

## Full Paper

# Microbial degradation of low-density polyethylene by *Neopestalotiopsis phangngaensis*

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Low-density polyethylene (LDPE) has been commercially used and accumulated as plastic solid waste. LDPE has also been found to be a non-degradable waste for decades and found as a pollution source in the environment. In this study, 65 fungi were screened for their biodegradation of LDPE. The fungi *Neopestalotiopsis phangngaensis*, *Alternaria burnsii*, *Alternaria pseudoeichhorniae*, and *Arthrinium sacchari* showed significant potential in LDPE biodegradation. These fungi were individually cultured with an LDPE sheet as a carbon source for 90 days. A maximum weight loss of the LDPE sheet was detected by the fungus *N. phangngaensis* (54.34%). This fungus also revealed the highest reduction rate of tensile strength of the LDPE sheet (0.33 MPa). The morphological surface of LDPE culturing with *N. phangngaensis* was crack, pit, and rough analyzed by scanning electron microscopy. The biodegradation of the LDPE sheet by *N. phangngaensis* was also confirmed by the Sturm test and analysis of enzymatic activities. The Sturm test showed the highest decomposition of the LDPE sheet by *N. phangngaensis* into CO<sub>2</sub> with 2.14 g/L after incubation. Enzymatic activities of laccase, manganese peroxidase, and lignin peroxidase enzymes were found by *N. phangngaensis* during the LDPE degradation. The volatile organic compounds in culture supernatant of *N. phangngaensis* were also investigated. The major compounds were 3Z-diethyl acetal hexenal, 2E,4E-decadienol, and 2Z-diethyl acetal hexenal. This study reveals the utilization of the fungus *N. phangngaensis* as the carbon source at a considerable biodegradation rate without any prior

treatment. Therefore, the fungus *N. phangngaensis* may be applied as an alternative degrader for LDPE degradation in the environment.

**Key Words:** Biodegradation; fungi; polyethylene; *Neopestalotiopsis*

## Introduction

The demand to develop eco-friendly materials which do not harm the environment is significantly raised due to increasing environmental problems. Plastic is known as a polymer that is difficult to degrade by biological methods. They have been accumulated as huge waste damage to human life and the environment (Idumah and Nwuzor, 2019). Among these plastic materials, using polyethylene has increased with the world's population growth (Chattopadhyay et al., 2016). The degradation of polythene is evaluated as a major challenge in waste management due to its nondegradable properties (Moharir and Kumar, 2019). The degradation of polyethylene by microbial enzymes could be an effective method to transform polymer into an oligomer and monomer, respectively, (Kurian and Das, 2021). The degradation is raised when microbes have grown by taking carbon from polyethylene (Nag et al., 2021).

Microbial degradation of polyethylene has been depicted in various microorganisms. Examples of polyethylene-degrading microbes are *Streptococcus*, *Pseudomonas*, *Staphylococcus*, *Micrococcus*, *Aspergillus glaucus*, *Aspergillus niger*, some species of *Fusarium* sp., *Aureobasidium* sp., and *Trichoderma* sp. fungi (Dhanraj and Hatha, 2022). In addition, some microbes have been

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Table 1. List of all fungal strains

