RESEARCH NOTE

Biodegradative Activities of Fungal Strains Isolated from Terrestrial Environments in Korea

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ABSTRACT

Polylactic acid (PLA) and polycaprolactone (PCL) are commercially available bioplastics that are exploited worldwide, and both are biodegradable. The PLA and PCL polymer-degrading activity of 30 fungal strains that were isolated from terrestrial environments were screened based on the formation of a clear zone around fungal colonies on agar plates containing emulsified PLA or PCL. Among them, five strains yielded positive results of biodegradation. Strains Korean Agricultural Culture Collection (KACC) 83034BP and KNUF-20-PPH03 exhibited PCL degradation; two other strains, KACC 83035BP and KNUF-20-PDG05, degraded PLA; and the fifth strain, KACC 83036BP, biodegraded both tested plastics. Based on phylogenetic analyses using various combinations of the sequences of internal transcribed spacer (ITS) regions, *RPB2*, LSU, *CAL*, and *β-TUB* genes, the above-mentioned strains were identified as *Apiotrichum porosum*, *Penicillium samsonianum*, *Talaromyces pinophilus*, *Purpureocillium lilacinum*, and *Fusicolla acetilerea*, respectively. Based on our knowledge, this is the first report on (i) plastic biodegraders among *Apiotrichum* and *Fusicolla* species, (ii) the capability of *T. pinophilus* to degrade biodegradable plastics, (iii) the biodegradative activity of *P. samsonianum* against PCL, and (iv) the accurate identification of *P. lilacinum* as a PLA biodegrader. Further studies should be conducted to determine how the fungal species can be utilized in Korea.

Petrochemical-based plastics are used in large volumes in various industries but cause severe damage to the environment, since they are not degradable [1]. Biodegradable plastics have been developed and commercialized during the past two decades as a potential solution to the problem that arises from the disposal of plastic waste [2]. Synthetic polymers vulnerable to biodegradation include polyesters, polyamides, polyurethanes, poly(amide-enamines), and polyanhydrides [3]. Aliphatic polyesters are biodegradable polymers due to their potentially hydrolyzable ester bonds and are the most representative examples of environmentally friendly polymeric materials [4]. Based on the bonding of constituent monomers, aliphatic polyesters, such as polycaprolactone (PCL), polylactic acid (PLA), and polyglycolic acid (PGA), are classified into polyhydroxyalkanoates [3]. Among them, PLA is the most popular biodegradable polymer, having broad potential applications in agricultural films, biomedical devices, packaging material, and others [5]. Due to its physicochemical properties and compatibility with various polymers, PCL is also considered to be an attractive substitute for nonbiodegradable polymers in commodity applications [6].

Biodegradation of this group of plastics is carried out by fungi, actinomycetes, and bacteria belonging to various taxa. Among known fungi, the members of a few genera were described as degraders of synthetic aliphatic polyesters, including *Aspergillus clavatus* [7], *Fusarium moniliforme* [8], *Trichoderma viride* [9], *Tritirachium album* [10], and *Penicillium oxalicum* [11]. The general approach for screening of fungal biodegraders is based upon the selective isolation of polymer-degrading strains from soil, plastic debris, or other environmental samples; however, in several cases, identification of active strains was performed only at the genus level [12–14]. Assessments of already known and deposited fungal species for polymer degrading activities were performed in rare cases, but the studies were usually limited to only few fungal strains [15]. Systematic evaluation of the biodegrading capabilities of a wider range of representatives of various fungal families and genera requires greater research focus to improve our understanding of the biodegradation potential of fungi.

This study was conducted to evaluate 30 fungal strains isolated from terrestrial habitats in Korea based on their ability to degrade synthetic aliphatic polyesters. PLA was selected as a widely used polymer that accounts for 24% of the global production capacity for biodegradable plastics [16]. Considering that degrading capability and rate of biodegradation are directly proportional to the molecular weight of plastics [17], PCL diol was selected as another representative of polyhydroxyalkanoates, as it has a relatively lower molecular weight than that of PLA. PCL diol (m.w. ∼530) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and poly-L-lactic acid was obtained from GFM Co., Ltd. (Suwon, South Korea; ME331050, film, 0.05 mm thickness). Plysurf A210G was purchased from Daiichi Kogyo Seiyaku Co. (Kyoto, Japan). All other compounds were standard commercial preparations that were used without further purification. The search for polymer-biodegradation agents was conducted among fungal strains collected from soil in Korea, all of which were identified at the genus level based on morphological characteristics and phylogenetic analysis (data are not shown). The halo zone technique was used to evaluate the polymer-degrading activity of the selected fungal strains. A mineral medium containing 1% (w/v) PCL or 0.4% emulsified PLA as the sole carbon source was prepared according to previous reports, with only slight modifications [18,19]. The pure colony was transferred onto the plate with minimal salt media

supplemented with PLA or PCL, which was solidified with agar. After the incubation period, the polymer-degrading activity of each fungal strain was screened by the formation of a clear (halo) zone around the growing colony on the opaque plate as a result of diffusing enzymes excreted by the fungal hyphae to degrade the plastic. The clear-zone formation test was performed on 30 fungal strains to determine their PCL- and PLA-degrading ability (Table 1). In the polymer degradation assays, halo zones were visible around the colonies of the five fungal strains, Korean Agricultural Culture Collection (KACC) 83034BP (=NIBR-WAE-1241), KNUF-20-PPH03, KACC 83035BP (=KNUF-20-PDG06), KNUF-20-PDG05, and KACC 83036BP (=KNUF-20-PBU01) after 7–8 days of incubation. As shown in Figure 1, strains KACC 83034BP and KNUF-20-PPH03 formed a clear zone around colonies on emulsified PCL plates within 7 days (the halo looks dark due to the black background), which indicates their ability to degrade this polymer. The diameter of the zones increased depending on the incubation time. Two other strains, KACC 83035BP and KNUF-20-PDG05, were incapable of degrading PCL, but both strains formed clear zones around colonies on agar mineral medium supplemented with emulsified PLA. As Figure 2 shows, halo zones were visible after 8 days of incubation; their diameters significantly increased in 18 days, and the polymer was completely degraded after 45 days of incubation. Among the above-mentioned

Table 1. Fungal strains tested for biodegradative activities on PCL and PLA.

