

Plastic degrading fungi *Trichoderma viride* and *Aspergillus nomius* isolated from local landfill soil in Medan

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Abstract. Plastic is a naturally recalcitrant polymer, once it enters the environment, it will remain there for many years. Accumulation of plastic as wastes in the environment poses a serious problem and causes an ecological threat. Alternative strategies to reduce accumulation of plastic wastes have been initiated and implemented from a different aspect including from microbiological view point. The study to obtain potential fungi in degrading plastic molecule has been initiated in our laboratory. Low density polyethylene (LDPE) plastic was used as a tested material. Candidate fungi were isolated from local landfill soil. The fungi were cultured in mineral salt medium broth containing LDPE powder. Two of nine isolates showed best growth response in broth media containing LDPE. These isolates (RH03 and RH06) were used in degradation test. Results showed that isolate RH03 and RH06 reduced the weight of LDPE film by 5.13% and 6.63%, respectively after 45 days of cultivation. The tensile strength of treated film even reduced significantly by 58% and 40% of each isolate. Analyses of electron micrograph exhibited grove ands rough were formed on the surface of LDPE film. These were not found in the untreated film. Furthermore, molecular analysis through polymerase chain reaction and DNA sequencing indicated that RH03 is *Trichoderma viride* and RH06 is *Aspergillus nomius* with 97% and 96% similarities, respectively.

Keywords: *Aspergillus nomius*, degradation of plastic, low density polyethylene, plastic degrading fungi, *Trichoderma viride*.

1. Introduction

Recently, various types of petroleum-based synthetic polymers are manufactured worldwide, and it reaches to approximately 140 million tons annually. Most of these polymers are returned to the environment as industrial waste products [1]. Alexander [2] most synthetic polymers are extremely resistant to microbial attack, due to their excessive molecular mass, a high number of aromatic rings, unusual bonds, or halogen substitutions. For this reason, the large-scale accumulation of waste plastics in the biosphere has given rise to the problem of severe environmental pollution. Recently Jambeck et al. [3] reported plastic debris in the marine environment of 192 countries published in Science. Surprisingly, Indonesia is the second country to dump most plastic waste to the ocean to the total of plastic marine debris 0.48-1.29 MMT/year (million metric tons), second after China (1.32-3.53), and the third country is Philippines (0.28-0.75 MMT/year). These numbers are predicted to escalate each year in parallel with the increase of the population and their utilization. Pavani and Rajeswari [4], contamination of plastic in the environment threatens human life. It causes immune and enzyme disorders, hormonal disruption leading to endocrinal disorders and it is also considered as



carcinogenic. It also dangerously affects other animal life and alters the environment (water and soil) sustainability causing serious pollution.

Many efforts have been tried to minimize the accumulation of plastic waste in the environment over the past four decades. Manufacturing of environmentally friendly polymers is considered an alternative way to minimize the effect of plastic waste on the environment on production point of view. Shima [1]; Scott [5]; Muller et al. [6], many types of aliphatic polyesters, including polyhydroxyalkanoates (PHAs), poly(ϵ -caprolactone) (PCL), and poly(l-lactide) (PLA), have been developed as biologically recyclable green polymers. The rate of polymer biodegradation in an ecosystem is affected by material processing, the inherent characteristics of the substrate to be degraded, and various microbiological and environmental factors.

Besides manufacturing degradable plastics, microbial biodegradation of plastic has been obtaining much attention of many scientists worldwide. Many promising works on searching potential microbes and modification of environmental condition to increase the degradation rate of plastic have been reported. Deepika and Jaya [7] and Mukherjee and Chatterjee [8] *Bacillus weihenstephanensis*, *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Diplococcus* spp. isolated from garbage soil shows their ability to degrade low density polyethylene (LDPE). While Hussein et al. [9] identifies some gram negatives *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Acinetobacter ursingii* isolated from plastic contaminated soil could degrade LDPE. *Moraxella* spp, *Burkholderia cepacia* and *Escherichia coli* are also able to degrade LDPE. Nwogu et al. [10] studies biodegradation of polyethylene films by selected exotic mushrooms. *Pleurotus tuber-regium*, *P. pulmonarius*, *Lentinus squarrosulus* and *Rigidoporus lignosus* can utilize polyethylene powder as sole carbon and energy. *P. tuber-regium* and *P. pulmonarius* reduces weight of polyethylene by 13.26% and 9.67%, respectively. Labuzek et al. [11] and Nowak et al. [12] examine the ability of filamentous fungi *Aspergillus niger*, *Aspergillus terreus*, *Aureobasidium pullulans*, *Paecilomyces varioti*, *Penicillium funiculosum*, *Penicillium ochrochloron*, *Scopulariopsis brevicaulis*, and *Trichoderma viride* in degrading LDPE. The weight loss increases in LDPE/ Bionolle polymer. Then, the objective of this study is to obtain potential fungal isolates in degradation of plastic. This paper describes the ability of fungi isolated from local landfill soil in Medan in degrading LDPE, the newly identified fungi are also described. The results are compared to other reported works.