No.	Strain	Accession No.	Gene	No.	Strain	Accession No.	Gene
1	<i>Acrocalymma ampeli</i>	MW063150	ITS	33	<i>Micropeltis ficina</i>	MW063190	ITS
2	<i>Alternaria burnsii</i>	KC584236	ITS	34	<i>Microthyrium fici-septicae</i>	MW063251	LSU
3	<i>Alternaria pseudoeichhorniae</i>	MH827030	ITS	35	<i>Muyocopron celtidis</i>	MW063194	ITS
4	<i>Arthrinium mori</i>	MW114313	ITS	36	<i>Muyocopron ficinum</i>	MW063197	ITS
5	<i>Arthrinium paraphaeospermum</i>	MW114315	ITS	37	<i>Muyocopron lithocarp</i>	MW063200	ITS
6	<i>Arthrinium sacchari</i>	MW114317	ITS	38	<i>Mycoleptodiscus alishanensis</i>	MW063203	ITS
7	<i>Cercophora fici</i>	MW114387	ITS	39	<i>Neofusicoccum moracearum</i>	MW063187	ITS
8	<i>Cladosporium tenuissimum</i>	MW063146	ITS	40	<i>Neopestalotiopsis phangngaensis</i>	MW114333	ITS
9	<i>Colletotrichum celtidis</i>	MW114362	ITS	41	<i>Nigrospora macarangae</i>	MW114318	ITS
10	<i>Conidiocarpus fici-septicae</i>	MW063143	ITS	42	<i>Ophioceras chiangdaoense</i>	MW114438	LSU
11	<i>Coniella quercicola</i>	MW114360	ITS	43	<i>Ophioceras ficinum</i>	MW114436	LSU
12	<i>Cylindrohyalospora fici</i>	MW063243	LSU	44	<i>Parawiesneriomyces chiayiensis</i>	MW063178	ITS
13	<i>Dematiocladium celtidicola</i>	MW114370	ITS	45	<i>Periconia alishanica</i>	MW063165	ITS
14	<i>Diaporthe celtidis</i>	MW114347	ITS	46	<i>Periconia byssoides</i>	MW063164	ITS
15	<i>Diaporthe fici-septicae</i>	MW114349	ITS	47	<i>Periconia celtidis</i>	MW063162	ITS
16	<i>Diaporthe millettiae</i>	MW114351	ITS	48	<i>Pestalotiopsis dracaenea</i>	MW114334	ITS
17	<i>Diaporthe pseudophoenicicola</i>	MW114353	ITS	49	<i>Pestalotiopsis papuana</i>	MW114337	ITS
18	<i>Diaporthesporella macarangae</i>	MW114354	ITS	50	<i>Pestalotiopsis parva</i>	MW114338	ITS
19	<i>Dictyocheirospora garethjonesii</i>	MW063152	ITS	51	<i>Phragmocapnia betle</i>	MW063142	ITS
20	<i>Dinemasporium parastrigosum</i>	MW114342	ITS	52	<i>Phyllosticta capitalensis</i>	MW063188	ITS
21	<i>Diplodia fici-septicae</i>	MW063180	ITS	53	<i>Pseudocercospora fici-septicae</i>	MW063147	ITS
22	<i>Discosia fici</i>	MW114330	ITS	54	<i>Pseudopestalotiopsis camelliae-sinensis</i>	MW114341	ITS
23	<i>Discosia querci</i>	MW114326	ITS	55	<i>Pseudopithomyces chartarum</i>	MK347741	ITS
24	<i>Fitzroyomyces cyperacearum</i>	MW293953	ITS	56	<i>Pseudopithomyces sacchari</i>	MW063156	ITS
25	<i>Hermatomyces biconisporus</i>	MW063161	ITS	57	<i>Pseudorobillarda phragmitis</i>	MW063204	ITS
26	<i>Lasiodiplodia chiangraiensis</i>	MW760854	ITS	58	<i>Sirastachys castaneda</i>	MW114379	ITS
27	<i>Lasiodiplodia thailandica</i>	MW063183	ITS	59	<i>Sirastachys pandanicola</i>	MW114381	ITS
28	<i>Lasiodiplodia theobromae</i>	MW063184	ITS	60	<i>Spegazzinia musae</i>	MW063160	ITS
29	<i>Leptodiscella sexualis</i>	MW293950	ITS	61	<i>Stachybotrys aloeticola</i>	MW114382	ITS
30	<i>Memnoniella alishanensis</i>	MW114372	ITS	62	<i>Strigula multiformis</i>	MW063175	ITS
31	<i>Memnoniella celtidis</i>	MW114374	ITS	63	<i>Wiesneriomyces laurinus</i>	MW063176	ITS
32	<i>Micropeltis fici</i>	MW063192	ITS	64	<i>Yunnanomyces pandanicola</i>	MW063236	LSU

ITS; internal transcribed spacer, LSU; large subunit

reported to enhance the biodegradation rate of polyethylene without any harm to the environment (Saravanan et al., 2021). The extracellular enzymes from microbes were penetrated deeply into the polymer material to break the polymeric chain into small compounds, carbon dioxide, and water (Saravanan et al., 2021). However, physical and chemical factors on culture conditions are affected degradation efficiency (Gaytán et al., 2021).

