BIODIVERSITAS
Volume 20, Number 3, March 2019
Pages: 867-871

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200334

Short Communication: Plastic degradation by *Coriolopsis byrsina*, an identified white-rot, soil-borne mangrove fungal isolate from Surabaya, East Java, Indonesia

NENGAH DWIANITA KUSWYTASARI[1,2,♥], ALFIA R. KURNIAWATI[1], NUR HIDAYATUL ALAMI[1], ENNY ZULAIKA[1], MAYA SHOVITRI[1], KYU MIN OH[3], NI NYOMAN TRI PUSPANINGSIH[4], NI'MATUZAHROH[2]

[1]Department of Biology, Faculty of Sciences, Institut Teknologi Sepuluh Nopember. Jl. Arif Rahman Hakim, Sukolilo, Surabaya 60111, East Java, Indonesia. Tel./fax. +62-31-5963857. ♥email: wyta2013@gmail.com
[2]Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Campus C, Mulyorejo Surabaya 60115, East Java, Indonesia
[3]Department of Chemical and Biological Engineering, Korea University, Anam-dong, Seongbuk-gu, Seoul, South Korea
[4]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Campus C Mulyorejo, Surabaya 60115, East Java, Indonesia

Manuscript received: 18 January 2018. Revision accepted: 26 January 2019.

Abstract. *Kuswytasari ND, Kurniawati AR, Alami NH, Zulaika E, Shovitri M, Oh KM, Puspaningsih TP, Ni'matuzahroh. 2019. Plastic degradation by* Coriolopsis byrsina*, an identified white-rot, soil-borne mangrove fungal isolate from Surabaya, East Java, Indonesia. Biodiversitas 20: 867-871.* The work deals with an important environmental issue, the disposal of plastic waste. The degradation of plastic by white-rot fungi of soil-borne mangrove isolate, T1P2, from Surabaya, East Java, Indonesia, was investigated using Minimal Salt Medium and the ability of the degradation was indicated as the amount of degradation efficiency. Analysis of the 18S rDNA sequence successfully identified the ligninolitic fungal isolated, T1P2, as Coriolopsis byrsina. The results have been revealed that *C. byrsina* have more potential to degrade plastic with maximum % DE was 22,7% for six weeks compared with the enzymatic plastic degradation reached 6,3% for two days. However, this study needs to do further investigation of extracellular enzyme that involved in degradation process.

Keywords: *Coriolopsis byrsina*, degradation, fungi, plastics

INTRODUCTION

Regarding their characteristics as strong, lightweight, durable, and inert materials, plastics have been applied to a wide range of commodities. Their bioinertness and resistance to deterioration have raised considerable ecological concerns about their increased production and accumulation in the environment. Thus, plastics waste become a major source of pollutants due to their durability and resistance to microbial attack (Esmaeili et al. 2013).

Growing concern over plastic waste has continued, including its handling through biodegradation approach. Biodegradation breaks down the plastic polymers into carbon dioxide, water and biomass as a result of the action of living microorganisms consumed the plastic materials as a carbon source (Ursa et al. 2003). Fungi is an organism that can be used as bioagents for a biodegradation procedure.

Fungi are the main decomposer in nature enable to form colonies in various environmental states. The long-lasting ability of the saprotrophic conditions and the ability to produce capillary enzymes capable of producing some of the recalcitrant enzyme degrading enzymes (Atiq et al. 2011). Some enzymes involved in plastic biodegradation include ligninolytic enzymes they are manganese, peroxidase, lignin peroxidase and laccase (Ehara et al. 2000; Premraj and Mukesh 2005; Ameen et al. 2015; Sowmya et al. 2015).

Degradation of plastic membrane by lignin-degrading fungi has been studied by several researchers. Ligninolitic enzyme can help in the oxidation of the hydrocarbon backbone of plastic. (liyoshi et al. 1998; Bhardwaj et al. 2012; Mahalakshmi and Siddiq 2015). White rot fungi that mostly include in a class Basidiomycetes, having their great ability to grow in a natural environmental condition where they degrade lignin. (Lee et al. 2014). Considering the ability in degrading lignin, white rot fungi is thought they also to be able to degrade plastic. . In the present study, white rot fungi, T1P2, a soil-borne mangrove isolated which subsequently identified as *Coriolopsis byrsina* was used as a model microorganism for the examination of plastic degradation. Identification of *C. byrsina* T1P2 was done by molecular characterization based on 18S rDNA. The 18S rDNA fragment is considered a useful marker in molecular genotype analysis (Duong et al. 2006). Further, the investigation of plastic degradation was examined by % degradation efficiency by dry weight plastic from biodegradation and enzymatic degradation, and the visual appearance of plastic.

