List of Publications


Papers presented at conferences


3. Prabhu, N.N., Santimano, M.C., Kowshik, M., Bhosle, S.N., Mavinkurve, S. and Garg, S. (2005) Diversity of PHA accumulating bacterial isolates belonging to *Bacillus* spp. obtained from marine and coastal environments" International conference on “Microbial Diversity: Current perspectives and potential applications” 16th to 18th April, University of Delhi, South Campus, New Delhi, India.
Native granule associated short chain length polyhydroxyalkanoate synthase from a marine derived Bacillus sp. NQ-11/A2

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Abstract A rapidly growing marine derived Bacillus sp. strain NQ-11/A2, identified as Bacillus megaterium, accumulated 61% polyhydroxyalkanoate by weight. Diverse carbon sources served as substrates for the accumulation of short chain length polyhydroxyalkanoate. Three to nine granules either single or attached as buds could be isolated intact from each cell. Maximum activity of polyhydroxyalkanoate synthase was associated with the granules. Granule-bound polyhydroxyalkanoate synthase had a $K_m$ of 7.1 x $10^{-5}$ M for DL-β-hydroxybutyryl-CoA. Temperature and pH optima for maximum activity were 30°C and 7.0, respectively. Sodium ions were required for granule-bound polyhydroxyalkanoate synthase activity and inhibited by potassium. Granule-bound polyhydroxyalkanoate synthase was apparently covalently bound to the polyhydroxyalkanoate-core of the granules and affected by the chaotropic reagent urea.

Keywords Polyhydroxyalkanoate (PHA) · Granule bound PHA synthase (GBPS) · Marine derived Bacillus · Short chain length—PHA (SCL-PHA)

Introduction

Bioplastics have received tremendous attention in the recent years due to their high molecular weight, thermoplastic/elastomeric properties, biodegradability, biocompatibility, non-toxicity and its production from renewable carbon sources. Polyhydroxyalkanoates (PHAs) are simple macromolecules synthesized by a wide variety of Gram-positive and Gram-negative bacteria, and members of family Halobacteriaceae of the Archaea (Philip et al. 2007; Hezayen et al. 2002). The synthesis of the polymer is initiated when acetyl-CoA is restricted from entering the tricarboxylic acid cycle due to the nutrient limitation, shutting the acetyl units from the TCA cycle into the production of polyhydroxybutyrate (PHB), which is an ideal carbon and energy storage polymer. Currently more than 150 different hydroxyalkanoic acids have been identified as substrates by PHA synthases, thereby determining the type of PHA produced by the organism.
PHAs Production Using Low-Cost Agro-Industrial Wastes by Bacillus sp. Strain COL1/A6

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Abstract: Recycling of wastes generated from agro-based industries for polyhydroxyalkanoate production is not only crucial for waste management but also in economizing and commercializing the polymer. In this study, the heterotrophic bacterium Bacillus sp. strain COL1/A6 isolated from humus was biologically characterized and explored for its potential to synthesize PHA using agroindustrial wastes. Qualitative analysis using Nile blue A staining revealed that starch, wafer residue, citrus pulp and cane molasses proved to be excellent carbon substrates for PHA accumulation. Growth and PHA producing ability of the isolate on cane bagasse and rice chaff improved after dilute acid hydrolysis. Highest cellular PHA content was obtained using wastes such as hydrolyzed wafer residue (62.41±1.04% of dry cell wt.) followed by cane molasses (54.68±1.36% of dry cell wt.) and hydrolyzed citrus pulp (47.5±1.01% of dry cell wt.). This is the first report wherein a Bacillus sp. has been reported to grow and utilize wastes such as wafer residue and citrus pulp as carbon feedstock for PHA production.

Key words: Bacillus sp., humus, low-cost, polyhydroxy alkanoate, agroindustrial wastes

INTRODUCTION

The extensive usage of petrochemical plastics due to their versatile properties especially durability is causing severe problem in waste management affecting the aesthetic quality of cities, water bodies and natural areas (Full et al., 2006). As a result, lot of research is now focused on the production of biodegradable plastics. Polyhydroxyalkanoates (PHAs) are the only naturally occurring polymers that are 100% biodegradable (Khanna and Srivastava, 2005). The wide spread use of this polymer is restricted only to areas where conventional plastics find limited applications such as the medical field due to its high production cost (Verlinden et al., 2007; Valappil et al., 2007a).