Recently, several researchers have reported several fungal genera having biodegradation ability of polyethylene consisting of *Aerobasidium*, *Candida*, *Chaetomium*, *Cladosporium*, *Ganoderma*, *Geotrichum*, *Paecilomyces*, *Phanerochaete*, *Phlebia*, *Penicillium*, *Sporotrichum*, *Talaromyces*, *Thermoascus*, *Thermomyces*, *Thielavia*, and *Trametes* (Sánchez, 2020). However, some microorganisms as above mentioned can break down carbon-carbon bonds in polyethylene at low rates (Kunlere et al., 2019). Our previous study has also reported the LDPE-degrading fungi from Thailand (Khuengsai et al., 2021). However, more than 100 new fungal species have been continuously discovered in Thailand per year by the Center of Excellence in Fungal Research, Mae Fah Luang University, and they have not been applied in this field. In addition, searching

for various polyethylene-degrading fungi is important for the further biodegradation development of low-density polyethylene (LDPE). Thus, this work is aimed to search for new alternative LDPE degrading fungi which may be applied to degrade LDPE with a considered biodegradation rate.

## Materials and Methods

**Fungal strains.** Sixty-five fungi were used in this work (Table 1). These strains were obtained from the culture collection of the Institute of Excellence in Fungal Research, Mae Fah Luang University, Thailand. All strains were identified by the Center of Excellence in Fungal Research, Mae Fah Luang University using the polymerase chain reaction technique with various genes. The fungus *Aspergillus niger* ATCC 10254 from the culture collection of the Thailand Institute of Scientific and Technological Research, Bangkok, Thailand is used as the reference fungal strain in this study.

**Preparation of LDPE sheets.** Five millimeters nominal size of white amorphous LDPE microplastic granules were used for biodegradability assessment. Each LDPE sheet

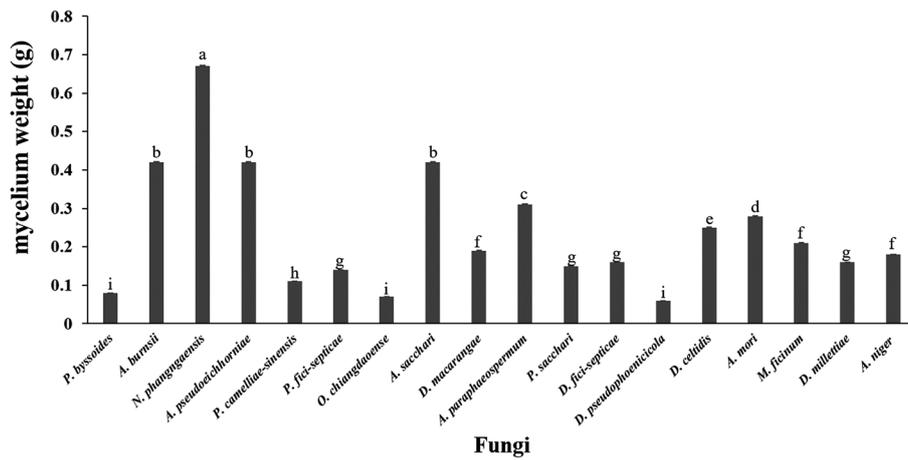


Fig. 1. Mycelium dry weight of fungi cultured on PDA plates containing LDPE sheets after 30 days of incubation.

Different letters above the bars indicate significant differences ( $p < 0.05$ ).

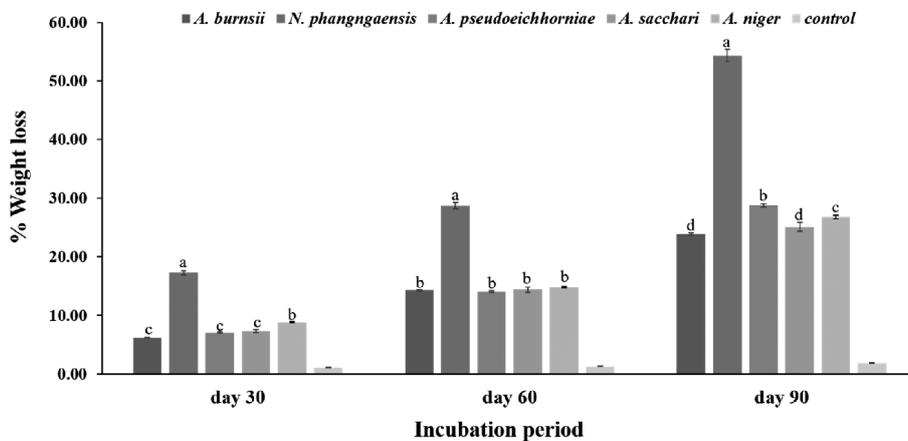


Fig. 2. Weight loss percentage of LDPE sheets cultured with *A. burnsii*, *N. phangngaensis*, *A. pseudo-eichhorniae*, *A. sacchari*, *A. niger*, and control after 30, 60, and 90 days of incubation.