MATERIALS AND METHODS

Organism and inoculum preparation

White-rot fungi were used in this study is T1P2 isolated from Wonorejo Mangrove soil, Indonesia. Culture was

maintained on Potato Dextrose Agar (PDA) at 30 °C. Mineral salt (MS) medium pH 7 following standard medium by Madella et al.(2015), consist of yeast extract 100 mg/L, $(NH_4)2SO_4$ 1 g/L, $MgSO_4.7H_2O$ 200 mg/L, $Na_2MoO_4.2H_2O$ 0.5 mg/L, NaCl 100 mg/L, $CaCl_2.2H_2O$ 20 mg/L, $FeSO_4.7H_2O$ 10 mg/L, $MnSO_4$ 0.5 mg/L, K_2HPO_4 1.6 g/L, and KH_2PO_4 200 mg/L. Medium of 90 mL place to 250 mL Erlenmeyer flask.

Table 1. Type and sequences of primer used in 18S rDNA amplification

Types	Sequences	References
NS1	5' GTAGTCATATGCTTGTCTC 3'	White and Lee (1990)
NS8	5' TCCGCAGGTTCACCTACGGA 3'	

DNA isolation

DNA isolation was performed by following a protocol from Promega Extraction Kit with slight modifications. Each of pure isolates was incubated in Potato Dextrose Agar (PDA) at 30 °C, 250 rpm for 5-7 days, 1 gram of mycelium was transferred to 1.5 mL microcentrifuge tube that containing 300 µl of nuclei lysis solution and 300 µl of glass bead. The mixture was vortex vigorously at 3000 rpm for 5 min, then added with 100 µl protein precipitation solution. The tube was inverted about 10 times and incubated on ice for 5 min. The sample was centrifuged at 13500 for 3 min, then the supernatant containing the DNA was transferred to a clean 1.5 mL microcentrifuge tube containing room temperature isopropanol in ratio 1:1. The sample was gently mixed by inversion until the thread-like strands of DNA have formed a visible mass. The tube was centrifuged at 13.500 for 2 minutes to precipitate DNA. The supernatant was discarded using pipette while the pellet was added by 70% ethanol and the tube was gently inverted to wash the DNA pellet, then centrifuged it for 1 minute. Ethanol was discarded and dried at 65 °C for 10 min. The pellet was added by 100 µl of DNA rehydration solution and 1 µl RNAse then incubated at 65 °C for 1 hour. The DNA concentration was calculated using a Nanodrop at 260/280 nm. Unused DNA was stored at -20 °C.

Amplification of 18S rDNA gene fragment

The polymerase chain reaction (PCR) is a relatively simple technique that amplifies a DNA template to produce specific DNA fragments in vitro. Two types of primer that used for amplification of 18S rDNA shows in Table 1. Reaction mix was prepared by combining 10 µl reaction buffer , 1.5 µl dNTP (dTTP, dCTP, dATP dan dGTP), 2.5 µl of each primer (100 pmol) forward and reverse (Table 1), 1 µl DNA template, 0.5 µl Q5® High-Fidelity DNA Polymerase and added sterile H_2O to get 50 µl final volume. PCR was run in 35 cycles, each cycle consists of denaturing 98° C for 15 seconds, annealing 57° C for 30 seconds, extending 72° C for 60 seconds.

PCR product was confirmed by loading sample in 0.8% gel electrophoresis. Electrophoresis was run at 135 Volts for 15 minutes. 5 µl of ladder and sample was mixed with loading dye and loaded into the wells. DNA migration result was observed under UV transilluminator after gel electrophoresis had been flooded in ethidium bromide solution for 15 minutes.

Purification was performed by following NucleoSpin® Gel and PCR Clean-up of Macherey-Nagel. 1 volume of sample was mixed with 2 volumes of buffer NTI. NucleoSpin® Gel and PCR Clean-up Column was placed into a collection tube (2 mL) and loaded up to 700 µL sample. The sample was centrifuged for 30 s at 13500 rpm. The flow-through was discarded and the column was placed back into the collection tube. 700 µL Buffer NT3 was added to the NucleoSpin® Gel and PCR Clean-up Column and centrifuged for 30 s at 13500 rpm. The flow-through was discarded and the column was placed back into the collection tube. The previous washing step was repeated to minimize chaotropic salt carry-over and improve A260/ A230 values. The sample was centrifuged for 1 min at 13500 rpm to remove buffer NT3 completely. the NucleoSpin® Gel and PCR Clean-up Column were placed into a new 1.5 mL microcentrifuge tube. 15-30 µL of buffer NE was added and incubated at room temperature (18°-25 °C) for 1 min, then was centrifuged for 1 min at 13500 rpm. The purified DNA was sequenced using Genetic Analyzer 3730xl (Applied Biosystems).

