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Review Article

Bioplastic Degradation, Production and Genetic Improvements of Bioplastic Producing Strains: A review

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ABSTRACT

Plastics have been polluting the environment for a long time due to their persistence in all ecosystems and especially in the marine ecosystem. Until now, the best candidate found to substitute these products are the bioplastics. This is due to the presence of bacteria that can degrade them in nature and the similarities they show in their structure to petroleum-based plastics. These polymers are represented by polyhydroxy alcanoates (PHAs) and are produced naturally by bacterial cells as reservoir compounds for carbon and energy. Many PHA-producing microbial strains have been isolated worldwide including bacterial and fungal genera which could be used to produce bioplastics commercially. However, the major obstacle behind preventing the prevalence of bioplastics is their raw material cost. As a result, many studies have been conducted to produce PHAs, and their derivatives from waste raw materials. This will help to eliminate the cost of bioplastic production and simultaneously save the environment from an unfavorable accumulation of such wastes. Genetics has played an essential role in reducing the cost of bioplastics by manipulating microorganisms to produce more efficient bioplastic producers. Disrupting some genes and introducing others has been found to be helpful for the accumulation of PHAs in microbial producers.

Keywords: Bioplastics, polyhydroxyalkanoates, Biodegradation.

INTRODUCTION

Plastics are one of the most important discoveries from human beings that interact with our lives in different ways. This invention has been designed to suit human needs through its ability to withstand different circumstances. Consequently, leading to their hard degradability by microorganisms (Vered and Shenkar, 2021). The accumulation of plastics has increased from 1.5-245 million tons during the period from 1950 to 2008 with an annual increase of 9%. Besides the environmental problems caused by plastics, their high usage has been threatened by the limited supply of crude oil, plastics main raw substrate, and the costs of recycling these products to ensure environmental safety (Phukon *et al*., 2012). The covid-19 pandemic had positive impacts on the environment by reducing CO_2 levels in the atmosphere, however, the pandemic itself has contributed to the increase of plastic waste worldwide. Recent plastic waste surveys showed that 129 billion face masks and 65 billion gloves are consumed each month worldwide, and a major percentage of them will end up transformed into microplastics and delivered to oceans and terrestrial environments (Zhang *et al.,* 2021). Microplastics have been detected at concerning levels in soil and marine environments and have been reported to cause different health issues, including obesity, organ miss-functioning, infertility, and late development in children. In addition, their presence in marine systems has been found to absorb inorganic chemicals leading to effects on their natural cycle in the environment (Bhatt *et al*., 2021). One of the promising substitutes for plastics is bioplastics. The term bioplastic is referred to any chemical product produced completely or partially from biological biomass, for instance, bacteria, yeast, or plants (Dilshad *et al*., 2021).

Microorganisms produce a variety of polymers carrying different chemical structures and one of them resembles petroleum-derived plastics and is called polyexoesters. This polymer includes polyhydroxyalkanoates (PHA), polymalic acid, cutin and suberin that are produced by microbial prokaryotes, microbial eukaryotes, and some plants. Due to the physical properties carried by PHAs, they have been recommended for different applications in various fields. These products are thermoplastic, insoluble in water, non-toxic, enantiomeric pure, biocompatible, piezoelectric, have a high degree of polymerization and carry molecular weights of up to several million Daltons (Steinbuchel and Lutke-Eversloh, 2003). These materials can act as substituents for ordinary petrol-derived plastics, however, the most significant trait of these PHAs is that they are biodegradable which makes them non-persistent compared to used plastics. Many studies have been conducted to produce PHAs from microorganisms by cultivating these microbes on different kinds of carbon sources under unfavorable conditions such as a surplus of carbon sources or limitation of an essential nutrient, for instance, nitrogen, phosphorous, and oxygen (Koller *et al*., 2005). This review focused on the natural degradation of bioplastics, the production of these polymers, and the efforts to genetically improve bioplastic production. Manipulating microbial genes has future promises in reducing the cost of bioplastics by manipulating microorganisms to produce more efficient bioplastic producers and thereby replace plastic with biodegradable plastics.

Degradation of bioplastics

PHAs are biodegradable thermoplastics that share many material properties with petroleum-derived plastics (Phukon *et al*., 2012). Due to their biodegradation ability and the similarities they show to petroleum-derived plastics, several studies have been conducted to substitute plastic materials with bioplastics (Koller *et al*., 2005). These polymers have reduced the negative effects of global warming by decreasing the amount of non-degradable plastics used (Atiwesh *et al*., 2021). PHAs are produced in bacterial cells as storage compounds under unfavorable circumstances and are degraded in the environment by different genera from bacteria and fungi. Consequently, helping to recycle carbon dioxide and organic compounds in the environment. This degradation occurs due to the ability of some microorganisms to excrete polyhydroxyalkanoate depolymerase that breaks the ester bond of this polymer to form smaller compounds that can be further metabolized to carbon dioxide and water by various microorganisms (Mbotho *et al*., 2021).

