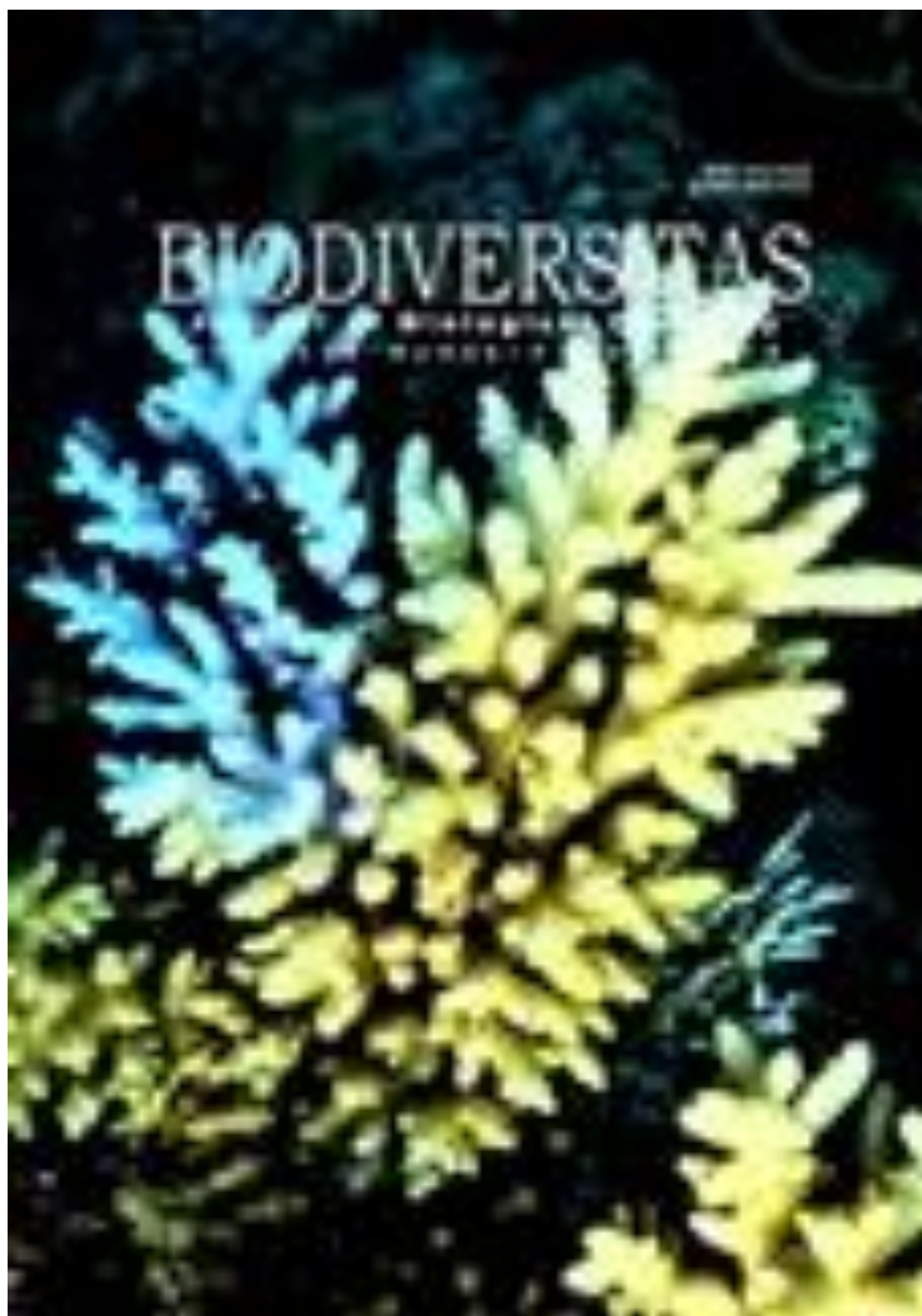


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### Table of Content

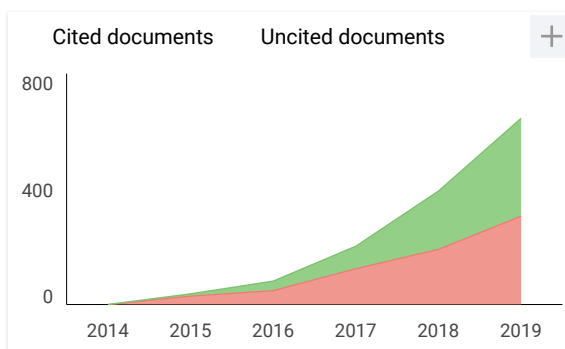
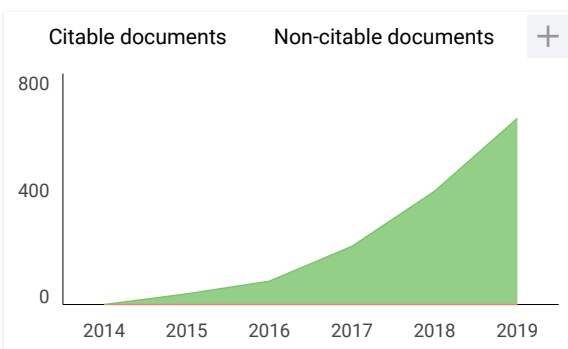
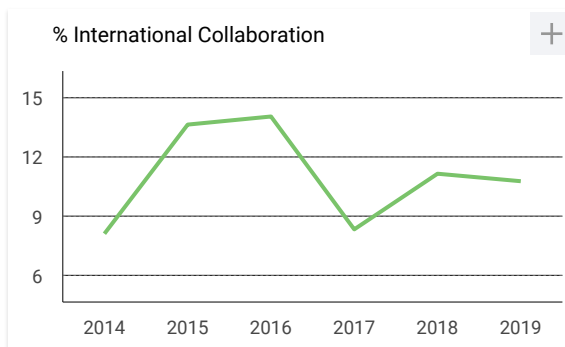