2. Method

2.1. Fungal isolation and Preparation of LDPE powder

Fungal candidates were isolated from local landfill soil in Medan. One of kg soil was collected using a clean shovel from three different sites (of plastic accumulated site) and mixed thoroughly. A serial dilution was prepared with NaCl 0.9% solution, 0.1 ml of sample was plated on Sabouraud Dextrose Agar (SDA) [13, 14]. Plates were kept at room temperature ($26 \pm 2^\circ \text{C}$) for 2-7 days. The growing colonies were transferred to fresh SDA plate to obtain pure isolates. The pure isolates were kept at 4°C for further use. LDPE powder was prepared by dissolving LDPE beads in xylene with continuous stirring. The clump LDPE was rinsed with 96% ethanol and dried completely in an oven at 50°C . The LDPE was cut into small pieces and smashed with blender to get powder form.

2.2. Screening of LDPE degrading isolates

Potential of isolates in degrading LDPE was screened in mineral salt medium broth (MSMB) containing LDPE powder as the only carbon source. Four agar plugs (\varnothing 5 mm) of the fully growing colony were inoculated into 100 ml of MSMB media in 250 ml erlenmeyer flask, control cultures did not contain LDPE powder. The cultures were incubated in agitated condition (100 rpm) at room temperature ($26 \pm 2^\circ \text{C}$) for 45 days. Cultures showing growth response were considered as potential isolates, and used for further analyses (modified method of Nwogu et al. [10]).

2.3. Biodegradation test of LDPE by potential isolates

Two isolates showing growth response from screening studies were used. Biodegradation studies were done in Mineral Salt Medium Agar (MSMA) media supplemented with 0.5% glucose. LDPE film was cut into 1 cm x 4 cm and placed on the surface of MSMA media aseptically. Two agar plugs (Ø 5 mm) of fully growing colonies were inoculated on each side of LDPE film. Cultures were incubated at room temperature (26 ± 2 °C) for 45 days. MSMA plate without agar plug fungus was used as the reference to confirm the degradation of LDPE film. Following the incubation, the film was taken from the culture using tweezers and rinsed with 70% ethanol and sterile distilled water. The film were air dried at ambient temperature for 24 hours prior to analysis [10].

2.4. Weight loss and tensile strength analyses of degraded LDPE and fungal identification

The weight of each film was measured using analytical balances. Reduction of the tensile strength of LDPE film following degradation test was identify using a Universal Testing Machine (UTM). The selected fungi were characterized microscopically. Furthermore, molecular analyses were done through polymerase chain reaction and DNA sequences to identify the species. The weight loss of LDPE was calculated on the following formula and the results obtained were compared with LDPE weight control.

$$\text{Weight loss (\%)} = \frac{(\text{Initial weight} - \text{Final weight}) \times 100}{\text{Initial weight}} \quad (1)$$

3. Results and Discussion

3.1. Fungal isolates and screening of LDPE potential

Fungi were isolated from landfill soil contaminated with plastic materials. Nine isolates were obtained by morphological characters including color, texture, and edge of colonies, identified as RH01 to RH09. Most isolates were white with green at center. From morphological appearances, there were indicated that most isolates were common contaminant fungi. Dineshraj and Ganesh [15] identified nine fungi from plastic contaminated sites in Cuddalore, in which most of them were belongs to the genus of *Aspergillus*, *Mucor*, *Penicillium*, and *Fusarium*. Williams and Hakam [16] isolated *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp. and *Saccharomyces* sp. from four landfill soil in Nigeria. Obire et al. [17] also reported *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium* sp., *Mucor* sp., dan *Penicillium* sp. from landfill soil. Two of nine isolates obtained in this study were identified molecularly, results are described at the end of this paper.

All isolates were screened for the ability to grow in LDPE containing MSMB medium. The medium was supplemented with 0.5% glucose to initiate the growth. Results showed that two isolates, RH03 and RH06, exhibited the slow growth, while the others did not until 45 days incubation. Figure 1 shows the mass of fungal mycelia absorbed LDPE powder.