DNA sequence and phylogenetic analysis

The resulting sequences were compiled using the Bioedit program and subjected to BLASTN analysis (www.ncbi.nlm.nih.gov/BlastN). The organisms were identified relying on the sequences available in the database presenting the highest homology. The sequences were aligned using CLUSTAL W (Drancourt et al. 2000). MEGA ver.6 was used to construct Neighbor-Joining (NJ) tree with Kimura 2 parameter model (Bootstrap value is 500).

Biodegradation test

Synthetic plastic was cut into (1 x 1 cm) strips and then washed with distilled water followed by rinsing in 70% ethanol for 30 min. Following dried under UV lamp, they situated in an incubator at 60 °C for 24 h. While, each isolate was cultivated in a tube containing 90 mL of mineral salt medium and 10 mL of fungal starter for 7 - 10 days (log phase) and 3 strips of plastic films were added. The tubes were incubated on a rotary shaker (120 rpm) at 37 °C. The tests were performed in triplicate. Biomass measured after incubation and percentage weight loss at day 0, 2, 4 and 6 weeks incubation period. The parameters used in the degradation test are % degradation efficiency of dry plastic weight and visual appearance.

Enzymatic degradation test

Isolate was co-cultivated in MS medium with synthetic plastic film pieces in replicates along with the controls. After ten days of incubation, enzyme level in the medium was assayed for controls and treatments. Cultures were centrifuged at 10,000 rpm, 4°C for 20 minutes; the pellet consisting of fungal cells was discarded and enzyme level

was determined in the extracellular fluids as crude plastic depolymerase. The enzymatic degradation test was performed by adding 1 x 1 cm plastic pieces into the mixture of crude enzymes and 100 mM tris-HCl buffer with a ratio of 1 : 1 and 2 : 1. The parameters used in the degradation test are % degradation efficiency of dry plastic weight and visual appearance.

Percentage of degradation efficiency

The degradation of these films was evaluated in terms of percentage of weight loss using the following formula:

$$\text{Percentage of weight loss} = \frac{Wi - Wf}{Wi} \times 100\%$$

Where:
Wi= Initial weight (gram)
Wf= Final weight (gram)

RESULTS AND DISCUSSION

As described in the procedure, plastic film was considered the only carbon and energy source used in fungal-aided biodegradation test. The fungal isolate, T1P2, gave a positive result for its potential in deteriorating plastic strips. A pure culture of T1P2 identified morphological characteristics pointed to the class Basidiomycota based on the microscopic examination.

Macroscopic features shown in Figure 1 revealed a white colony with a cotton-like surface, Pileus -10 cm in radius, sessile, often connate, annual to perennial, hispid-tomentose, narrowly zoned, somewhat rugulose, spicular-rugose at the base, yellowish ochraceous to light cinnamon buff, then brownish to greyish brown.

The incorporation of morphologic and molecular-based identification can assist the fungi taxonomy in the differentiation of species and even varieties. Molecular characterization using 18S rDNA amplification assay followed by the analysis of the sequences for intraspecies similarity with existing data on GenBank NCBI using the Local Alignment Search Tool (BLAST) algorithm has successfully identified the genotype of the isolate. The amplificons from the 18S rDNA area varies ± 1500 bp (Figure 2). Sequence analysis results are shown in Table 2. The overall similarity of queries with data on GenBank reached 99%.

Similarity and relationship between sequences of the soil-borne mangrove isolates can be seen through the phylogenic trees constructed using neighboring distance calculation method. The advantage of using this method is the position of the sequence of isolate to the nearest sequence (sequence having small base pair difference) can be shown, (Baum 2008). The construction of the phylogenic tree from *Coriolopsis byrsina* T1P2 can be seen in Figure 3.

