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Bacillus megaterium as potential producer for Polyhydroxybutyrates

Neelam K.Naranje[1*], Bharat J.Wadher[1], Hemant J.Purohit[2], Manohar G. Muddeshwar [3]
[1](*P.G.T. Department of Microbiology, R.T.M. Nagpur University, Nagpur, India)*
[2](*Department of Environmental Genomic Division, NEERI, Nagpur, India)*
[3](*Department of Biochemistry, Govt. MedicalCollege, Nagpur, India)*
**(Corresponding Author: Neelam K. Naranje (PhD Research scholar)*
Address: P.G.T. Department of Microbiology, R.T.M. Nagpur University, Nagpur, India Email: neelamnaranje@gmail.com Mob: +91 80875 38333

Abstract: *Poly3-hydroxybutyrate (PHB) or bioplastic, is a most common natural biopolymer, produced by a range of microorganisms. It is accumulated as storage materials allowing microbial survival under stressed conditions. The viability of microbial large scale production of bioplastics is dependent on the development of a low cost process that produces biodegradable plastics with properties similar or superior to petrochemical plastics. Certain unique properties of Bacillus megaterium such as ability to produce large quantities of intracellular PHB with more efficient utilization of relatively cheaper substrates, lack of the toxic lipo-polysaccharides, structural and segregational stability of recombinant plasmids enable it to compete as potential candidate for commercial production of PHB. We are working with twenty potential isolates, which were selected on HiCrome Bacillus Agar and getting them screened by RAPD-PCR analysis.*

Key words: *Bacillus megaterium, PHB, RAPD-PCR*

I. Background

Plastic materials have made entry in every sphere of human life because of its superior characteristics such as durability, strengths, shape and moldable property, since these plastics have high molecular weight & tightly bonded together these are non-degradable , rendering their disposal difficult, which inversely leads to negative impact on the environment.

Thus the concept of biodegradable plastic came as a solution which could be produced and degraded by microorganism in the environment when proper condition such as sunlight, moisture, oxygen etc are available. (Abe and Doi, 2002).

To overcome this problem, the production and applications of eco-friendly products such as bio-plastics becomes inevitable. poly 3-hydroxy butyric acid (PHB) is a biopolymer also known as bioplastic which is produced by microorganism.

The viability of microbial large scale production of Bioplastic is dependent on the development of a low cost process that produces biodegradable plastics with properties similar or superior to petrochemical plastics.

In order to make the process economically attractive, many goals have to be addressed simultaneously. Recombinant microbial strains are being developed to achieve both a high substrate conversion rate and close packing of PHB granules in the host cell. A more efficient fermentation process, better recovery ⁄ purification, use of genetically modified organism and the use of inexpensive substrates can also substantially reduce the production cost[17].

II. What are Bioplastic?

Bioplastic are naturally occurring biodegradable polymers made from polyhydroxyalkanoates (PHA) of which PHB is the most common. They are polyesters, produced by a range of microbes, cultured under different nutrient and environmental conditions.[2] These polymers, which are usually lipid in nature, are accumulated as storage materials (in the form of mobile, amorphous, liquid granules), allowing microbial survival under stress

conditions.[3,4] The number and size of the granules, the monomer composition, macromolecular structure and physico-chemical properties vary, depending on the producer organism[5.] They can be observed intracellularly as light-refracting granules or as electronlucent bodies that, in overproducing mutants, cause a striking alteration of the bacterial shape.

Bacillus megaterium

Several unique properties of Bacillus megaterium enable it to compete as potential candidate for commercial production of PHB.

Natural PHB Producer

Highest polymer producer in bacillus genus Gives fast growth on diversity of cheap substrates

It has an advantage that no alkaline protease is present that enables excellent production & secretion of foreign protein without degradation. (Meinhardt *et al., 1989; Rygus and Hillen, 1991)*

lack of the toxic lipo-polysaccharides

structural and segregational stability of recombinant plasmids

We are working with twenty potential isolates, which were selected on HiCrome Bacillus Agar and getting them screened by RAPD-PCR analysis.