No	Strains	Region of isolation	Identification at the genus level	Biodegradative activity PCL	PLA
1	KACC 83034BP	Ulleung-do	*Apiotrichum* sp.	+	−
2	KNUF-20-PDG02	Daegu	*Penicillium* sp.	−	−
3	KNUF-20-PDG03	Daegu	*Arthrinium* sp.	−	−
4	KNUF-20-PKY02	Daegu	*Perenniporia* sp.	−	−
5	KNUF-20-PDG06	Daegu	*Talaromyces* sp.	−	+
6	KACC 83036BP	Chungbuk province	*Fusicolla* sp.	+	+
7	KNUF-20-PPH03	Pohang	*Penicillium* sp.	+	−
8	KNUF-20-PDG05	Daegu	*Purpureocillium* sp.	−	+
9	KNUF-19-PYH05	Yeongduk-gun	*Aspergillus* sp.	−	−
10	KNUF-20-PUH07	Uljin-gun	*Mortierella* sp.	−	−
11	KNUF-19-POD13	Geochang-gun	*Chaetomium* sp.	−	−
12	KNUF-19-PDG22	Daegu	*Aspergillus* sp.	−	−
13	KNUF-20-PUD92	Daegu	*Ilyonectria* sp.	−	−
14	KNUF-20-PUD72	Daegu	*Penicillium* sp.	−	−
15	KNUF-20-PST42	Daegu	*Fusarium* sp.	−	−
16	KNUF-19-PPH05	Pohang	*Trichoderma* sp.	−	−
17	KNUF-19-PPH04	Pohang	*Bipolaris* sp.	−	−
18	KNUF-19-PUH02	Uljin-gun	*Trichoderma* sp.	−	−
19	KNUF-19-PYH02	Yeongduk-gun	*Talaromyces* sp.	−	−
20	KNUF-19-PYH01	Yeongduk-gun	*Aspergillus* sp.	−	−
21	KNUF-19-PSM04	Daegu	*Penicillium* sp.	−	−
22	KNUF-19-PDG01	Daegu	*Aspergillus* sp.	−	−
24	KNUF-19-PDG11	Daegu	*Penicillium* sp.	−	−
25	KNUF-20-PDG12	Daegu	*Penicillium* sp.	−	−
26	KNUF-20-PGW19	Gunwi-gun	*Didymocrea* sp.	−	−
27	KNUF-20-PGC27	Geochang-gun	*Chaetomium* sp.	−	−
28	KNUF-19-PCS19	Cheongsong-gun	*Gonytrichum* sp.	−	−
29	KNUF-19-PGW01	Gunwi-gun	*Penicillium* sp.	−	−
30	KNUF-19-PGC16	Gimcheon	*Humicola* sp.	−	−