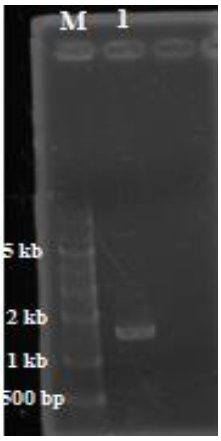

Figure 2. Amplicon of 18S rDNA using primer universal NS1 and NS8. M= Marker, 1 = T1P2

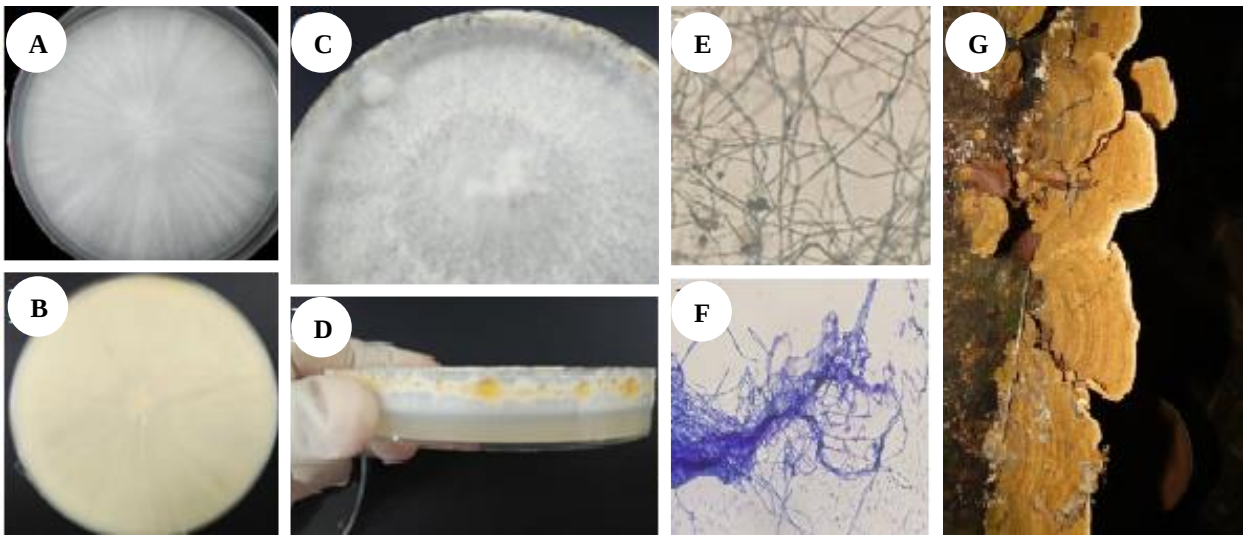

Figure 1. Morphological characteristic of *Coriolopsis byrsina* T1P2. A. Surface colony; B. Reverse colony; C. Initial fruiting body in surface medium; D. Initial fruiting body in marginal disk; E. Microscopic feature; F. Microscopic feature with lactophenol blue; G. Mushroom form of *C. byrsina* (Carranza-Morse 1991)

Table 2. BLAST result of *Coriolopsis byrsina* T1P2

Closest Match NCBI Database	No. Accession	Max Score	E-Value	% Similarity
Coriolopsis byrsina	AY336773.1	3018	0	99
Coriolopsis byrsina	KY418163.1	2929	0	99

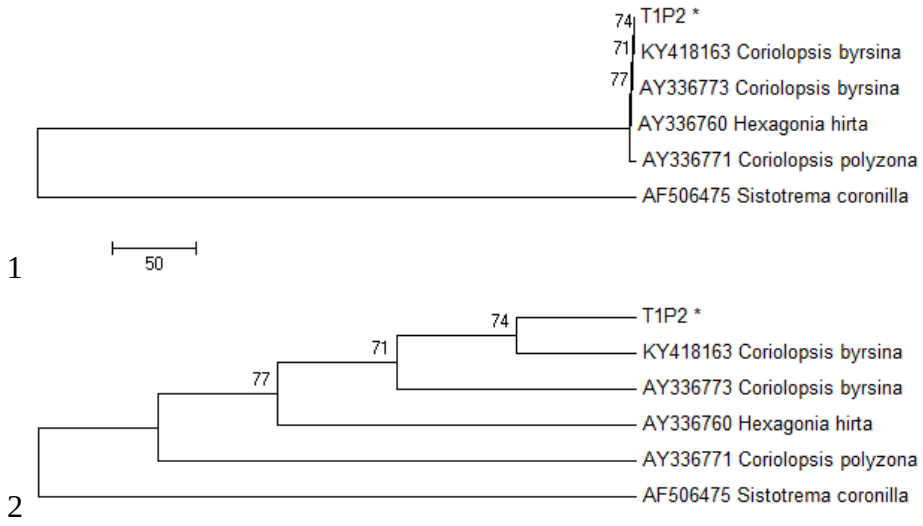

Figure 3. Phylogenetic tree of T1P2 using Neighbor-joining Method. The value in the branch represents the bootstrap value (percentage of 500 x replication) and the bar scale indicates the length of the branch

