The Dawn of Novel Biotechnological Applications of Polyhydroxyalkanoates

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Abstract

The synthetic polymers – plastics have been applied in a wide range of activities of our daily routine. However, extensive usage and their non-biodegradable nature have led to their accumulation in quantities, which are difficult to manage and a major cause of environmental pollution. Bacteria have the ability to accumulate Carbon (C) as biopolymers especially under stress conditions. The biopolymers – polyhydroxyalkanotes (PHAs) are biodegradable and have properties quite close to those possessed by plastics. PHAs have been explored in diverse fields including agriculture and medical. In the field of medicine, PHAs hold greater promise because of their usage in producing high value products, in addition to their biodegradable, biocompatible, and non-toxic nature. PHAs have been explored for their role as implants, drug carriers, tissue engineering, biocontrol agents, inhibitors of cancerous growth, and memory enhancing molecules.

Keywords

Biopolymers · Polyhdroxyalkanoates · Tissue engineering · Drug carriers · Biocontrol agents

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1.1 Introduction

Microbes have unique abilities to divert or even curtail their metabolic pathways, as soon as they encounter major modifications in their immediate vicinity. A unique case which clearly exemplifies this phenomenon is the diversion in their highly energy efficient metabolic pathway during stress conditions. Bacteria sense the nutritional imbalance, such as excess of Carbon (C) compounds and limited quantities of nitrogen, phosphorus, potassium, oxygen, and magnesium. Instead of metabolizing C compounds to generate energy through the Tricarboxylic acid cycle (TCA), bacteria divert the acetyl CoA towards polyhydroxyalkanoates (PHA) synthetic pathway (Porwal et al. 2008; Singh et al. 2009; Kumar et al. 2013; Ray and Kalia 2016, 2017). As far as microbes are concerned, they seem to store C as PHA granules and use them as energy reservoirs (Patel et al. 2011, 2012, 2015a, b, 2016; Singh et al. 2013; Kumar et al. 2014, 2015a, b, c; Bhatia et al. 2015a,b, 2016, 2017, 2018, Kalia et al. 2016; Koller et al. 2017). However, because of the unique physicochemical properties, PHAs have been explored for their potential to replace synthetic plastics. The direct usage of PHAs has been proving uneconomical. This has forced researchers to look for high value derivatives of PHAs. Hence, the emphasis has shifted towards use of PHA catabolic pathway products and their chemical modifications, which confer unique properties for biomedical applications (Hazer et al. 2012; Martinez et al. 2014; Ke et al. 2017).

1.2 Antimicrobials, Biocontrol and Anticancer Agents

Catabolic activity of PHAs results in intermediate like 3-Hydoxy acids (3HAs). It primarily involves depolymerase enzyme resulting in monomers. These intermediates can be modified to synthesize antimicrobials (Gallo et al. 2014; Kalia et al. 2019). Hydroxycarboxylic acids: 2-alkylated 3HB and β-lactones produced by transforming 3HAs, can be used as oral drugs. Antibiotics carbapenem or macrolide can be used to treat *Staphylococcus aureus* infections (Dinjaski et al. 2014). Medium chain length 3HAs prepared by *Streptomyces* strains from *Jatropha curcas* as antimicrobial agent against pathogens such as *Salmonella typhimurium, Listeria monocytogenes* and *E. coli* (Allen et al. 2012). PHA co-polymer poly (3-hydroxybutyrate-co-70%4-hydroxybutyrate) produced by *Cupriavidus* sp. also proved to have antimicrobial properties against pathogens such as *S. aureus* (Hema et al. 2013). Chlorhexidine (CHX), an efficient antifungal agent was carried through PHB/PEO fibres, showed 99–100% reduction in *E. coli* and *S. aureus* population at 1 wt% concentration (Fernandes et al. 2014). Tetracycline encapsulated in polymeric microspheres showed 85% reduction in periodontitis-causing bacteria *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (Panith et al. 2016). P3HB and P4HB can be exploited for treating skin infections and healing wounds (Shishatskaya et al. 2016). Combining 3HAs with D-peptide is effective against cancers (O'Connor et al. 2013; Sangsanoh et al. 2017). Aquaculture and livestock industry employ antibiotics at quite low doses along with the feed. This regular supplementation has turned out to be harmful to gastrointestinal microflora. This selection pressure is likely to cause evolution of drug resistant bacteria. PHAs as food supplement have been shown to act as anti-pathogenic in the intestine of giant tiger prawn (Defoirdt et al. 2007, 2009; Halet et al. 2007; Dang et al. 2009; Liu et al. 2010; Ludevese-Pascual et al. 2016).