+, positive for halo zone formation; −, negative for halo zone formation.
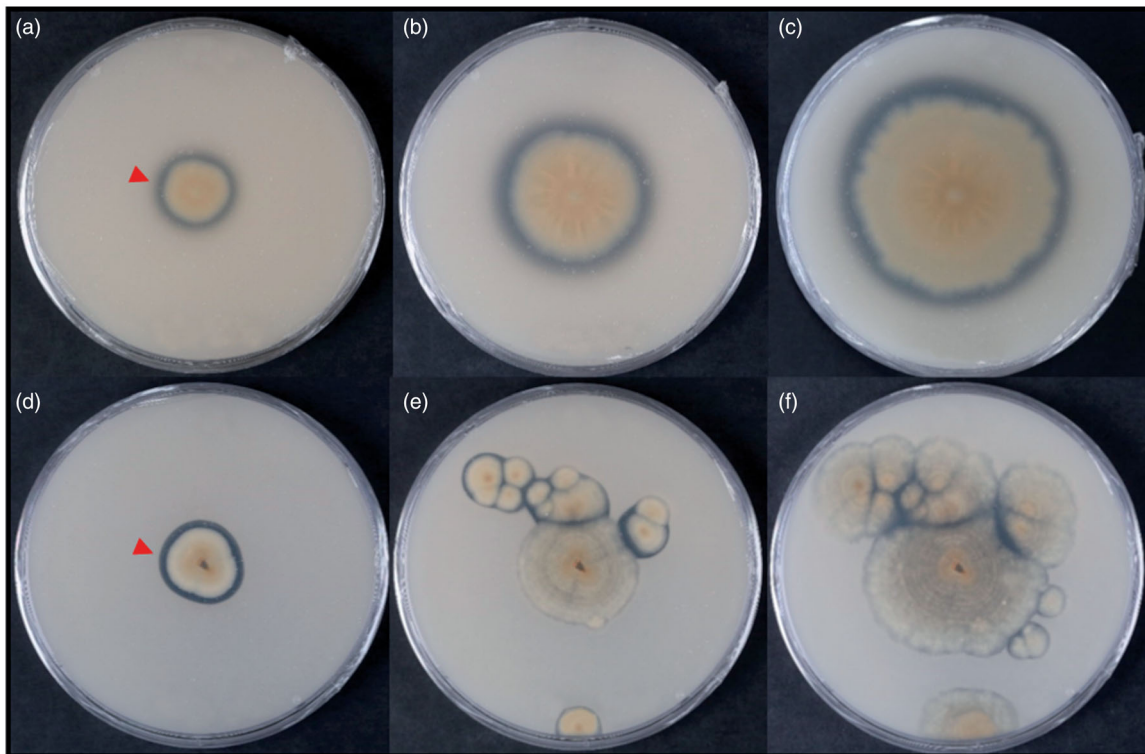
Figure 1. Clear zones around the colonies of strain KACC 83034BP (a, b, and c) and KNUF-20-PPH03 (d, e, and f) on a mineral media containing emulsified PCL. (a, d) 7 days after inoculation (DAI); (b, e) 14 DAI; (c, f) 21 DAI. The red arrowhead indicates a clear zone around the colony. The reverse side of the colonies is shown.
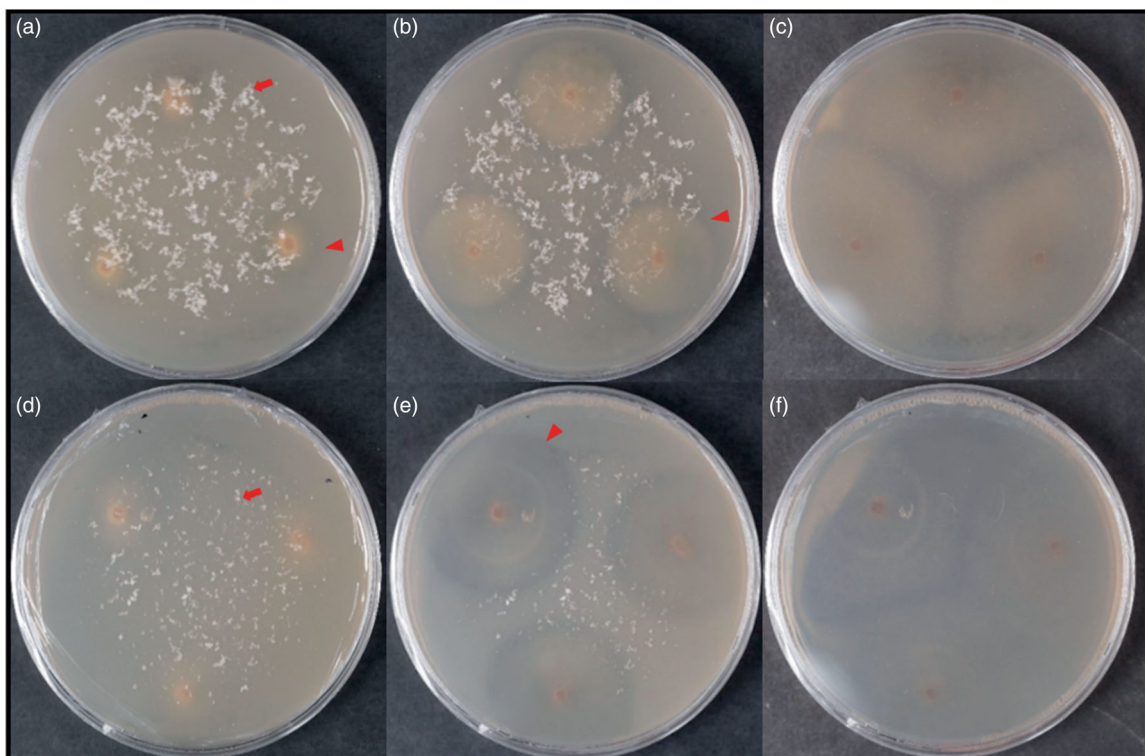

Figure 2. Clear zones around the colonies of strain KACC 83035BP (a, b, and c) and KNUF-20-PDG05 (d, e, and f) on a mineral media containing emulsified PLA. (a, d) 8 days after inoculation (DAI); (b, e) 18 DAI; (c, f) 45 DAI. The red arrowhead indicates a clear zone around the colony. The red arrow indicates PLA particles. The reverse side of the colonies is shown.

