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Low-Density Polyethylene (LDPE) Degradation by Malaysian *Rhodococcus spp.* Using Weight Reduction Test

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Abstract: As known, plastic accumulation worldwide is unbearable and becoming a huge environmental threat as they are very hard to be degraded. Low-Density Polyethylene (LDPE) is one of most common material used to produce plastic. This paper presents the investigation of the ability of twenty-three Malaysian *Rhodococcus* isolates in degrading low-density polyethylene (LDPE) through weight reduction test. Shake-flask incubation was performed for the investigation. Through the study, all isolates showed the ability to degrade the LDPE supplemented in the culture media but at different degradation rate. Among the 23 isolates, *Rhodococcus* UCC0018 demonstrated the highest degradation with 8.69 % of LDPE weight reduction. This finding shows the promising potential to explore this bacteria isolate as an agent for plastic waste biological treatment.

Keywords : Low-density Polyethylene (LDPE) microbial degradation, Malaysian *Rhodococcus spp.*, fermentation, plastic biodegradation

1. Introduction

Low-Density Polyethylene (LDPE) is a thermoplastic that are commonly used in manufacturing plastics bags, trays containers and plastic wraps. It is a polymer made of long monomers of ethylene (Nanda and Sahu, 2010). Due to its benefits and low production cost, it becomes extremely popular in all the sectors of the industry (Lee et al., 2020; Montazer et al., 2018). The annual production of petroleum-based plastics exceeded 300 million tons in 2015 (Emadian et al., 2017). Malaysians generated 38,142 tonnes of waste daily in 2018. In per capita terms, there was a relatively large increase in daily waste generated per capita in 2018 compared to previous years at 1.18kg (Jasmin and Wong, 2019). Up to 64% of the plastic are castoff within a short period after use, resulting in massive and rapid accumulation in our environment (Harshvardhan and Jha, 2013) and caused an enormous effect in the environment, food safety and quality, human health and contributes to climate change. This is because LDPE is not organic materials, and most bacteria cannot degrade them naturally. Even with the help of UV light from the sun, it will take many years to break it down.

The characteristics of polyethylene such as molecular weight, crystallinity, functional groups, mobility, substituent present in the structure and the additives added to the polymers play a significant role in its degradation (Gu et al., 2000). The common media used during incubation of treatment are M1 (Das & Kumar, 2014), synthetic media (SM) (Hadad et al., 2005) and liquid minimal salt medium (Hadar & Sivan, 2004) with some modifications (Orr, Hadar & Sivan, 2004). There are many ways to degrade LDPE which are chemical degradation, photodegradation and biological degradation (Da et al., 2014). However, biological degradation is the most popular method because it does not cause harm to the environment.

Research on microbial degradation of plastic wastes, especially polyethylene which is highly resistant to biodegradation has been steadily growing (Harshvardhan and Jha, 2013; Muenmee *et al.*, 2015; 2016; Veethavyaa *et al.*, 2016). Some microorganisms have been shown to produce enzymes capable of polyethylene plastic degradation, including molds such as *Aspergillus* and *Fuzarium* (Sahebnazar *et al.*, 2010), yeast such as *Cryptocucus* (Sen & Raut, 2015; Thirunawakaru *et al.*, 2016) and bacteria in the genera *Pseudomonas*, *Bacilius* and *Rhodococcus* (Abrusci *et al.*, 2013; Sawadago *et al.*, 2014; Nanda & Sahu, 2010). Some researchers have been working on improving microbial-degradation of plastics by changing in physical and chemical conditions of microbial growth (Jeon & Kim, 2015; Vimala & Mathew, 2015) while others attempted to enhance degradation overproducing plastic-degrading enzymes in exogenous microorganisms (Jeon & Kim, 2016; Watanabea *et al.*, 2015). Discovering the latent ability of new synthetic-polymer degrading strains from different environments is another important strategy (Sawadago *et al.*, 2014; Sung *et al.*, 2016). The isolation and characterization of novel polyethylene degrading microorganisms can improve and accelerate the slow degradation of plastics that are recalcitrant to biodegradation in landfills.