Different letters above the bars indicate significant differences ( $p < 0.05$ ).

(1.0 cm × 4.0 cm) with a thickness of 0.11 mm was prepared according to a modified method (Khruengsai et al., 2021). They were washed with water, sterilized with 70 % ethanol, and air-drying. The dry sheets were kept inside the desiccator until use. The weight of the dried LDPE sheet was recorded as the initial weight.

**Primary screening of LDPE-degrading fungi.** All fungi were screened for their degradation potential on the LDPE sheet using the solid medium assay. A solid medium was prepared following the study of Khruengsai et al. (2021). Four LDPE sheets were spread on a potato dextrose agar (PDA) plate, 0.5 cm apart from each sheet. The agar plug of each fungus was placed on the medium at the midpoint of the plate. Then, all plates were sealed with plastic paraffin film and incubated at room temperature for 30 days. The plates plugged with *A. niger* were used as the positive control. The LDPE-degrading activity of the fungi was evaluated by inspecting colony diameter after incubation. After incubation, mycelium was dried and weighed by a digital weighing balance.

**Biodegradation analysis of LDPE sheets.** Five agar plugs of the selected fungus and *A. niger* were added to 250 mL of mineral salt medium broth supplemented with four sterilized LDPE sheets (Khruengsai et al., 2021). The cultures were incubated at room temperature for 30, 60, and 90 days. After incubation, the LDPE sheets were rinsed with 70% ethanol and sterile distilled water prior to drying at room temperature for 24 h while their mycelium was dried and weighed by a digital weighing balance.

**Characterization of the degraded LDPE sheets.** After incubation, the weight loss of LDPE sheets was calculated by using this using the formula: % weight loss = [(initial weight-final weight)/initial weight] × 100. The tensile strength of the LDPE sheets was also determined using a Universal Testing Machine (UTM; 8800 servo-hydraulic testing machine, Instron) in displacement-controlled mode according to the modified method of Amjadi et al. (2020).

**Determination of CO<sub>2</sub>.** The amount of CO<sub>2</sub> in the medium broth was determined using a modified Sturm test following our previous study (Khruengsai et al., 2021). The

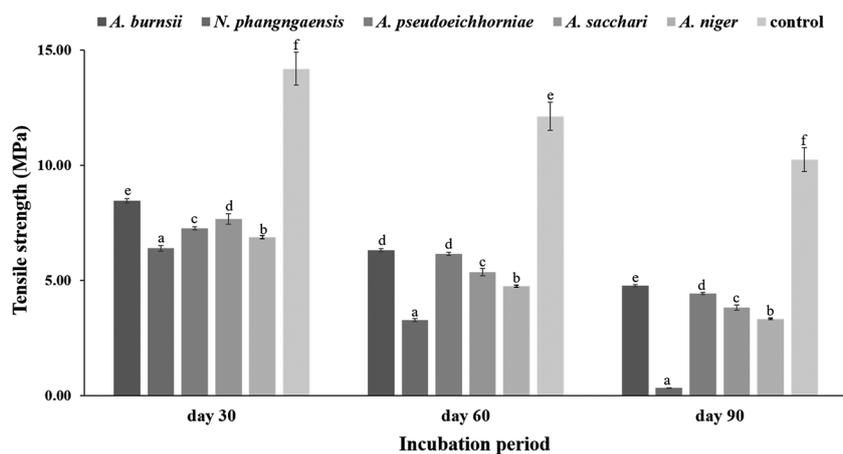


Fig. 3. Tensile strength of LDPE sheets cultured with *A. burnsii*, *N. phangngaensis*, *A. pseudoeichhorniae*, *A. sacchari*, *A. niger*, and control 30, 60, and 90 days of incubation.

Different letters above the bars indicate significant differences ( $p < 0.05$ ).

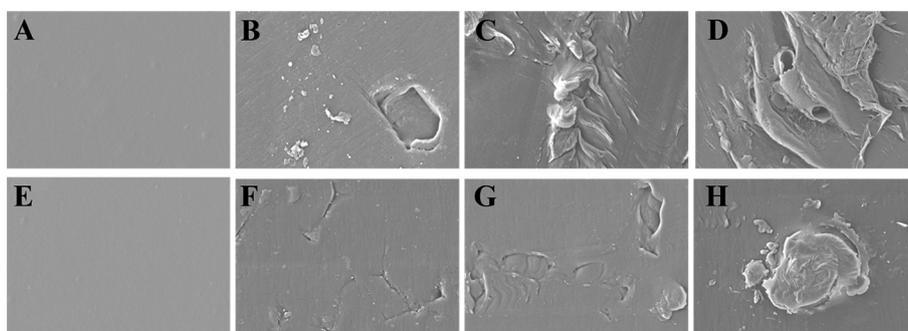


Fig. 4. Scan electron micrographs of LDPE sheets (5000 $\times$ ) cultured with *N. phangngaensis* (A-D), and *A. niger* (E-H) after 0, 30, 60, and 90 days of incubation, respectively.