Process economics reveal that the use of inexpensive and renewable carbon substrates viz. agro industrial wastes and byproducts as PHA feedstock can contribute to as much as 40-50% reduction in the overall production cost (Choi and Lee, 1999; Kim, 2000). Other parameters which also influence the total production cost are bacterial strains, fermentation strategies and recovery processes. Currently, Gram negative microorganisms such as Cupriavidus necator, Alcaligenes latus and recombinant Escherichia coli are used for commercial polymer production (Valappil et al., 2007a). They contain Lipopolysaccharide (LPS) endotoxins which co-purify with PHA. This limits the application of the polymer in medical field as LPS can elicit severe immunological reactions (Valappil et al., 2007a; Chen and Wu, 2005). Synthesis of LPS free polymer requires additional purification step increasing the production cost. Gram positive bacteria such as Bacillus sp. are ideal candidates for industrial scale PHA production due to the lack of LPS layer. Members of this genus are known to grow rapidly, possess various hydrolytic enzymes and produce copolymers from structurally unrelated carbon sources (Valappil et al., 2007b; Halami, 2007). These very characteristics of Bacillus sp. can be exploited for the production of PHA with desirable material properties from various low-cost agricultural feedstocks.

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A simple method for PHA production by Bacillus sp. COL1/A6 utilizing waste coconut oil cake

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ABSTRACT

The bacterial strain, COL1/A6 used in this study was phenotypically characterized and identified as Bacillus sp. Ability of this isolate to utilize waste coconut oil cake as carbon feedstock for polyhydroxyalkanoate (PHA) production was exploited. The innovative method developed for polymer synthesis yielded 41.92 % PHA of total cell dry weight. The polymer purity was confirmed by detecting the presence of a single crotonic acid peak at 235 nm. This is the first report on the use of waste coconut oil cake as sole source of carbon for PHA production.

Keywords: Bacillus, humus, polyhydroxyalkanoate, biodegradable, carbon feedstock, coconut oil cake

INTRODUCTION

The global production of oil seeds for the year 2007-08 is estimated to be about 392.6 million tonnes with India being one of the world’s leading oil seed producers (FAO 2008). During the oil extraction process, the major by-product generated is the oil cake which contributes to almost 50 % of the discarded waste. These cakes can be used as fertilizers, fuel or animal feed due to their rich protein content, but are mostly disposed off as waste in the environment (Ramachandran et al. 2007).

Utilization of oil cakes as potential raw materials in bioprocesses is advantageous since they can serve as excellent substrates for the growth of microorganisms. With increasing emphasis on cost reduction of industrial processes, attempts are now being made to exploit such agro-industrial wastes for production of value added commodities such as enzymes, amino acids, organic acids and mushrooms (Joo and Chang 2005; Pandey 2003; Sircar et al. 1998; Shashirekha et al. 2002). In the recent past, attempts have been made for producing polyhydroxyalkanoates (PHAs) using these wastes (Singh and Mallick 2008; Oliveira et al. 2004).
Studies on polyhydroxyalkanoate production by a marine *Bacillus* sp. NQ-11/A2 isolated from continental shelf sediment

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ABSTRACT

The marine bacterial strain *Bacillus* sp. NQ-11/A2 isolated from continental shelf sediment exhibited bright orange fluorescence indicating PHA production. Optimum temperature for growth and PHA accumulation was found to be 30 °C. Out of the five diverse carbon rich wastes tested as substrates for PHA production, maximum growth and PHA accumulation was obtained using sugarcane molasses. Among the different inorganic nitrogen sources supplied, ammonium chloride supported excellent PHA production. The polymer accumulated by the isolate using glucose as sole carbon source was characterized. Fourier transform-infrared (FT-IR) spectroscopy confirmed the aliphatic nature of the polymer with a low Crystallinity Index and a thermal melting temperature \( T_m \) of 167 °C.

**Keywords:** Polyhydroxyalkanoate, Bacillus, continental shelf sediment, glucose, carbon rich wastes, feedstock.

INTRODUCTION

Biodegradable plastics are gaining tremendous attention globally not only due to the rising oil prices but also because of the problems associated with the disposal of conventional plastics. Polyhydroxyalkanoates (PHAs) are polyesters synthesized by a wide range of bacteria when a carbon source is present in excess and one of the essential growth nutrients is limiting (Rehm 2007). The basic unit and the most common member of PHA is poly-3-hydroxybutyric acid \([P(3HB)]\), a homopolymer made up of repeating units of (R)-3HB. It is hard and brittle unlike P(3HB-co-3HV), a copolyester containing randomly arranged units of (R)-3-hydroxybutyrate and (R)-3-hydroxyvalerate, which is an elastomer. The copolymer possesses superior material properties and finds application in many fields including manufacture of consumer products such as plastics, films and fibres (Rehm 2003). This copolymer was produced at an industrial scale for the first time in the year 1982 by Imperial Chemical Industries Ltd. (ICI), England using the Gram-negative bacterium, *Alcaligenes eutrophus* and the commercialized product was trade named “Biopol” (Lenz and Marchessault 2005).