Degradation of PHA has been reported by many researchers in Gram-negative and Gram-positive bacteria, and some other methanogenic archaea (Phukon *et al*., 2012). A thermotolerant and halotolerant *Bacillus sp*. JY14 was isolated from marine environments for its ability to degrade PHB in solid and liquid media. Results were confirmed by gas chromatography and scanning electron microscopy and showed that these bacteria degraded 98% of PHB after 14 hours of incubation. This strain was also found to degrade P(3HB-co-4HB) and P(3HB-co-3HV) at similar efficiencies (Cho *et al*., 2021).

A study by Mabrouk and Sabry (2001) revealed that the marine bacterium *Streptomyces sp* SNG9 was able to degrade poly3-hydroxybutyrate (PHB) and its copolymer poly3-hydroxybutyrate-co-3-hydroxy valerate (P3HB-co-HV) as a sole carbon source in 4 days. Other microorganisms such as *Pseudomonas lemoignei*, *Pseudomonas pseudomallei*, *Acidovorax facilis*, *Acidovorax delafieldii*, *Comamonas testosterone*, *Variovorax paradoxus, Zoogloea ramigera*, and *Bacillus* have also been reported to degrade P(3HB) by extracellular enzymes (Phukon *et al*., 2012).

Production of bioplastics by different microorganisms

The biodegradable thermoplastic PHAs can be produced by many strains of bacteria (Palmeri *et al.*, 2012). These PHAs can be categorized into two major groups, short-chain-length (SCL) PHA which contains five or fewer carbon atoms, and medium-chain-length (MCL) PHA that contains six to fourteen carbon atoms (Chee *et al*., 2010). PHA granules could be viewed by the fluorescence-microscope (1000x) after staining the cells with Nile blue A stain. The existence of PHA granules in bacteria could be noticed by the strong orange fluorescence emission of these granules (Phukon *et al*., 2012). One of these biodegradable polymers is the polyhydroxy butyrate (PHB) which is synthesized by bacteria as a carbon and energy storage compound under unbalanced nutrient conditions. Commonly, PHBs are found in bacterial cells either as inclusion bodies distributed in the cytoplasm or the cytoplasmic membrane (Palmeri *et al*., 2012). In addition to poly (3HB) some researchers have detected other hydroxyl alkanoic acids as constituents of poly (3HB). Recently, there are about 150 different constituents occurring in PHAs alone as homopolyesters or in combination as copolyesters. Production of these PHAs is due to the synthesis of polyhydoxy alkanoate synthases that show a broad substrate range (Steinbuchel and Lutke-Eversloh, 2003).

To produce PHAs industrially, it is better to develop strains that can reach the highest final cell density in a reasonable time and produce PHAs at high levels from simple, inexpensive raw materials (Chee *et al*., 2010). One of the popular raw materials that could be used is biodiesel fuel which is helping to protect the world from the dangers of global warming. This raw material is produced by the methyl esterification process of oil and fat which generates intermediate waste products such as glycerol that is useful to synthesize bioplastics (Katawa, 2010). This has been achieved by Palmeri *et al*. (2012) as they used three concentrations of glycerol (1.0, 2.0 and 5.0%) from the biodiesel process as a source of carbon in batch fermentation processe, to enhance the production of medium-chain length poly (3HA) from *Pseudomonas mediterranea*. The best PHA/dried cell ratio obtained was 60% by using crude glycerol as a carbon source. Wong *et al.* (2000) found that using malt wastes as a carbon source for the production of PHB in *Staphylococcus epidermidis* was more efficient than using other wastes such as ice cream, sesame oil, and soya wastes. PHB gained was 1.76g/L and 3.5g/L in shaking cultures and fermentation cultures, respectively.

Each year, 40,420,800 tons of whey is produced from the industry of cheese in the EU which still contains 619, 250 tons of sugar. These amounts of sugar are lost without being further metabolized. Koller *et al*. (2005) grew highly osmophilic organisms on this resource and were able to produce PHAs. These organisms were able to produce PHAs at a concentration of 5.5g/L.

Genetic improvements of PHA producers

The cost behind the production of PHA has been the major drawback for delaying the replacement of petroleum-derived plastics with bioplastics. Even though some scientists are producing PHA from waste products, petroleum raw materials such as polyethylene and polypropylene cost less than $1/Kg. As a result, scientists are searching for new bacterial strains and manipulating others to obtain recombinant cells that could produce more PHAs to further reduce their cost (Chee *et al*., 2010).

