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The Diversity of Fungi in Landfill and their Potential to Degrade Plastic

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Abstract: Plastic has been known as a recalcitrant material and is very difficult to degrade in nature, resulting in its accumulation and threatening the environment if it is not managed properly. Studies on the degradation of plastics have been obtaining much attention recently. This study aimed to determine the diversity of fungi isolated from plastic wastes in landfills and to identify the potential plastic-degrading ability of the isolates. Plastic waste samples were collected from Terjun Landfill, Medan Marelan, Indonesia. Fungi were isolated directly on potato dextrose agar medium and characterized macroscopically and microscopically. Plastic-degradation potential was screened by growing the isolates on mineral salt medium agar containing 0.5% plastic powder. Plastic sheets of low-density polyethylene and linear low-density polyethylene were used for testing the biodegradation ability of the fungi. Twenty-four different fungal morphotypes were successfully purified from plastic wastes, in which five isolates showed better growth. Molecular identification indicated that the five potential isolates belong to different species of *Fusarium solani* (LDPE5), *Botryosphaeria laricina* (LLDPE10), *Aspergillus fumigatus* (HDPE1), *Aspergillus flavus* (HDPE3) and *Aspergillus niger* (PP5). The biodegradation test showed that isolate LDPE5 exhibited the best activity with a 20.83% weight reduction of the plastic sheet after 45 days followed by isolate LLDPE10 with a 6.49% weight reduction. Scanning electron micrographs showed the surface of a degraded sheet of the plastic sheet became rough and wavy. Fourier transform infrared analysis showed the formation of new functional groups on the plastic sheet. Then, it indicates that fungi colonizing plastic material in landfills plays an important role in the biodegradation process.

Keywords: Diversity, Fungus, Identification, Landfill, Plastic Degradation

Introduction

Plastic is a supporting material composed of synthetic polymers with stable physical and chemical characteristics, good mechanical properties, and low production costs. On the other hand, the emerging contaminant including microplastic was reported to be present in mineral water bottles (Akhbarizadeh *et al.*, 2020). Materials containing plastic are recalcitrant and very difficult to degrade in the environment because the C atoms in plastic materials do not have functional groups, making them difficult to hydrolyze (Inderthal *et al.*, 2021). The main-chain structures of plastic polymers can only be broken down through high-energy oxidation reactions, resulting in the processing of plastic waste that has not been handled chemically and thus accumulates in landfills and can cause health problems and environmental damage (Sanchez *et al*., 2020).

Chemical and physical treatments of waste tend to be expensive and produce persistent organic pollutants in the form of furans, dioxins, CO_2, nitrogen oxides, SO_2, polychlorinated dibenzodioxins, etc. which are known to be toxic to biota, including humans (Thiounn and Smith, 2020). Plastic accumulation on land can lower soil fertility, reduce water infiltration into the soil, and threaten animal populations (Ojha *et al*., 2017). Microorganisms contribute to every ecological niche both on land and in water and some microorganisms can use plastic as an energy source in microhabitats with abundant plastic materials, known as plastispheres (Kyaw *et al*., 2012).

Landfills are potential habitats for exploring and exploiting various biological agents (such as fungi and bacteria) in applications involving the biodegradation of plastic waste (Amaral-Zettler *et al*., 2020). Fungi show the most potent biological plastic-degrading agents because they can colonize various substrates (such as soil, water, and air) by producing extracellular enzymes (such as laccase, peroxidase, and esterase) that directly promote attachment to PE as a substrate and its subsequent biodegradation (Muhonja *et al*., 2018). In addition, the degradation mechanisms of fungi are also assisted by biosurfactants and other secondary metabolites, although the quality of the compounds produced may differ at the strain level, which requires further investigation (Wei and Zimmermann, 2017). Some important fungo, including basidiomycetes (i.e., *Agaricubisporus*, *Pleurotus abalones*, and *Pleurotus ostreatus*) and ascomycetes (i.e., *Aspergillus*, *Penicill*ium, and *Trichoderma*) can degrade plastic via laccase production (Sánchez, 2020). Some bacteria, including Pseudomonas members such as *Enterobacteriaceae* and *Moraxella*, can degrade plastic polymers. The biodegradation process involves the hydroxylation of C-C bonds to release polymer alcohols or secondary groups, which are then oxidized to ketones (Yuan *et al*., 2020).