amount of  $\text{CO}_2$  was determined using the following equation  $[A \times B \times 50 \times 1000] / V$  Where A = volume of NaOH in mL, B = normality of NaOH, and V = volume of sample in mL.

**Screening of enzymes production.** Enzyme activity of fungi cultured with LDPE sheets after different incubations was investigated following previous studies (Khandare et al., 2021; Khruengsaï et al., 2021). The culture supernatant was tested for the enzymatic activity of laccase, manganese peroxidase, and lignin peroxidase. The enzymic activity obtained from all enzymes was expressed as U/mL.

**Analysis of volatile organic compounds.** The volatile organic compounds in culture supernatants of *N. phangngaensis* and *A. niger* after incubation of 90 days were analyzed using gas chromatography-mass spectrometry following our previous report (Khruengsaï et al., 2021).

**Statistical analysis.** The obtained data were shown as the mean  $\pm$  standard deviation. Data were subjected to Analysis of Variance (ANOVA) followed by posthoc multiple pairwise comparisons using Duncan's multiple range tests with a reference treatment ( $\alpha = 0.05$ ). The statistical analyses were conducted using the SPSS 20.0 software (IBM Corp). All experiments were performed in triplicates.

## Results and discussion

Seventeen fungi were able to grow on plates containing LDPE sheets while other fungi were not able to grow in the same conditions compared to *A. niger* (Fig. 1). The fungus *N. phangngaensis* showed the highest growth rate with 0.68 g of mycelium weight while *A. burnsii*, *A. pseudoeichhorniae*, and *A. sacchari* showed similar growth rates ranging from 0.41-0.43 g of mycelium weight. However, the control fungus, *A. niger* showed a lower growth rate with 0.18 g of mycelium weight. The results showed that LDPE sheets were used as a carbon source for fungal growth (Mewada et al., 2021). The biodegradation of LPDE by different fungi has been reported such as some species of the *Aspergillus* and *Penicillium* genera (Mewada et al., 2021). It was found that there are rare reports on polyethylene degrading fungi from *Neopestalotiopsis* genera. Therefore, this is the first study reporting biodegradation of LDPE sheets by *N. phangngaensis*.

Due to the high growth rate of *N. phangngaensis*, *A. burnsii*, *A. pseudoeichhorniae*, and *A. sacchari*, these fungi were cultured with LDPE sheets in different incubation periods to determine weight loss percentage compared to those obtained from *A. niger*. A significant difference in weight loss was obtained among these fungi

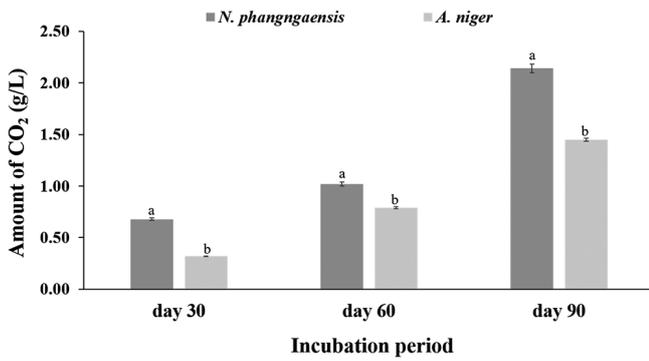


Fig. 5. Amount of CO<sub>2</sub> dissolved in a medium broth of *N. phangngaensis* and *A. niger* after 30, 60, and 90 days of incubation, respectively. Different letters above the bars indicate significant differences ( $p < 0.05$ ).

