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PLA Degradation and PLA-Degrading Bacteria: A Mini-Review

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Abstract. Polylactic acid (PLA) is not new to the world of science, since the application of PLA can be found in various industries such as biomedical, agricultural, and packaging. Despite the amazing properties shown by PLA, it still has a setback in terms of waste disposal of PLA. Since PLA is more resistant towards bacterial attack, it prolonged the decomposition of PLA disposed in the environment. Therefore, PLA microbial degradation and enzymatic degradation needs to be highlighted since most PLA waste will end up in the landfill. Most PLA-degrading can be found in the genus family *Amycolatopsis*, and a few can be found in the genus *Lentzea, Kibdelosporangium, Paecilomyces, Thermomonospora*, and *Thermopolyspora*. The enzymatic degradation of PLA is mostly studied relating to enzyme proteinase K, serine protease, and even hydrolase. This review paper aims to discuss the microbial degradation mechanism of PLA as well as the types of microorganisms and enzymes that involve in the biodegradation of PLA.

Introduction

The scarcity of oil and the global population's high reliance on it has heightened the interest and motivation of researchers and industry to look towards bioplastics as an alternative to compensate the shortage of fossil-fuel in the future of plastic industry [1, 2]. The usage of conventional plastics has been in favoured due to their low cost, lightweight, high durability, and ease of processing [3]. This, however, draws several setbacks such as the majority of plastics consists of packaging and single-use plastics with brief usage life before being discarded. A study by Smith et al. [4] found fewer than 20% of plastic trash is currently recycled, with even less being incinerated, while around 70 million tonnes out of 90 million tonnes of plastic manufactured by humans end up in the environment, where they are eventually degrading into microplastics, posing major health risks. In addition to this problem, the uncontrollable release of greenhouse gases such as carbon dioxide (CO_2), catalyze the issue of global warming.[5]. According to the 15th session of the Conferences of Parties (COP 15) meeting hosted in Copenhagen in 2009 [6], due to the 18th century industrial revolution, the threat of global warming had become more alarming when the concentration of CO_2 had increase above 390 ppm while the global temperature had spiked more than 0.9 °C.

Global warming and plastic pollution, in addition to other environmental issues, have led to a significant increase in demand for renewable materials. Therefore, renewable bioplastic has been in the spotlight as a potential replacement for conventional polymers derived from petroleum, in order to reduce the overall CO_2 emissions, due to the raw material for bioplastics ability to absorb CO_2. Moreover, the main purpose that bio-degradable plastics are initially manufactured and developed is to regulate and decrease plastic pollution since bioplastic have the property to decompose much faster than traditional plastics in the environment.

Bioplastics research has increased exponentially with the growing environmental consciousness due to petroleum-derived polymer polluting the earth [7]. Bioplastics, which are created from renewable resources like plants or microbes, are a viable substitute to petroleum polymers. Polylactic acid (PLA) is one of the most commercially successful bioplastics in the world due to its exceptional processability and high mechanical performance. The PLA monomer, lactic acid, is produced by fermentation of starch, sugarcane, or corn, which is an accessible renewable resource. Within 2019, the global PLA manufacturing volume is predicted to be approximately 190,000 tonnes. In terms of mechanical strength, durability, and transparency, PLA outperforms other bio-based plastics in the marketplace. Polylactic acid (PLA) is among the most widely utilized bioplastics due to its excellent renewability potential, which are contributed by the usage of agricultural by-product in its production and decreases the dependency of petroleum-based plastic in the future [8, 9]. Aside from that, in recent decades, PLA has gained remarkable scientific interest due to its compelling characteristics. like outstanding biocompatibility, great gas barrier, UV resistant, and high tensile strength and modulus [10, 11, 12].

The usage of PLA plastic ranges from packaging for common goods with short shelf life, as well as single-use items in the medical, food, and agricultural industry. PLA, on the other hand, typically requires months to optimally breakdown at elevated temperature (between 48°C and 60°C), which are common conditions in industrial composting facilities. In addition, compared to regular thermoplastics, this bioplastic has a low thermal deformation temperature and poor water barrier properties. Despite its promising biodegradation capability, recent research has found a number of issues with PLA's disposable practices and waste management. [13-15].