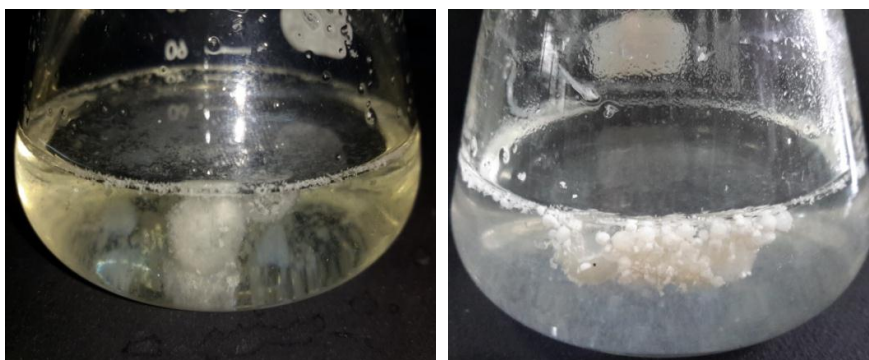


Figure 1. Growth of fungi isolates in MSMB media containing LDPE after four days incubation. Right isolate RH03; Left isolate RH06.

The growth of RH03 and RH06 in LDPE containing media until the end of cultivation indicated that the isolates could utilize LDPE molecule for the growth. These two isolates were considered as potential fungi and used for further study. The other seven isolates could not survive the growth; the growth was only observed until week two. From the results, it was assumed that once glucose was used up, fungi decreased their metabolic activity since they could not use LDPE molecule. Figure 1 also clearly shows that powder of LDPE was absorbed by fungal mycelia, which further indicates and suggests that exopolymer substances on the surface of fungi might involve in the process. Then, since LDPE is not soluble, hydrophobicity of EPS on the mycelial surface is essential in the absorption process. This phenomenon is our current concern and its role in absorption of color material in decolorization of textile wastewater have been reported [18]. Seneviratne et al. [19] fungi were considered to have the higher capability in degrading LDPE because they secrete hydrophobic protein and bind to the polymer surface. Furthermore, Kim and Rhee [20] fungi grew much faster than bacteria in soil environment, and the hype can penetrate various substrates. Singh and Gupta [21] obtained *Aspergillus flavus*, *A. niger*, *A. japonicus*, *Mucor* sp., *Penicillium* sp. and *Fusarium* sp. from polyethylene contaminated soil on the basis of clear zone forming in *Czapek Dox* Agar plate containing 0.1% LDPE powder. While Nwogu et al. [10] obtained *Pleurotus tuber-regium* dan *Pleurotus pulmonarius* from Mineral Salt Medium Agar plate containing 3 g/L LDPE powder. Both culture incubations were carried out under room temperature, as also done for this study ($26 \pm 2^\circ\text{C}$).

3.2. Biodegradation of LDPE film by selected isolates

Potential fungal isolates (RH03 and RH06) as described above were tested for their capability in degrading LDPE. LDPE film (1 cm x 4 cm) was placed on preculture isolate in MSMA plate media. The following figure shows the growth of isolates after 45 days incubation under room temperature ($26 \pm 2^\circ\text{C}$). Even the growth was observed much slower that when fungus grew on sabouroud dextrose agar, macroscopic observation as shown in Figure 2 shows tested isolates colonized and covered the surface of LDPE film.

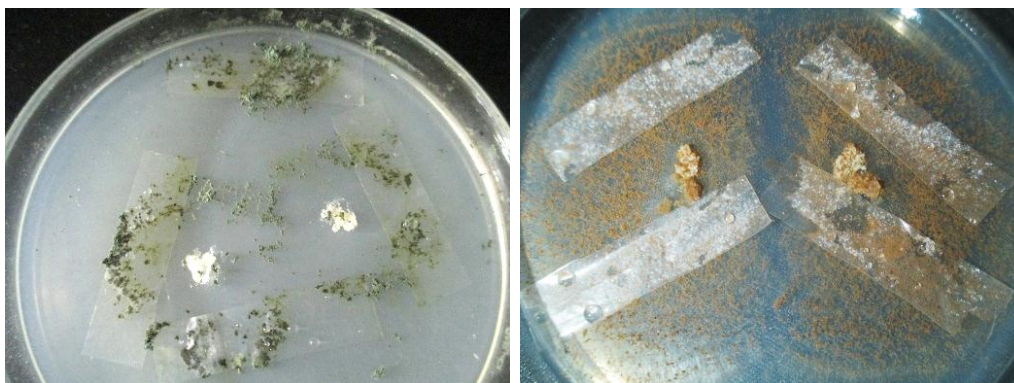


Figure 2. Biodegradation test of LDPE by selected isolates after 45 days of incubation. Left, isolate RH03; right, isolate RH06.