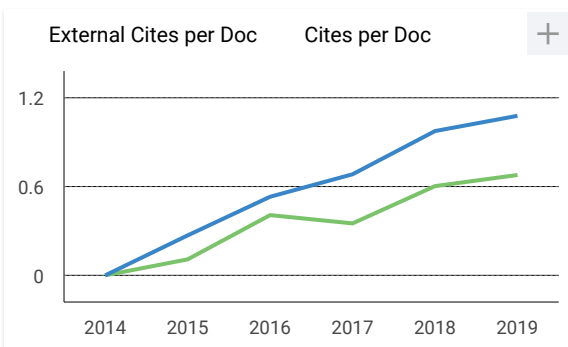
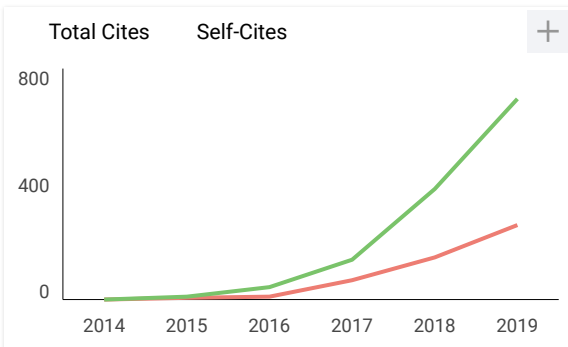
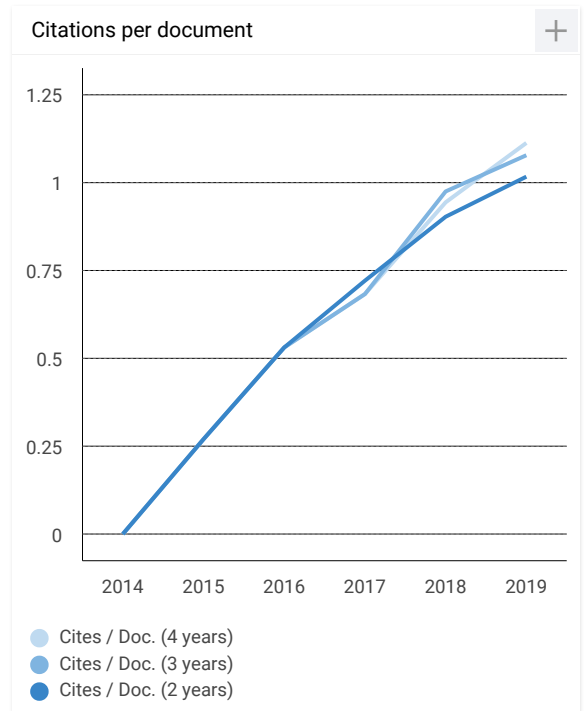
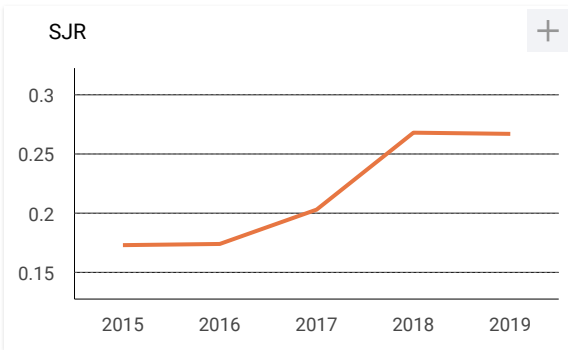
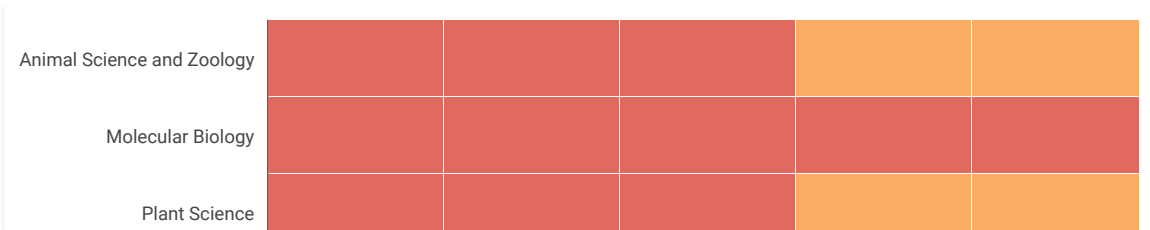
Cover [\[PDF\]](#)Editorial Board & Guidance for Authors [\[PDF\]](#)