degradative active strains, only KACC 83036BP formed clear zones around colonies on agar medium supplemented with emulsified PLA or PLC, which demonstrated the degradative ability of KACC 83036BP against both polymers (Figure 3). After at least 4 weeks of incubation, there were no signs of halo zones around the inoculum or growth of the inoculum for the remaining 25 strains in the degradation assays, which indicated that they were incapable of biodegrading PCL and PLA.
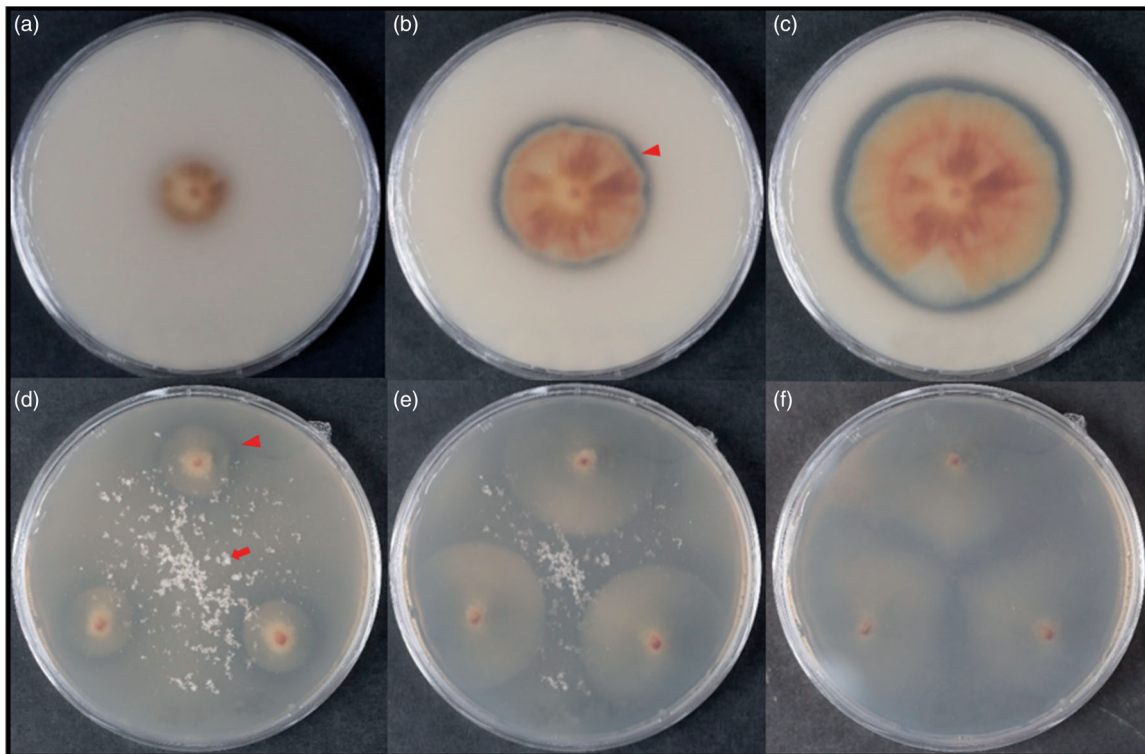
Figure 3. Clear zones around the colonies of strain KACC 83036BP on a mineral media containing emulsified PCL (a, b, and c) and PLA (d, e, and f). (a) 7 days after inoculation (DAI), (b) 14 DAI, (c) 21 DAI, (d) 8 DAI, (e) 18 DAI, (f) 45 DAI. The red arrowhead indicates a clear zone around the colony. The red arrow indicates PLA particles. The reverse side of the colonies is shown.
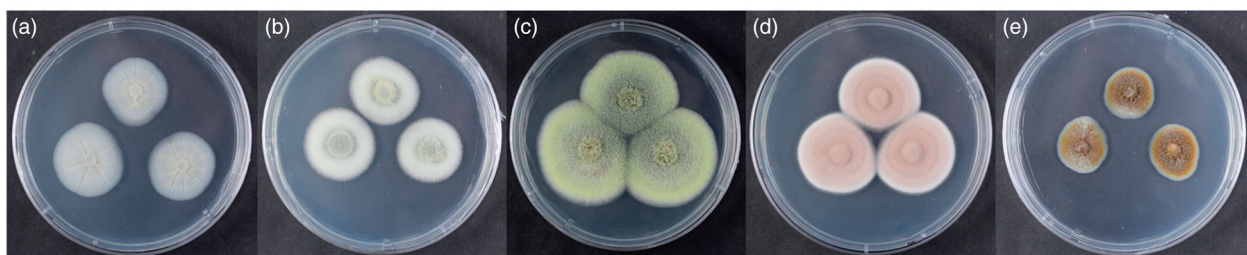

Figure 4. The photographs of selected five fungal strains cultured on PDA for 7 days in 25 °C. (a) KACC 83034BP; (b) KNUF-20-PPH03; (c) KACC 83035BP; (d) KNUF-20-PDG05; (e) KACC 83036BP.

The identification of all selected fungal strains at the genus level was previously performed based on their morphological characteristics and phylogenetic analysis using the sequences of the internal transcribed spacer (ITS) regions (data are not shown). A more precise identification at the species level was conducted on the strains that could degrade PCL and PLA. The selected five fungal strains; KACC 83034BP, KNUF-20-PPH03, KACC 83035BP, KNUF-20-PDG05, and KACC 83036BP were cultured on potato dextrose agar (Difco) for 7 days in 25 °C (Figure 4). Genomic DNA of the five strains was extracted from growing mycelia on PDA using the Solg^{TM} Genomic DNA Prep Kit (Solgent, Daejeon, Korea) following the manufacturer's instructions. Molecular identification based on polymerase chain reaction (PCR) amplification was conducted using ITS regions, calmodulin (*CAL*),