1.3 Drug Carriers

Efficiency of the drug for treating diseases is dependent up on their delivery to the target (Nigmatullin et al. 2015). Since, the Drugs can be delivered through intravenous, subcutaneous and oral routes. The delivery system to be opted depends upon the nature and dose of the drug to be administered. The drug release is also influenced by the composition of the polymer (Kamaly et al. 2016). Nano-particles and scaffolds can prove effective for eluting drugs from PHA derived monomers (Mokhtarzadeh 2016). Nanoparticles of poly (4-hydroxybutyrate)-monomethoxypoly(ethylene glycol) were used for delivering anticancer drug cisplatin in to hippocampal HT22 cells of mouse (Shah et al. 2014). Monomers of PHA such as 3-hydroxybutyrate (3HB) can prove helpful for synthesizing novel biodegradable polymers. Dendrimers – tamsulosin, ketoprofen and clonidine, have high monodispersity and surface-functional moieties, which help these molecules to play the role of drug carriers (Parlane et al. 2016a, b). Microspheres made up of PHAs, in combination with rifampicin behave as drug carriers and hemoembolizing agents Implants such as rods made up of PHA co-polymers have efficient ability to deliver antibiotics. Nanoparticles based on docetaxel loaded with PHA copolymer – poly (3-hydroxybutyrate-co-3-hydroxyvalerate) was used for its pharmacokinetic evaluation. These nanoparticles were reported to have stability with reference to drug content and physical characteristics. Nanoparticles helped to increase the efficacy in inhibiting human breast cancer cell line (Vardhan et al. 2017).

1.4 Engineering Tissues

Chemically modified PHAs can be helpful in tissue engineering (Goonoo et al. 2017). These can be used as therapeutics and for other medical applications such as: (i) grafts, (ii) cardio-vascular valves, and (iii) nerve tissues (Chen 2011). They also find use as films, pins, sutures, screws, and scaffolds for repairing skin, cartilage and liver tissue engineering (Levine et al. 2015; Ching et al. 2016; Insomphun et al. 2016; Shishatskaya et al. 2016; Rașoga et al. 2017).

1.5 Medical Implants and Devices

The use of PHAs for medical devices is improved by developing co-polymers. These specific PHAs are relatively quite strong, and highly biocompatible (Qu et al. 2006a). The biotechnological application range gets broader since their ability to resist bacterial infections is high, they lack immunogenicity and have been found to be non-toxic. Potential medical devices developed have been implants: rivets and tacks, orthopaedic pins, stents, cardiovascular grafting, meniscus repair, cartilage repair, staples, mesh, sutured fastener, repair patches (Lobler et al. 2002; Qu et al. 2006b; Rodríguez-Contreras et al. 2017). PHA films embedded with lysozyme inhibit bacterial biofilm formation and are useful in wound dressing (Kehail and Brigham 2017).

1.6 Anti-osteoporosis Agent

3HB improves growth of osteoblasts and proves useful as an anti-osteoporosis agent. The serum alkaline phosphatise activity and ability to improve calcium deposition process are the properties by which 3HB helps in prevention of lowering of bone density and serum osteocalcin (Tokiwa and Calabia 2007; Zhao et al. 2007; Chen 2011).

