Isolation of a *Nocardiopsis chromatogenes* strain that degrades PLA (polylactic acid) in pig waste-based compost

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Abstract

A new *Nocardiopsis* species that degrades polylactic acid (PLA) was isolated from pig dung-based compost from a municipal composting facility in Japan. To obtain strains capable of efficient PLA degradation, the effect of non-enzymatic degradation of PLA was minimized by maintaining the temperature at or below 37 °C. Screening 15 animal waste-based compost samples, consisting of pig dung, cow dung, horse dung, or chicken droppings, revealed that compost derived from pig dung was most efficient for degradation of PLA films. Hence, pig waste-based compost was used to isolate PLA-degrading microorganisms by screening for PLA-degrading microorganisms in compost using an agar plate-based method in which an emulsifier was omitted to avoid selecting strains that assimilated the emulsifier instead of PLA in the medium. Repeated enrichment obtained six strains. The one that exhibited stable PLA degradation on agar plates was subjected to genomic analysis and identified as *Nocardiopsis chromatogenes*, an actinomycete.

Keywords Polylactic acid · Compostability · Biodegradability · Actinomycete · *Nocardiopsis chromatogenes* · Circular economy

Introduction

Plastic products contribute to many aspects of daily life, and the global community is harnessing their benefits. However, the increased use has led to four major social issues: resource depletion, increased greenhouse gas (GHG) emissions, inadequate treatment after use, and environmental pollution. To realize a resilient and sustainable economy, bioplastics are expected to play an important role from two perspectives (Kawashima et al. 2019). First, bio-based plastics help reduce GHG emissions and secure fossil resources. Second, biodegradable or compostable plastics support appropriate and efficient after-use treatment in compost or a specific open environment (Kawashima 2021).

Polylactic acid (PLA) exhibits both characteristics and has recently attracted renewed attention. PLA is commercially used in packaging, containers, agriculture, hygiene, and durables (Kawashima et al. 2004; Gruber 2005; Iwata 2015; Castro-Aquirre et al. 2016). A pilot-scale composting study of PLA products confirmed that there were no adverse effects on the composting process or plant growth cultivated using the obtained compost (Kawashima et al. 2021).

Previous studies have discussed the degradation of PLA in compost (Kawashima et al. 2004; Castro-Aquirre et al. 2016; Karamanlioglu and Robson 2013). Generally, degradation occurs in two phases. PLA is initially disintegrated and fragmented via chemical or enzymatic hydrolysis. Then, the fragmented PLA is further hydrolyzed and digested by microorganisms. Some studies, however, have reported that PLA degradation is mostly performed chemically, with a limited contribution from microorganisms (Husárová et al. 2014). A previous study (Agarwal et al. 1998) concluded that degradation depended on temperature, not the presence of microbes.

To construct a feasible framework for PLA waste management, further studies on the factors and mechanism influencing biodegradability are necessary. The factors to consider include the types of compost, microorganisms, the...
enzymes involved in degradation, the physical properties of PLA, and the treatment conditions such as temperature, pH, and time. The composition of the compost depends on the types of the waste and the composting facility. Furthermore, even at the same composting facility, the degradability of PLA changes if the composting conditions, including the microbiota, are different.

Pranamuda et al. (1997) isolated and identified *Amycolatopsis*, a genus of PLA-degrading actinomycetes. They subsequently isolated various PLA-degrading microorganisms from environmental sources, soil, and compost. Some of these microorganisms belong to the genera of *Rhizobium, Bacillus*, and *Tuberibacillus* (Sangwan and Wu 2008). Others belong to the family *Pseudonocardia*ceae and related genera such as *Amycolatopsis, Lentzea, Kibdelosporangium, Streptoalloteichus*, and *Saccharothrix* (Tokiwa and Calabia 2006). These discoveries have been subsequently reviewed (Pathak and Navneet 2017; Butbunchu and Pathom-Aree 2019).

A study attempted to improve the efficiency of PLA degradation in the compost by spraying a mixture of potent PLA-degrading bacterial strains in 2016. In these experiments, a mixture of four strains, classified as *Penicillium chrysogenum, Cladosporium sphaerospermum, Seratia marcescens*, and *Rhodotorula mucilaginosa*, was sprayed onto compost made of vegetable waste, wood chips, and fruit peels. Adding a bacterial cocktail facilitated PLA degradation, demonstrating the importance of microorganisms in PLA degradation during composting (Nair et al. 2016).