Recently, a group of scientists from China were able to engineer *E. coli* by reprogramming its metabolic pathway for β-oxidation and incorporating a ω-oxidation pathway. The engineered strain was capable of metabolizing waste cooking oil as a sole source of carbon and energy to produce building blocks for bioplastics particularly, medium-chain α,ω-dicarboxylic acids (Li *et al*., 2021). A second study to reduce the cost of bioplastics was conducted by Jian *et al*. (2010) who manipulated the strain *Aeromonas hydrophila* 4AK4 by deleting its polyhydroxyalkanoate synthase gene $phaC_{ah}$ and replacing it with the *Pseudomonas stutzeri* strain 1317 $phaC1_{ps}$ gene which prefers 3-hydroxyhexanoate (3HHx) and longer chain monomers. In addition to inserting the gene $phaC1_{ps}$, the genes *fadD* encode *E. coli* acyl-CoA synthase and *fadL* which encodes a fatty acid transporter in *Pseudomonas putida* KT2442 were also inserted into the genome of the new manipulated strain. The new genetically modified strain was able to produce more than 50% PHA in cell dry weight that contained 80-94 mol% 3HHx and which was much higher than the natural production of the wild type which was less than 20 mol% 3HHx. Cloning of the fatty acid-related genes *fadD* and *fadL* increased the ability of this strain to use short-chain length fatty acids such as hexanoate and octanoate (Scheel *et al*., 2016).

Insertional mutagenesis using the transposon Tn5 also had a beneficial effect on the production of bioplastics in the isolate *Pseudomonas putida*. This method was used by Olivera *et al*. (2001) to obtain mutations ($fadA^-$, $fadB^-$) or deletions (Δ*fadA* or Δ*fadAB*). These genes were involved in the β-oxidation pathway of these bacteria leading to the formation of some morphological changes in bacteria when grown on solid media due to the overproduction of homo and co-polymer bioplastics. Removing the genes *fadBA* was further studied by Sandoval *et al*. (2005) who mentioned that *Pseudomonas putida* used these genes in the β-oxidation pathway to catabolize fatty acids. They also found that these mutants can still catabolize fatty acids although both genes were deleted. This was due to the occurrence of a second β-oxidation pathway (β_{II} oxidation) that was only induced when the first β-oxidation pathway (β_{I} oxidation) was blocked. The β_{II} oxidation catabolizes n-alkanoic acids but cannot metabolize other compounds that contained aromatic rings attached to the acyl moiety (n-phenylalkanoic acids). As a result, *P. putida* Δ*fadBA* mutant was able to produce and accumulate various types of PHA. This strain was considered a bioplastic overproducing strain due to its ability to accumulate more than 90% of the bacterial cytoplasm by bioplastic polymers. To ascertain these results, Sandoval and his co-workers inserted the gene *phaZ* responsible for coding the enzyme poly-3-hydroxy-n-phenylalkanoate depolymerase responsible for avoiding the accumulation of the polymer storage granules. They found that the colonies that overproduced *phaZ* gene lost their cereous morphology when grown on solid media.

CONCLUSION

Plastics are one of the best human discoveries that cannot be dispensed, however, their degradation in the environment is extremely slow. This makes it a persistent contaminant in the environment. Bioplastics are the best candidates found to date that have promising features to substitute plastics and eliminate plastic waste worldwide. Metabolic engineering of bioplastic-producing strains have enabled better production and thereby gives an advantage to the industry of bioplastic production.

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التحلل الحيوي للبلاستيك الحيوي، انتاجها والتحسين الوراثي للسلالات المنتجة

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الملخص

لقد تلوثت البيئة منذ فترات طويلة بالمواد البلاستيكية وذلك بسبب مقاومتها للتحلل في جميع النظم البيئية وخاصة في النظام البيئي البحري. حتى وقتنا الحاضر فإن أفضل بديل تم العثور عليه للمواد البلاستيكية هو البلاستيك الحيوي وذلك لوجود بكتريا تتمكن من تحليله في الطبيعة وأوجه التشابه التي تظهرها مع المواد البلاستيكية المستخلصة من البترول. هذه البوليمرات تعرف بالالكان متعدد الهيدروكسيل (PHA) والتي تنتج بشكل طبيعي من قبل الخلايا البكتيرية كمركبات خازنة للكربون والطاقة. تم عزل العديد من السلالات المنتجة للـ PHA في جميع أنحاء العالم بما في ذلك الأجناس البكتيرية والفطرية التي يمكن استخدامها لإنتاج البلاستيك الحيوي تجاريًا. على اي حال، العقبة الرئيسية أمام منع انتشار البلاستيك الحيوي هي تكلفة المواد الخام، نتيجة لذلك ، تم إجراء العديد من الدراسات لإنتاج PHA ومشتقاتها من نفايات المواد الخام التي ستساعد على التخلص من تكلفة إنتاج البلاستيك الحيوي وفي نفس الوقت إنقاذ البيئة من التراكم غير المواتي لهذه النفايات. لعبت الوراثة دورًا أساسيًا في تقليل تكلفة البلاستيك الحيوي من خلال التلاعب الوراثي بالكائنات الحية الدقيقة لإنتاج سلالات لها القدرة على انتاج بلاستيك حيوي بكفاءة أكثر. اذ وجد أن حذف بعض المورثات وإدخال أخرى كان مفيدا لتراكم PHA في الجراثيم المنتجة.

الكلمات الدالة: البلاستيك الحيوي، الالكان متعدد الهيدروكسيل، التحلل الحيوي.