Poly(lactide) Degradation By *Pseudonocardia alni* AS4.1531<sup>T</sup>
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ABSTRACT
Twenty actinobacterial strains belong to the genus *Pseudonocardia* were screened for their ability to degrade poly(lactide) plastic. *Pseudonocardia alni* AS4.1531<sup>T</sup> was the only strain that decreased PLA film weight by more than 70% in eight days. This strain could degrade 35.8 mg out of 50 mg PLA films in liquid culture containing 0.1% (w/v) gelatin. In addition, *Pseudonocardia alni* AS4.1531<sup>T</sup> assimilated the major degradation product, lactic acid.

Keywords: Poly(lactide), Degradation, *Pseudonocardia alni* AS4.1531<sup>T</sup>.

1. INTRODUCTION
Plastic wastes are currently a serious environmental problem of concern. Bioplastics have been considered as a solution to this problem. Several kinds such as poly(e-caprolactone)(PCL), poly(tetramethylene succinate)(PTMS), poly(l-hydroxybutyrate)(PHB) and poly(lactide)(PLA) are now commercially available with potential applications as food containers, foamed sheets and textiles [1,2]. PLA, an aliphatic polyester and biocompatible thermoplastic is currently the most promising biodegradable material since it can be produced from renewable resource such as starch, corn, cassava, through fermentation process [3]. PLA is being used in packaging material, films, fiber and non-woven fabrics [4].

Many soil microorganisms were able to degrade PLA plastics such as fungi *Trichirachium album* [5], *Fusarium moniliforme* and *Penicillium roqueforti* [6] or bacteria such as *Bacillus smithii* [7], *Bacillus brevis* [8], and *Paenibacillus amylyolyticus* [2]. Members of actinobacteria are also known to degrade plastics and polyester [9]. *Amycolatopsis* strain HT-32 was the first isolated PLA-degrading microorganism [10].
PLA-degrading actinobacteria were reported to belong phylogenetically to the *Pseudonocardiaeae* family and related genera, including *Amycolatopsis*, *Saccharothrix*, *Lentzea*, *Streptoalloteichus* and *Kibdelosporangiium* [11]. *Pseudonocardia* is the type genus of the family *Pseudonocardiaeae* which accommodates 25 genera [12]. However, no information is available for PLA degradation by *Pseudonocardia*.

In this study, the degradation of PLA film in liquid culture by selected actinobacteria in the genus *Pseudonocardia* was investigated.

2. MATERIALS AND METHOD

2.1 Materials

Poly(lactide)(PLA),4042D (Mw=74,000) was purchased from NatureWorks® LLC (U.S.). A PLA film was prepared by casting 100 mg of PLA in 10 ml chloroform. The resultant transparent film was dried under vacuum for 2 days at room temperature.

2.2 Microorganisms and Culture Media


2.3 Degradation of PLA films

Degradation of PLA was carried out in duplicates using 250 ml Erlenmeyer flasks containing 50 ml liquid basal medium with 0.1% (w/v) gelatin and 50 mg PLA films. The seed cultures were grown for 8 days in liquid basal medium containing 0.1% (w/v) gelatin. The cell pellets were inoculated at 10% (v/v) into the medium. Two control experiments were carried out: one was a film control, which cells were not inoculated; the other was a culture control in which the film was not added. Flasks were incubated at 30°C with shaking at 180 rpm for 8 days.

2.4 Analytical Methods

The culture broth was taken 10 ml every 2 days for measurement of pH and lactic acid. Lactic acid was measured by titration method with NaOH according to AOAC (1998): Method 936.16 [14]. For dry cell weight, the culture broth was filtered through Whatman No.1 filter paper and dried to constant weight at 105°C.

The residual films were efficiently recovered from culture broth and washed to remove the bacterial cells then dried at room temperature for 2 days and weighed.