Microbial proteases and lipases are the enzymes that degrade PLA (Panyachanakul et al. 2019). These enzymes have subsequently been characterized (Nakamura et al. 2001; Sukkhum et al. 2009; Hanphakphoom et al. 2014; Lomthong et al. 2015). Proteases catalyze proteolysis by breaking proteins into smaller polypeptides or single amino acids. Some even degrade PLA because they recognize the α-ester bond of PLA (Pranamuda et al. 1997; Nakamura et al. 2001; Jarerrat et al. 2004; Lim et al. 2005). Tokiwa and Calabia (2006) found that protease degrades PLA and is secreted by actinomycetes such as *Amycolatopsis* sp., *Lentzea waywayandensis, Kibdelosporangium aridum* and a fungus, *Tririchium album*. Moreover, lipases, which are a family of enzymes, catalyze the hydrolysis of fats by cleaving the ester bonds in a polymer substrate such as PLA. Lipase is secreted by *Bacillus sinithii* and *Cryptococcus* species (Tokiwa and Calabia 2006). Some studies on the enzymatic degradation of PLA have focused on the biological recycling of PLA products after use. Jarerrat et al. (2006) reported the production of a PLA-degrading enzyme by *Amycolatopsis orientalis*.

This study investigated the compost composition of 15 different animal waste-based composts. These consisted of pig dung, cow dung, horse dung, or chicken droppings together with organic house wastes. Then, efficient PLA degradation was screened at a relatively low temperature (e.g., 37 °C). On-Farm Composting Methods, which contains guidance published by the Food and Agriculture Organization, states that in the aerobic composting process, microorganisms decompose organic matter to generate heat, which accelerates the breakdown of proteins (Misra et al. 2003). The resulting ammonia accelerates hydrolysis of PLA at higher temperatures. To focus on the isolation of microorganisms capable of degrading PLA, the temperature was regulated at 37 °C to avoid promoting chemical decomposition. Emulsifiers such as those developed for PLA by Pranamuda et al. (1997) are often used to isolate PLA-degrading microorganisms. In the current study, emulsifiers are removed afterwards to avoid the possibility of selecting microorganisms that assimilate these compounds. In this study, a strain of a microorganism was isolated from the most efficient pig-dung compost, which was 99.2% homologous with *N. chromatogenes* in 16S rRNA gene analysis. Furthermore, our strain exhibited physiological characteristics of *Nocardiopsis* species, which was reported previously (Li et al. 2006). This is the first study focusing on screening animal waste-based composts from farms and facilities, leading to the isolation of a strain of *Nocardiopsis chromatogenes*.

**Materials**

**Compost**

Fifteen different types of matured compost were collected. They originated from nine operating municipal composting facilities in Japan. Among them, eight were based on pig dung, four on cow dung, one on horse dung, one on chicken droppings, and one on a mixture of cow dung and chicken droppings along with organic house wastes.

**PLA**

A PLA cast film was used in the compost screening test. The film measured 3 × 5 cm with a thickness of 20–30 µm. The film was produced by laboratory-scale T-die extrusion using the PLA resin LACEA™ (Mitsui Chemicals). LACEA™ is a BPS-certified Green Plastic (PL#40701). The PLA resin in our study was derived from our pilot facility using a direct polymerization process of lactic acid (Kawashima et al. 2004; Ajioka et al. 1995) with a Mw of 134,000, Mw/Mn = 3.0, D ratio of 1.2%, and residual lactide > 0.01%. It showed Tm:165 °C and Tg:58 °C. These properties are very similar to commercially available PLA produced by Nature-Works (Plymouth, MN, USA). PLA powder (40–50 µm radius) was used to prepare PLA microspheres (1–5 µm radius).
Reagents

Plysurf A 210G, which was produced by Dai-ichi Kogyo Seiyaku or DKS (Kyoto, Japan), was used as an emulsifier to disperse PLA microspheres.

Methods

Screening of composts for PLA film degradation (primary screening)

Fifteen composts from nine municipal composting facilities were placed in separate jars with PLA films. The jars, which had a dimension of 10 × 10 × 11.5 cm³ and contained PLA films, were placed in an incubator at 37 °C for three months to suppress non-enzymatic degradation of PLA. Two-thirds of the 5-cm PLA film was inserted vertically into the jar with PLA. The jars, which were placed in an incubator at 37 °C for three months to suppress non-enzymatic degradation of PLA. Two-thirds of the 5-cm PLA film was inserted vertically into the jar with the compost (Fig. S1).