translation elongation factor 1-alpha (*TEF1-α*), the large subunit of the nuclear ribosomal RNA (LSU), *β-tubulin* (*β-TUB*), and DNA-directed RNA polymerase II second largest subunit (*RPB2*) genes (separately or in various combinations). The ITS regions of ribosomal DNA (including ITS1, 5.8S, and ITS2) were amplified using the primers ITS1/ITS4 as described by Park et al. [20]. Partial regions of *β-TUB*, *TEF1-α*, and *CAL* genes were amplified using the primer pair T1/Bt2b [21,22], EF1-728F/EF2 [23], and CAL-228F/CAL-737R [23], respectively. The primers LR0R and LR5 [24] were used for LSU amplification, and *RPB2* gene was amplified with primers RPB2-5F2 and fRPB2-7cR [25,26]. The amplified PCR products were purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA) and sequenced by Macrogen (Daejeon, Korea). The sequences of allied species were retrieved from
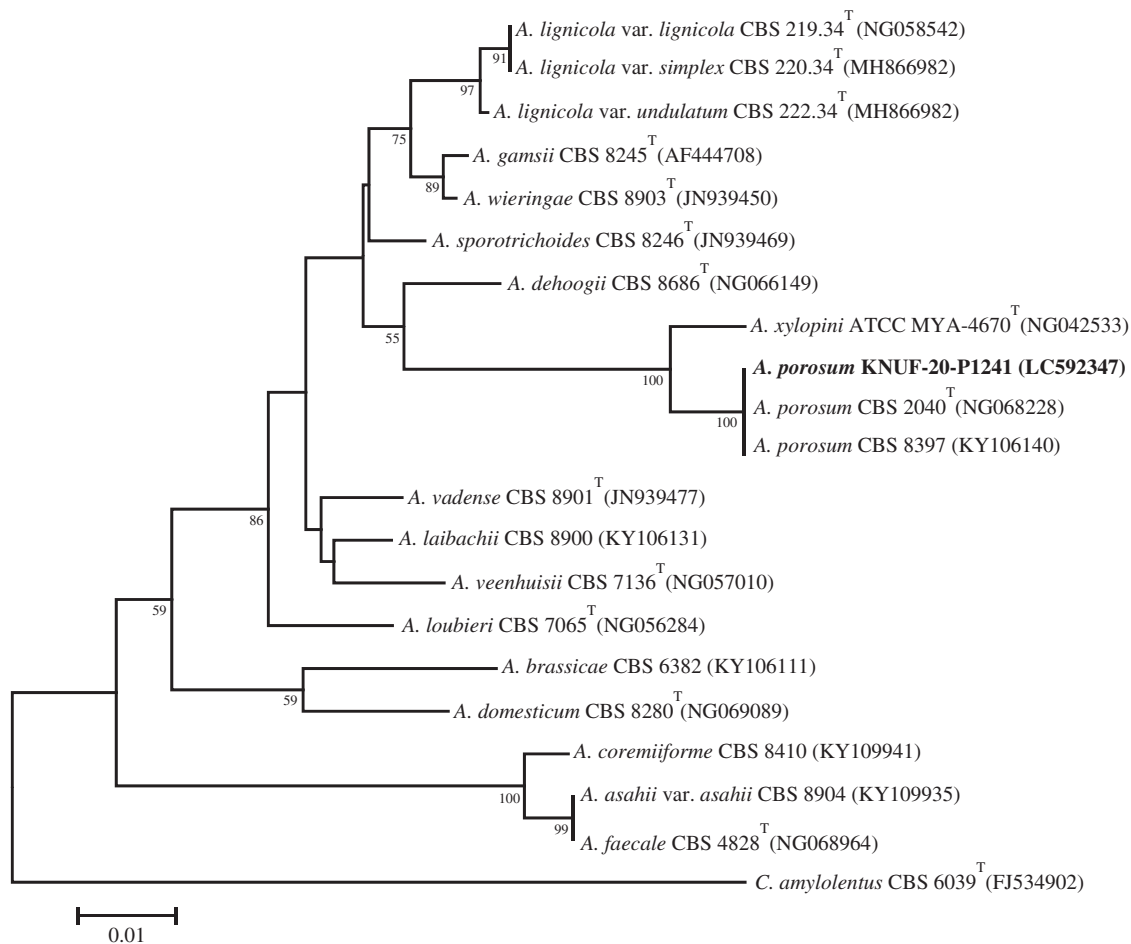
Figure 5. Neighbor-joining phylogenetic tree based on sequences of the LSU gene showing the affiliation of KACC 83034BP with *Apiotrichum porosum* among the closest *Apiotrichum* spp. Accession numbers are shown in parentheses. Bootstrap values (based on 1,000 replications) greater than 50% are shown at the branch points. The tree was rooted using *Cryptococcus amylolentus* CBS 6039[T] as an outgroup. The isolated strain of this study is indicated in bold. Scale bar = 0.01 substitutions per nucleotide position.

the National Center for Biotechnology Information (NCBI) GenBank. Phylogenetic trees were constructed based on concatenated nucleotide sequences of the ITS regions and the partial sequences of selected genes using the neighbor-joining (NJ) method [27], as implemented in MEGA7 [28]. NJ analysis was performed using the Kimura two-parameter distances with gaps excluded from the analysis.

A BLAST search of the NCBI database revealed similarities of 100% between the LSU sequences of KACC 83034BP and *Apiotrichum porosum* CBS 2040[T] (NG_068228), *A. porosum* CBS 8397 (KY106140), and several other strains of this species. A phylogenetic tree based on the LSU gene sequence (Figure 5) confirmed the affiliation of KACC 83034BP with *Apiotrichum porosum*; this strain was clustered together with the two above-mentioned neighbors in a monophyletic clade with the maximum bootstrap value. The *RPB2*, *CAL*, and *β-TUB* gene sequences of strain KACC 83035BP, respectively shared 100, 99.7–99.8, and 99.7%–99.8% identities with those of *Talaromyces pinophilus*