Terjun Landfill is a city-wide landfill located in Medan City, North Sumatra. The landfill uses an open dumping system in which all types of wastes, organic and inorganic including plastic materials, are disposed to that site. Here, we aimed to characterize the plastic waste-associated fungal community in this landfill and to identify specific fungal isolates with plastic-degrading potential.

Materials and Methods

Sampling Site and Isolation of Fungi from Plastic Wastes

Collections of plastic-waste samples of various types, namely linear Low-Density Polyethylene (LDPE), Linear Low-Density Polyethylene (LLDPE), High-Density Polyethylene (HDPE), and Polypropylene (PP) were obtained from the Terjun Landfill (Medan Marelan, Medan City, North Sumatra. Samples were taken in sufficient quantities from three different randomly selected sites and kept in the cool box before transporting to the laboratory. The PE-based organic waste was cut into small square shapes (1×1 cm). Fungi were isolated from the cut plastic-waste samples by direct cultivation on Potato Dextrose Agar (PDA) medium. Each culture was incubated at 37°C for 48 h. Fungi growing around the plastic were then purified to obtain single colonies and were characterized as described previously (Spina *et al*., 2021).

Screening for Potential Plastic-Degrading Fungal Isolates

Powder from each type of plastic (LDPE, LLDPE, HDPE, and PP) was prepared in Mineral Salt Medium Agar (MSMA) medium at a concentration of 0.5% and used as the main C source for different fungal isolates. An agar plug was prepared for each isolate using a cock borer and inoculated into a petri dish containing MSMA medium. As the positive control, 0.5% glucose was used as the main C source. As the negative control, each agar plug was incubated in the absence of plastic powder. Each condition was tested with 3 replicates. The radial diameters of the colonies were measured and compared to those of the positive control at the experimental endpoint (Munir *et al*., 2018a).

Biodegradation Testing with Plastic LDPE and LLDPE Sheets

Biodegradation tests were conducted on MSMA medium supplemented with 0.5% glucose. LDPE sheets were cut into 1×2 cm pieces and weighed. A plastic sheet was aseptically placed on the surface of the MSMA medium. Two agar plugs of the growing fungal isolates were inoculated on each side of the LDPE sheet. The culture was incubated at room temperature (26±2°C) for 45 days. MSMA medium without a fungal-isolate agar plug was used as a reference to confirm the degradation of LDPE plastic sheets. After incubation, the plastic sheets were removed from the culture using tweezers and rinsed with 70% ethanol and sterile distilled water.

The plastic sheets were air-dried at room temperature for 24 h and weighed. The LDPE and LLDPE plastic sheets that showed a large change in their final weight were selected for testing at the next stage (Munir *et al*., 2018b).

Scanning Electron Microscope (SEM) and FOURIER Transform Infrared (FT-IR) Analysis of Degraded Plastic Sheets

Analysis of detection of the surface texture of the plastic before and after degradation test with the fungi of interest was conducted using an SEM at 5000 magnification. Prior to analyses, samples were treated with a series of glutaraldehyde and alcohol solutions for fixation (Hadar and Sivan, 2004). Analyzing and detecting functional groups in the LDPE and LLDPE plastic sheets before and after degradation testing with the fungi of interest were conducted using FT-IR at wavenumbers between 650^{-1} and 4000 cm^{-1} (Hadar and Sivan, 2004).