The result of biodegradation test revealed the ability of T1P2 isolate that identified as C. byrsina in degrading plastic. The plastic used in this study is a white plastic bag with anonymous components. The biodegradation process begins with the formation of biofilms and the penetration of hyphae into plastic polymers. Percentage of degradation efficiency obtained from dry weight loss of plastic. The % DE outcome of *C. byrsina* showed an increasingly high value until week 6 (Figure 4).

C. byrsina has well known as filamentous Basidiomycota produced ligninolytic enzyme that involved in PAH (pyrene) degradation (Agrawal and Shahi 2017) and dye degradation (Sanchez-Lopez et al. 2008; Verma et al. 2010; Daassi et al. 2014; Chen and Ting 2015).

The potential of a crude enzyme in degrading plastic strips was visualized in Figure 5. The ratio enzyme: buffer formulation, 2:1 shows more optimum to use. The % DE decreased in day-2 presumably caused by enzyme degradation. This result indicated that *C. byrsina* have the potential to degrade plastic with value of % DE enzymatic plastic degradation reached 6,3% (day-1).

To determine physical deterioration, plastic films were placed in MS medium and incubated at 30°C. The films were recovered after 2, 4, and 6 weeks (Figure 6). For comparative purposes, films were placed together and analyze the change of films. Initially, the films had a smooth surface. After incubation at in MS medium for 6 weeks, the surfaces of films appeared rough; but there are no visible cracks appeared (Figure 2-6).

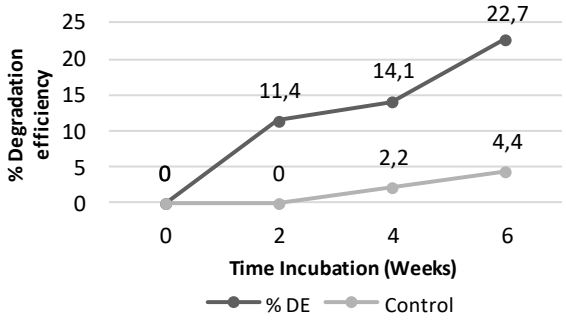

Figure 4. Biodegradation result after 6 weeks incubation

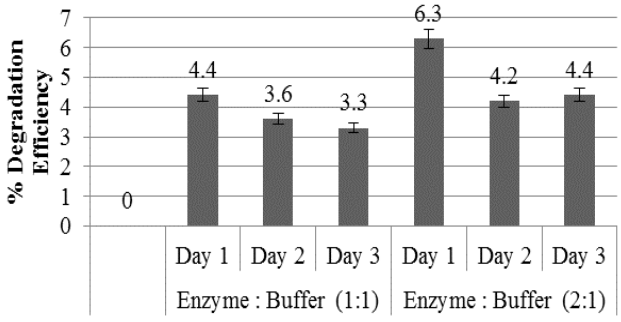

Figure 5. Enzymatic degradation by *Coriolopsis byrsina* T1P2

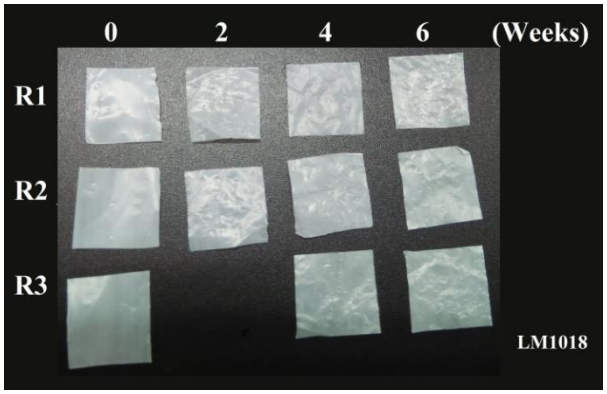

Figure 6. Visual appearance of plastic after biodegradation

The *C. byrsina* T1P2 isolate is a novel fungi for plastic degradation study because of its ability and efficiently degrade plastic up to 22,7% after six weeks incubation. The visual appearance of plastic after biodegradation test was not shown significance. Further research addressing the purification of the enzyme from the isolated is needed to optimize its degradation potential.