NRRL 62136 (MH793131, MH793004, MH792940, respectively) and *T. pinophilus* CBS 631.66[T] (KM023291, KF741964, JX091381, respectively), and 99.3–99.9% with several other closely related strains of this species. The NJ phylogenetic tree (Figure 6) based on the concatenated *β-TUB*, *CAL*, and *RPB2* sequences clearly demonstrated that strain KACC 83035BP is identical to *Talaromyces pinophilus* and that all above-mentioned closest relatives occupy a distinct position from other *Talaromyces* species. The *RPB2* gene region of strain KACC 83036BP revealed a similarity of 100% with that of *Fusicolla acetilerea* BBA 63789 (HQ897701) but lower than 94% with *Fusicolla bharatavarshae* PUFD71 (MK157022), *Fusicolla betae* BBA 64317 (HQ897781), and other *Fusicolla* species. Based on the ITS region and partial LSU nucleotide sequence similarities, the closest relatives of strain KACC 83036BP were identified as two representatives of *Fusicolla acetilerea*, strains IMI 181488[T] (NR_111603) and RF3 (LC333211), which showed 100% similarity. The NJ phylogenetic tree (Figure 7) that was constructed using the concatenated
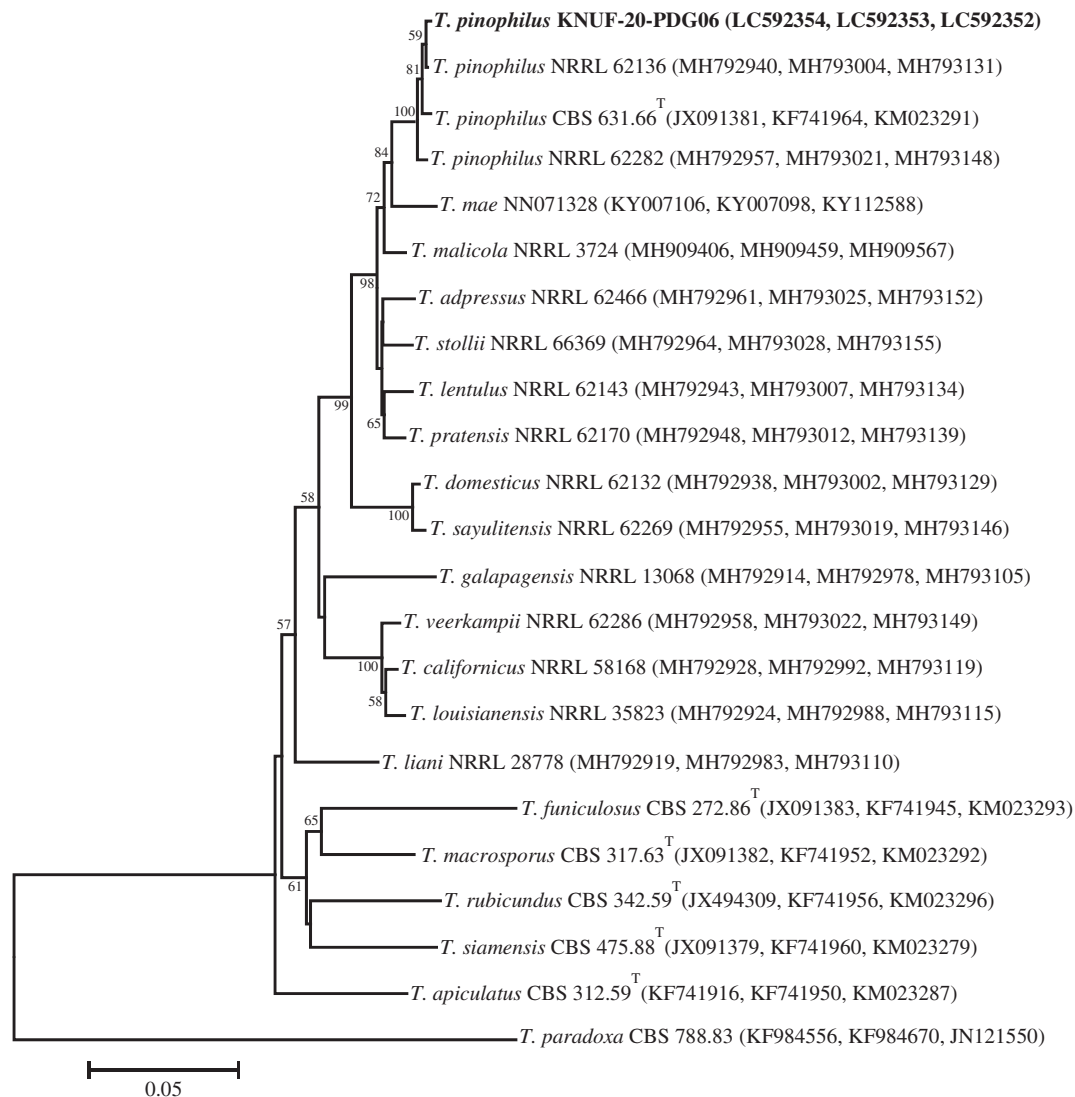
Figure 6. Neighbor-joining phylogenetic tree based on the concatenated sequences of *β-TUB*, *CAL*, and *RPB2* genes showing the affiliation of KACC 83035BP with *Talaromyces pinophilus* among the closest *Talaromyces* spp. Accession numbers of *β-TUB*, *CAL*, and *RPB2* sequences are, respectively, shown in parentheses. Bootstrap values (based on 1,000 replications) greater than 50% are shown at the branch points. The tree was rooted using *Trichocoma paradoxa* CBS 788.83 as an outgroup. The isolated strain of this study is indicated in bold. Scale bar = 0.05 substitutions per nucleotide position.

ITS and LSU sequences clearly supported the affiliation of strain KACC 83036BP with *Fusicolla acetilerea.*

A BLAST search of the NCBI database revealed similarities of 100% between the *CAL* sequence of KNUF-20-PPH03 and several strains belonging to *Penicillium samsonianum*, such as AS3.15406 (KJ668583), DTO 327D6 (KT698891), and DTO 187G1 (KT698890). The *RPB2* gene region of strain KNUF-20-PPH03 revealed a similarity of 99.73% with that of *Penicillium samsonianum* AS3.15403 (KT698899) and *P. samsonianum* DTO 327D6 (KT698901). The *β-TUB* gene sequence of KNUF-20-PPH03 also demonstrated 99.29%–99.74% identity to several strains of *P. samsonianum,* including AS3.15406 (KJ668579), PSF373 (MK800137), and SFC100899 (MK682878). The NJ phylogenetic tree (Figure 8) based on the concatenated *β-TUB*, *CAL*, and *RPB2* sequences showed that strain KNUF-20-

PPH03 clustered with representatives of *Penicillium samsonianum* indicating its affiliation with this fungal species.