Molecular Identification of Fungal Isolates

The Internal Transcribed Spacer (ITS) DNA sequence of each potential isolate was analyzed for species identification. The DNA sequences were then aligned with sequences deposited in the fungal ITS DNA database of the National Center for Biotechnology Information (NCBI). The sequences were further analyzed through BLASTn searches against the rRNA/ITS database. The sequences obtained were then aligned using the muscle algorithm of MEGA X software and phylogenetic trees were constructed using the neighbor-joining method.

Results and Discussion

Fungi Isolated from the Plastic Waste

We isolated 24 distinct fungal morphotypes from four types of plastic wastes of LDPE, LLDPE, HDPE, and PP from a landfill in Medan Marelan on a PDA medium. The isolates had different shapes, textures, elevations, margins, colors, and conidia (Table 1). With these samples, we obtained five fungal isolates from LDPE as the primary C source, 10 with LLDPE, three with HDPE, and six with PP. Overall, the fungal colonies had circular and irregular shapes. Texturally, the dominant fungi among all isolates were cottony and velvety, and whole and wavy margins were the most common. These results indicate that plastic material dumped in landfills might have been infected by fungal mycelia or by spore or fungal conidia. Furthermore, the difference in the number of fungi isolated from each plastic waste might be caused by the different plastic materials.

Previously, nine isolates were identified with different colors and shapes in soil samples from the Palembang landfill, which predominantly showed an overall blackish-brown color (Russell *et al*., 2011). The involvement of fungi in the degradation of waste material including plastics in landfills has been reported by some groups. Various microorganisms (including fungi and bacteria) can colonize the waste, resulting in the degradation of the material (Gajendiran *et al*., 2016). A previous study also identified several fungal species, including *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Trichoderma pseudokoningii*, *Paecilomyces lilacinus*, *Cogronella* sp. and *Acremonium recifei*, in a landfill (Folino *et al*., 2020).

Screening Fungi for Plastic-Degrading Activity

By selecting for plastic-degrading ability among the fungal isolates from the landfill in Medan Marelan on MSMA medium containing plastic powder, we found that five isolates grew well on the medium (Table 2).

Twenty-one fungal isolates grew successfully on MSMA medium with various types of added plastic powder. We used a medium with added glucose as a positive control medium lacking glucose or plastic powder as a negative control. The screening was performed on a plate containing plastic powder (up to 0.5% by volume). The fungal strains capable of plastic degradation showed increases in their colony diameters during the screening. However, not all fungal isolates survive the screening. Five fungal isolates (LDPE5, LLDPE10, HDPE1, HDPE3, and PP5) grew to diameters of >57 mm and showed constant mycelial growth.

Table 1: Macroscopic and microscopic characteristics of fungi from the landfill site of Medan Marelan

	Morphological characteristics of the fungal isolates in this study					
	Macroscopic					Microscopic
Isolate code	Shape	Texture	Elevations	Margins	Surface color	
LDPE						
LDPE1	Circular	Granular	Raised	Entire	Black	Round conidium heads attached to conidiophores
LDPE2	Circular	Cottony	Raised	Entire	Gray	Branched conidiophores
LDPE3	Filamentous	Cottony	Raised	Filiform	Brown	Ellipsoid vesicle head of conidium
LDPE4	Circular	Velvety	Rugose	Entire	Black	Rounded conidium head
LDPE5	Circular	Cottony	Raised	Entire	White	Ellipsoid vesicle head of conidium
LLDPE						
LLDPE1	Circular	Granular	Raised	Entire	Black	Ellipsoid vesicle head of conidium
LLDPE2	Irregular	Glabrous	Flat	Undulate	Black	Round conidium head
LLDPE3	Circular	Cottony	Raised	Entire	White	Ellipsoid vesicle head of conidium
LLDPE4	Circular	Velvety	Raised	Entire	Black	Branched conidiophores
LLDPE5	Irregular	Cottony	Raised	Undulate	White	Branched conidiophores
LLDPE6	Circular	Granular	Raised	Entire	Green	Short cylindrical vesicles on conidium head
LLDPE7	Circular	Cottony	Raised	Entire	White	Conidium head hemispherical
LLDPE8	Irregular	Glabrous	Flat	Undulate	Dark green	Typical conidium head was spherical, then collapsed
LLDPE9	Irregular	Granular	Raised	Undulate	Red	Typical conidium head was spherical, then flattened out like a fan
LLDPE10	Filamentous	Cottony	Raised	Filiform	Gray	Branched conidiophores
HDPE						
HDPE1	Circular	Velvety	Raised	Entire	Green	Typical conidium head was spherical, then collapsed
HDPE2	Filamentous	Cottony	Raised	Filiform	White	Round shape of conidium head
HDPE3	Circular	Glabrous	Umbonate	Entire	Blue	Typical conidium head was rounded like a fan
PP						
PP1	Filamentous	Cottony	Raised	Filiform	Gray	Hemispherical columella with a funnel-shaped apophysis
PP2	Irregular	Glabrous	Umbonate	Undulate	White	Round conidium head shape
PP3	Irregular	Cottony	Raised	Undulate	Black	Conidium heads were round and attached to conidiophores
PP4	Filamentous	Cottony	Raised	Filiform	Gray	Columella hemispherical with funnel-shaped apophysis
PP5	Circular	Velvety	Raised	Entire	Black	Typical conidium head was spherical, then collapsed
PP6	Irregular	Velvety	Raised	Undulate	Black	Typical conidium head was rounded like a fan

