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Short communication

Isolation of mesophilic bacterium for biodegradation of polypropylene

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ABSTRACT

A mesophilic bacterium capable of polypropylene (PP) biodegradation was isolated from soil using a culture medium containing low molecular weight polypropylene (LMWPP) as a carbon source. The biodegradation of LMWPP and high molecular weight polypropylene (HMWPP), by the isolated bacterium, was measured under compost conditions. As produced PP has a broad molecular weight distribution so that it contains molecules with a wide spectrum of chain length, ranging from extremely low molecular weight to very high molecular weight. As the extremely low molecular weight fraction of PP may produce misleading results on biodegradability of PP, Soxhlet extraction using toluene was performed to obtain HMWPP, by dissolving out the extremely low molecular weight fractions from PP as produced from a commercial scale polymerization reactor. Previous research on PP biodegradation used PP compounded with pro-oxidants or with natural polymers, such as starch, or pretreated commercial-grade PP containing various additives, such as heat and light stabilizers. In contrast, the present study employed PP without any additives, to examine the true biodegradability of neat PP, excluding any effects of the additives.

1. Introduction

Polypropylene (PP) is being used for various applications, ranging from single use disposables to long lasting durables, owing to its excellent mechanical properties and good processability, together with price competitiveness. Its weak impact strength, especially at low temperatures, has been improved greatly due to its favorable compatibility with polyolefin rubbers, widening its application field (Arutchelvi et al., 2008; Jeyakumar et al., 2013). PP has a melting point around 160 °C, which makes it possible to sterilize perishable ingredients after packing, thereby, dramatically extending the shelf life of the contents. Another advantage is that the freshness of the packed contents can be monitored, because the transparency of PP sheet has been increased significantly by controlling processing conditions. Accordingly, the amount of PP waste is increasing, as it is being used as various disposable packing materials.

PP, just like polyethylene (PE), consists of hydrocarbons and its hydrophobicity is very high. Apart from this, it has no available functional groups for hydrolysis or for microbes to attack on, hence, its degradation in natural environment is extremely slow (Sepperumal and Markandan, 2014). In addition, biodegradation of isoparaffin having side chains is more difficult than that of linear n-paraffin (Atlas and Bartha, 1972). Therefore, PP, with methyl side chains on every repeating unit, is expected to undergo a more difficult biodegradation process than PE (Arkatkar et al., 2009).

Some research attempted to increase the rate of biodegradation of PP by making it easier for microbes to form biofilms on its surface by adding biodegradable natural polymers, such as starch or natural fibers. However, its efficacy proved to be very limited. There have been numerous research aiming at increasing biodegradability of PP by creating polar groups, such as carbonyl, carboxy, and ester groups, through addition of pro-oxidants or by UV irradiation. The degree of PP biodegradation by microbes is examined by either observing the change in the morphology, measuring the mass reduction (Kaczmarek et al., 2005; Khoramnejadian, 2013; Fontanella et al., 2013; Sepperumal and Markandan, 2014) or monitoring decrease in the molecular weight and tensile strength (Gu, 2003).

Microbes, capable of biodegradation of neat PP as the sole carbon source, have never been reported yet. However, Iwamoto and Tokiwa (1994) reported that *Rhizopus arrhizus* can biodegrade PP/polycaprolactone (PCL) blend, and Khoramnejadian (2013) determined that PP/starch blend was biodegraded by *Pseudomonas aeruginosa*. In addition, Sepperumal and Markandan (2014) isolated *Actinomycetes* sp. and *Pseudomonas* sp. that can biodegrade PP

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treated with UV or nitric acid, whereas Pang et al. (2013) buried PP in soil to directly expose it to various types of microbes. Gu et al. (1996) reported microbial degradation of various fiber reinforced plastics.

In the present study, isolation of a mesophilic bacterium that can degrade PP was attempted using soil harvested from open storage yard for municipal solid waste. Identification was performed through analysis of 16S rRNA sequence and the biochemical and morphological properties using API 20E, API 20NE kits and scanning electron microscope (SEM). In addition, the activity of the isolated bacterium was measured toward biodegradation of PP having different molecular weights.

2. Materials and methods

2.1. Polymer characterization

YUHWA that was manufactured in a commercial scale polymerization reactor was used as PP. Low molecular weight polypropylene (LMWPP), LMWPP-1 having number average molecular weight (Mn) and weight average molecular weight (Mw) of 2800 and 10,300, respectively, and LMWPP-2 having Mn and Mw of 3600 and 19,700, respectively, were also used. The LMWPPs were prepared by thermal degradation of a commercial PP under a strict nitrogen atmosphere.

YUHWA includes PP molecules with both high and low molecular weights, as its polydispersity index is 5.7. It is expected that biodegradability of YUHWA relies heavily on the PP fraction with low molecular weights. In this regard, Soxhlet extraction with toluene was applied to YUHWA to examine biodegradability of high molecular weight PP, excluding the impacts of PP fraction with low molecular weights. PP of very low molecular weights is removed as a result of the Soxhlet extraction, and the PP fraction that is not dissolved in hot toluene, remaining in the thimble after the Soxhlet extraction was dried in a vacuum oven at 40 °C, until a constant weight was attained, and was named as high molecular weight polypropylene (HMWPP).