The sequences of the ITS regions and the *TEF* gene of strain KNUF-20-PDG05 shared 100% identity with those of several strains of *Purpureocillium lilacinum*, including CBS 128764 (MH865073), IFM 64709 (LC317747), KU20018.18 (MT487832), DTO 39B5 (JF896010), and ER148 (KU738451). In addition, based on the *β-TUB* gene sequence, KNUF-20-PDG05 had a 99.67% similarity with *P. lilacinum* CBS 248.36 (JQ965114). The NJ phylogenetic tree (Figure 9) that was constructed using the concatenated ITS and *β-TUB* sequences clearly supported the affiliation of strain KNUF-20-PDG05 with *Purpureocillium lilacinum.*

In this study, 30 fungal strains were selected and tested for their ability to biodegrade PCL and PLA. Among the fungal species belonging to Ascomycota
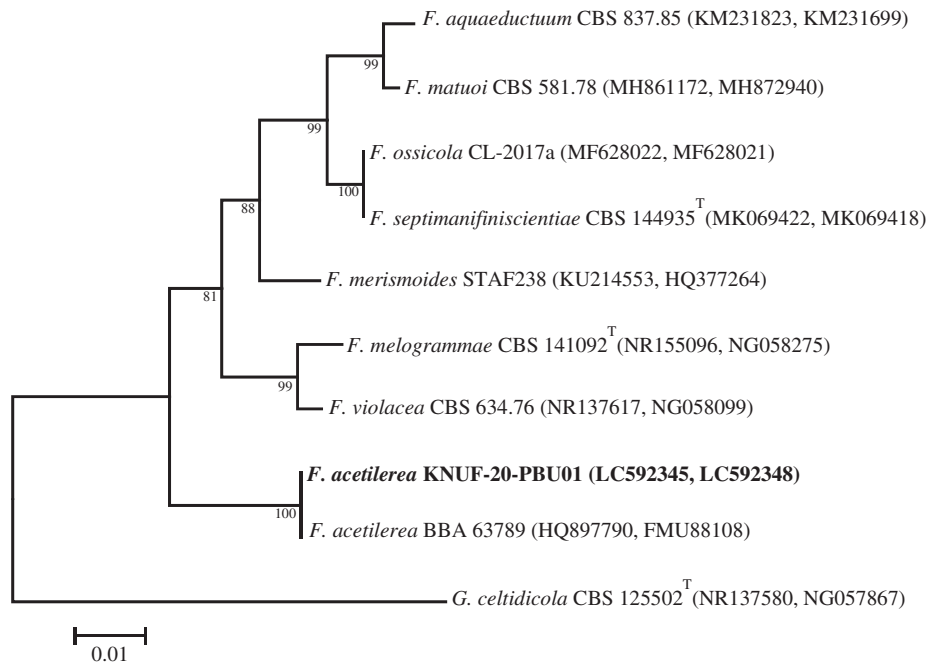
Figure 7. Neighbor-joining phylogenetic tree based on the concatenated sequences of the ITS regions and the LSU gene showing the affiliation of KACC 83036BP with *Fusicolla acetilerea* among the closest *Fusicolla* spp. Accession numbers of ITS and LSU sequences are respectively shown in parentheses. Bootstrap values (based on 1000 replications) greater than 80% are shown at branch points. The tree was rooted using *Geejayessia celtidicola* CBS 125502^T as an outgroup. The isolated strain of this study is indicated in bold. Scale bar = 0.01 substitutions per nucleotide position.
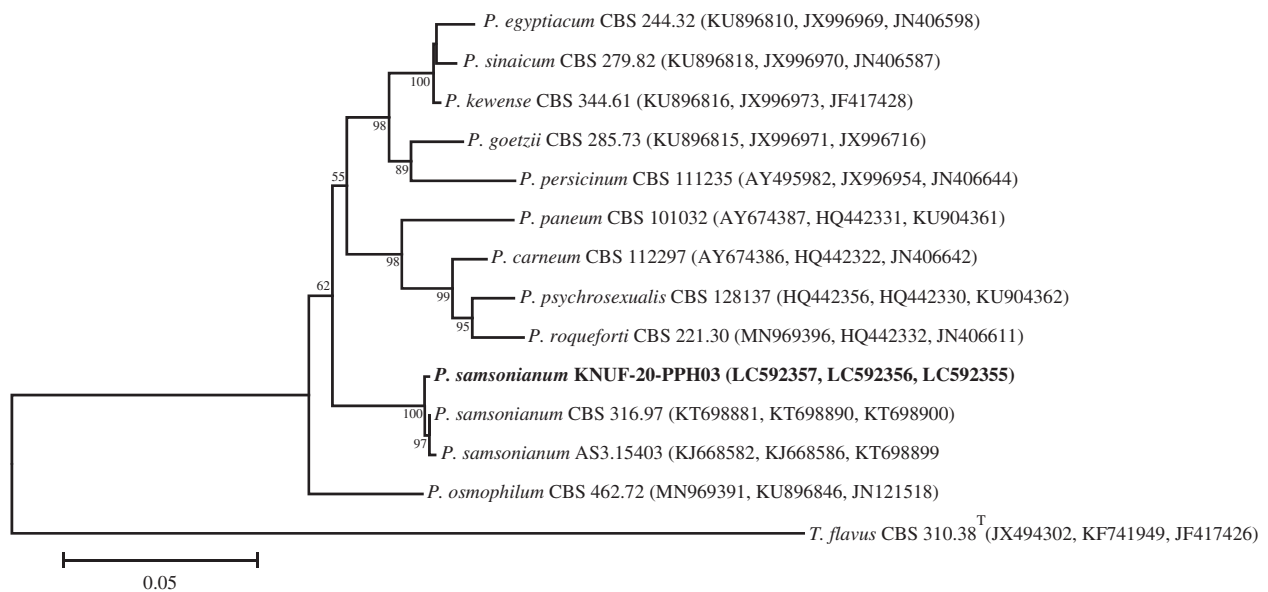

