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# Poly-Beta-Hydroxybutyrate (phb) from Bacillus Cereus Production

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## Abstract

Plastics associated polymers area unit an integral a part of our daily existence. However, thanks to their properties of sturdiness and resistance to degradation, they accumulate within the surroundings at the speed of concerning twenty-five million tons per year. These type 8 May 1945 by weight and 2 hundredth by volume of the landfills. They inhibit catalyst activities because of extremely hydrophobic in nature. The low area of plastics with inherent high mass additional compounds the problem. Environmental issues related to usage and particularly spread of plastics has stirred the formulation of legislations regulation compound use. With increasing public and political awareness, and to satisfy the environmental imperatives large analysis has been directed towards finding appropriate substitutes that area unit perishable besides holding the required properties of the standard plastics. perishable polymers from renewable purpose of sources area unit of specific interest, that work into the ecological cycle. Since the potential for utilizing biological systems as a supply of perishable materials is turning into more and more engaging, polyhydroxyalkanoates have gained importance, as they possess properties getting ready to plastic.

**Key words:** Bacillus cereus; Screening; Endospore; Biopolymer.

## INTRUDUCTION

Plastics associated polymers area unit an integral a part of our daily existence. However, thanks to their properties of sturdiness and resistance to degradation, they accumulate within the surroundings at the speed of concerning twenty-five million tons per year [1,2]. These type 8 May 1945 by weight and 2 hundredth by volume of the landfills [3]. They inhibit catalyst activities because of extremely hydrophobic in nature. The low area of plastics with inherent high mass additional compounds the problem [4]. Environmental issues related to usage and particularly spread of plastics has stirred the formulation of legislations regulation compound use. With increasing public and political awareness, and to satisfy the environmental imperatives large analysis has been directed towards finding appropriate substitutes that area unit perishable besides holding the required properties of the standard plastics. perishable polymers from renewable purpose of sources area unit of specific interest, that work into the ecological cycle [5]. Since the potential for utilizing biological systems as a supply of perishable materials is turning into more and more engaging, polyhydroxyalkanoates have gained importance, as they possess properties getting ready to plastic.

The chemical nature of those biopolymers is 1st studied in eubacteria megaterium that exist as inclusion bodies [6,7] and named them as poly-3-hydroxy-butyricacid P (3HB). This compound became a lot of in style because of studies on many eubacteria strains [8] and phototropic bacteria [9]. The identification of polyhydroxyalkanoates apart from P (3HB) particularly poly-3-hydroxyvalerate P (3HV) and poly-3-hydroxyhexanoate P (3HHx) [10]. Accumulation of ninety fifth P(3HB), three-d poly-3-hydroxyheptanoate P(3HHp), two of Associate in Nursing 8-carbon hydroxyalkanoate (HA) and trace amounts of 3-other hour angle compounds by batch big eubacteria megaterium mistreatment capillary gas action was reported [11].

Poly-3-hydroxy-butyric acid has restricted applications because of its brittle nature. Incorporation of different 3HA considerably increased the biopolymer properties that significance the start of the second stage of analysis on PHAs. Production of P (3HB-co-3HV), a co-polymer of business importance, passed throughout this stage. the stress currently shifted to identification of all the 3HAs that might be related to the microorganism polyesters. throughout this era, it became clear that not solely Gram negative however conjointly a good vary of Gram-positive microorganism, true bacteria (aerobic photosynthetic), non-sulfur and sulfur phototrophic bacteria (anaerobic photosynthetic), archaea will synthesize and accumulate these 3HAs.

The present study reports on isolation Associate in Nursing identification of an economical biopolymer manufacturing microorganism.

## MATERIALS AND METHODS

### Screening for Biopolymer Producing Bacteria

1 gram of soil samples, collected from starch industrial effluent sites around Hyderabad, India, were suspended in numerous a hundred milliliter sterile water flasks and serially diluted up to 10-8. The samples were agitated for ten minutes on a shaker and zero.2 milliliter of samples were unfold on mineral salts medium plates containing (g/l) disaccharide a pair of0.0; $KH_2PO4$1.0; $(NH_4)2SO_4$ a pair of.0; $MgSO_4$ $7H_2O$ 0.2; $CaCl_2$ $2H_2O$ 0.02; $FeSO_4$ $7H_2O$ 0.01. To the on top of answer, one milliliter of trace answer containing (g/l) $ZnSo_4$ $7H_2O$ 0.2; $H_3BO_3$ 0.6; $MnCl_2$ $4H_2O$ 0.06; $CoCl_2$ $6H_2O$ 0.4; $CuSO_4$ $4H_2O$ 0.02; $NaMoO_4$ $2H_2O$ 0.06 was additional. The hydrogen ion concentration of the medium was adjusted to 6.5 ± 0.2. The plates were incubated at $35^0$C for forty-eight hours. The isolated colonies with totally different colony morphologies were picked and therefore the cultures were sublimate by recurrent streaking on mineral salts medium plates and at last pure cultures of isolates were maintained on medium slants at $4^0$C.

