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Research Paper

ISOLATION AND OPTIMIZATION OF CULTURE CONDITIONS FOR POLY-β-HYDROXYBUTYRATE (BIO-POLYMER) PRODUCING BACTERIA

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Abstract

Polyhydroxybutyrates are biodegradable polyesters synthesized by many bacteria.. PHB is produced variety of microorganisms under appropriate conditions. Due to their biological origin it is an advantage of PHB is, they are degraded naturally and completely to carbon dioxide and water under natural environment by the enzymatic activities of microbes. The present study reports the isolation and screening of soil bacteria and subsequent PHB production under normal conditions. Out of a total of 54 isolated strains two strains were selected for PHB production in different conditions. PHB production conditions were optimized with different carbon sources. The highest PHB production was observed with sucrose and fructose. The isolates were characterized biochemically as *Bacillus species.* The present study provide the useful data about the optimized conditions for PHB production by *Bacillus species* that can be utilized for industrial production of PHB, a fast emerging alternative of non biodegradable plastics.

Key words: Polyhydroxybutyrate (PHB), *Bacillus species*.

INTRODUCTION

Polyhydroxybutyrate (PHB) is a suitable source for biodegradable polymer material because their fully degradability and nonpollutant characteristics[1] PHB is a natural, biodegradable polymer, which is accumulated in the form of intracellular granules by large number of bacteria. These granules acts as energy reserve materials when nutrients such as nitrogen and phosphorus sources are available in limiting concentrations in the presence of excess carbon sources [2,**3**]**.** Among PHAs, PHB is the best known polyester, due to its similarity to synthetic petroleum–based plastics such as polypropylene. PHB has many applications in medicine, veterinary practice, tissue engineering materials, food packaging and agriculture [**4**].

In spite of the advantages of PHAs compared with petroleum-derived plastics, their use is currently limited due to their high production costs [**5, 6**]. The low price of crude carbon substrates such as cane and beet molasses, cheese whey, plant oils and hydrolysates of starch, cellulose and hemicellulose make them attractive substrates for producing PHAs by several bacteria utilizing them [**7**]**.** More than 250 different microorganisms synthesizing PHAs, only several of these, such as *Alcaligenes eutrophus, Alcaligenes latus, Azotobacter vinelandii, methylotrophs, Pseudomonas oleovorans and recombinant Escherichia coli* are suitable for the production of PHAs to a high concentration with high productivity [**8,9**].Keeping this mind, the

present study was planned to isolate PHB producing microorganisms and optimization the culture conditions for maximum PHB production.

MATERIALS AND METHODS

Isolation of PHB producing bacteria from different samples

Isolation of PHB producing bacteria form different soil, sewage and solid waste were collected from Noida Industrial Waste and Saharanpur, 1 gm of soil sample was suspended in 9 ml of sterilized distilled water and was shaken vigorously for 2 minutes. Soil samples were heat treated for 20 minutes at 50°C in order to enrich the sporulating bacteria belonging to the genus *Bacillus.* After that the suspension was serially diluted from 10^{-3} to 10^{-7} on nutrient agar plates. Plates were incubated at 37°C for 24 to 48 hrs.PHB producing bacteria was detected using the lipophilic stain Sudan Black B [9, 10]. The positive isolates were transferred to nutrient agar slants.

Quantification of PHB production and selection of isolates

All the Sudan Black B positive isolates were subjected to quantification of PHB production as per the method of John and Ralph 1961. The bacterial cells containing the polymer were pelleted at 3,000 rpm for 10 min and the pellet washed with acetone and ethanol to remove the unwanted materials. The pellet was resuspended in equal volume of 4% sodium hypochlorite and incubated at room temperature for 30 min. The whole mixture was again centrifuge and the supernatant discarded. The cell pellet containing PHB granules were dissolved in hot chloroform. The chloroform was filtered and to the filted, concentrated 10 ml of hot sulfuric acid was added. The addition of sulfuric acid converted the polymer into crotonic acid which is brown colored. The solution was cooled and absorbance read at 235 nm against a sulfuric acid blank. By referring to the standard curve, the quantity of PHB produced was determined. Based on the PHB yield, promising bacterial isolates was selected [11]. Standard curve of PHB was prepared following the method of Law and Slepecky (1969). Pure PHB (Sigma,USA) was used to prepared the standard curve.