In 2019, the Institute of Bio-IT Selangor had embarked on a national megaproject in collecting Malaysian *Rhodococcus* strains which resulted in the isolation and identification of 23 Malaysian *Rhodococcus* isolates and they were maintained at the Unisel Culture Collection (Hasdianty *et al.*, 2020). Since *Rhodococcus* has been proven to be able to degrade LDPE, a study was performed to evaluate the ability of local *Rhodococcus* isolates in degrading LDPE. Therefore, this paper presents the findings from the stated study.

2. Materials and Methods

2.1 Bacterial strain and culture conditions

The 23 Malaysian *Rhodococcus* isolates were obtained from Unisel Culture Collection maintained at the Institute of Bio-IT Selangor Laboratory. Nutrient agar (NA) and nutrient broth (NB) media were used to maintain the bacterial cultures. The liquid cultures (25 ml) were incubated in falcon tubes (50 ml) in an incubator shaker (150 rpm) at 30^{o}C for 24 hours before treatment of polyethylene.

2.2 Pre-treatment of Polyethylene

The LDPE were cut into small strips, washed with 70% ethanol, distilled water and air-dried. The strips were grinded with crystalline NaCI by using mortar and pestle until become soft and ruptured strips. The mixture was transferred into a conical flask with the distilled water and mixed well in a shaker for 1 hour. The solution was filtered using Whatman no. 1 filter paper and air-dried.

2.3 Treatment of Polyethylene

The *Rhodococcus* isolates were inoculated individually in 25 ml of nutrient broth in the 50 mL of falcon tubes. The falcon tube containing only the nutrient broth acted as a control. After 24 hours, 1 mL of *Rhodococcus* inoculum was added into the 50 mL falcon tube containing 25 mL of nutrient broth and 0.2 g polyethylene. The treatments were incubated in incubator shaker at 150 rpm, 30^{o}C for 24 hours. The treatments were conducted in triplicates.

2.4 Removal of bacterial biofilm from the polyethylene surface

The polyethylene strips were recovered from nutrient broth after 24 hours by filtering using Whatman No. 41 filter paper. The strips were washed with 2% aqueous sodium dodecyl sulphate (SDS) solution for 3 hours and finally rinsed with distilled water until the bubbles from SDS were gone. The washed polyethylene strips were dried overnight in drying oven at 50^{o}C before weighing. The weight after drying was measured and recorded. The same procedures were repeated for all the 23 *Rhodococcus* isolates.

3. Results and Discussion

Figure 1 shows the percentage of LDPE weight reduction by *Rhodococcus* isolates using the method as suggested by (Nanda & Sahu, 2010). The highest percentage was demonstrated by *Rhodococcus pyridinivorans* strain UCC0018 as registered in Genbank (Hasdianty *et al.*, 2020) with 8.69 % of weight reduction, followed by *Rhodococcus pyridinivorans* strain UCC0017 with 7.86 % in 24 hours of incubation. UCC0021 demonstrated the third highest activity with 7.04 % of weight reduction. While other isolates showed lower degradation activities with weight reduction ranging from 6.57 % (UCC0014) to 0.16 % by UCC001. The degradation activities by all isolates were verified by ANOVA analysis where all the results are found significant (<0.05).

The ability of these Rhodococci in degrading LDPE is due to their capability in producing polyethylene-degrading enzymes such as lipase. A few studies on enzymatic plastic degradation reported lipases as the responsible enzyme for the degradation activity (Danso *et al.*, 2019; Carniel *et al.*, 2017). Screening on the lipase activity by Malaysian *Rhodococcus spp*. by Jayasudha et al. (2014) confirmed the presence of this enzyme in the local strains. (**describe the properties of UCC0018 strain that contribute to the highest degradation activity)

Each microorganism reacts differently towards polyethylene. According to Das & Kumar (2014), *Fusarium* sp. and *Aspergillus* sp. can degrade 9% and 8% polyethylene in 60 days. *Brevibacillus borstelensis* degrade polyethylene 30% higher than *Rhodococcus ruber* (8%), *Rhizopus oryzae* NS 5 (8.4±3%) despise the same incubation period (30 days) (Hadad, Geresh & Sivan, 2005; Orr, Hadar & Sivan, 2004; Awasthi, Srivastava, Singh, Tiwary & Mishra, 2017). Sowmya, Ramalingappa, Krishnappa & Thippeswamy (2014) showed that the pre-treatment of polyethylene affect the degradation by *Penicillium simplicissimum* as the degradation of UV-treated, autoclaved and surface-sterilized polyethylene have different percentage of degradation, 38%, 16% and 7.7% in 90 days.