1. Diversity of medicinal plants and their uses by the Sanger tribe of Sangihe Islands, North Sulawesi, Indonesia  
DINGSE PANDIANGAN, MARINA SILALAH, FARHA DAPAS, FEBBY KANDOU [\[PDF\]](#)
2. The diversity and physiological activities of weeds in land cultivated with various corn cultivars and fertilized with various nitrogen doses  
MUJI RAHAYU, PRAPTO YUDONO, DIDIK INDRADEWA, EKO HANUDIN [\[PDF\]](#)
3. Delineation of the selected *Cucumis* L. species and accessions using leaf architecture characters  
LAILANI A. MASUNGSONG, MARILYN M. BELARMINO, INOCENCIO E. BUOT JR. [\[PDF\]](#)
4. Short Communication: Molecular identification of White Sea Squirt *Didemnum* sp. (Tunicata, Ascidiacea) colonies growing over corals in Raja Ampat Islands, Indonesia  
LUTHFI ANZANI, HAWIS H. MADDUPPA, I WAYAN NURJAYA, P. JOANA DIAS [\[PDF\]](#)
5. Vegetation analysis of tree communities at some forest patches in North Sulawesi, Indonesia  
MUSTAID SIREGAR, HENDRA HELMANTO, SRI ULIE RAKHMAWATI [\[PDF\]](#)
6. Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of *Curcuma aeruginosa* accessions growing in West Java, Indonesia  
NURUL KHUMAIDA, MUHAMAD SYUKUR, MARIA BINTANG, WARAS NURCHOLIS [\[PDF\]](#)
7. Evaluation of *Salacca sumatrana* as soil conservation crop in South Tapanuli, North Sumatra, Indonesia  
YUSRIANI NASUTION, AZWAR RASYIDIN, YULNAFATMAWITA, AMRIZAL SAIDI [\[PDF\]](#)
8. Short Communication: Molecular characteristic of taro white cattle based on DNA microsatellite markers  
LUH GDE SRI SURYA HERYANI, I NENGAH WANDIA, I WAYAN SUARNA, I KETUT PUJA, NI NYOMAN WERDI SUSARI, KADEK KARANG AGUSTINA [\[PDF\]](#)
9. Short Communication: Estimation of the above- and below-ground carbon stocks in University of Lampung, Indonesia  
IRWAN SUKRI BANUWA, RIZKI AFRILIYANTI, MUHAJIR UTOMO, SRI YUSNAINI, MELYA RINIARTI, PURBA SANJAYA, ERDI SUROSO, WAHYU HIDAYAT [\[PDF\]](#)
10. Isolation and characterization of endophytic bacteria from roots of *Piper nigrum* and their activities against *Fusarium oxysporum* and *Meloidogyne incognita*  
WIRATNO, MUHAMMAD SYAKIR, IRWANTO SUCIPTO, ANKARDIANSYAH PANDU PRADANA [\[PDF\]](#)
11. New record of *Grateloupia taiwanensis* S.-M. Lin et H.-Y. Liang in Vietnam: Evidence of morphological observation and rbcL sequence analysis  
XUAN-VY NGUYEN, TRUNG-HIEU NGUYEN, VIET-HA DAO, LAWRENCE LIAO [\[PDF\]](#)
12. The genetic diversity and population structure of shallots (*Allium cepa* var. *aggregatum*) in Indonesia based on R gene-derived markers  
LINA HERLINA, REFLINUR, SOBIR, AWANG MAHARIJAYA, SURYO WIYONO [\[PDF\]](#)
13. Seed germination of *Carex lainzii* Luceno, E. Rico & T. Romero: An endemic Spanish endangered species  
KAROLINE APARECIDA FELIX RIBEIRO, CRISTIAN MADEIRA DE MEDEIROS, JOSE ANGEL SANCHEZ AGUDO, JOSE SANCHEZ SANCHEZ [\[PDF\]](#)
14. Population study and habitat preferences of Pinang Jawa (*Pinanga javana*) in Mt. Slamet, Central Java, Indonesia  
RIZMOON NURUL ZULKARNAEN, NISYAWATI, JOKO RIDHO WITONO [\[PDF\]](#)
15. Short Communication: Activity budget and diet in silvery lutung *Trachypithecus cristatus* at Gunung Padang, West Sumatra, Indonesia  
MUHAMMAD AZHARI AKBAR, RIZALDI, WILSON NOVARINO, DYAH PERWITASARI-FARAJALLAH, YAMATO TSUJI [\[PDF\]](#)
16. Local knowledge on landscape sustainable-hydrological management reduces soil CO<sub>2</sub> emission, fire risk and biomass loss in West Kalimantan Peatland, Indonesia  
DWI ASTIANI, MOHAMMAD J. TAHERZADEH, EVI GUSMAYANTI, TRI WIDIASTUTI, BURHANUDDIN [\[PDF\]](#)
17. Ecology of *Zanthoxylum acanthopodium*: Specific leaf area and habitat characteristics  
DECKY INDRAWAN JUNAEDI, YATI NURLAENI [\[PDF\]](#)
18. Detection and identification of *Begomovirus* infecting Cucurbitaceae and Solanaceae in Yogyakarta, Indonesia  
APRILIA SUFI SUBIASTUTI, SEDYO HARTONO, BUDI SETIADI DARYONO [\[PDF\]](#)
19. Short Communication: Protein profiles of Giant Mudskipper and its potential use as biomarker candidate for heavy metal contamination in Barito Estuary, Indonesia  
HIDAYATURRAHMAH, MABRUR, HERI BUDI SANTOSO, RANI SASMITA, UMMY SHALIHA AULIA RAHMY, BADRUZSAUFARI [\[PDF\]](#)