1.7 Memory Enhancer

PHAs can rapidly diffuse to improve cardiac efficiency and prevent brain damage, by acting as source of energy. Parkinson and Alzheimer diseases can be cured through PHA monomers such as 3HB. It acts by preventing neuronal cell death (Camberos-Luna et al. 2016). Modified PHA monomers such as methyl esters of 3-hydroxybutyrate can be employed as drugs to protect mitochondrial damage (Zhang et al. 2013). HA can stimulate Ca^{2+} channels, which acts as an aid in enhancing memory especially patient with dementia – Alzheimer's disease (Cheng et al. 2006; Xiao et al. 2007; Zou et al. 2009; Magdouli et al. 2015).

1.8 Packaging

Use of plastics as packaging material is quite prevalent. Their use for packing food material need special attention. The specific requirements include: (i) protection from dust, contaminants, dehydration, etc., (ii) food grade quality, (iii) food stability, and (iv) degradation during (Prasad and Kochhar 2014). PHAs have the potential to meet the requirements of food grade packing material, especially material properties and permeability (Chen 2010; Chanprateep 2010; Rai et al. 2011; Wang and Chen 2017). Copolymers of PHA having high hydroxyvalerate and mcl-PHA content helps to reduce brittleness and Young's modulus, allowing it to achieve higher flexibility (Fu et al. 2014; Albuquerque and Malafaia 2018).

Packaged food needs to retain its aroma for a long storage period. PHB made films show higher barrier to aromatic compounds. Limonene, which is commonly used for testing the loss of aroma during storage was found to be retained for longer period in PHBV copolymers (Sanchez-Garcia et al. 2007). Nanocomposites of PHB or PHBV with organo-modified montmorillonite Cloisite® 30B or halloysite (HNT), bacterial cellulose nano-whiskers allowed variation in morphology, thermal and mechanical properties (Wang et al. 2005; Carli et al. 2011; Martínez-Sanz et al. 2014; Arrieta et al. 2015). More recently, polymer films with desirable characteristics such as odorless, high flexibility, nontoxicity, antimicrobial and antioxidant activities have been developed by using: (i) PHB: nanomelanin: glycerol polymer film (Kiran et al. 2017), (ii) PHBV along with natural vermiculite and organoclay (Reis et al. 2016, 2017).

1.9 Agriculture

The usage of PHA in agriculture has been exploited only to a limited extent in comparison to that in the medical field. The obvious reason for his biased attitude is the high cost associated with medical applications. Among the few fields where PHAs have found some application are: (i) mulching, (ii) nets, and (iii) bags. Mulching helps to improve and maintain good soil structure, control contamination, and regulate weeds. PHA copolymer (PHBHHx) based NodaxTM has been used to prepare agricultural mulch (Hassan et al. 2006). Another mulch being produced at commercial level is made from PHA based Mirel™ resin, Metabolix Inc. (Andrews 2014). Nets are used in greenhouse and for protecting crops from insects, birds, hails, and for creating special environmental conditions (Castellano et al. 2008; Niaounakis 2015; Guerrini et al. 2017; Ojanji 2017). PHA based bags are used for seedlings, retaining water and regulating temperature (Lu et al. 2014; Schrader et al. 2016). PHA nanomaterials specifically microspheres have found its application as nanoherbicide which have lower genotoxicity and high biodegradability increasing the herbicide efficacy (Grillo et al. 2010; Lobo et al. 2011).

1.10 Challenges in Customizing PHAs

Despite the wonderful and unique characteristics of PHAs, their real-life applications are limited (Singh et al. 2015). The major challenges include: (i) selecting a host organism to express genes involved in PHA synthesis (Singh et al. 2009), (ii) regulating co-polymer composition and production (Kumar et al. 2015c; Ray and Kalia 2016), (iii) manipulate feed composition, (iv) improving physicochemical properties, and (v) develop techniques to modify the products generated from metabolism of PHAs (Singh et al. 2015).

1.11 The Future

PHAs have the necessary potential for being applied in diverse fields. The major limitation has been the economic – feasibility of this product. Application of PHAs and their metabolic products in the field of medicine can circumvent the economic issue. The synthetic biology approach to produce these biochemical in a cell-free system has been envisaged as a viable alternative to limit costs (Opgenorth et al. 2016).

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