The extensive research heavily focused on PLA degradations is due to the previous research showing the persistence of PLA in soil at ambient temperature and causes it to accumulate and contaminate the environment [16]. PLA is different from other biodegradable thermoplastics whereby it can degrade in two stages: hydrolytic breakdown followed by microbial predation. Other bioplastics decompose in a single stage. [17]. The examination of methods utilized to decompose PLA has been the topic of many research due to its relevance, and the amount of work published has steadily rises from 2010 to 2019 [18].

Biodegradation and Biodegradability

Two qualities must be addressed when a novel biodegradable polymer is presented to the market: biodegradability and biodegradation [19]. There is a natural anticipation that innovative polymers are made from renewable sources and can degrade as well. Even if this assumption is plausible, there is no assurance that these polymers will decompose completely. Changes in bonding density, copolymerization with non-biodegradable composites, can often result in non-biodegradable materials. Therefore, biodegradability studies are necessary for establishing the long-term ecological effects of bio-based products.

The exposure of PLA to various environmental condition will cause the degradation of the polymer which produce irreversible changes and could lead to property damage. Depending on the surrounding condition of PLA, such as moisture, oxygen, pH and microbial activity, The bioplastics will breakdown into carbon dioxide, water, and a small number of harmless compounds. [20]. However, the rates of biodegradation are strongly influenced by different of variables, including ratio of the isomers, surrounding temperature, pH level of soils, burial period, moisture content, oxygen concentration, and material size. [21, 22].

The process of PLA decomposition can occur through multiple mechanisms, which can cause main and side chain scissions in the bioplastics. PLA degrades via a variety of processes, including hydrolytic, thermal, microbiological, enzymatic, chemical, photodegradation, and oxidative. However, most research are mainly focusing on the enzymatic degradation and microbial degradation of PLA as shown by Zaaba and Jaafar [18]. The high density of research related to microbial and enzymatic degradation is very likely due to the consideration of the waste management facilities of PLA in the future.

Microbial Degradation Mechanism

In the laboratory or in the natural environment, microbial degradation is defined as the microbial conversion of organic molecules to less hazardous or more advantageous [23]. The schematic diagram of PLA-degradation by bacteria are shown in Figure 1 where the microbial degradation begins after a high-molecular wight PLA undergoes hydrolysis. According to Tokiwa and Calabia, [24], the bioplastic degrading bacteria will secrete extracellular depolymerases to decompose the

PLA. This method commonly introduces inducers such as silk fibroin, and other suitable amino acids and peptides [25], to further stimulate the degradation process. Oligomers, dimers, as well as monomers are products formed after a depolymerase targets intramolecular ester linkages in PLA. This enables microbial membranes to penetrate low molecular weight molecules. Intercellular enzymes break down the bioplastic into carbon dioxide, water, or methane consequently [14].

In lieu of all the advantages shown by this bioplastic, there is still opportunity for improvement due to the fact that PLA is more resistant to microbial attack than other bioplastics, resulting in a slower degradation rate in soil. [26]. In addition, PLA-degrading bacteria are not as abundant in the environment as other aliphatic thermoplastic, such as Polyhydroxy butyrate (PHB), Polycaprolactone (PCL), and Polybutylene succinate (PBS) [27]. This concur that PLA is less resistant towards bacterial attack when discarded in the environment compared to other plastic degrading microorganisms [15, 28-29].