20. The local knowledge of the plant names and uses by Semende tribe people in Kaur District, Bengkulu Province, Indonesia  
WIRYONO, YUSUF WANANDI, AISYAH KURNIA ILAHI, DESELINA, GUNGGUNG SENOAJI, SISWAHYONO [PDF]
21. Population and distribution of Sunda porcupine (*Hystrix javanica* F. Cuvier, 8) in designated area of Cisokan Hydropower, West Java, Indonesia  
IRINA A. MUSTIKASARI, SUSANTI WITHANINGSIH, ERRI N. MEGANTARA, TEGUH HUSODO, PARIKESIT [PDF]
22. Genetic variations of strawberry cultivars of *Fragaria x ananassa* and *Fragaria vesca* based on RAPD  
GANIES RIZA ARISTYA, RINA SRI KASIAMDARI, RACHMI SETYONINGRUM, BENING LARASATI [PDF]
23. Exploration culturable bacterial symbionts of sponges from Ternate Islands, Indonesia  
AGUS TRIANTO, OCKY KARNA RADJASA, AGUS SABDONO, SAKTI IMAM MUCHLISSIN, RACHMAT AFRIYANTO, SULISTIOWATI, SEPTHY KUSUMA RADJASA, PHILLIP CREWS, ERIN MCCAULEY [PDF]
24. Genetic diversity of Ongole Grade, Aceh, and Sumbawa cattle based on polymorphism on ND-5 fragment mitochondrial DNA using PCR-RFLP technique  
SUTARNO, SYARIFA ZAHRAH, OKID PARAMA ASTIRIN, ELISA HERAWATI, AHMAD DWI SETYAWAN [PDF]
25. Source, harvesting, conservation status, threats and management of indigenous plant used for respiratory infections and related symptoms in the Limpopo Province, South Africa  
SEBUA SILAS SEMENYA, ALFRED MAROYI [PDF]
26. Impact of Bukit Dua Belas rainforest transformation to oil palm plantation on phylogenetic of soil bacterial communities in Sarolangun, Jambi, Sumatra, Indonesia  
MARINI WIJAYANTI, ARIS TRI WAHYUDI, MUNTI YUHANA, MARTIN ENGELHAUPT, ANJA MERYANDINI [PDF]
27. Bird diversity in transition zone of Taka Bonerate, Kepulauan Selayar Biosphere Reserve, Indonesia  
RADISTI A. PRAPTIWI, RONNA SAAB, TATANG MITRA SETIA, GUSTI WICAKSONO, PRAWESTI WULANDARI, JITO SUGARDJITO [PDF]
28. Diversity wild banana species (*Musa* spp.) in Sulawesi, Indonesia  
HASTUTI, PURNOMO, I. SUMARDI, BUDI S. DARYONO [PDF]
29. Short Communication: Investigation of leaf architectural patterns: Implications in delineating taxonomically controversial *Hoya merrillii* Schlechter and *Hoya quinquenervia* Warburg  
DIANA P. PAGUNTALAN, INOCENCIO E. BUOT JR. [PDF]
30. Short Communication: The composition of mangrove species in coastal area of Banggai District, Central Sulawesi, Indonesia  
RAMLI UTINA, ABUBAKAR SIDIK KATILI, NURAIN LAPOLO, TALHA DANGKUA [PDF]
31. Genetic diversity of *Rhizopus microsporus* from traditional inoculum of tempeh in Indonesia based on ITS sequences and RAPD marker  
