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Isolation and characterization of Polyhydroxy butyrate (PHB) producing Bacillus species from agricultural soil

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Abstract

Polyhydroxy alkanets(PHAs), which is produced by several bacteria, is a biodegradable polymer that has many industrial and medical applications. The present study deals with the isolation, and characterization of biopolymer (PHB) producing bacteria from the agricultural soil. The biopolymer producing bacteria was isolated from soil samples like crop, nursery, and fodder fields. The bacteria were screened by morphological and biochemical characteristics. The bacteria were further identified by Sudan black- B staining and molecular characterization. High and efficient PHB accumulation bacteria was selected by quantification and extraction method. Further bacteria were confirmed by FTIR analysis to know the functional groups present in the bacteria. A total of six bacillus species were isolated, the strain BBKGBS12 and BBKGBS6 are gram positive and other isolated bacteria were gram negative. Black colour granules of bacteria BBKGBS6 were seen by Sudan Black-B staining. Also granules of isolated bacteria were fluorescence by acridness orange staining . BBKGBS6 identified to be a strain of Lysinibacillus sphaericus BBKGBS6 by 16S r- RNA sequencing and high PHB accumulation. FTIR analysis gave prominent functional groups like CH3, CH2, C=O, C-O, CH, and OH were identified and the results revealed that the poly-3-hydroxybutyrate compound was produced by Lysinibacillus sphaericus BBKGBS6 (Accession number KP403811). However the nursery field soil has showed high amount of PHB accumulation bacteria.

Keywords

Polyhydroxy butyrate(PHB), Polyhydroxy alkanets(PHAs), Fourier Transform Infra-Red Spectroscopy (FTIR)

INTRODUCTION

Polyhydroxy butyrate(PHB) is a biodegradable and biocompatible thermoplastic, there are a class of bacterial polyesters collectively called Polyhydroxy alkanets(PHAs), accumulated intracellular as reserve granules by many bacteria in harsh environmental conditions. PHB was first isolated and characterized in 1925. 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. 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 applications. Poly-β- hydroxy butyrate (PHB) is synthesized as an intracellular storage material and accumulates as distinct black granules during unbalanced growth in the cell; these are clearly visible in the cytoplasm of the cell. During the adverse conditions PHB is used by the cell as an internal reserve of carbon and energy.

MATERIALS AND METHODS

Sample collection and Isolation of PHB producing bacteria: The bacteria used in this study was collected from University of Agricultural sciences, Dharwad crop, Nursery and Fodder field soils for screening of high PHB producing bacteria and the below mentioned media is used as a nutrient source. Various samples which are collected were serially diluted and 10-5 dilution was plated on nutrient media (g/l), with Peptone- 2, Beef extract-2, NaCl-1, Agar-4, Distilled water- 1lt, the media was then autoclaved. These plates containing soil sample were incubated overnight.

Screening for PHB producing bacteria: All the bacteria isolates were tested for PHB production using Sudan black –B stain and also the PHB produces bacterial granules were detected by fluorescent staining methods using acridine orange as suggested by Senthilkumar and Prabhakaran. The bacterial granules were observed by Carl Zeiss and fluorescent microscope under 100X.

Morphological characterizations of isolated bacteria: Colony and cell morphology based on their colour, shape, margin, elevation, surface and arrangement of bacteria were studied.

Biochemical characterizations of isolated bacteria: Gram staining, Nitrate reduction test, MR, VP test, in dole test, citrate utilization test, oxidise test, gelatine liquefaction test, catalyse activity, H2S production, starch hydrolysis, D- fructose, sodium malonate, sodium acetate, D-fucose, D-Sorbitol, D- glucose, L-alanine, salicin, rhamnose, propinoic acid, valeric acid, trisodium citrate, Lproline, L-rhamnose, D-ribose, inositol, glycogen, capric acid, L-histidine, L-arabinose, hydroxyl butyric acid tests were carried out using standard protocols proposed by Cappuccino and Sherman, (14), for the biochemical characterization of isolates.

RESULTS AND DISCUSSION

Black colour granules were taken as a positive result. A number of Bacillus sp. has been reported to accumulate 9– 44.5% DCW PHB (17). By comparison, Bacillus sp.BBKGBS6 produced 70% PHB from glucose. A relatively high yield of PHB was obtained in these strains, hence these strains were considered as potent organisms for industrial application. Black colour colonies were taken as a positive result. Out of 50 soil samples three samples were shown positive results. The appearance of black coloured granules in the cell indicates PHB production.

Conclusion

In the present study showed that isolation of Biopolymer producing bacteria Lysinibacillus sphaericus BBKGBS6 which can utilize glucose in simple media containing only peptone as nitrogen source for PHB production has been identified and characterized from the soil. Among the three soil samples which were used Fodder field soil and nursery field soil gave the number of isolated single positive and high amount of PHB accumulation colonies respectively. Further bacterial biopolymer was confirmed by FTIR analysis gives prominent functional groups like CH3, CH2, C=O, C-O, CH, and OH were identified and the results suggest that the poly-3-hydroxybutyrate compound was produced by Lysinibacillus sphaericus BBKGBS6 (Accession No. KP403811). The production of PHB was found to increase along with the increase in the biomass.

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