Optimization of culture conditions for maximum PHB production

Effect of different carbon sources at 1% use as substrates for PHB production

PHB production by the selected bacterial isolates NIW13 and SWS6 were grown in 250 ml conical flasks containing 100 ml MSM broth with best waste at different 5% and different carbon sources like glucose, sucrose, fructose, and maltose at 1%. The flasks were incubated at 30°C for 48 hours. After incubation PHB produced by the isolates were quantified spectrometerically following the method of John and Ralph (1961).

RESULTS

Isolation and quantitative screening of the isolates for PHB production

The microbial isolates positive for Sudan Black B stain were quantitatively screened for PHB production and results obtained (Fig 1) showed that the isolates (NIW13 from Noida Industrial Waste and SWS6 from Saharanpur Waste Sample). The organisms were grown in MSM broth was used for the quantification. Based on PHB yields, 2 promising isolates were selected (Fig 1). They were NIW13 and SWS6. They were selected from samples by quantification. NIW13 yielded PHB of 6.8 mg/100 ml and SWS6 yielded PHB of 9.2 mg/100 ml.

Selection of different carbon sources use as substrates for PHB production

The data in Table 1 depicts the effect of different carbon sources for PHB yield. The results interpreted significantly differences for the isolates, waste materials and their interactions as well. Among the different carbon sources tested to evaluate their effects for PHB yields.During selection of various carbon sources for PHB production, Out of them sucrose and fructose were to be best carbon source for NIW13 and SWS6. NIW13 yielded 10.2 mg/100 ml and SWS6 yielded 10.8 mg/100 ml PHB in whey.

Identification of Bacteria

On the basis of PHB screening method and PHB production, two isolates frorm Noida Industrial Waste and Saharanpur Water Sample were selected for further identification. ***Bacillus cereus***

and ***Bacillus subtilis*** were taken as a positive standard. Biochemical identification of the isolates was done by the use of Hi-media identification kit

DISCUSSION

Most of the plastics and synthetic polymers are produced from petrochemicals. Because of their persistence in the environment, several communities are more sensitive to the impact of discarded plastics on the environment. Consequently, for the past two decades, there have been growing public and scientific interests in the development and use of biodegradable polymers as an ecologically useful alternative to plastics.

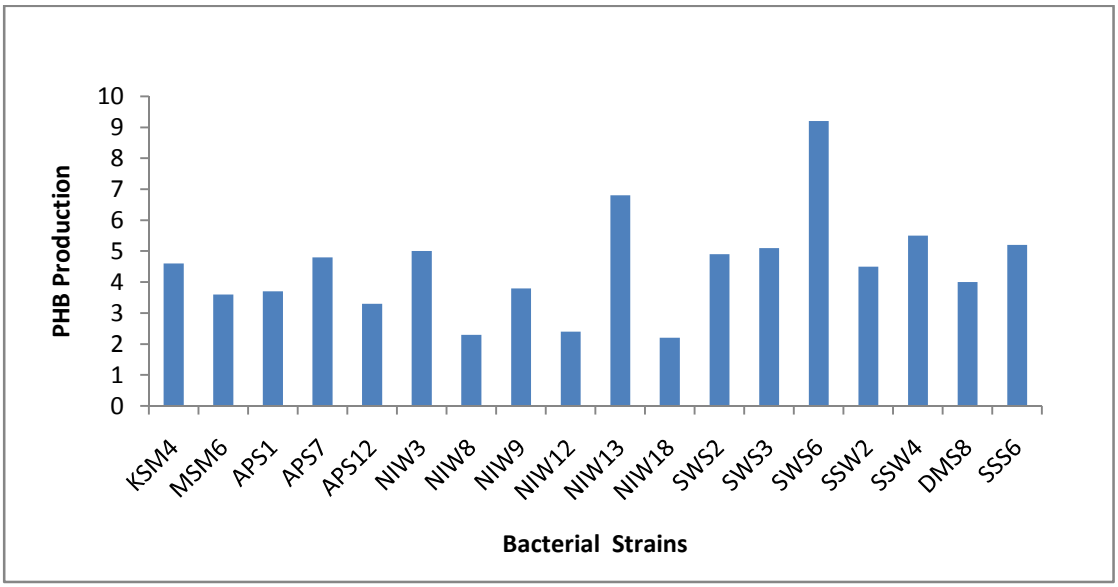

Fig 1 : PHB production of different bacterial strains

Table:-1 Effect of different carbon sources used as substrates for PHB (mg/100ml) produced by selected bacterial isolates

Different carbon sources	PHB yields (mg/ 100 ml)	
	NIW13	**SWS6**
Glucose	7.2	9.3
Fructose	7.8	**10.8**
Sucrose	**10.2**	9.8
Maltose	8.8	10.0

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