TATI BARUS, RONALDO HALIM, ANASTASIA TATIK HARTANTI, PAULUS KEVIN SAPUTRA [PDF]
32. An exploration of gender equity in household: A case from a peatland-based community in Riau, Indonesia  
TUTI HERAWATI, DEDE ROHADI, MAMAT RAHMAT, BONDAN WINARNO [PDF]
33. The diversity of morphological characteristics and chemical content of *Celosia cristata* plantlets due to gamma ray irradiation  
IZZATUL MUHALLILIN, SYARIFAH IIS AISYAH, DEWI SUKMA [PDF]
34. Short Communication: Plastic degradation by *Corioloopsis byrsina*, an identified white-rot, soil-borne mangrove fungal isolate from Surabaya, East Java, Indonesia  
NENGAH DWIANITA KUSWYATASARI, ALFIA R. KURNIAWATI, NUR HIDAYATUL ALAMI, ENNY ZULAIKA, MAYA SHOVI TRI, KYU MIN OH, NI NYOMAN TRI PUSPANINGSIH, NI'MATUZAHROH [PDF]
35. Assessment of benthic and coral community structure in an inshore reef in Balok, Pahang, Malaysia  
MUHAMMAD FAIZ MOHD HANAPIAH, SHAHBUDIN SAAD, ZUHAIRI AHMAD, MUHAMMAD HAMIZAN YUSOF, MOHD FIKRI AKMAL KHODZORI [PDF]
36. The Bornean orangutan (*Pongo pygmaeus wurmbii*) density in a logging concession of Hulu Belantikan, Central Kalimantan, Indonesia  
IMAN SAPARI, DYAH PERWITASARI-FARAJALLAH, SRI SUCI UTAMI ATMOKO [PDF]
37. Distribution and abundance of the giant clams (Cardiidae: Bivalvia) on Kei Islands, Maluku, Indonesia  
TEDDY TRIANDIZA, NEVIATY P. ZAMANI, HAWIS MADDUPPA, UDHI E. HERNAWAN [PDF]
38. A mutant variant of *Nix* gene on  $\gamma$ -irradiated *Aedes aegypti* as a male-determination factor  
BENI ERNAWAN, HARRY NUGROHO EKO SURNIYANTORO, IRAWAN SUGORO, USMAN SUMO FRIEND TAMBUNAN [PDF]
39. Analysis of the condition of forest interior and edge in Mount Papandayan, West Java, Indonesia based on floristic composition and structural characteristics of tree community  
ENDAH SULISTYAWATI, EKA FATMAWATI TIHURUA [PDF]
40. Morphological and phytochemical characterization of susceptible and resistant sengon (*Falcataria moluccana*) tree to gall rust disease  
DEWI RAHMAWATI, NURUL KHUMANDA, ULFAH J. SIREGAR [PDF]
41. Short Communication: Species confirmation of juvenile cloudy grouper, *Epinephelus erythrurus* (Valenciennes, 1828), based on a morphologic analysis and partial CO1 gene sequencing  
YANTI ARIYANTI, ACHMAD FARAJALLAH [PDF]
42. Morphological variation and heritability studies of *Lagenaria siceraria* landraces from northern KwaZulu-Natal, South Africa  
LUNGELO GIVEN BUTHELEZI, SYDNEY MAVENGHAMA, NONTUTHUKO ROSEMARY NTULI [PDF]