Table 2: Colony growth of Medan Marelan fungi grown on MSMA medium for 2 weeks

No.	Isolate code	Positive control	Negative control	Plastic powder
1.	LDPE1	+++	+	++
2.	LDPE2	+++	+	+
3.	LDPE3	++	-	+
4.	LDPE4	+++	++	+++
5.	LDPE5	+++	+	+++
6.	LLDPE1	++	++	++
7.	LLDPE2	++	-	-
8.	LLDPE3	+++	++	++
9.	LLDPE4	++	++	+++
10.	LLDPE5	++	++	+
11.	LLDPE6	+++	+	+++
12.	LLDPE7	+++	++	+++
13.	LLDPE8	+++	++	++
14.	LLDPE9	++	+	+
15.	LLDPE10	+++	++	+++
16.	HDPE1	+++	++	+++
17.	HDPE2	+++	+	-
18.	HDPE3	+++	++	+++
19.	PP1	+++	+	++
20.	PP2	+	+	+
21.	PP3	+	+	+
22.	PP4	+++	+	+++
23.	PP5	+++	++	+++
24.	PP6	+++	+	+

-, no growth; +, growth (7.0-30.0 mm); ++, good growth (31.0-57.0 mm); +++, excellent growth (>57.0 mm)

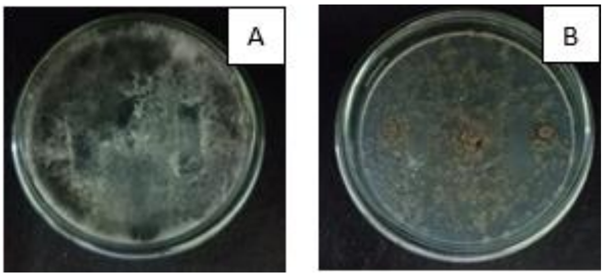

Fig. 1: Fungal isolates capable of growing on the surface of LDPE and LLDPE plastic sheets. (A) Isolate LLDPE10, (B) Isolate PP5

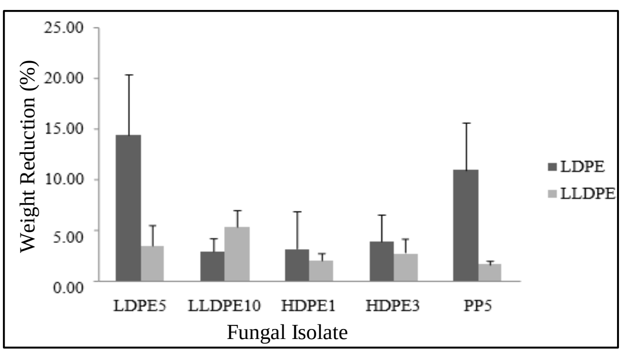

Fig. 2: Percentage of weight loss of plastic sheets on MSMA medium after 45 days of incubation