RAPD-PCR: RAPD (Random Amplified Polymorphic DNA) It is a type of PCR reaction, but the segments of DNA that are amplified are random. RAPD is an

inexpensive yet powerful typing method for many bacterial species. DNA fragments amplified by

PCR using short synthetic primers (generally 10 bp) of random sequence. These oligonucleotides serve as both forward and reverse primer, and are usually able to amplify fragments from 1-10 genomic sites simultaneously. Amplified fragments, usually within the 0.5-5 kb size range, are separated by agarose gel electrophoresis, and polymorphisms are detected, after ethidium bromide staining, as the presence or absence of bands of particular sizes. These polymorphisms are considered to be due to variation in the primer annealing sites.

III. Characteristics of PHB:

It is a polyester synthesized and catabolized by numerous microorganisms processing glucose[5], corn starch or wastewater. Its characteristics are similar to those of the petroplastic polypropylene. PHB is distinguished primarily by its physical characteristics. It produces transparent film at a melting point higher than 130 degrees Celsius, and is biodegradable without residue.

IV. Properties of PHB

Water insoluble and relatively resistant to hydrolytic degradation. This differentiates PHB from most other currently available biodegradable plastics, which are either water soluble or moisture sensitive.

Good oxygen permeability.

Good ultra-violet resistance but poor resistance to acids and bases. Soluble in chloroform and other chlorinated hydrocarbons. Biocompatible and hence is suitable for medical applications. Tensile strength 40 MPa, close to that of polypropylene.

Sinks in water (while polypropylene floats), facilitating its anaerobic biodegradation in sediments.
Nontoxic.
Less 'sticky' when melted, making it a potentially good material for clothing in the future.

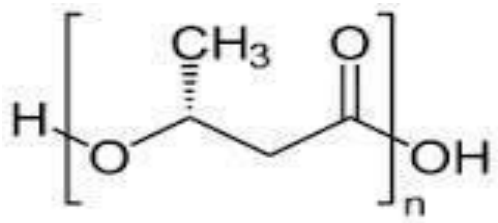


Fig : Chemical Structure of PHB

Advantages of Bioplastics over Petroleum-based polymers:

PHB and other bioplastics are natural polymers.

Thermoplastic or elastic properties with melting-points ranging from 40° to 180°C. Degradation in microbe active environments in 5-6 weeks.

Degradation process ultimately leave behind carbon dioxide and water, which are environmental friendly byproducts.

The synthesis and biodegradation of bioplymers are totally compatible to the carbon-cycle.

Bioplastics can be produced from renewable carbon resources. As long as there is fuel shortage & rise in crude oil prices traditional energy sources are safe.

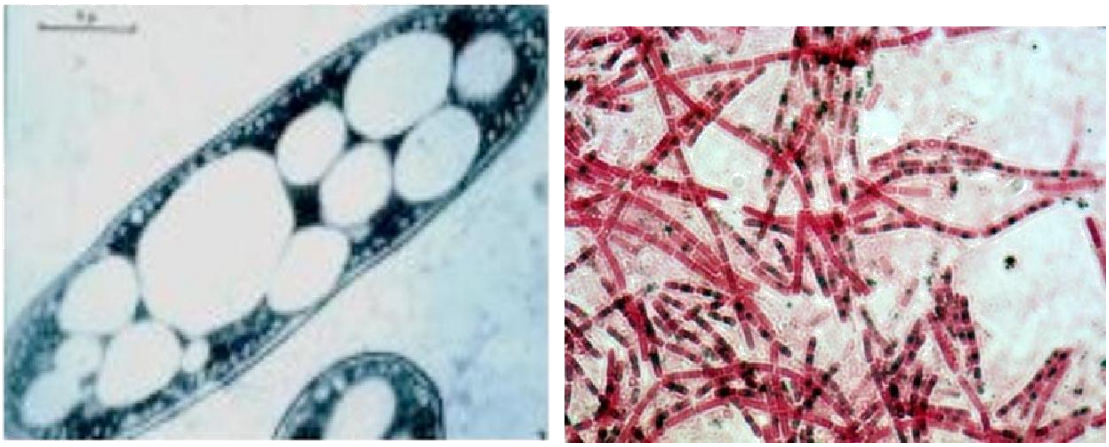
The wider use of bioplastics in daily life will solve the increasing problem of organic waste.