(Fig. 2). The weight loss of LDPE sheets after 30 days of incubation were 6.20, 17.32, 7.07, 7.32, 8.83, and 1.10%, after 60 days of incubation were 14.29, 28.73, 14.05, 14.39, 14.83, and 1.30%, and after 90 days of incubation were 23.90, 54.34, 8.78, 25.12, 26.78, and 1.90% for fungus *A. burnsii*, *N. phangngaensis*, *A. pseudoeychhorniae*, *A. sacchari*, *A. niger*, and LDPE sheets without any treatment, respectively. The result indicated that the fungus *N. phangngaensis* showed the highest percentage of weight loss compared with other fungi. This result confirmed the consumption of the carbon from the LDPE sheets by *N. phangngaensis*. The weight loss of the LDPE sheets is probably associated with enzymatic degradation by these fungi. Our previous study demonstrated that some fungi including *Diaporthe italiana*, *Thyrostroma jaczewskii*, *Colletotrichum fructicola*, and *Stagonosporopsis citrulli* showed an average 40% dry weight loss of LDPE during degradation within three months (Khruengsai et al., 2021). Similarly, the percentage of degradation for used polyethylene by *Alcaligenes faecalis* was increased over 2-10 weeks (Nag et al., 2021). El-Sayed et al. (2021) showed an excellent weight loss percentage of LDPE sheets after incubation with a mixed culture of *Aspergillus carbonarius* and *Aspergillus fumigates* compared to those obtained from a single isolate. In another study, a significant difference in weight loss of LDPE was detected by *Paenibacillus* sp. from a landfill in Brazil (Bardaji et al., 2019). Moreover, El-Sayed et al. (2021) also reported the biodegradation of LDPE using various treatments. It was found that greater degradation was achieved by 39.1%, 17.76%, and 5.79% from thermal, HNO<sub>3</sub>, and  $\gamma$ -irradiation treatment, respectively. Our present study demonstrated the biodegradation by fungi without any pretreatment such as UV photo-oxidation, and thermal or chemical treatment. Therefore, the *N. phangngaensis* was evaluated to be an LDPE-degrading fungal candidate that was able to reduce the LDPE weight significantly compared to the control strain of *A. niger*.

The tensile strength of LDPE sheets cultured with *A. burnsii*, *N. phangngaensis*, *A. pseudoeychhorniae*, *A. sacchari*, and *A. niger* after incubation is determined and shown in Fig. 3. *N. phangngaensis* showed the highest reduction rate of tensile strength of the LDPE sheet after 30, 60, and 90 days of incubation to 6.39, 3.28, and 0.33

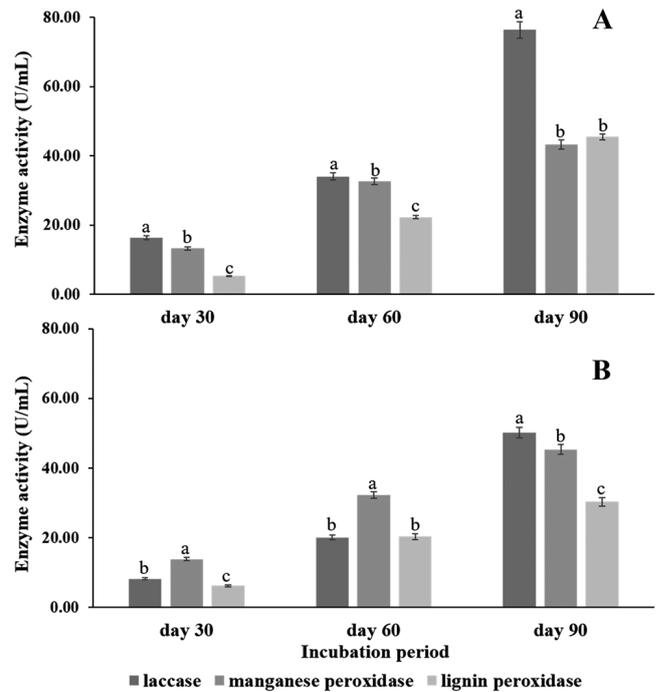


Fig. 6. Enzymic activity in the culture supernatant of *N. phangngaensis* (A) and *A. niger* (B) after 30, 60, and 90 days of incubation, respectively.

Different letters above the bars indicate significant differences ( $p < 0.05$ ).

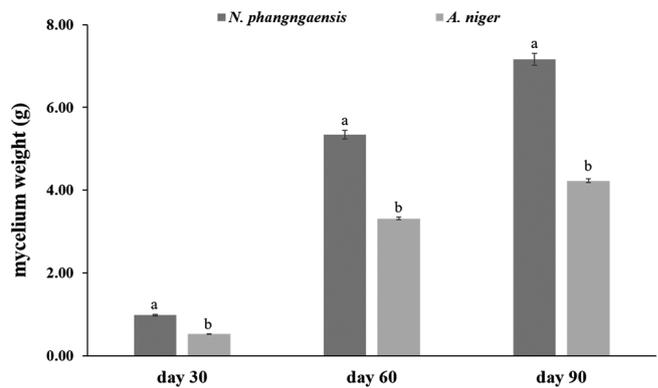


Fig. 7. Mycelium dry weight of *N. phangngaensis* and *A. niger* cultured in liquid medium after 30, 60, and 90 days of incubation.

