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SHORT COMMUNICATION **OPEN ACCESS**

Isolation and Screening of the Polyhydroxy Butyrate (PHB) Producing Bacteria from Soil

Jayashri. P. Nanaware*, Shubham V. Nasale, Gulzar B. Shaikh, Pathade G. R.
Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth, "Deemed to be University", Karad

ABSTRACT

Poly-B-hydroxybutyrate (PHB) is a biodegradable and biocompatible thermoplastic produced by various microorganisms. It can be made into films, fibers, sheets, even molded to the shape of bag and bottle. Poly-B-hydroxybutarate (PHB) and poly-hydroxylvaleric acid (PHV) are being developed a variety of applications. The aim of this study was to isolate and characterize the bacteria from soil sample and the production, purification and determination of amount of PHB by dry weight determination. The six PHB producing bacteria from fertile soil samples where isolated and characterized for their morphological, and biochemical properties and they were designated as PHB1, PHB2, PHB3, PHB4, PHB5, & PHB6. The bacteria were further screened for PHB production by Sudan Black B staining out of six bacteria the three bacteria designated as PHB1, PHB2 & PHB3 showed the positive result for Sudan Black B staining. The highest PHB production was found in PHB1 and PHB2 and was 50% on dry weight basis and tentatively identified as Bacillus cereus, and Bacillus subtilis. These two strains seem to be promising for the PHB production and further optimization work is in process.
***Key words**: Polyhydroxybutyrates (PHB), Bioplastic, Soil, PHV.*

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INTRODUCTION

Polyhydroxybutyrate (PHB) is a biodegradable and biocompatible thermoplastic, there are a class of bacterial polyesters collectively called polyhydroxyalkanoates (PHAs), accumulated intracellularly as reserve granules by many bacteria in harsh environmental conditions. PHB was first isolated and characterized in 1925 [2]. PHB is primarily a product of carbon assimilation and is used by micro-organisms as a form of energy storage molecule. It can be made into many forms and shapes. PHB & PHV (polyhydroxy valeric acid) are being developed for a variety of applications [3]. PHB differentiates itself from other biodegradable plastics it has unique properties like insoluble in water, highly resistant to hydrolytic degradation, oxygen permeability, UV resistant, other biodegradable plastics are moisture sensitive and water soluble.PHB is poor resistance to acids and bases, soluble in chloroform and other chlorinated hydrocarbons and biocompatible and hence it is suitable for medical application.Production of PHB has most commonly been studied on microorganisms belonging to the genera *Alcaligenes, Azoto bacter, Bacillus and Pseudomonas*[10]. The rapid phenotypic screening methods are applied to the screening of PHB producers, including Sudan Black B, Nile red and Nile blue A staining [11]. The production cost of PHB could be lowered by enhancing bacterial cells growth to produce a large amount of polymer by using cheaper raw materials or waste products as carbon sources; for example, molasses and crude glycerol. Many efforts have been devoted to reducing the production costs by isolating bacterial strains capable of growing and producing PHB from inexpensive raw materials and also optimization fermentation conditions for PHB production [12]. The aim of this study was to screen PHB producing bacteria from soil samples in Karad, Maharashtra, India.

MATERIALS AND METHODS

Collection of Soil Samples

Fertile soil samples from various places were collected from nearby karad,. Maharashtra, India.
Soil samples collected were in clean polythene bag and taken into laboratory for further studies.

Isolation of PHB Producing Bacteria from Soil

Serial dilution of the collected three soil samples were carried out according to the method of [4]. 1mL of diluents were spread inoculated on nutrient agar plates containing 1% glucose and glycerol and plate were incubated at 30˚C for 48h. Morphological appearance of the bacterial colonies on petri plates were

observed and well isolated colonies were further identified by morphological and Biochemical characterization (catalase, oxidase, carbohydrate fermentation) with reference to [5, 14].

Screening of PHB Producing Bacteria

The isolates were screened for PHB producing potential by staining with Sudan Black B stain and observed under microscope. isolates with PHB producing potential were stained light to deep red in color. The isolates were further screened for PHB production using submerged fermentation process. The medium for fermentation was nutrient agar containing 1% glycerol,1% glucose[6].Three isolates with high PHB producing potential were selected for further work.

Production of PHB Granules

For the production of PHB the simplified medium (g/100mL) was used: Glucose - 1g, Glycerol-1%, Peptone - 0.25g, Yeast extract – 0.25g, NaCl – 0.01g and pH at 7 [6].The medium was prepared and sterilized at 121°C and 1% inoculums(10^8 CFU/mL suspension of each isolate separately) was added to each flask to carry out fermentation. The flasks were incubated at 30°C for 48h.

Cell Dry Weight

After 48h incubation at 30^0C, culture medium was collected and centrifuged at 10,000 rpm for 15min. Supernatant was discarded and the cell pellet was dried at 30^0C to estimate the dry cell weight (DCW) in units of g/mL [7].