Observations of the fungal isolates revealed that the sizes of the fungal colonies increased, indicating that they used plastic powder as their main C source. In a previous study, fungal isolates used the C source in the medium to support growth (Ghosh *et al*., 2013). In this study, the growth diameter of the fungal isolates ranged from 7.0-84.00 mm. Microorganisms such as fungi can adapt to almost any environment and have the potential to degrade various compounds, including plastics (Wang *et al*., 2021). However, some fungi were not able to grow consistently during 2 weeks of incubation, such as the LDPE4, LLDPE3, and PP1 isolates. When using growth media lacking added glucose and plastic powder, we found that the fungi generally did not grow well, although several fungal isolates grew to a diameter of up to 36.8 mm. The underlying assumption is that the only source of fungal nutrients originated from the agar plug (Sivan, 2011).

Plastic-Degradation Potential of Selected Fungal Isolates

We performed 45-day degradation tests involving LDPE and LLDPE plastic sheets with the five fungal isolates selected during screening. Their plastic-degradation potentials on MSMA medium are shown in Fig. 1.

On day 45, the final weight of the plastic sheet in the plastic sheet-degradation test was measured using an analytical balance. The plastic sheets are indicated with arrows in Fig. 1. The results of plastic degradation on MSMA medium showed that all five fungi grew and reduced the weight of the plastic. The fungal isolates inoculated into the medium degraded the plastic to obtain C as a nutrition source. Isolate LDPE5 showed the highest degradation of LDPE plastic in the medium, with a weight reduction of 20.83% (Fig. 2). Isolate LLDPE10 showed the highest degradation rate for LLDPE (approximately 6.49%).

Soil fungus *Fusarium* sp. can degrade plastics by up to 20% was previously reported by Sánchez (2020). *Fusarium solani* degraded 4.41% of the LLDPE plastic. In addition to weight reductions on the plastic sheets, the fungal mycelia also attracted plastic powder on the MSMA medium indicating that plastic powder binds to fungi mycelia (Sharma and Sharma, 2004). Fungi can degrade plastics when under nutrient stress by producing extracellular enzymes that can depolymerize plastic polymers (Thiounn and Smith, 2020).

plastic polymers (Thiounn and Smith, 2020). The degradation of plastics in the study might involve enzymatic and non-enzymatic hydrolysis by microorganisms as reported in other work. Zhang *et al*. (2022) reported that *Penicillium funiculosum* and *Streptomyces* enabled to degradation of polyhydroxybutyrate.

SEM and FT-IR Analysis of Degraded Plastics

We analyzed the plastic surface texture and visualized mold adhesion on LDPE and LLDPE before and after the degradation tests via SEM tool at 5000× magnification (Fig. 3). Isolate LDPE5 degraded LDPE plastic sheet, resulting in a change in the surface texture on the LDPE plastic. Isolate LLDPE10 also degraded LLDPE, causing a change in the surface texture from smooth to rough. The attachment of the LLDPE10 fungal isolate to the plastic was observed via SEM at 5000 magnification. The presence of textural changes on the surfaces of plastic sheets after fungal treatment was due to enzymatic activity, which (after a 45-day incubation period), can break down the C in plastic into a nutrient source for fungal growth (Akhbarizadeh *et al*., 2020).

Morphological analysis of the surfaces of LDPE and LLDPE showed that the molecular structure was not dense. We hypothesize that the cracks appearing in the plastic were caused by extracellular enzyme activity. The less-dense the structure or cracks in the plastic, the more water was absorbed. The image also shows a less smooth and porous surface after fungal degradation. The uneven surfaces indicate that the plastic was degraded. We also observed wavy surfaces, as reported previously by Axmalia and Mulasari (2020).