ACKNOWLEDGEMENTS

This research was partly supported by Korea University in collaboration with Chemical and Biological Engineering Departement, Korea University, Seoul, South of Korea which supports the facilities for molecular analysis.

REFERENCES

Agrawal N, Shahi KS. 2017. Degradation of polycyclic aromatic hydrocarbon (pyrene) using novel fungal strain *Coriolopsis byrsina* strain APC5. Intl Biodeterior Biodegrad 122: 69-81.

Ameen F, Moslem M, Hadi S, Ahmed EA. 2015. Biodegradation of Low-Density Polyethylene (LDPE) by mangrove fungi from the Red Sea Coast, progress in rubber. Plastics Recycl Technol 31 (2): 125-143.

Atiq N, Safia A, Ishtiaq A, Saadja A, Bashir A, Geoffery R. 2011. Isolation and identification of polystyrene biodegrading bacteria from soil. African J Microbiol Res 4: 1537-1541.

Baum D. 2008. Reading a phylogenetic tree: the meaning of monophyletic groups. Nature Educat 1 (1): 190.

Bhardwaj H, Gupta R, Tiwari R. 2012. Microbial Population Associated with Plastic degradation. Open access Sci Rep 1 (5): 1-4.

Carranza-Morse J. 1991. Pore fungi of Costa Rica I. Mycotaxon 41: 345-370.

Chen SH, Ting ASY. 2015. Biodecolorization and biodegradation potential of recalcitrant triphenylmethane dyes by *Coriolopsis* sp. isolated from compost. J Environ Manag 150: 74-280.

Daassi D, Rodriguez-Couto S, Nasri M, Mechichi T. 2014. Biodegradation of textile dyes by immobilized laccase from *Coriolopsis gallica* into Ca-alginate beads. Intl Biodeter Biodegrad 90: 71-78.

Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clinic Microbiol 38 (10): 3623-30.

Duong LM, Jeewon R, Lumyong S, Hyde KD. 2006. DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. Fungal Divers 23: 121-138.

Ehara K, Iiyoshi Y, Tsutsumi Y, Nishida T. 2000. Polyethylene degradation by manganese peroxidase in the absence of hydrogen peroxide. Journal of Wood Science 46 (2): 180-183.

Esmaeili A, Pourbabaee AA, Alikhani HA, Shabani F, Esmaeili E. 2013. Biodegradation of Low-Density Polyethylene (LDPE) by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in Soil. Plos One 8 (9): e 71720. DOI: 10.1371/journal.pone.0071720.

Lee H, Jang Y, Choi YS, Kim MJ, Lee J, Lee H, Hong JH, Lee YM, Kim GH, Kim JJ. 2014. Biotechnological procedures to select white rot fungi for the degradation of PAHs. J. Microbiol Methods 97: 56-62.

Liyoshi Y, Tsutsumi Y, Nishida T. 1998. Polyethylene Degradation by Lignin-Degrading Fungi and Manganese Peroxidase. J Wood Sci 44: 222-229.

Madella NR, Scalvenzi L, Perez M, Montera C, Gooty JM. 2015. Efficiency of indigenous filamentous fungi for biodegradation of petroleum hydrocarbons in medium and soil: Laboratory study from Ecuador. Bull Environ Contam Toxicol 95: 385-394.

Mahalaksmi V, Siddiq SA. 2015. Enhanced biodegradation of polyethyene by development of consortium. Adv Appl Sci Res 6(4):183-189.

Premraj R, Mukesh D. 2005. Biodegradation of polymers. Indian J Biotechnol 4: 186-193.

Sanchez-Lopez MI, Vanhulle SF, Mertens V, Guerra G, Figueroa SH, Decock C, Corbisier A, Penninckx MJ. 2008. Autochthonous white rot fungi from the tropical forest: potential of Cuban strains for dyes and textile industrial effluents decolourisation. Afr J Biotechnol 7: 1983-1990.

Sowmya HV, Ramalingappa B, Nayanashree G, Thippeswamy B, Krishnappa M. 2015. Polyethylene degradation by fungal consortium. Intl J Environ Res 9 (3): 823-830.

Ursa K, Jozefa F, Andrej K. 2003. Polyamide - 6 fibre degradation by lignolytic fungus. Polymer Degrad Stabil J 97: 99-104.

Verma AK, Raghukumar C, Verma P, Shouche YS, Naik CG. 2010. Four marine-derived fungi for bioremediation of raw textile mill effluents. Biodegradation 21: 217-233.