Further evaluation of selected composts and collection of microorganisms (secondary screening)

One compost selected from the primary screen was subject to PLA film degradation at 37 °C for 6 months. During the test period, the sample was occasionally sprayed with water to avoid drying.

Single-colony isolation of PLA-degrading microorganisms on agar plates

Strain screening used a glycerol/asparagine-based medium, which was previously reported (Nishida and Tokiwa 1993). Two-layer agar plates were prepared. The bottom layer was prepared using a glycerol/asparagine-based medium with a composition of 0.5 g/L glycerol, 0.5 g/L l-asparagine, 0.5 g/L K₂HPO₄, 0.5 g/L MgSO₄·7H₂O, 0.5 g/L NaCl, 1 mg/mL FeSO₄·7H₂O, 1 mg/mL MnCl₂·4H₂O, and 1 mg/mL ZnSO₄·7H₂O at a pH of 7.0. To the medium was added 20 g of Bacto agar. The resultant mixture was autoclaved and resuspended in TE buffer (TE: Tris and EDTA, Tris: 2-Amino-2-(hydroxymethyl) propane-1,3-diol; EDTA: 2,2′,2‴-(ethane-1,2-diylidinitrilo) tetraacetic acid) to extract the total genomic DNA. The cells were disrupted by a vortex with glass beads. Then a clear lysate was prepared via centrifugation (7000g) to remove the debris. The lysate was subsequently subject to PCR (polymerase chain reaction) amplification using the primers 27f (5′-GAGT TTGAGTCTGCTCAG-3′) and 1525r (5′-AGAGGTGATCCAGCC-3′). The portion coded for 16S rRNA was obtained with an initial denaturation at 95 °C for 5 min and 30 cycles at 95 °C for 1 min, annealing at 54 °C for 1 min, a primer extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The PCR product was purified using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The 16S rRNA gene sequences were obtained using the dRhodamine Dye Terminator Cycle Sequencing Kit on an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA) with Genetyx software. The 16S rRNA gene sequences were manually assembled from a combination of separate fragments generated with the following primers: 27f (5′-GAGTTTAGATCTGCTCAG-3′), 357f (5′-CTCCTGCGGGAGGCCAGCAG-3′), 536f (5′-CCACGACGCGCCGTTATAC-3′), 803f (5′-GATTAGATACCTGGTGATAG-3′), 1114f (5′-GCAACGAGCGCAACC-3′), 1525r (5′-GTATTACCCGCGCTGTGTGCTGCGC-3′), 907r (5′-CCGTCAATTCCTTGAGTTT-3′), 1385r (5′-CGGTTGTGACCCAGGCC-3′), and 1525r (5′-AGAGGTGATCCAGCC-3′). The complete sequence was identified using the BLAST (Basic Local Alignment Tool) program (NCBI; http://www.ncbi.nlm.nih.gov/) and aligned against reference strain sequences. Phylogenetic analysis was
performed using the neighbor-joining algorithm of MEGA5 software (Tamura et al. 2011) with bootstrap analysis based on 1000 re-samplings of the dataset. The obtained sequence data was submitted to the DNA Data Bank of Japan (DDBJ) with the accession number LC648363.

**Results and discussion**

**Screening of composts for PLA film degradation (primary screening)**

The films were inspected visually every 2 weeks during the screening for PLA film degradation in the 15 composts. The pig dung-based composts No. 2, 3, 9, 12, 13, and 15 degraded the PLA film, whereas two composts of the same type, No. 11 and 14, did not. The cow dung-based composts (No. 1, 5, 7, 8), the horse dung-based compost (No. 4), the chicken dropping-based compost (No. 6), and the mixture (No. 10) did not degrade PLA film. The PLA film degraded in six composts derived from pig dung after three months of composting in an incubator at 37 °C (Tables 1, 2).

Since the temperature was controlled at 37 °C and ammonia generation by the protein decomposition was suppressed, the hydrolysis of PLA was not accelerated. It is speculated that degradation of PLA occurs only if the microorganisms to degrade PLA exist in the composts. In this experiment, no microorganisms capable of degrading PLA at 37 °C were present in compost derived from cow dung, chicken droppings, or horse dung, but such organisms were present in compost derived from pig dung.