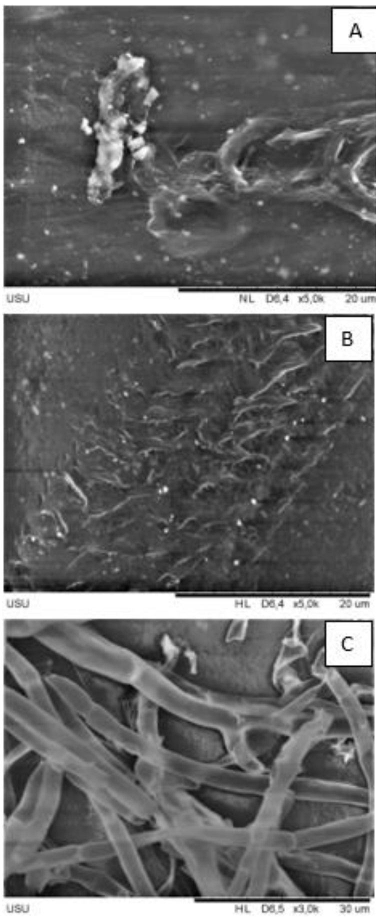

Fig. 3: (A) LDPE sheet surface degraded by isolate LDPE5; (B) LDPE sheet surface degraded by isolating LLDPE10; (C) Mycelial growth of LLDPE10 isolate on an LLDPE sheet

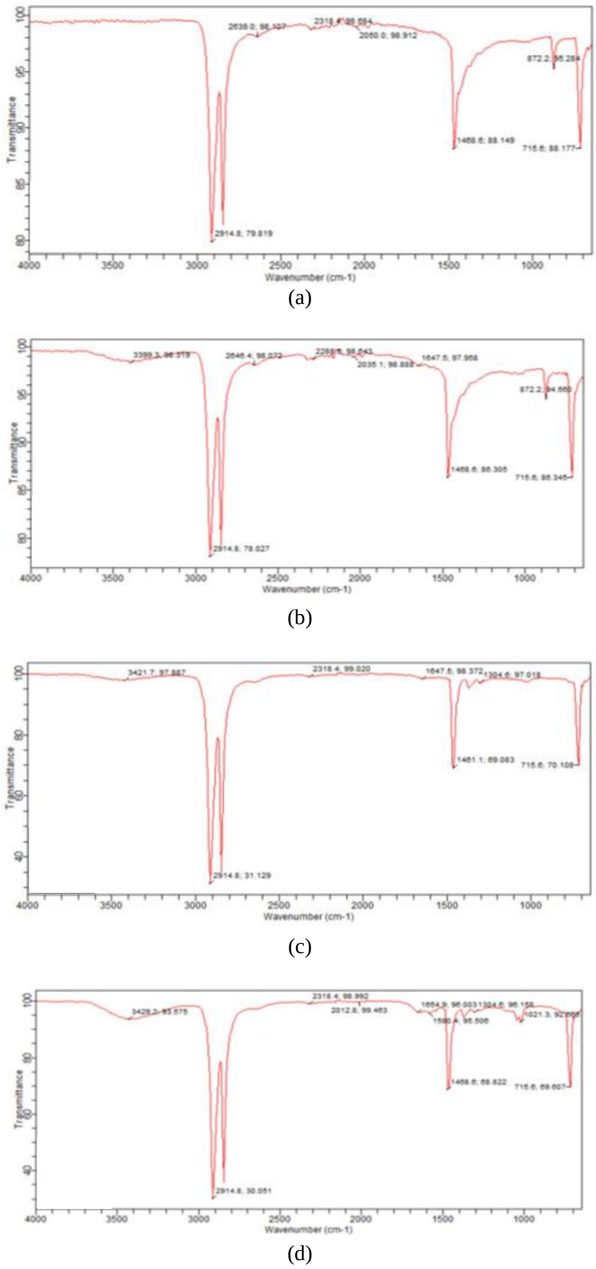

Fig. 4: (A) FT-IR results of control LDPE sheet; (B) LDPE sheet degraded by isolate LDPE5; (C) Control LLDPE sheet; (D) LLDPE sheet degraded by isolate LLDPE10

