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Bacillus and biopolymer: Prospects and challenges

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ABSTRACT

The microbially derived polyhydroxyalkanoates biopolymers could impact the global climate scenario by replacing the conventional non-degradable, petrochemical-based polymer. The biogenesis, characterization and properties of PHAs by *Bacillus* species using renewable substrates have been elaborated by many for their wide applications. On the other hand *Bacillus* species are advantageous over other bacteria due to their abundance even in extreme ecological conditions, higher growth rates even on cheap substrates, higher PHAs production ability, and the ease of extracting the PHAs. *Bacillus* species possess hydrolytic enzymes that can be exploited for economical PHAs production. This review summarizes the recent trends in both non-growth and growth associated PHAs production by *Bacillus* species which may provide direction leading to future research towards this growing quest for biodegradable plastics, one more critical step ahead towards sustainable development.

1. Introduction

In developing countries several activities are transforming local problems into international issues in this global village. Plastics with favourable mechanical integrity and excellent durability have been one of the fall-outs of the rapid progress in material science technology. Having its utility in diverse sectors, plastics have became an essential part of the modern life. In the global commodity petrochemical based plastic production has grown two hundred fold from 1.5 million tons in 1950 to 299 million tons with an annual growth rate of 9% in 2013 [1,2]. These are typical petroleum-based, non-biodegradable polymers gather or aggregate around our ecosystem which is a far cry from few years back ecosystem [2]. Degradation of such solid wastes is a global concern. Even though it is difficult to completely ban the use of plastics due to their versatile utilities, it is possible to replace or reduce their use with alternative biodegradable polymers with similar properties.

Among the entire bio-based and bio-degradable polymer, polyhydroxyalkanoates (PHAs) are well-known. These are bio-based and biodegradable without waste and also recycled to CO_2 and water. The endocellular PHAs are biosynthesized hydroxy-fatty-acids stored as lipid inclusions when carbon source is in abundance and nutrients like nitrogen, phosphorus, oxygen or sulphur are limited. These are secondary metabolites produced by various microbes in response to environmental stress. Such microorganisms can be located in diverse ecological niches like costal water body sediments, marine region, rhizospheric soil, water sediments and sludge [3]. These environments are often brimming over with organic nutrients and poor in other nutrients to support active growth and meet the metabolic requirements of the starving PHAs accumulating microbial population [4]. Extensive research provides a clear vision on several PHAs producers, that these microbes synthesize PHAs inclusions in the late log phase of growth cycle. Then, in later stage of their life cycle they use it as a carbonosoms [5,6]. Through metabolic activities, PHA is normally depolymerized to D-hydroxy-butyrate on demand, and then metabolized to acetoacetate and acetoacetyl-CoA [7] to provide energy to the cell.

Though these carbonosoms accumulation has been investigated in various bacterial isolates, *Bacillus* species are extensively studied in PHAs world since the exploration of poly-β-hydroxybutyrate (PHB) in the cytosol of *Bacillus megaterium* by the French Lemoigne, in 1926 [8]. Some *Bacillus* species have been reported to produce as much as 90% (w/w) PHAs of dry cells during nutrients imbalance [9]. *Bacillus* species becoming model organisms in industry and academic world attributed primarily to its genetic stability [10]. Apart from higher growth rate compared to other bacteria, the use of *Bacillus* species to produce PHAs is advantageous over others due to the absence of lipopolysaccharides external layer in them which makes PHAs extraction much simple [11]. *Bacillus* species are also capable of producing PHAs copolymers utilizing the relatively simple, inexpensive and structurally unrelated carbon

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sources. Moreover, the isolates possess the ability to secrete number of hydrolytic enzymes that can be exploited for cost affordable PHAs production by utilizing, for instance, agro-industrial and other waste materials [12].

The major drawback of *Bacillus* species in PHAs production is their sporulating nature. Practically the fact of sporulation and deposition of PHAs granules provoked due to stress factors [13]. To overcome the predicament research on pilot scale PHB productions by *B. cereus* in the media that depresses sporulation, under acidic pH [14] and potassium deficiency [15] conditions. These pores over strategies not only inhibit spore formation in *Bacillus* but also can enhance the PHAs productivity. Several studies of PHAs are dealing with mostly on upstream and downstream process, its applications [16,17] and with genetic modifications or mutations to increase the yield [9,18]. Now these expertises become an impediment, being economically nonfeasible to market. This review summarizes these recent trends in PHAs production by *Bacillus* species as an effort to provide direction and leads to future research and development towards the growing quest for biodegradable plastics, one more critical step ahead towards an eco-sustainable development.

2. Biogenesis and chemistry

2.1. Diversity and synthesis of biopolymers by Bacillus

The genus *Bacillus* is capable of producing organic and inorganic intracellular spherical inclusion bodies enclosed by phospholipid-protein membrane in the cytosol. The inorganic inclusion bodies are magnetosomes surrounded by iron oxide core and the organic hydrophobic inclusion is PHAs surrounded by polyester core [19]. Evidently, the presence of PHAs granules in the microbial cytosol have also been served as a chemotaxonomic signature for detection of various isolates [20]. A wide array of PHAs producer *Bacillus* species (Table 1a) are recorded in the last few years with diverse biosynthetic mechanism, structural, thermal and functional properties.