For the microscopic observation of the degraded PLA films, the film samples(from Day 4) were directly retrieved from the cultures, washed with distilled water to remove attached cells, and dried for 2 days. The films were coated with gold using a JEOL, JFC-1200 fine coater and observed using a JEOL scanning electron microscope (SEM), Model JSM-5410LV, operating at 15 kV.

3. RESULTS AND DISCUSSION

3.1 PLA Film Degradation by *Pseudonocardia alni* AS4.1531T

Among the 20 strains of *Pseudonocardia* tested only *Pseudonocardia alni* AS4.1531T could...
degrade PLA when grown in liquid basal medium containing 0.1% (w/v) gelatin. This observation suggested that PLA degrading ability was not widely distributed in this genus unlike their taxonomically related taxa *Amycolatopsis*. Several *Amycolatopsis* strains were reported as PLA degrader [1, 10, 11, 15, 16, 17]. However, PLA-degrading microorganisms were reported to be not widely distributed in natural environment [15, 18]. Similar effects of gelatin on the induction of PLA degrading activity were also observed in other actinomycetes, *Saccharothrix wasyuyamensis* [3] and *Kibdelosporangium aridum* [19]. PLA degrading ability was also reported to increase by other proteins e.g., silk fibroin [1].

To study the degradation of PLA and assimilation of PLA by *Pseudonocardia alni* AS4.1531\(^T\), the time course of PLA film degradation was further investigated by culturing this strain in liquid basal medium containing 50 mg PLA film and 0.1% (w/v) gelatin. After 4 days of cultivation, degradation was evident as the film sample was disintegrated with an increase in cell growth (Figure 1). *P. alni* AS4.1531\(^T\) degraded 35.8 mg out of 50 mg film (71.5%) within 8 days. PLA degradation by *P. alni* AS4.1531\(^T\) was faster than previously reported *Amycolatopsis* strains. For instance, *Amycolatopsis* strain HT32 degraded 60% of PLA film within 14 days [10] or *Amycolatopsis* strain No. 3118 degraded 50% of PLA film in 8 days [16]. A significant cell growth as indicated by high dry cell weight (50.5 mg) suggested that *P. alni* AS4.1531\(^T\) could assimilate the degradation products (Figure 1). Most of the PLA-degrading actinomycetes were found to assimilate the degradation products [1, 2]. The degradation products could be used by these strains for their growth and eventually metabolized to CO\(_2\) and H\(_2\)O [15]. The pH increased during 4 days of cultivation, generally due to the ammonium ions formation by microbial metabolisms of gelatin. Thereafter, the amount of lactic acid increased as a result of PLA film degradation and accumulated in the culture broth leading to a decreased pH value (Figure 1).

![Figure 1. PLA degradation by *Pseudonocardia alni* AS4.1531\(^T\) in liquid culture containing 0.1% (w/v) gelatin.](image-url)
3.2 SEM observation of PLA films

SEM was used to observe the changes of the remaining PLA film after 4 days of cultivation with the strain. As shown in Figure 2B, all over the surface of film sample became rough and many irregular holes were observed within the early period of degradation. In contrast, the surface of film remained smooth in the control without inoculation of the tested strain (Figure 2A).

![Figure 2. Scanning electron micrographs of PLA films (magnification x 1,000); (A) film control without inoculation and (B) after cultivation with *Pseudonocardia alni* AS4.1531\(^T\) for 4 days at 30°C (bar = 10 μm).]

4. CONCLUSION

Only 1 of the 17 *Pseudonocardia* reference strains was found to degrade PLA. *Pseudonocardia alni* AS4.1531\(^T\) could degrade 71.5 % of PLA within 8 days in liquid basal medium containing 0.1% (w/v) gelatin. The information obtained from this study provides further evidence that members of the family *Pseudonocardiaceae* are good source for PLA degraders. PLA degradation by *Pseudonocardia alni* AS4.1531\(^T\) under extreme condition such as high salt concentration is under investigation in our laboratory.

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REFERENCES