The FT-IR spectrum between 650 and 4000 cm^{-1} indicated the presence of different functional groups in the degraded LDPE sheet than in the control LDPE sheet. Analysis of the degraded LDPE sheet revealed primary aliphatic and secondary amine groups at a wavenumber of 3339.3^{-1}, alkyne bonds at a wavenumber of 2288.6^{-1}, and isothiocyanate groups at a wavenumber of 2035.1^{-1}. With the degraded LLDPE sheet, CO_2 groups were detected at 2318.4^{-1}, isothiocyanate groups were detected at 1654.9^{-1}, and aromatic amines were detected at 1304.6^{-1}. The functional groups formed with each plastic sheet are shown in Fig. 4.

Table 3: BLASTN results of five fungal isolates with potential plastic-degrading ability from a landfill in Medan Marelan

No.	Isolate code	Species	Query cover %	Kemiripan %	Accession number
1.	LDPE5	*Fusarium solani*	97	92,46	MW216969.1
2.	LLDPE10	*Botryosphaeria laricina*	84	97,18	KC509580.1
3.	HDPE1	*Aspergillus flavus*	81	97,58	KY233188.1
4.	HDPE3	*Aspergillus fumigatus*	97	96,17	CP084969.1
5.	PP5	*Aspergillus niger*	96	95,27	DQ374422.1

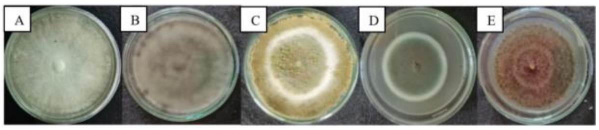

Fig. 5: Potential fungal isolates in plastics degradation; (A) *F. solani* (LDPE5); (B) *B. laricina* (LLDPE10); (C) *A. flavus* (HDPE1); (D) *A. fumigatus* (HDPE3); (E) *A. Nige*r (PP5)

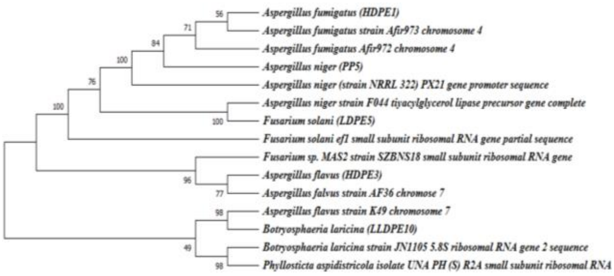

Fig. 6: Phylogenetic tree of fungi from a landfill in Medan Marelan, constructed using MEGA X software and the Kimura 2-parameter method and a bootstrap value of 1000×

The FT-IR results showed that the plastic sheet degradation involved physical interactions between hydrogen chains. Hydrogen bonding occurs when O or N atoms interact with H atoms. Our findings clearly indicate that the plastic products retained chemical groups like those present in the constituent materials. The degraded plastic had hydrophilic properties, i.e., water entered the plastic and degradation occurred. The hydrophilic nature of this plastic was also evidenced by the presence of OH- groups in bioplastics found at wavenumber 2630.30 cm^{-1}. In addition to Hydroxide groups (OH), ester groups (-RCOOR) were also detected.

The presence of these functional groups indicates that the plastic was properly degraded. The FT-IR spectrum showed the response of the detector as percent transmission (%T) on the Y-axis and the IR frequency as wavenumber (cm^{-1}) on the X-axis. The radiation passing through the sample was measured as the % T and wavenumber (Rånby, 1989). FT-IR analysis of the membrane revealed breakage of the PE polymer chains and the formation of PE-oxidation products during the degradation (Du *et al*., 2022). Bonding sites and frequencies indicate the presence of specific functional groups in a material. Bonds in the functional group region (4000-1500 cm^{-1}) are usually easy to interpret, whereas assigning a bond to a specific functional group in the 1500-500 cm^{-1} region can be more difficult due to the large number of functional groups that absorb at the same wavenumber (Elahi *et al*., 2021).
During degradation, LDPE is first broken down into its monomers and then the monomers are mineralized. LDPE is too large to cross microbial cell membranes, so it must first be depolymerized into smaller monomers before it can be absorbed and degraded by microbial cells (Nwogu *et al*., 2012).