Figure 8. Neighbor-joining phylogenetic tree based on the concatenated sequences of *β-TUB*, *CAL*, and *RPB2* genes showing the affiliation of KNUF-20-PPH03 with *Penicillium samsonianum* among the closest *Penicillium* spp. Accession numbers of *β-TUB*, *CAL*, and *RPB2* sequences, are respectively shown in parentheses. Bootstrap values (based on 1000 replications) greater than 50% are shown at branch points. The tree was rooted using *Talaromyces flavus* CBS 310.38^T as an outgroup. The isolated strain of this study is indicated in bold. Scale bar = 0.05 substitutions per nucleotide position.

that are responsible for plastic's biodegradation, *Aspergillus*, *Fusarium*, and *Penicillium* were mainly encountered [29]. In particular, *Penicillium oxalicum* DSYD05-1 [11], *P. chrysogenum* VKM F-227 [15], and *P. funiculosum* [30] were observed to have the capability to degrade PCL. Based on the above-mentioned results, *P. samsonianum* KNUF-20-PPH03 is

considered to be the novel representative of this genus confirming its ability to degrade PCL. In contrast with the *Penicillium* species, among members of *Apiotrichum*, *Fusicolla*, *Talaromyces*, and *Purpureocillium* species, there are only two representatives, namely *Talaromyces verruculosus* [31] and strain MCM_HumanF8-1, closely related to
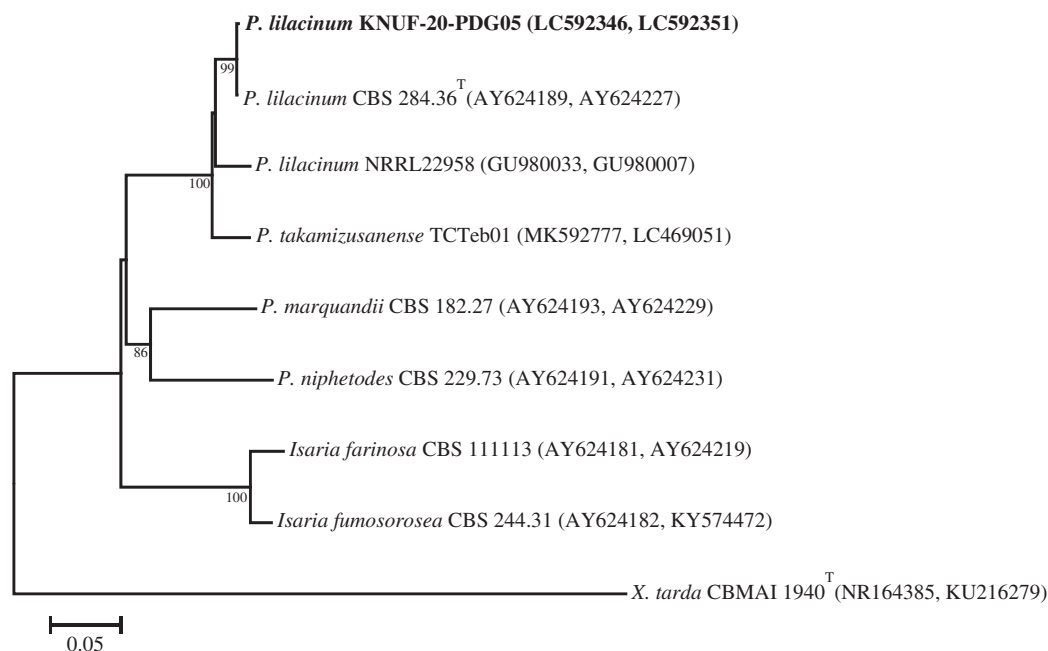
Figure 9. Neighbor-joining phylogenetic tree based on the concatenated sequences of the ITS regions and the β-TUB gene showing the affiliation of KNUF-20-PDG05 with *Purpureocillium lilacinum* among the closest *Purpureocillium* spp. and other closely related taxa. Accession numbers of ITS and β-TUB sequences are respectively shown in parentheses. Bootstrap values (based on 1000 replications) greater than 80% are shown at branch points. The tree was rooted using *Xenopenidiella tarda* CBMAI 1940[T] as an outgroup. The isolated strain of this study is indicated in bold. Scale bar = 0.05 substitutions per nucleotide position.

Purpureocillium lilacinum [32], that were reported to be degraders of polyhydroxybutyrate and poly (butylene succinate), PCL, and PLA, respectively. Our accurate identification of strain KNUF-20-PDG05 as *P. lilacinum* clearly confirmed the ability of this fungal species to biodegrade PLA. Based on our knowledge, this is the first report of polymer biodegraders among known species of the genera *Apiotrichum* and *Fusicolla* and is the first report of *Talaromyces pinophilus* as a plastic-degrading microorganism. Among the studied fungal species, *Fusicolla acetilerea* KACC 83036BP, which degraded both PCL and PLA, has a broader spectrum of degradation activity against biodegradable plastics and is considered to be a potential candidate for the treatment of biodegradable plastic wastes. Further research is needed to understand the mechanism of PCL and PLA biodegradation by the five studied strains at the molecular level.

Disclosure statement

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