### Analytical Ways

Bacteria obtained from the on top of study were screened for PHB accumulation capability. The PHB accumulated altogether isolates were assayed mistreatment chemical analysis method[12].The isolates were on an individual basis inoculated in to fifty millilitre mineral salts medium broth in 250 milliliter round shape flasks and were incubated on rotary shaker at 350C with 225 rate /min for forty eight hours. a pair of milliliters of culture was collected at intervals of twenty-four and forty-eight hours and centrifuged at ten thousand rates for quarter-hour. The supernatant was discarded; the pellet was washed doubly with water and centrifuged at ten thousand rates for quarter-hour. the ultimate pellet therefore obtained was dried in hot air kitchen appliance at 600C for twenty-four hours. once drying, the full microorganism Leclanche cell weight determined. The dried cells were lysed by incubating with antimicrobic for one hour at 600 C. Lysed samples were then finally subjected to natural process at ten thousand rates for quarter-hour and supernatant was collected in an exceedingly sterile tube. To the collected supernatant, five milliliters of ninety-six grain alcohol and five milliliters of dissolving agent (1:1) was additional. ten milliliter Chloroform was additional into every tube to extract PHB. The chloroform was then gaseous at temperature in order that, the PHB granules seem as crystals. Finally, ten milliliter of Conc. $H_2SO_4$ was additional and heated for one hour at 600 C to convert PHB into crotonic acid. Crotonic acid shaped was measured at 235 nm against sulfuric acid blank mistreatment UV- visible photometer (UV-160A Shimadzu). the number of PHB per gram dry weight of microorganism cells determined employing a normal curve of PHB (Sigma).

Morphological and Organic Chemistry Characterization

Preliminary characteristics of the microorganism capable of accumulating PHB were studied by perceptive morphological characteristics and therefore the economical microorganism among them was any known by finding out organic chemistry characteristics as per Bergey's manual of determinative bacteriology [13]. PHB granules accumulated within the microorganism were discovered by sudan black –B staining.

## RESULTS AND DISCUSSION

Screening of soil samples resulted in obtaining 18 colonies, of which, six colonies showed different colony morphologies and were selected for further characterization. Preliminary morphological characterization and the PHB accumulation efficiency of these six bacterial isolates is given in Table 1. PHB accumulation of a Gram-positive endospore forming Bacillus was observed to be more than the other five isolates in Table 1. Cocci isolated during this study, failed to accumulate PHB under the tested conditions. The morphological and some other growth characteristics of efficient PHB producing isolate is given in Table 2.

**Table 1:** Preliminary characterization of bacterial isolates obtained during this study.

| S.No | Grams Nature | PHB production |
|---|---|---|
| 1 | Gram positive *Bacilli* | 7.69% |
| 2 | Gram negative *Bacilli* | 5.0% |
| 3 | Gram positive *Bacilli* with endospore | 20.28% |
| 4 | Gram positive *Cocci* | Not detected |
| 5 | Gram negative *Cocci* | Not detected |
| 6 | Gram negative short *Bacilli* | 6.0% |

**Table 2:** Morphological and some physiological studies of efficient PHB producing Bacteria isolated during this study.

| S.No | Morphological characters | Results |
|---|---|---|
| 1 | Cell shape | Rod |
| 2 | Motility | Random Motility |
| 3 | Endospore | Positive, subterminal and oval |
| 4 | Colony shape | Minute, mucoid, round and raised |
| 5 | Optimum temperature | 350–370 C |
| 6 | Optimum pH | 6.5–7.0 |

The isolate gave positive result for methyl red, citrate, lysine decarboxylase and ornithine decarboxylase and negative result for urease, nitrate reduction, tryptophanase and Voges-proskauer test. Based on the morphological and biochemical characterizations, the efficient PHB producing bacterial Isolate was identified as Bacillus cereus.