3.3. Weight loss and tensile strength reduction LDPE film

Biodegradation of LDPE was evaluated on the basis of weight loss and reduction of tensile strength of the treated film compared to the untreated one. As shown in Figure 2 above, the isolates colonized and covered the surface of LDPE film; it is assumed that the fungi have modified the film by secreting degradative enzymes and utilized polyethylene for the growth. However, Esmaeli et al. [22] hydrophobicity and the limited functional group of LDPE have limited the attachment of degrading microbes to molecule surface, and fungal growth was also much slower. Results of biodegradation analyses in this study as shown in Figure 3, confirmed that the tested isolates have an ability to degrade the plastic (LDPE).

As shown in Figure 3, both isolates reduced the weight of LDPE and the ability was relatively the same values. RH03 reduced the weight of LDPE film to a total loss of 5.13% and isolate RH06 by 6.3% (the value was an average of two measurements). The ability of selected isolates in degrading LDPE in this study was very comparable with previously reported work. Das and Kumar [23] found that *Aspergillus* reduced the weight of LDPE film by 5-8%, and *Fusarium* by 9% after 60 days incubation in MSMB under shaking condition (at 130 rpm). A much higher total weight loss of LDPE has also been reported. Singh and Gupta [21] reported *Aspergillus flavus* reduced the weight by 30%, *Aspergillus niger* 20%, *Aspergillus japonicus* 36%, *Mucor* sp. 16%, *Penicillium* sp. 24%, and *Fusarium* sp. by 32%, after 30 days cultivation.

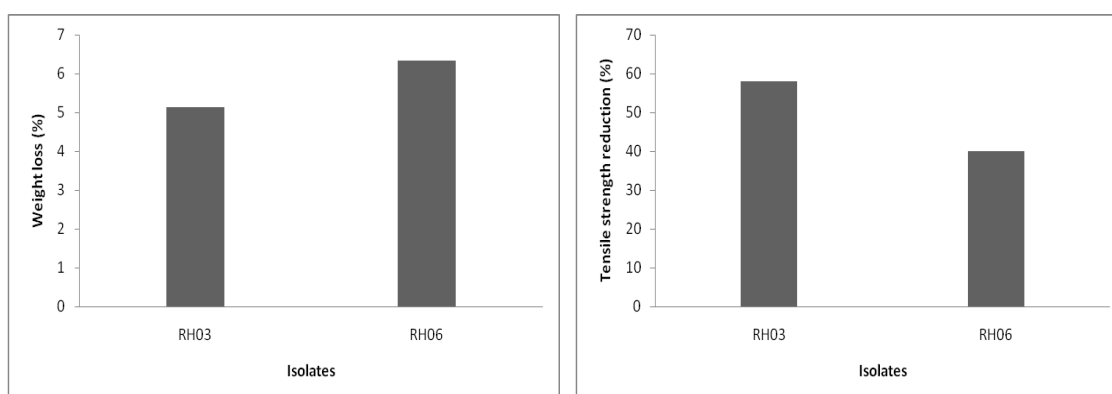


Figure 3. Profile of weight loss (left) and tensile strength (right) of LDPE film after incubation with tested isolates for 45 days.

Furthermore, tensile strength analyses, results as shown in Figure 3 right picture, showed that both isolates reduced the tensile strength of LDPE film compared to control film (5.292 MPa). The strength was reduced from 2.45 MPa to 1.96 MPa in RH03 culture with the average reduction of 58% and from 3.92 MPa to 2.646 MPa in RH06 culture or 40% reduction. This means that LDPE film treated with these isolates became fragile. Electron micrograph of treated film showed the formation of the crack, groove, and uneven surface of LDPE film (data not shown in this paper). Reduces of the tensile strength of treated LDPE film were previously reported. Vijaya dan Reddy [24] studied biodegradation of polyethylene film in natural condition. LDPE film was buried with solid waste in compost soil. He found no significant biodegradation process of LDPE after four months. However, there was a reduction of tensile strength up to 10.5-11.6% after 12 months. On the other hand, a significant decrease of tensile strength was reported by Nowak et al. [25] *Penicillium feniculosum* enabled to reduce the tensile strength of LDPE film by 70% after incubation of 84 days.

3.4. Identification of potential isolates

Selected isolates used in the study were identified through molecular analyses. Sequencing of amplified DNA showed that potential isolates RH03 and RH06 are resembled to *Trichoderma viride* and *Aspergillus nomius* with 97% and 96% similarities, respectively. As far literatures have been surveyed on LDPE degrading fungi, limited work has been reported on the ability of these two fungi in degrading plastic molecule. Most reported fungi capable of degrading LDPE are another type *Aspergillus*, *Penicillium*, and *Mucor* as described above.

In conclusion, fungi isolated from landfill soil have the capability in degrading LDPE. Weight and tensile strength of LDPE film reduced after incubation with selected isolates. Two potential isolates obtained in the study have been successfully identified as *Trichoderma viride* and *Aspergillus nomius*. Then, this results enriched the information of plastic degrading fungi.

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