A previous report examined pig dung- and cow dung-based composts for degradation of food wastes (Adegunloye and Adetuyi 2009). That study compared the process of composting between cow dung/food waste and pig manure/food waste. The results revealed that the number of bacteria was about two orders of magnitude higher in the pig dung system than in the cow dung system, and the pH was also lower. This result relates to our assumptions regarding the comparison of cow and pig dung.

| Municipal composting facility\(^a\) in Japan | Main compost component | Degradation\(^b\) |
|-----------------------------------------------|------------------------|-----------------|
| Hokkaido A (Hayakita)                          | #1                     | Cow dung-based  | Y   |
| Hokkaido B (Kita-Hiroshima)                    | #2                     | Pig dung-based  | Y   |
| Aomori                                        | #3                     | Pig dung-based  | Y   |
| Aomori                                        | #4                     | Horse dung-based| N   |
| Yamagata                                      | #5                     | Cow dung-based  | N   |
| Yamagata                                      | #6                     | Chicken dropping-based | N |
| Niigata                                       | #7                     | A cow dung-based| N   |
| Niigata                                       | #8                     | B cow dung-based| N   |
| Niigata                                       | #9                     | Pig dung-based  | Y   |
| Chiba                                         | #10                    | Cow dung- and chicken dropping-based | N |
| Nagano                                        | #11                    | Pig dung-based  | N   |
| Nagano                                        | #12                    | Pig dung-based  | Y   |
| Aiichi                                        | #13                    | Pig dung-based  | Y   |
| Aiichi                                        | #14                    | Pig dung-based  | N   |
| Kagoshima                                     | #15                    | Pig dung-based  | Y   |

\(^a\)Composting facilities provided the compost at the authors’ request

\(^b\)Y: degraded, N: not degraded

| Main compost component                  | Number of composting facilities | Numbers of compost samples and the ID numbers of samples capable of degrading PLA films |
|-----------------------------------------|---------------------------------|----------------------------------------------------------------------------------------|
| Pig dung                                | 8                               | 6 (#2, #3, #9, #12, #13, #15)                                                             |
| Cow dung                                | 4                               | 0                                                                                       |
| Chicken droppings                       | 1                               | 0                                                                                       |
| Horse dung                              | 1                               | 0                                                                                       |
| Cow dung and chicken droppings          | 1                               | 0                                                                                       |
The standard test method for evaluating biodegradable plastic with compost (ISO14855) is performed at 58 ± 2 °C. Many studies have evaluated the degradability of PLA products in compost on pilot or commercial scales. Examples include compost with fiber, fat, and protein in animal fodder at 58 °C (Yang et al. 2005); compost with cow manure and wood waste at 60–65 °C (Kale et al. 2006, 2007); and compost with green yard waste as a main component around 60 °C (Green 2007; Zhang et al. 2017; Sintim et al. 2019). These studies employed temperatures of 58 °C or higher. In addition, a pilot-scale PLA degradation test employed compost containing horse manure and plants (Kawashima et al. 2021). Because the sample was fresh and had a total weight of 110 kg, the internal temperature was 70 °C or higher at the beginning of the composting process. Around 10 days, the pH exceeded 9.0 as the ammonia concentration increased. Degradation proceeded because hydrolysis was promoted at the early stage. Controlling the temperature to avoid hydrolysis was critical to identify microorganisms capable of degrading PLA.

The compost derived from pig dung (No. 2) provided by a municipal composting facility in Kita-hiroshima, Hokkaido, Japan showed the highest PLA degradation activity. This was a mature compost with a pH of 7. Accordingly, pig dung (No. 2) was selected for further study, which involved evaluation of selected composts and collection of microorganisms (secondary screening). The selected compost (No. 2) was subject to further evaluation of PLA degradation. PLA film was placed in the compost, and degradation was monitored. After incubating at 37 °C for six months, films placed in the selected compost (No. 2) were degraded (Fig. 1).

Isolation of the strain

A cluster of microorganisms was observed on the degraded portions of the films. The cluster was collected with an inoculation loop to isolate the strain responsible for the degradation of PLA film. The cluster was streaked on agar film, and clear zones were spotted after 1.5 months. To avoid isolating microorganisms that assimilate and grow on the emulsifier, the emulsifier was removed during plate prep. As described in the “Methods”, after PLA powder was dissolved in CH2Cl2, the emulsifier was added, and the resultant emulsified solution was warmed to evaporate the CH2Cl2. PLA microspheres were collected by repeated filtration and washing to remove the emulsifier. Conversion of PLA powder to microspheres was predicted to increase contact with the microorganisms.

Previous studies have used emulsifiers to generate microspheres (Pranamuda et al. 1997; Jarerat et al. 2006; Nakamura et al. 2001; Nair et al. 2016). However, microspheres can also be generated without emulsifiers. For example, after dissolving PLA pellets in chloroform, methanol (Jarerat et al. 2004; Panyachanakul et al. 2019) or ethanol (Husárová et al. 2014) can be added for homogenization. Then microspheres are obtained via filtration.