Sudan black –B staining of PHB accumulated in Bacillus cereus was observed as round stained granules, considered to be fat bodies prominently in almost all the rods from the samples collected at 24 hours (Figure 1). However, samples collected at 48th hour showed more prominent PHB granules (Figure 2). Transmission electron micrograph of the PHB accumulated in the bacterium is shown in (Figure 3).

Polyhydroxyalkanoates (PHAs) are aliphatic polyesters naturally produced via microbial process on Carbohydrates based medium, where they act as carbon and energy storage material in bacteria. They were the first biodegradable polyesters to be utilized in plastics. The two main members of the PHA family are polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV). Poly β-hydroxybutyrate (PHB) accumulates as energy reserve material in many micro-organisms like Alcaligenes, Azotobacter, Bacillus, Nocardia, Pseudomonas, Rhizobium, etc. PHB is accumulated during the stationary phase of growth by these organisms and used later as an internal reserve of carbon and energy.

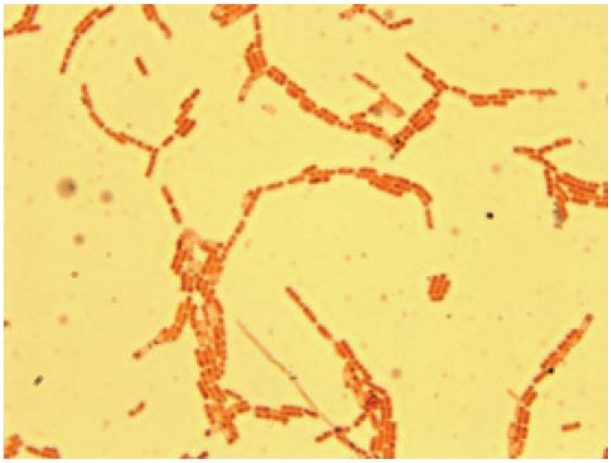

**Figure 1:** Sudan black staining of PHB accumulated in *Bacillus cereus* in 24 hours.

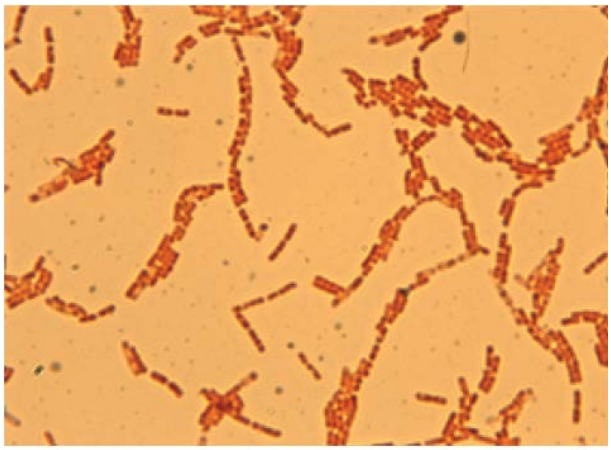

**Figure 2:** Sudan black staining of PHB accumulated in *Bacillus cereus* in 48 hours.

**Figure 3:** Transmission electron micrograph of PHB accumulated in *Bacillus cereus*.

PHB is an energy storage material produced by a variety of bacteria in response to environmental stress and nutrient imbalance. PHB exists as discrete granules in the cell cytoplasm with an average number of 8-12 granules per cell and are typically of 0.2-0.5μm in diameter and possess a membrane coat of 2-3nm thick. Because PHB is biodegradable, there is considerable interest in using PHB for packaging purposes as opposed to other plastic materials in order to reduce the environmental impact of human garbage.

## CONCLUSION

Many B species are reportable to accumulate PHB at 9%–44.5 dry out cell weight similarly; within the gift study we've got isolated B Cereus that has the capability of accumulating twenty.28% PHB. The isolate PHB accumulation potency may be increased by optimizing physiological conditions. The PHB yield can even increase by subjecting the isolate to varied mutagens and choosing a mutant with higher PHB accumulation than the wild kind. B. Cereus is present in nature associated a timeserving infective agent, typically related to 2 kinds of human illness, characterized by either symptom and abdominal distress or nausea and unconditioned reflex. The organism produces associate remedy poisonous substance and enter poisonous substance to blame for these symptoms. To the simplest of our data, we tend to area unit initial to report a unique strain of B Cereus with significant potency in accumulating PHB. any improvement studies area unit understudy which might promise higher yields of PHB from these bacteria.

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