Extraction and Quantification of PHB

After 48h incubation at 30^0C, culture was collected and centrifuged at 10,000 rpm for 15min and lyophilized. The lyophilized pellet was digested with 4% sodium hypochlorite solution at 30^0C for 20min. Then itwascentrifuged at 10,000 rpm for 15min, the pellet was washed with water, acetone, ethanol, respectively. Finally, polymer was dissolved in chloroform and kept for complete evaporation [8]. Dry weight of extracted PHB was estimated as g-% /g dry weight. Residual biomass was estimated as the difference between dry cell weight and dry weight of PHB [13]. The percentage of intracellular PHB accumulation is estimated as the percentage composition of PHB present in the dry cell weight

PHB accumulation (%) = Dry weight of extracted PHB (g/mL) × 100 / DCW (g/mL)

RESULTS AND DISCUSSION

Isolation and Identification of PHB Producing Bacteria

The soil samples collected from various sources were used for isolation ofPHB producing bacteria. In all six PHB producing isolates were obtained. Which were further studied for PHB producing granules by Sudan Black B staining method. Out of six isolates three isolates showed positive results for Sudan Black B staining and they were designated PHB1, PHB2 and PHB3 (Photoplate-1). The isolates were identified based on morphological observation and biochemical characterization (Table-1 and 2). The identification of isolates was done with the help ofBergy's Manual of Determinative Bacteriology (1994). The isolates were identified as*, Bacillus cereus, Bacillus subtilis, Bacillus megaterium.*

Identification of Isolates

In present study, the three PHB producing bacterial isolates that produced PHB granules were characterized and were tentativelyidentified as *Bacilluscereus, Bacillus subtilis and Bacillus megaterium* (Table 3).

Quantification of PHB

The percentage of intracellular PHB accumulation was estimated as the percentage composition of PHB present in the dry cell weight from these results(Table-4). isolate PHB1 and PHB2 showing high PHB production(50%) whereas the PHB3 showing less PHB production (10%) compared with other PHB1 and PHB2[9].

Table 1: Morphological, cultural and Biochemical characterization of PHB producing Isolates

IsolateNo.	Size	Shape	Colour	Margin	Opacity
PHB1	1mm	Circular	White	Irregular	Opaque
PHB2	2mm	Circular	White	Irregular	Opaque
PHB3	1mm	Circular	White	Irregular	Opaque

Table-2: Biochemical Tests of Isolates

Test	PHB1	PHB2	PHB3
Indole production	+	-	-
Methyl red	-	+	-
Vogus-Proskauer	+	+	-
Citrate utilization	+	+	-
Urease production	+	+	+
Gelatin hydrolysis	+	+	+
Starch hydrolysis	+	+	+
Sugar Fermentation			
Glucose	+	+	+
Arabinose	-	+	D
Xylose	-	+	D
Mannitol	-	+	D

(+ =positive test) (- = negative test) D= differential

Table 3: Identification of Isolates of Bacteria

Isolates	Identification
PHB1	*Bacillus cerus*
PHB2	*Bacillussubtilis*
PHB3	*Bacillusmegaterium*

Table 4: Dry Cell Weight and Yield of PHB of Isolates

Isolate	Dry cell weight (g/L)	PHB (g/L)	Yield of PHB %
PHB1	**2**	**1**	**50%**
PHB2	**2**	**1**	**50%**
PHB3	**1**	**0.1**	**10%**

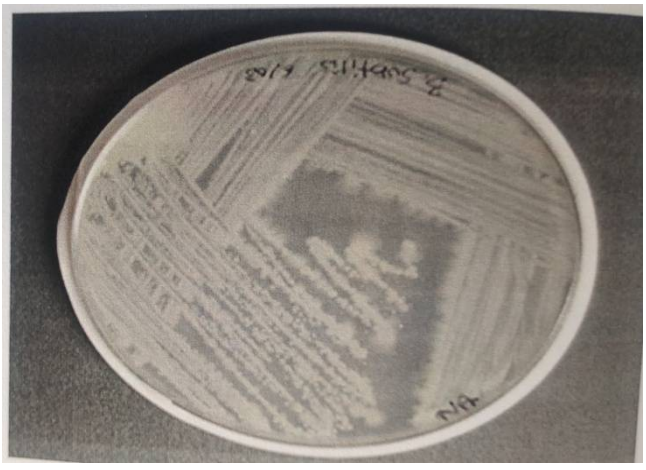

Fig. 1: Photoplate of Colonial Morphology of Isolateproducing PHB

CONCLUSIONS

The present study demonstrated that soil samples were collected from different areas of karad region for the isolation of PHB producing bacteria. In the present study Six PHBtproducing bacterial isolates were obtained out of which three isolates designated as PHB1, PHB2 and PHB3 showed positive result for Sudan Black B staining and produced highest PHB. The isolate PHB1 and PHB2 which was genera of *Bacillus cerus, Bacillus subtilis*. Was found to potent of PHB yield 50%. Hence the isolate PHB3 was genera

of *Bacillus megaterium* produce PHB yied 10%. Therefore, the study concludes that the isolate PHB1 & PHB2 can be used for to commercially for PHB Production.

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