To further purify the microorganisms, the clear zone was scraped and streaked onto a new plate. This process was repeated four times. Ultimately, six colonies were isolated that produced a clear zone on the plate. The large halo formed (Fig. S3) suggested that enzymes were secreted. One strain, MT-24107, was selected for further analysis and identification. Phase-contrast microscopy showed
that a halo formed on the edge of the white fungus (Fig. S4). The thread-like spreading pattern is characteristic of actinomycetes.

The reason for using the two-layer screening system in this experiment is as follows. Because it takes about one month to decompose PLA, it is difficult to detect the decomposition halo in a one-layer system in which PLA microspheres are dispersed over the entire agar medium. By adopting a two-layer system, when the upper microspheres decompose and halos are generated, it is easier for light to pass through, and detection is easier. PLA microspheres were employed in our plate screening for PLA-degrading microorganisms in order to shorten the overall time required. Although PLA powder (40–50 µm radius) took four weeks to form a clear zone (i.e., halo), the process took only two weeks when PLA microspheres (1–5 µm radius) were used.

MT-20147 was suspended in medium with the same composition as during agar plate testing after secondary screening, except for the addition of Bacto agar. The suspended liquid was poured onto a PLA film placed in a Petri dish. After incubation for several days, the film surface was washed and observed by electron microscopy. A characteristic degradation path was observed on the film surface, as shown in Fig. 2.

**Identification of the strain**

As a result of physiological characterization shown in Table 3, the strain was presumed to be *Nocardiopsis* and was classified as an actinomycete because of its cell wall and quinone types (Williams et al. 1989). However, spore formation was not observed, and the genus could not be confirmed from this result alone. Colonies of isolated strain MT-20147 were scraped and collected. The harvested cells were physically disrupted by glass beads and the genomic DNA was extracted.

Sequencing started with the primer 27f. Using the resultant sequence data, the new primers 519r, 357f, and 536f were designed. Sequence analysis was performed for the region read by these primers, and subsequent primers were designed in the same manner to cover a 1489-bp fragment in the 16S rRNA gene by assembling contigs. The sequence of the assembled contigs was analyzed using BLAST. The

| Items                                | Results                  |
|--------------------------------------|--------------------------|
| Cell wall composition                | Type III                 |
| Quinone system                       | MK-10 (H₆), -10 (H₈)    |
| Substrate mycelium                   | +                        |
| Aerial mycelium                      | +                        |
| Colony color                         | Grayish orange           |
| Production of water-soluble pigments | + Brownish orange        |
| Nitrate reduction                    | +                        |
| Growth at 30 °C/40 °C/50 °C          | +/-                      |
| Carbon source utilization            |                          |
| Aesculin                             | +                        |
| Casein                               | +                        |
| DNA                                  | –                        |
| Gelatin                              | –                        |
| Guanine                              | –                        |
| Hypoxanthine                         | –                        |
| Starch                               | +                        |
| Testosterone                         | –                        |
| Tyrosine                             | –                        |
| Xanthine                             | –                        |
| DNA GC content (mol%)                | 73                       |

*a*According to the HPLC method.

![Fig. 2](image-url) Photo of PLA film degradation by suspended media of strain MT-20147. Surface of PLA film before suspension in the media of strain MT-20147. Surface of PLA film after suspension in the media of strain MT-20147.
contigs showed a similarity with Nocardiopsis chromatogenes (99.2%), N. baichengensis (98.8%), and N. halophila (98.7%). A phylogenetic tree was created with the MEGA5 software as shown in Fig. 3.

**Isolation of N. chromatogenes**

This study isolated and identified PLA-degrading microorganisms using animal-based composts (pig, cow, horse, and chicken). Multiple reports have described the isolation of PLA-degrading microorganisms, but to date no study has identified N. chromatogenes as a PLA-degrading microbe. In the 1990s, actinomycetes isolated from the natural environment (e.g., soil) were identified as PLA-degrading microorganisms, and multiple actinomycetes have been described not only in soil but also in compost. Furthermore, Bacillus subtilis and fungi capable of degrading PLA have also been reported.