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11

H Index

<b>Country</b>	Indonesia - <a href="#">IIII</a> SIR Ranking of Indonesia
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<b>Publisher</b>	<a href="#">Biology department, Sebelas Maret University Surakarta</a>
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<b>Scope</b>	"Biodiversitas, Journal of Biological Diversity" or Biodiversitas encourages submission of manuscripts dealing with all biologi aspects of plants, animals and microbes at the level of gene, species, and ecosystem.
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	<a href="#">How to publish in this journal</a>
	<a href="#">Contact</a>
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## Short Communication:

# Plastic degradation by *Corioloopsis byrsina*, an identified white-rot, soil-borne mangrove fungal isolate from Surabaya, East Java, Indonesia

NENGAH DWIANITA KUSWYASARI<sup>1,2,\*</sup>, ALFIA R. KURNIAWATI<sup>1</sup>, NUR HIDAYATUL ALAMI<sup>1</sup>, ENNY ZULAIKA<sup>1</sup>, MAYA SHOVI TRI<sup>1</sup>, KYU MIN OH<sup>3</sup>, NI NYOMAN TRI PUSPANINGSIH<sup>4</sup>, NI'MATUZHROH<sup>2</sup>

<sup>1</sup>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

<sup>2</sup>Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Campus C, Mulyorejo Surabaya 60115, East Java, Indonesia

<sup>3</sup>Department of Chemical and Biological Engineering, Korea University, Anam-dong, Seongbuk-gu, Seoul, South Korea

<sup>4</sup>Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Campus C Mulyorejo, Surabaya 60115, East Java, Indonesia

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**Abstract.** Kuswytasari ND, Kurniawati AR, Alami NH, Zulaika E, Shovitri M, Oh KM, Puspaningsih TP, Ni'matuzhroh. 2019. Plastic degradation by *Corioloopsis 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 ligninolytic fungal isolated, T1P2, as *Corioloopsis 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:** *Corioloopsis 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. Ligninolytic enzyme can help in the oxidation of the hydrocarbon backbone of plastic. (Iiyoshi 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 isolate which subsequently identified as *Corioloopsis 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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1 g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O 200 mg/L, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 0.5 mg/L, NaCl 100 mg/L, CaCl<sub>2</sub>·2H<sub>2</sub>O 20 mg/L, FeSO<sub>4</sub>·7H<sub>2</sub>O 10 mg/L, MnSO<sub>4</sub> 0.5 mg/L, K<sub>2</sub>HPO<sub>4</sub> 1.6 g/L, and KH<sub>2</sub>PO<sub>4</sub> 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' TCCGCAGGTTACCTACGGA 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<sub>2</sub>O 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](http://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:

$$\text{Percentage of weight loss} = \frac{W_i - W_f}{W_i} \times 100\%$$

Where:

W<sub>i</sub> = Initial weight (gram)

W<sub>f</sub> = Final weight (gram)

## RESULTS AND DISCUSSION

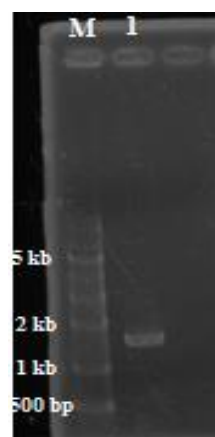
As described in the procedure, plastic film was considered the only carbon and energy source used in fungal-aided biodegradation test. The fungal isolate, T1P2, gave a positive result for its potential in deteriorating plastic strips. A pure culture of T1P2 identified morphological characteristics pointed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

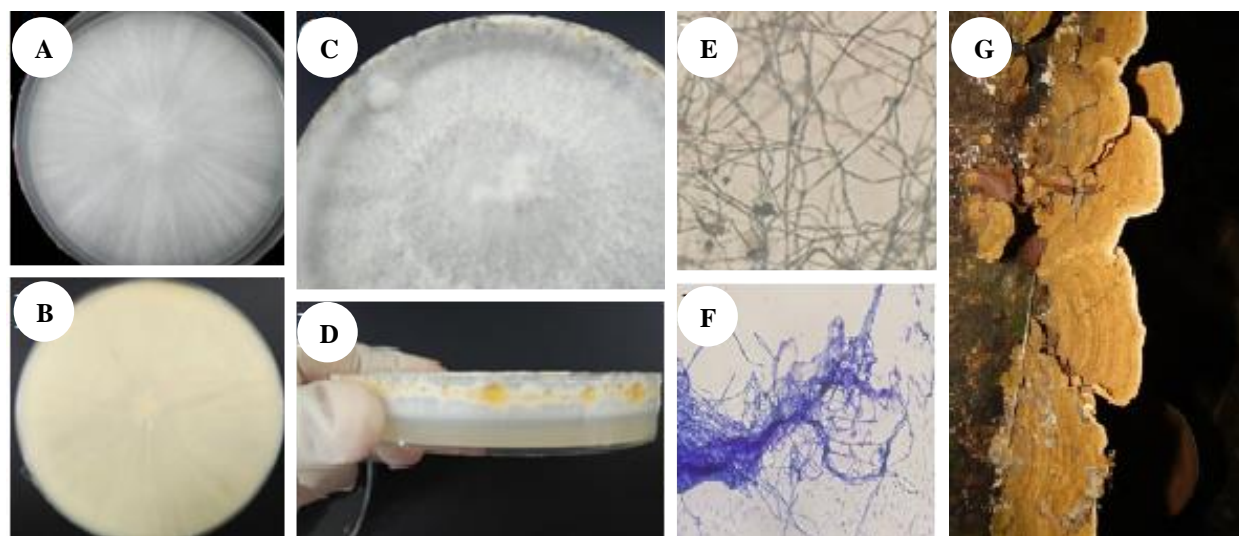
The incorporation of morphologic and molecular-based identification can assist the fungi taxonomy in the

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 amplicons from the 18S rDNA area varies  $\pm 1500$  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 phylogenetic 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 phylogenetic tree from *Corioloropsis byrsina* T1P2 can be seen in Figure 3.