Molecular Identification of Potential Plastic-Degrading Fungi

We identified five potential plastic-degrading fungal isolates at the species level (Table 3, Fig. 5). Their DNA sequences were then used to construct a phylogenetic tree to determine their degrees of relatedness and evolutionary distances using MEGA × software (Fig. 6). The phylogenetic tree showed genetic variations between *Fusarium solani* (LDPE5) cultivated with LDPE as the primary C source, *Botryosphaeria laricina* (LLDPE10) cultivated with LLDPE, *Aspergillus fumigatus* (HDPE1) and *Aspergillus flavus* (HDPE3) cultivated with HDPE and *Aspergillus niger* (PP5) cultivated with PP.

Fungal isolates are abundant in plastic waste. Previous data showed that 30 species of fungal isolates were obtained from plastic waste heaps. Importantly, *Fusarium solani*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* isolates obtained from landfills in Chennai City showed potential for degrading plastic waste (Khan *et al*., 2022). *Aspergillus* sp. and *Lynisibacillus* sp. were also found in plastic piles. These findings can be attributed to different factors. *Aspergillus* sp. comprises a group of fungi that spread in metropolitan areas with different shapes and colors. Fungal spores are easily dispersed by the wind and grow easily on organic or inorganic materials such as PE (De Souza Machado *et al*., 2018). The percentage of similarities between the five fungal isolates was quite high (>90%). This finding indicates that the fungal species closely match existing genetic data deposited in NCBI. *Fusarium solani* and *Aspergillus* sp. isolates can grow on and degrade plastic (Muhonja *et al*., 2018). The fungus *Botryosphaeria laricina* has not previously been reported to degrade plastics. *Botryosphaeria laricina* fungi are commonly found in plants. *Botryosphaeria laricina* fungi isolated from agricultural soils previously exposed to endosulfan were tolerant to endosulfan and could degrade toxins and harmful metabolites such as endosulfan sulfate, alpha-endosulfan, and beta-endosulfan by using them as C and energy sources (Akhtar and Mannan, 2020).

Conclusion

All types of plastic waste were colonized by fungi with different numbers of morphotypes; 5 isolates from LDPE, 10 from LLDPE, 3 from HDPE, and 6 from PP. The potential isolate from each plastic waste was successfully identified to the species level as *Fusarium solani* (LDPE5), *Botryosphaeria laricina* (LLDPE10), *Aspergillus fumigatus* (HDPE1), *Aspergillus flavus* (HDPE3) and *Aspergillus niger* (PP5). The *F. solani* and *B. laricina* successfully reduced the weights of plastic sheets of LDPE and LLDPE by 20.83 and 6.49%, respectively, after 45 days of degradation test. SEM the sheets showed erosion and physical damage on their surface. Then, the result of the study indicated that fungi colonizing the plastic materials at the landfill might play an important role in the process of plastic degradation.

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Author’s Contributions

Erman Munir: Formulated the initial concept, managed financial resources, designed the study, meticulously revised and proofread the manuscript, and oversaw the submission and subsequent revisions.

Yitro Pasaribu: Spearheaded experimental development, conducted data analyses, performed literature reviews, and prepared the manuscript.

Syahira Mubtasima: Prepared equipment, conducted fieldwork for microbial isolation.

Ahmad Faisal Nasution: Monitored material and equipment engagement during laboratory work.

Ethics

This article is original and it contains unpublished data. The corresponding author certifies that all authors have read and accepted the work and there is no ethical contradiction.

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