PLA-degrading microorganisms have been reviewed based on the classification of the cited references. The species identified in this study has yet to be reported. Tokiwa et al. (1999) isolated and identified Amycolatopsis, a genus of actinomycete whose members are capable of degrading PLA, from 45 types of soil samples in Tsukuba City, Japan (Pramanuda et al. 1997). This bacterium also degraded silk (Tokiwa et al. 1999). Using actinomycetes obtained from public institutions, Kibdelosporangium aridum (Jarerat et al. 2003) and Saccharothrix waywayandensis (Jarerat and Tokiwa 2003) degraded PLA, and Amycolatopsis orientalis produced PLA-degrading enzymes (Jarerat et al. 2006). Furthermore, Tokiwa and Jarerat (2004) reviewed the importance of actinomycetes as PLA-degrading microorganisms in conjunction with active enzymes. The same type of microorganism, Amycolatopsis sp., was isolated from 300 soil samples (Nakamura et al. 2001).

In addition to actinomycetes, Laceyella sacchari isolated from forest soil (Hanphakphoom et al. 2014) and strains of Pseudomonas and Bacillus, both isolated from sludge (Kim et al. 2017), were capable of PLA degradation. One study showed that three out of four microbes isolated from 300 soil samples from various sources were fungi: Penicillium chrysogenum sp., Cladosporium sphaerospermum sp., and Rhodotorula mucilaginosa sp.; the exception was Serratia marcescens (Nair et al. 2016).

PLA-degrading microorganisms in compost are diverse. The raw materials used in the compost test for isolation and identification of PLA-degrading microorganisms are mainly animal feed, food waste, and plant residue, and animal manure has been used in only a few cases. Bacillus smithii of the order Bacillales was obtained from a garbage fermenter, and the PLA-degrading enzyme was identified as a serine protease (Sakai et al. 2001). Bacillus licheniformis of the order Bacillales was isolated from compost made from animal fodder and identified as a PLA-degrading microorganism (Kim et al. 2008).

Microorganisms that form biofilms were shown to degrade PLA in compost (Walczak et al. 2015). These organisms were of the genera Acidovorax, Aeromonas, Arthrobacter, and Chryseobacterium. All four are Bacteria, and Arthrobacter is a type of actinomycete. Thermopolyspora flexuosa, another actinomycete, was identified from lab-scale compost (Husárová et al. 2014). As reviewed above, it is clear that PLA-degrading microorganisms range from prokaryotes such as actinomycetes to eukaryotes such as fungi.

Butbunchu and Pathom-Aree (2019) published a comprehensive review on PLA-degrading microorganisms. That paper described five families, eleven genera, and twenty-five species for which enzyme classification was also available. Furthermore, a unique review article on microorganisms, which degrade fossil-based plastics and biodegradable plastics, including PLA, was published (Pathak and Navneet 2017).

In the long history of research on PLA-degrading microorganisms, Nocardiopsis chromatogenes has not been identified. Recent studies have examined microorganisms that degrade PET have been conducted. Among them, Nocardiopsis chromatogenes was shown to be a PET-degrading microorganism.
species (Joo et al. 2018). Although PET is aromatic and PLA is aliphatic, and the two compounds have different chemical structures, it is noteworthy that microorganisms from the same genus are involved in the degradation of both polyesters.

As previously described, the PLA degradation mechanism consists of hydrolysis, enzymatic degradation, microbial degradation, and combinations thereof. However, the enzymes secreted from the isolated microorganism in this study, Nocardiopsis chromatogenes, have yet to be identified. A future direction includes identifying and evaluating its degradation mechanism. To carry out the degradation in compost more efficiently and economically, future work should elucidate the mechanisms, microbes, and enzymes secreted during the process of degradation.

Conclusions

This study employed two distinct approaches to isolate PLA-specific degrading microorganisms. The first approach attempted to eliminate PLA degradation via hydrolysis. Specifically, the compost temperature, which was derived from various types of livestock dung, was kept below 37 °C because ammonia generation or a higher pH due to proteolysis at high temperatures may promote hydrolysis. Suppressing the temperature inhibited hydrolysis in the initial stage of degradation. Consequently, non-enzymatic degradation was avoided. Furthermore, to avoid the possibility of selecting microbes that assimilate the emulsifier, it was removed after the screening process.

As a result, a microorganism capable of degrading PLA was isolated from pig dung-based compost. Based on the properties of the microorganism and a genetic analysis, it was identified as Nocardiopsis chromatogenes, an actinomycete of order Streptosporangiales. This is the first time that N. chromatogenes has been shown to degrade PLA. Future studies include characterizing the PLA degradation, identifying the enzymes involved, and elucidating the degradation mechanism.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03144-w.

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Conclusions

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