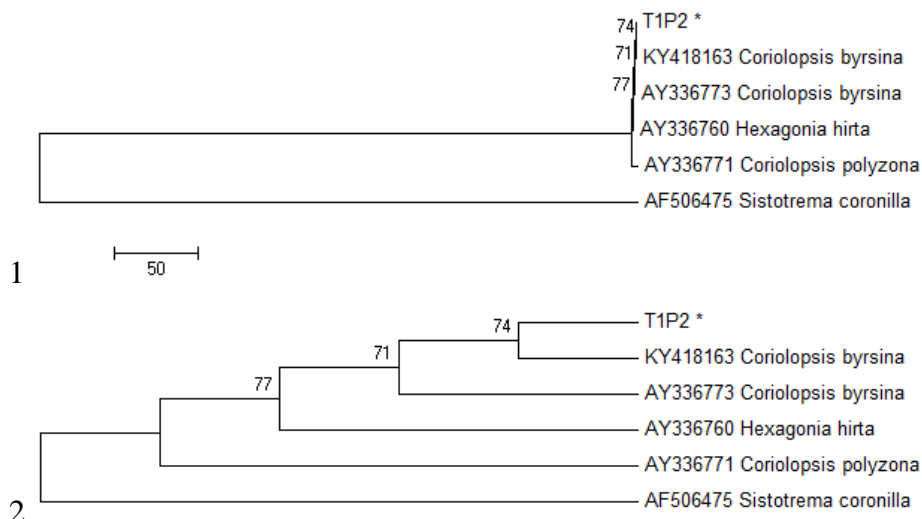
**Figure 2.** Amplicon of 18S rDNA using primer universal NS1 and NS8. M= Marker, 1 = T1P2



**Figure 1.** Morphological characteristic of *Corioloropsis 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 *Corioloopsis byrsina* T1P2

Closest Match NCBI Database	No. Accession	Max Score	E-Value	% Similarity
<i>Corioloopsis byrsina</i>	AY336773.1	3018	0	99
<i>Corioloopsis byrsina</i>	KY418163.1	2929	0	99

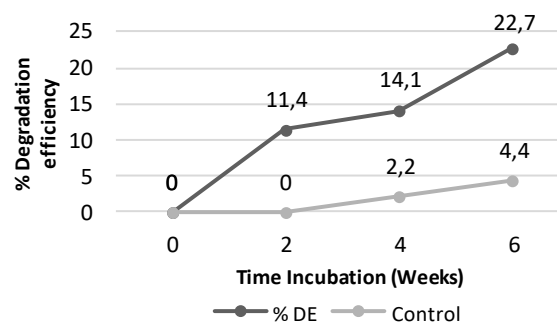
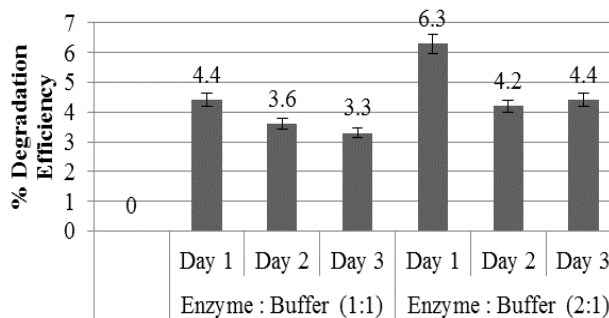
**Figure 3.** Phylogenetic tree of T1P2 using Neighbor-joining Method. The value in the branch represents the bootstrap value (percentage of 500 x replication) and the bar scale indicates the length of the branch

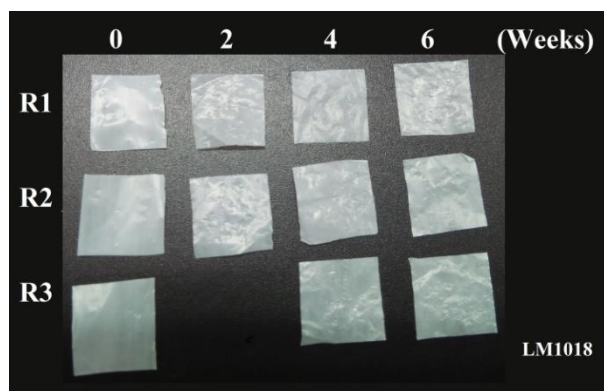
The result of biodegradation test revealed the ability of T1P2 isolate that identified as *C. byrsina* in degrading plastic. The plastic used in this study is a white plastic bag with anonymous components. The biodegradation process begins with the formation of biofilms and the penetration of hyphae 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 *Corioloopsis 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.

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