Low molecular weight polyethylene (LMWPE), LMWPE-1 (Mn: 790, Mw: 1700) and LMWPE-2 (Mn: 5,200, Mw: 23,700) were manufactured by thermal degradation of a commercial high density polyethylene (HDPE) and low density polyethylene (LDPE), respectively, under a strict nitrogen atmosphere.

2.2. Culture media

Enrichment medium, used for cultivation of microbes, was prepared according to the method suggested by Kim and Park (2010) (K_2HPO_4, 2.34 g l^{-1}; KH_2PO_4,1.33 g l^{-1}; $MgSO_4 \cdot 7H_2O$, 0.2 g l^{-1}; $(NH_4)_2SO_4$, 1 g l^{-1}; NaCl, 0.5 g l^{-1}; yeast extract, 0.06 g l^{-1}; and trace element solution ($CoCl_2$, 11.9 mg l^{-1}; $NiCl_2$, 11.8 mg l^{-1}; $CrCl_2$, 6.3 mg l^{-1}; $CuSO_4$, 15.7 mg l^{-1}; $FeCl_3$, 0.97 g l^{-1}; $CaCl_2$, 0.78 g l^{-1} and $MnCl_2$, 10.0 mg, l^{-1}), 1 ml pH 7.0).

Agar plate, prepared according to the method suggested by Hadad et al. (2005) after a slight modification, was used to select bacterium that is active toward biodegradation of PP. 0.1 g of detergent (Plysurf A210G) was added to 1L of enrichment medium and mixed using an ultra-sonicator (280 W) for 1 h 40 ml dichloromethane solution containing 1 g of LMWPP-1was added to the enrichment medium and then stirred for 2 h using the same ultra-sonicator. Agar plat was made by adding 15 g of agar to the solution.

2.3. Isolation of PP degrading bacterium

PP degrading bacterium was isolated according to the method suggested by Kim and Park (2010). Soil (10 g) harvested from an open storage yard for municipal solid waste (Anyang-si, Gyeonggi-do, Korea) was mixed with 90 ml of sterilized distilled water for 10 min followed by keeping static for 30 min. Its supernatant was inoculated into 20 ml of enrichment medium containing 0.1 g of LMWPP-1. The mixture was incubated for a week in the shaking incubator, at 37 °C and 120 rpm. After five times of subculture, the culture medium was spread onto the agar plate containing LMWPP-1. After incubation at 37 °C, the bacteria forming clear zone on the plate were collected and used for the microbial selection. Isolation of PP degrading strain was not successful when yeast extract was excluded from the medium, and thereby yeast extract was added to the medium as appeared in the literatures for the isolation of strains degrading hydrophobic polymers such as PE, polystyrene, poly(vinyl chloride) and poly(ethylene terephthalate) (Hadad et al., 2005; Zahra et al., 2010; Patil and Bagde, 2012; Devi et al., 2015; Yoshida et al., 2016; Tang et al., 2017).

2.4. Identification of PP degrading bacterium

After extracting genomic DNA of isolated PP degrading bacterium with DNeasy Blood and Tissue Kit (Qiagen), 16S rRNA analysis was conducted using universal primers 518F (5′-CCAGCAGCCGCGGTAATACG -3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). The 16S rRNA sequence of the isolated bacterium was compared with similar sequences in EzTaxon (http://www.ezbiocloud.net/eztaxon) database using CLUSTAL W (Thompson et al., 1994), and a phylogenetic tree was drawn by neighbor-joining method using MEGA (Kumar et al., 2004).

The cultural and biochemical characteristics of the isolated bacterium were compared to those of the reference strains that had similar 16S rRNA sequence to the isolated bacterium.

The isolated bacterium cultured in Luria Bertani (LB; Difco) agar was incubated at different temperatures (25, 37, 42, and 45 °C) for 5 days. Its growth behavior was assessed at various pH (5, 6, 7, 8, 9, 10 and 11) in LB broth at 30 °C and its biochemical characteristics were examined using API 20E and API 20NE kits (bioMerieux, France). The morphology of the isolated strain was observed using SEM (JEOL, Tokyo, Japan, Model JSM-5600LV).

2.5. Biodegradation of PP and PE by isolated bacterium

Biodegradability of PP and PE was examined according to KS M3100-1:2002; MOD ISO 14855:1999, under compost condition. Compost was sterilized in an autoclave at 121 °C for 30 min, and dried in a dry oven at 100 °C for 24 h. Two hundred gram (wet weight) of sterilized compost was blended with 3.5 g of PP or PE powder. The isolated bacterium was incubated in nutrient broth (Difco) at 37 °C and 120 rpm overnight. The culture broth of the isolated bacterium was centrifuged at 3700 rpm for 20 min and the pellet was washed with sterilized D.W. after removing the supernatant. This process was repeated twice. Sterilized D.W. was added to the pellet of the isolated bacterium until the optical density (O.D.) reached about 0.7–0.8 at 600 nm. Thus prepared inoculum (20 ml) was inoculated into the compost.

The biodegradability tests were performed at 37 °C for 90 days. The amount of CO_2 released from the compost was measured by absorbing the effluent gas through solution of 0.4 N KOH and 2 N $BaCl_2$ and by titration with 0.2 N HCl solution. The air flow rate into the compost was kept at 40 ml min^{-1} and the moisture content of the compost was maintained at 65%.

2.6. PP sortation

The compost/PP mixture was suspended in water and the