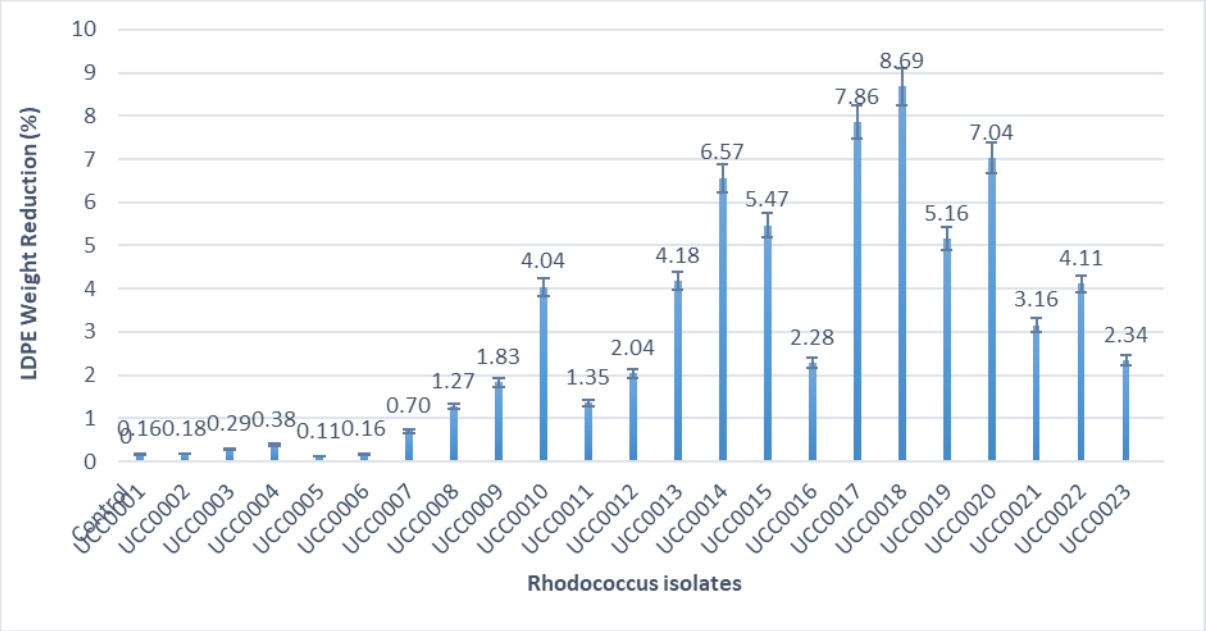

Figure 1 The result of LDPE degradation by 23 Malaysian *Rhodococcus* isolates measured using weight reduction method (Nanda and Sahu, 2010) at 30 °C for 24 hours incubation. The error bars are based on the standard deviation which are <0.05

Other polyethylene degradation studies reported the activity in a few *Pseudomonas* genera demonstrated different rate of degradation; 20% of degradation by *Pseudomonas aeruginosa* (PA01) (B1), 11% by *Pseudomonas aeruginosa* (ATCC) strain (B2), 9% by *Pseudomonas putida* (B3) and 11.3% in *Pseudomonas syringae* (B4) strain in 120 days incubation periods(Kyaw *et al.*, 2012). The mentioned studies were conducted using the similar approach with the current study except for the pre-treatment methods.

(Are these strains were evaluated using the same approach as mentioned in this study?, if possible compare the outcome of previous studies that applied weight reduction technique because different approach would give different outcome)

4. Conclusion

This study has successfully demonstrated the ability of 23 Malaysian *Rhodococcus* isolates obtained from Unisel Culture Collection to degrade low-density polyethylene (LDPE) with 8.69 % of highest degradation activity showed by *Rhodococcus pyridinivorans* strain UCC0018. Eventhough the reported species in the literature exhibited higher degradation percentage than demonstrated by our local *Rhodococcus* isolates, the degradation activity by our isolates occurred at a much shorter incubation period, which is only within 24 hrs. While other strains required at least 30 to 120 days of incubation period to achieve the degradation activity. Hence, this finding shows the promising potential to explore this bacteria strain as an agent for plastic waste biological treatment. In future work, it is suggested to maximize the degradation activity by optimizing a few growth parameters using statistical methods such as Response Surface Methodology (RSM).

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