2.2. Forms and taxonomy of biopolymers from Bacillus

The accumulated biopolymer PHAs comprises of 3-hydroxy fatty ester representing not only divergence but also complexity in their monomer classes. It is fascinating and the largest group of biopolyesters with more than 150 monomer compositions exhibiting diverse physical and chemical properties, and functionalities [43,44]. Till now PHAs are grouped into three different categories based on the size, arrangements and number of carbon atom in the polymer, such as short chain length (scl-PHAs with C5 monomer), medium chain length (mcl-PHAs; with C6–C14 monomers) and long chain length (lcl-PHA; with ≥ C14 monomers) respectively [45].

Moreover, the homo and heteropolymers of PHAs corresponds to the presence of more than one type of hydroxyalkanoate monomers. The molecular weight of the polymer ranges from 2×10^5 to 3×10^6 Da, which is based on the type of microbial strain, upstream and downstream processing employed in the production method [46]. *Bacillus* species are also reported to accumulate heteropolymers of scl- to mcl-PHAs including P(3HB-co-3HV), P(3HB-co-3HHx) and P(3HB-*co*-4HB) with c-butyrolactone or e-caprolactone as C-source in the production media [47]. Though various PHA monomers are produced by *Bacillus* species *in vitro*, very few such as PHB, PHBV and PHBH have en routed for pilot-scale production [48].

2.3. Biochemical pathway of PHAs synthesis

Bacteria have the ability to synthesize PHAs in the stationary as well as exponential growth phases. Non-growth associated PHAs accumulation occurs in the stationary phase of bacterial growth with limitation of N, P, Mg and oxygen and excess carbon sources; however growth associated PHAs production takes place under balanced condition. Notably, most of the *Bacillus* species accumulate PHAs by adopting growth associated and non-growth associated mechanism [6,49] as compared to other genera. Biosynthetic pathway of PHAs production varies among microbial groups. So far eight different pathways of microbial PHAs synthesis have been reported [13]. PHB, the most common homopolymer of PHAs synthesis starts from metabolism of glucose to generate acetyl-CoA and NADPH through the glycolytic and pentose phosphate pathways. Then, the two acetyl- CoA molecules condensed by β-ketothiolase (*PhaA*) into acetoacetyl-CoA and subsequently reduced to 3-hydroxybutyryl-CoA by acetoacetyl-CoA dehydrogenase (*PhaB*) using NADPH as a cofactor and finally polymerized into PHB by P(3HB) polymerase (*PhaC*) [6,45,50]. Thus, the NADPH is involved in reduction of acetoacetyl-CoA to 3-hydroxybutyryl-CoA due to over expression of the *zwf* and *gnd* genes that encode glucose 6-phosphate and 6-phosphogluconate dehydrogenase respectively [51]. As a matter of fact, the PHB production has been increased by raising the ratio of NADPH to $NADP^+$.

Carbon sources in bacteria are metabolized differentially. So far three pathways for the synthesis of monomers of PHAs in bacteria have been well-studied (Fig. 1). Pathway I utilize sugars like glucose and fructose to yield PHB homopolymer. Copolymers are produced through pathway II and III [53,54]. A contemporary hypothesis for the reaction mechanism of PHA synthases was proposed based on a model by

Table 1a
PHAs produced from synthetic substrate by different species of *Bacillus*.

Bacillus sp.	Substrate	PHAs yield (% of DCW)	Fermentation	PHAs type	Reference
Bacillus aryabhattai	Sucrose, glucose & fructose	57.62	Batch	PHAs	[40]
Bacillus cereus SPV	Glucose	38.00	Batch	3HB & 3HV	[14]
Bacillus cereus	Glucose	13.77	–	PHB-3HHX	[34]
Bacillus licheniformis	Glucose	53.01	Batch	PHB	[42]
Bacillus megaterium uyuni S29	Glucose	70.00	Feed Batch	PHB	[37]
Bacillus mycoides DFC1	Glucose	57.20	Batch	PHB	[28]
Bacillus mycoides DFC1	Glucose	76.32	–	PHB	[35]
Bacillus sp.	Glucose	68.85	–	PHB	[26]
Bacillus sp.	Raffinose	60.57	Batch	P(3HB)	[27]
Bacillus sp.	Glucose	80	–	PHA	[57]
Bacillus sp.	Sucrose	51.49	Batch	PHAs	[41]
Bacillus sp. SW1-2	Glucose	36.00	Feed Batch	PHB	[36]
Bacillus sp. Ti3	Starch	58.73	Batch	PHB	[12]
Bacillus subtilis	Glucose	69.01	Batch	PHB	[3]
Bacillus thuringiensis	Glucose	11.30	Batch	PHB	[32]
Bacillus thuringiensis IAM12077	Glucose	64.16	–	PHB	[38]
*Lysinibacillus*sp. 3HHX	Glucose	80.94	Batch	PHB	[1]
Paenibacillusdurus BV-1	Fructose	0.93 g/l	–	PHB	[39]

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