MPa, respectively, while other fungi reduced the tensile strength of the LDPE sheets ranging 8.46 to 3.81 MPa. The fungus *A. niger* showed the lowest reduction rate of the tensile strength of the LDPE sheets from 6.87 to 3.32 MPa. The obtained results were in agreement with previous studies (Khruengsai et al., 2021; Sanniyasi et al., 2021). The tensile strength of LDPE depended on the incubation period. The fungi induced brittleness and decreased tensile strength by transforming the LDPE into smaller molecules (Hsu et al., 2012).

The biodegradation of LDPE sheet culturing with *N. phangngaensis* was also confirmed by SEM, Sturm test, and enzyme activity. The change of LDPE surface after incubation with *N. phangngaensis* and *A. niger* was monitored by SEM (Fig. 4). Most surfaces of LDPE sheets cultured with *N. phangngaensis* were damaged, fragile,

**Table 2.** Volatile organic compounds in culture supernatants of *N. phangngaensis* and *A. niger* after 90 days of incubation.

No.	compound	<i>N. phangngaensis</i>	<i>A. niger</i>
1	hexadienal	1.85	0.82
2	methyl-1,4-cyclohexadiene		0.23
3	octene		0.57
4	3,3,5-trimethyl-cyclohexene	1.71	
5	3,5,5-trimethyl-cyclohexene		0.38
6	2E-methyl-3-octen-5-yne		1.07
7	2E,4E-hexadienol	1.45	
8	4-methyl-3-heptanone	3.09	
9	dimethyl acetal hexanal	0.21	
10	decene	0.54	0.58
11	decane	0.92	0.96
12	1,2,4-trimethyl benzene	1.63	
13	2-methoxyethyl-benzene	3.83	
14	2Z-diethyl acetal hexenal	10.43	
15	3E-diethyl acetal hexenal	2.73	
16	3Z-diethyl acetal hexenal	22.70	
17	4Z-diethyl acetal heptenal	2.50	
18	4E-diethyl acetal heptenal	2.63	
19	1,4-dimethoxy-benzene		23.15
20	2E,4E-decadienal	2.12	
21	2E,4E-decadienol	12.50	
22	2E-undecenal	5.98	1.28
23	1,1-dimethoxy decane		50.18
24	2E,6Z-diethylacetal-nonadienal	0.88	14.16
25	isobutyl phenylacetate	1.93	1.25
26	1,3-dimethoxy-5-(1-methylethyl)-benzene	0.99	1.45
27	dodecanal	1.24	
28	2E,4E-undecadienal	3.98	
29	2Z,6E-dodecadien-1-al	6.92	
30	4Z-diethyl acetal decenal	4.87	
Total of % area		97.63	96.08

cracked, and scratched compared to those obtained from *A. niger* while LDPE sheets without fungal culturing remained smooth, intact, and clear. Previous studies reported the degradation of LDPE sheets by various fungi using SEM (Bardaji et al., 2019; Khruengsaï et al., 2021; Nag et al., 2021; Khandare et al., 2021). From SEM analysis, it is confirmed that *N. phangngaensis* degraded the LDPE by consuming carbon from LDPE sheets.

The Strum test showed the degradation of the LDPE sheet by culturing with *N. phangngaensis* into CO<sub>2</sub> on 30, 60, and 90 days was 0.68, 1.02, and 2.14 g/L, respectively, while CO<sub>2</sub> released by *A. niger* on 30, 60, and 90 days was 0.32, 0.79, and 1.45g/L, respectively (Fig. 5). As a result, *N. phangngaensis* showed the highest amount of CO<sub>2</sub> after incubation compared to those obtained by *A. niger*. The results of CO<sub>2</sub> production supported the biodegradation of the LDPE sheet according to CO<sub>2</sub> and H<sub>2</sub>O production and changing of polymers into small molecules and simple compounds by microorganism cells (Montazer et al., 2020).

