Production of Poly-(3-hydroxybutyrate) by *Bacillus* sp. 112A, Utilizing some Waste Starchy Materials

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Production of poly-(3-hydroxybutyrate) by *Bacillus* sp. 112A isolated from activated sludge sample using flours of ten different pulses and cereals, such as, pigeon pea, red lentil, black gram, bengal gram, green gram, corn, soya bean, wheat, rice and sorghum available in abundant as spills was in a range of 1.20 g/L to 0.14 g/L. *Bacillus* sp. 112A was able to accumulate a maximum of 1.20 g/L PHB within 30 h of incubation when corn flour (25 g/L) was used as carbon substrate suggesting a very faster rate of polymer synthesis when compared to all other substrates.

Key words: *Bacillus* sp., GC, Polyhydroxybutyrate, Production, Waste starchy materials.

At present, from an environmental view point, better biodegradable polymers are widely sought to replace synthetic polymers in the material industries and Polyhydroxyalkanoates (PHA) seems to be one of the favourable source for such polymers based on its favourable characteristics. Poly (3-hydroxybutyrate) (PHB) is a typical PHA and has been investigated in detail. PHB is biodegradable thermoplastic polyester that can be considered analogous to many conventional petrochemical-derived plastics currently in use (Holmes, 1985). Due to potential applications in medicine, agriculture and marine fields (Lee *et al.*, 1999), the production of PHB by microorganisms has drawn much attention in recent years. However, one of the most important factors that would popularize the use of these polymers as conventional plastics is its production cost (Chen *et al.*, 1999). Therefore, less expensive substrates, improved cultivation strategies and easier downstream processing methods are required for reducing the cost (Ahn *et al.*, 2001).

In this paper, we describe PHB production from starchy waste materials by *Bacillus* sp. 112A, a bacterium isolated from activated sludge sample in a previous investigation and deposited at Institute of Microbial Technology, Chandigargh, India as MTCC 9719. (Thirumala *et al.*, 2010).

MATERIALAND METHODS

For the production of PHB from *Bacillus* sp. 112A, the isolate was cultivated in E2 mineral broth, a nitrogen limiting media (Lageveen *et al.*,

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1988), with 2 % (w/v) carbon source. After the inoculation with overnight grown 4% (v/v) inocula, the flasks were incubated at 30° C for 48 h on an orbital shaker at 150 rev min^{-1}. PHB was extracted from the *Bacillus* isolate 112A by using the hypochlorite method (Rawte and Mavinkurve, 2002). The polyesters content of the cell was determined by the using gas chromatography (GC) analysis (Reddy *et al.*, 2008).

Flours of ten different pulses and cereals, such as, pigeon pea, red lentil, black gram, bengal gram, green gram, corn, soya bean, wheat, rice and sorghum available in abundant as spills were collected from local flour mills and tested as cheap substrates for PHB production. These different substrates were used in different concentrations (5-25 g/L) in the E2 media substituting the carbon substrate for PHB production (Table 1). The experiments were done with all other optimal conditions and in triplicates. The means of the results of experiments conducted in triplicates were presented in this paper.

Table 1. PHB produced (g/l) by the *Bacillus* sp. 112A from various concentrations of flours

Conc. of flour (g/l)	Bengal gram	Pigeon pea	Soya bean	Red lentil	Corn	Wheat	Sorghum	Rice	Black gram	Green gram
5	0.44	0.34	0.44	0.22	0.51	0.40	0.14	0.44	0.22	0.51
10	0.53	0.38	0.91	0.33	0.43	0.53	0.28	0.51	0.33	0.43
15	0.54	0.47	0.69	0.47	0.52	0.64	0.47	0.69	0.47	0.52
20	0.33	0.27	0.52	0.66	0.42	0.77	0.37	0.52	0.66	0.42
25	0.36	0.59	0.29	1.05	1.20	0.86	0.59	0.29	0.54	0.20

RESULTS AND DISCUSSION

The highest yield of PHB was found with corn flour as the carbon substrate as evident in the Transmission Electron Microscopic (TEM) photograph (Fig. 1). Time dependent studies on the production of biomass and PHB by *Bacillus* sp. 112A in the corn flour containing medium was shown in Fig. 2. *Bacillus* sp. 112A was able to accumulate a maximum of 1.20 g/L PHB within 30 h of incubation when corn flour (25 g/L) was used as carbon substrate suggesting a very faster rate of polymer synthesis when compared to all other substrates (Table 1). This was followed by red lentil flour, soya bean flour, wheat flour, rice flour, black gram flour, pigeon pea flour, sorghum

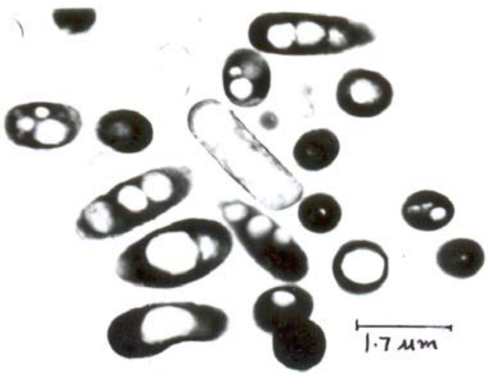

Fig. 1. TEM showing polymer granules in *Bacillus* sp. 112A (corn flour)

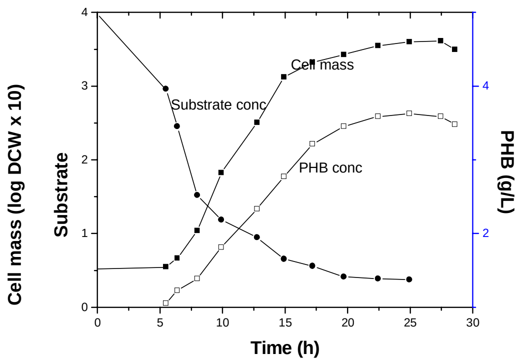

Fig. 2. Time dependent production of biomass and PHB by *Bacillus* sp. 112A (corn flour)

flour, bengal gram flour and green gram flour. Same concentration of PHB (0.59 g/L) was produced with pigeon pea flour and sorghum flour (25 g/L) as carbon substrates. All these cereal and pulse flours yielded PHB in a range of 1.20 g/L to 0.14 g/L at different (5-25 g/L) carbon source concentrations. Though, the PHB concentration from these flours was less when compared to PHB concentration from other carbon and nitrogen substrates, this could be a significant investigation because as mentioned in many earlier reports (Law *et al.*, 2001), the cost of carbon substrate is very much important in the economical production of PHB from bacteria.

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