The use of biodegradable plastics will decrease the country's dependence on other countries for fossil fuels.

Biocompatible and low oxygen permeability makes them suitable for medical applications. Therefore can be implanted in the body without causing inflammations. Some possible bioplastics applications include: biodegradable carriers that demonstrate the ability to deliver drugs for a given time within the individual's body, surgical needles, suture materials, bone tissue replacement, etc. The advantage of using biodegradable plastics is that it does not require surgical removal.

V. Application of Bioplastic:

Many different applications have been described for bioplastics since the first industrial production of Biopol1 by ICI Ltd in 1982. Initially, they were used for the fabrication of bottles, fibres, latex and several products of agricultural, commercial or packaging interest.[9,10] Currently, these polyesters have been employed for medical applications such as sutures, implants, urological stents, neural- and cardiovascular-tissue engineering, fracture fixation, treatment of narcolepsy and alcohol addiction, drug-delivery vehicles, cell microencapsulation, support of hypophyseal cells, or as precursors of molecules with anti-rheumatic, analgesic, radiopotentiator, chemopreventive, antihelmintic or anti-tumuoral properties (those containing aromatic monomers or those linked to nucleosides)



(A) (B)
Electron Micrographs Sudan Black Staining
Fig: Bioplastic particles produced in bacteria

VI. Current Scenerio

The occurrence of PHB in bacteria has been known since 1920s, when Lemoigne reported the formation of poly 3-hydroxybutyrate (PHB) inside bacteria.

During recent years, intensive research has investigated the bacterial production of PHB and a great effort is underway to improve this procedure .In order to make the process economically attractive, many goals have to be addressed simultaneously. Recombinant microbial strains are being developed to achieve both a high substrate conversion rate and close packing of PHB granules in the host cell.

A more efficient fermentation process, better recovery ⁄ purification, use of genetically modified organism and the use of inexpensive substrates can also substantially reduce the production cost. Additionally, further research is required to enhance the physical properties of PHB. Global Bioplastics demand is forecast to reach 884,000 tones by 2020.

References

[1]. T.Charen1 , P.Vaishali1 , M.Kaushalya1 , K.Amutha: Isolation and identification of Polyhydroxybutyrate producing bacterial strain (Bacillus thuringiensis GVP) from chlorine contaminated and D.Gowdhaman1,* 1 (2014)
[2]. Gulab Singh,1 Anish Kumari,1 Arpana Mittal,1 Anita Yadav,2 Poly β-Hydroxybutyrate Production by Bacillus subtilisNG220 Using Sugar Industry Waste Water(2013)
[3]. Kanokphorn Sangkharak, Poonsuk Prasertsan: The production of polyhydroxyalkanoate by Bacillus licheniformis using sequential mutagenesis and optimization (2013).
[4]. Rodrıguez-Contreras1 , M. Koller2 , M. Miranda-de Sousa Dias2 , M. Calafell-Monfort3 :High production of poly(3-hydroxybutyrate) from a wild Bacillus megaterium Bolivian strain (2013)
[5]. Nur Ceyhan1* and Guven Ozdemir : Poly-_-hydroxybutyrate (PHB) production from domestic wastewater using Enterobacter aerogenes 12Bi strain,(2011).
[6]. Paramjit Singh and Nitika Parmar: Isolation and characterization of two novel polyhydroxybutyrate (PHB)-producing bacteria, (2011).
[7]. Mirel: PHAs grades for Rigid Sheet and Thermoforming, (2011)
[8]. Denise Knobloch, Andre Clemens,Kai Ostermann,Gerhard Ro¨delThe xylA promoter of Bacillus megaterium mediates constitutive gene expression in Escherichia coli.(2011)
[9]. Nur Ceyhan1* and Guven Ozdemir : Poly-_-hydroxybutyrate (PHB) production from domestic wastewater using Enterobacter aerogenes 12Bi strain,(2011).
[10]. Akaraonye E, Keshavarz T, Roy : Production of polyhydroxyalkanoates: the future green materials of choice. J. Chem. Technol. Biotechnol., (2010).