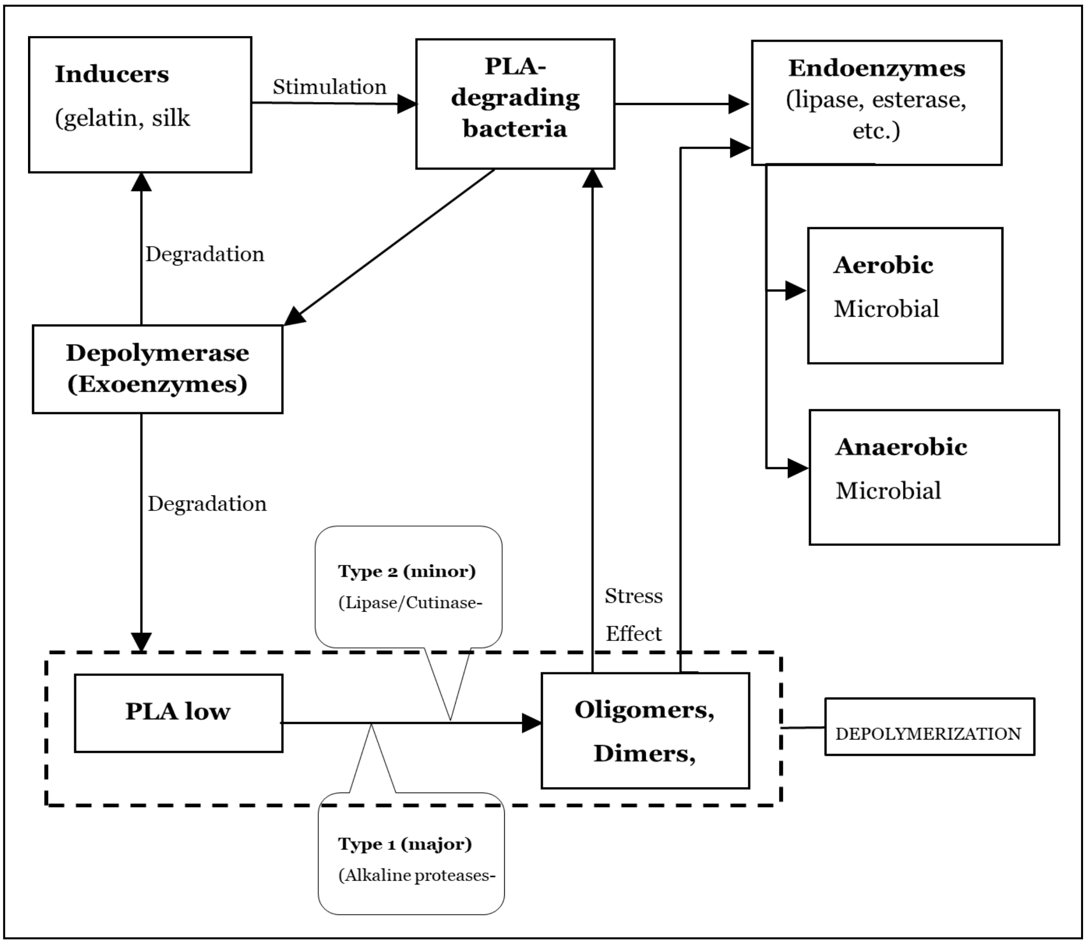

Fig. 1 The schematic diagram of PLA biodegradation process by bacteria

PLA-Degrading Microorganisms

Pranamuda et al. [28] were the first one to discover *Amycolatopsis sp.*, namely *Amycolatopsis sp.* strain HT-32, as PLA-degrading bacteria. Then it was discovered that PLA-degrading activity was prevalent in this genus [30-34]. *Amycolatopsis sp.* strain K104-1 has a PLA-degrading enzyme that has been isolated, purified, and described. The isolated enzyme has a molecular weight of 24kDa and similar characteristics to alkaline serine protease [33].

There are also studies focusing on PLA-degrading capability in other genera besides *Amycolatopsis*. Among them are *Lentzea waywayandensis* and *Kibdelosporangium aridum*, strains from the genera *Lentzea* and *Kibdelosporangium* respectively, are also discovered to have the ability to breakdown PLA. These bacteria can degrade PLA film by over than 90 percent and ingest the breakdown product. [35, 36].

It is expected that members of the genera *Paecilomyces, Thermomonospora*, and *Thermopolyspora* will also breakdown PLA. This is because molecular ecology approaches have identified their gene sequences in the aerobic compost for PLA degradation. [37, 38]. In addition, *Pseudonocardia alni* is recently discovered to be able to breakdown PLA as well [39].