Enzymatic activities of *N. phangngaensis* and *A. niger* were also determined after incubation. The results are shown in Fig. 6. Moreover, the growth of *N. phangngaensis* and *A. niger* were investigated based on their mycelium after incubation (Fig. 7). The results were compared to those obtained by culturing each fungus in same culture medium without LDPE sheets. Both fungi were able to grow in liquid medium containing LDPE sheets as carbon

source while no growth was detected in the controls. The mycelial weight of *N. phangngaensis* was higher significantly than those obtained by *A. niger* ranging from 0.98-7.16 g and 0.53-4.23 g, respectively. In addition, it was revealed that the fungus *N. phangngaensis* had the ability to produce laccase, manganese peroxidase, and lignin peroxidase enzymes. The laccase activity was significantly higher in the supernatant of *N. phangngaensis* while similar enzyme activity of manganese peroxidase and lignin peroxidase activities were detected in *N. phangngaensis* supernatant after 90 days of incubation. Our results were similar to those reported in previous studies demonstrating the enzyme activity involved in the degradation of polyethylene. Santo et al. (2013) reported the biodegradation of polyethylene by the laccase enzyme produced by *Rhodococcus ruber*. Iiyoshi et al. (1998) also depicted the biodegradation of high-density polyethylene using manganese peroxidase from *Phanerochaete chrysosporium*. The laccase and manganese peroxidase enzymes released by *Bacillus cereus* were evaluated to contribute to the polyethylene degradation after 9-week incubation (Sowmya et al., 2014). Enzymes produced from microorganisms are known to enhance the biodegradation rate of LDPE without causing any harm to the environment (Saravanan et al., 2021). The extracellular enzymes penetrate deeply into the polymer material and induce an erosion process only on the polymer surface (Kumar et al., 2013).

The volatile organic compounds in culture supernatants of *N. phangngaensis* and *A. niger* were also analyzed after incubations. The results showed that the peaks of volatile organic compounds were detected and identified after 90 days of incubation. These compounds in culture supernatants of *N. phangngaensis* and *A. niger* after 90 days of incubation are shown in Table 2. GC-MS detected 24 and 13 compounds in the culture supernatants of *N. phangngaensis* and *A. niger*, respectively. The major compounds of culture supernatant of *N. phangngaensis* were 3Z-diethyl acetal hexenal (22.70%), 2E,4E-decadienol (12.50%), and 2Z-diethyl acetal hexenal (10.43%) while 1,1-dimethoxy decane (50.18%), 1,4-dimethoxy-benzene (23.15%), and 2E,6Z-diethylacetal-nonadienal (14.16%) were detected as main volatile organic compounds in *A. niger* culture supernatant. This result indicated that the composition in the LDPE sheets could be released into the culture medium. This study was in agreement with previous studies reporting hydrocarbons were major biodegradation products of LDPE sheets (Pramila and Ramesh, 2015; Park and Kim, 2019; Khruengsaï et al., 2021). Although several countries have deployed microorganisms for composting processes, few applications of biodegradation of polyethylene are employed (Ahmed et al., 2018). The breakdown of carbon-carbon bonds in polyethylene structure requires a huge amount of energy due to slow degradation in the environment by microorganisms (Ahmed et al., 2018). Moreover, natural degradation under natural environmental conditions is used for a long time for breaking the polymer structure. Although, *N. phangngaensis* can break down carbon-carbon bonds in LDPE at considered rates as above mentioned, genetically engineered microorganisms with high degradation rates will be improved prior to application in the future.

## Conclusion

This study carried out the screening of LDPE degrading fungi. Biodegradation of LDPE sheets by fungi was evaluated based on weight loss percentage, tensile strength, SEM, CO<sub>2</sub> and enzyme analysis. This finding revealed that *N. phangngaensis* has the ability to degrade LDPE sheets without any additional chemical or photodegradation. Morphological changes on the LDPE surface culturing with *N. phangngaensis* were observed through SEM images including cracks, pits, and roughness resulting from the consumption of the LDPE sheet as a carbon source for growth. The amount of CO<sub>2</sub> and enzymatic activities also confirmed the LDPE degradation by the fungus *N. phangngaensis*. It can be concluded that *N. phangngaensis* is efficient in the biodegradation of the LDPE sheet. This augmented understanding of the biotechnological application of fungi may be expanded for plastic waste treatment.

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## Conflict of interest

The authors declare that there is no conflict of interest.

## Author Contributions

SK and TS performed the experiments. SK analyzed and interpreted data as well as wrote the manuscript including figures. All authors reviewed the manuscript. PP supervised the work and edited the manuscript.

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