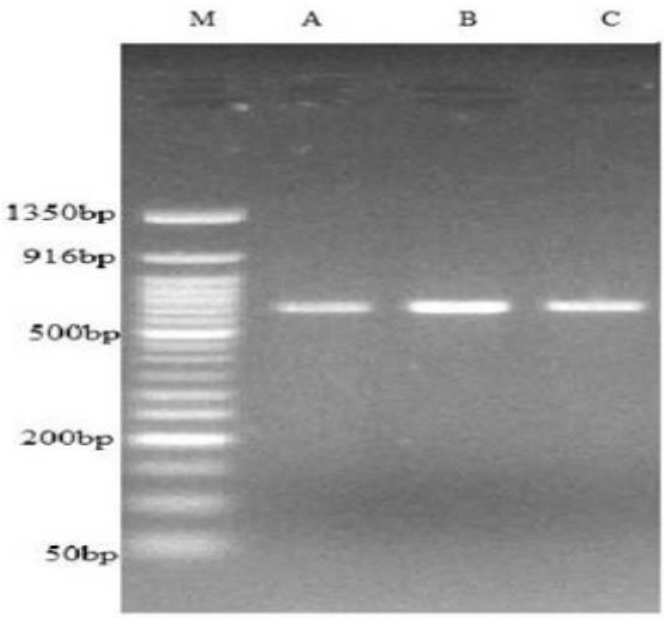

Fig. 5. PCR products obtained from fungi isolates using ITS primer pair

4. DISCUSSION

The degree of biodegradation depends on polymer traits and the specific microorganisms in the environment. Fungi are capable of growing in the dumped site soil. The isolated fungi *(Candida fungus, Aspergillus flavus and Aspergillus niger)* are inhabitants of soil and plant decayed matter organisms. biodegradable polymers are designed to degrade quickly by the microbes due to their ability to degrade organic and inorganic materials, such as lignin, starch, cellulose, and hemi-cellulose [15]. The mechanism of biodegradation involves the action of microbial enzymes on the surface of the plastics Montazer., et al., [16].

Different strains of fungi from dumped site soil were isolated and identified by morphological and microscopic characteristics and are presented in Table 1. Three (3) fungi were isolated from dump site soil. Their macroscopic and microscopic characteristics confirmed the species as *A. niger, A. flavus and C. fungus* respectively [17,18].

Table 2 indicates to have uniform initial weight of the polythene bag (Black nylon) which is 0.06g for all the days of the treatment. There was slight decrease in the final weight of the polythene bag (black nylon) with Day 5 and 10 having 0.05g as the final weight of the polythene bag (black nylon) which indicates a slight weight loss and also in correspondence with weight loss measurement is a global analysis, which constitutes the first global approach for studying the biodegradation of polymer by Magnin, A., *et al.,* 2018 and Day 15 and 20 had 0.06g as the final weight of the polythene bag (Black nylon) which was same with the initial weight.

Table 3 demonstrates a consistent initial weight of the polythene bag (white nylon) at 0.06g throughout the treatment period. There was a slight decrease in the final weight on Day 5, remaining at 0.06g. However, on Days 10 and 15, the final weight decreased to 0.04g, aligning with the observations made by Magnin, A., et al., in [19]. Day 20 showed a decrease with a final weight of 0.05g, further supporting Magnin, A., et al.'s [19] findings.

PCR was used to amplify genetic material from fungi isolates using specific primers. In the gel, Lane M represents molecular weight markers, Lane C is the negative control, and Lanes A and B show PCR products from isolates A and B. In Panel A, the PCR results for the fungal universal region are displayed. M represents a 50 base pair molecular weight marker and it's indicate the rate of biodegradation being proportional to the molecular weight of the plastic targeted which is inline with Muthukumar A, Veerappapillai S. 2015 study. The samples labeled Iso18, Iso19,

and Iso20 are all identified as *Candida guilliermondii.*

Additionally, it's noted that Isolate 19 shares a high similarity of 98.97% with a *Candida intermedia* strain called JYC551. This suggests a close genetic relationship between Isolate 19 and this particular *Candida intermedia* strain.These results showed that the primers are specific, highly sensitive and targeted fungal rDNA. They were reported to be on the conserved regions that identified ITS/rDNA region for *Aspergillus* species. The presence of these three *Aspergillus* species on the dumped site soil environment shows their ability to produce spores capable of resisting and spreading in harsh conditions such as tropical bed bugs in peninsular Malaysia and this potential suggests they are good for bioremediation study [20-22].

5. CONCLUSION

The increase in plastic pollution exerts a notable influence on various forms of life. Fungal biodegradation of plastics offers a promising solution to mitigate this issue. This area of research has garnered substantial attention in addressing plastic pollution. Fungi, specifically *Aspergillus flavanus, Aspergillus Niger, and Candida fungus,* were isolated from soil at a dump site and a mechanic workshop. The findings revealed that the microbe tested was identified as *Candida fungus* through molecular analysis. This finding carries substantial promise for efficiently breaking down and purifying soil in dump sites.This review offers valuable insights into the diverse range of fungi that play a role in breaking down various types of plastic polymers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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