Consecutively, in the study conducted by Sukkhum et al. [40], several genera of bacteria were identified as a PLA-degrader which were widely dispersed in numerous families of actinomycetes and bacilli. This include the *Bacillus licheniformis, Laceyella sacchari, Thermoactinomy- ces vulgaris, Nonomuraea fastidiosa, Nonomuraea terrinata, Micromonospora viridifaciens, Micromonospora echinospora,* and *Actinomadura keratinilytica*. These bacteria demonstrate PLA degradation at temperature range 40°C until 60°C, and pH level 6.8 – 8.8. It is concluded that the highest PLA-degrading is exhibited by *Actinomadura keratinilytica* which produces serine protease enzyme that can degrade both peptide bond and ester bond in bioplastics.

PLA-Degrading Enzyme

The role of enzymes in the breakdown of PLA has been a source of debate. Some research supports enzymatic breakdown, while others look at the nonenzymatic role of PLA hydrolysis in natural conditions. Although enzymes may not be fully responsible for the breakdown of PLA, they do play a significant part in its decomposition [15]. The maximum activity of these enzymes is influenced by pH levels, temperatures, chain stereochemistry, and material crystallinity [41]. PLA-degrading enzymes are usually hydrolases like esterase, lipase, and protease, which can catalyse the hydrolysis of the ester bonds that connect PLA monomers together [42-44].

In PLA-degradation, there are two type of enzymes that are used, which are intracellular and extracellular enzymes. If internal enzymes are used to breakdown PLA, the presence of whole microbial cells at the contaminated site is necessary. Extracellular enzymes, on the other hand, may require the presence of whole cells because of their metabolic pathway, or cell-free enzyme alone may enough for the biodegradation process [45].

1. *Amycolatopsis sp*. bacteria enzyme

The genus *Amycolatopsis* have been reported to produce several PLA-degrading enzymes in various studies. In Pranamuda et al. [28] study, a novel depolymerase was obtained from soil isolate, purified and characterized from *Amycolatopsis sp.* Strain 41. The enzyme was found to have a higher substrate selectivity compared to Proteinase K. The enzyme was able to degrade casein, silk fibroin, and succinyl-(L-alanyl-L-alanyl-L-alanine)-p-nitroanilide (Suc-(Ala)3-pNA). Similarly, Nakamura et al. [33] was also able to isolate a bacterial PLA-degrader from *Amycolatopsis sp.* K104-1, that has a molecular weight (MW) of 25kDa. This enzyme shown to have degradation activity on casein and fibrin and to be an elastase-like protease [14].

2. Serine Protease

A number of previous studies shows that serine protease was found in PLA degrading microorganisms such as *Tritirachium album* [48], and *Actinomadura keratinilytica* strain T16-1 [40], and *Laceyella sacchari* LP175 [49]. Meanwhile other studies indicate the presence of alkaline protease in PLA-degrading bacteria, including *Bacillus lentus*, *Bacillus subtilis* and *Bacillus licheniformis* [46].

3. Proteinase K

Numerous research using proteinase K, lipase, and cutinase-type enzymes have been reported on the enzymatic breakdown of PLA [47]. William [48] published the first known study of enzymatic degradation by proteinase K on PLA and it was extracted from *Tritirachium album* strain ATCC 22563. Soon after, the study of enzymatic degradation is highly focused and enables 56 types of proteases to be established commercially worldwide [50]. This enzyme is also used to study the break down mechanisms of PLA [51, 52], copolymers of PLA [53], and PLA blends [54]. Furthermore, enzymatic breakdown of PLA in the presence of proteinase K revealed that the rate of degradation decreased as crystallinity of PLA increased [51, 54-56].

Conclusion

The introduction of bioplastic has been highlighted in the past several decades as a mean to substitute petroleum-based plastic and combat the challenges surrounding plastic waste pollution. Despite the many research conducted regarding PLA and all its advantages, the implementation and introduction of this bioplastic is yet to breach the norm usage of the industry and society. This paper serves to review the biodegradation mechanisms of polylactic acid in terms of bacterial and enzymatic breakdown and highlighting the pre-existing microbes and enzymes involved. By reviewing the biodegradability potential of PLA, it aims to signify the solution for the waste management of the future of bioplastic industries and raising awareness to combat plastic waste problem in the future.

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