INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY ADVANCED RESEARCH TRENDS
ISSN : 2349-7408
VOLUME II, ISSUE I, FEBRUARY 2015

POLY- β -HYDROXYBUTYRATE - A POTENTIAL BIOMATERIAL PRODUCTION BY *Azotobacter vinelandii*

Dr.C.MADHAVI
Lecturer, Dept. of Microbiology, Govt. Degree College (W), Guntur, A.P.

ABSTRACT

Poly-β-hydroxybutyrates (PHB) which are produced mainly by bacteria as carbon and energy reserves provide a degradable alternative to petrochemical plastics. So this study aimed to focus on production of bio-plastics as they are the promising technology that can change the scenario of plastic waste management. In this present study *Azotobacter vinelandii*, the PHB producing bacteria under optimized conditions was isolated from the marine soil collected from the surya lanka beach, Guntur District. The isolate was tested qualitatively for PHB production following the Fluorescence Staining method and identified by TLC. Quantification of PHB production was determined by spectrophotometry at 235nm. The present study provide the useful data about the carbon source for PHB production by *Azotobacter vinelandii* that can be utilized for industrial production of PHB, a fast emerging alternative of non biodegradable plastics.

Keywords: PHB, *Azotobacter vinelandii*, Fluorescence Staining method, TLC, spectrophotometry.

Introduction:

Many prokaryotes, including aerobic methylotrophic bacteria, accumulate poly- hydroxybutyrate (PHB) intracellularly as a carbon and energy reserve material. Methylotrophic bacteria employing the serine cycle for formaldehyde assimilation are able to accumulate up to 80% of PHB by dry weight, while methylotrophic bacteria with the Calvin-Benson-Bassham cycle of C1 assimilation can synthesize up to 20% of PHB by dry weight.

Applications of Biopolymers:

Expansion of plastic production and consumption is having a significant impact both visibly and invisibly on the environment and society. Improper disposal of plastics has threatened natural environment worldwide since long time ago. Conventional petrochemical plastics are recalcitrant to microbial degradation. Excessive molecular size might be mainly responsible for the resistance of these chemicals to

biodegradation and their persistence in soil for a long time. These non-degradable petrochemical plastics accumulate in environment at a rate of 25 million tons per year. To overcome this problem, the production and applications of eco-friendly products such as bio-plastics becomes inevitable. Decades have been invested on extensive research to develop biodegradable polymers as a substitute for petrochemical based polymers due to their eco-friendly nature.

Polyhydroxyalkonoates (PHA) are polyesters of various β - hydroxyalkanoates and are considered as a good alternative amongst other biodegradable polymers developed, due to their biodegradability, biocompatibility, use of renewable resources as raw material, plastic and elastomeric material properties similar to petrochemical based polymers . PHA's are accumulated as intracellular inclusion bodies by many Gram-positive and Gram-negative bacteria to levels as high as 90% of dry cell weight when carbon source is in excess but other nutrient supply (O, P, N and S) are in limiting. As being biodegradable and biocompatible, a large number of PHA's and its copolymers have been exploited as bioplastics, biomedical applications such as drug delivery, tissue engineering and in food packaging etc. Various researchers have isolated the promising micro-organisms from different environment such as municipal sewage sludge, marine microbial mats and marine environments.

Materials and methods

Collection of sample:

Under optimized conditions bacteria was isolated from the marine soil sample collected from the surya lanka beach, Guntur district. For rapid screening of PHB producers, nutrient agar medium supplemented with 1 % arabinose was sterilized by autoclaving at 121°C for 20 minutes and cooled to 45°C. The medium was poured into sterile Petri plates and allowed for solidification. The plate was divided into 6 equal parts and in each part soil sample was spotted. The plates were incubated at 30°C for 24 hours at pH 6. After incubation growth and cell dry weight was determined.

Identification:

The bacterial isolate was identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology based on the characters such as morphology, physiology and nutritional, cultural characteristics and biochemical tests.

Detection of PHB production by Fluorescence Staining Method (Acridine Orange):

Detection of PHB production was also done following fluorescent staining method using acridine orange. 5 µl of 48 hr old culture of the isolate was transferred to an eppendorftube containing 50 µl of acridine orange (Himedia) and incubated for 30 minutes at 30°C. After the incubation period, the culture was centrifuged at 4000 rpm, for 5 min. The pellet was collected and resuspended in distilled water. A smear was prepared on a clean microscopic slide and observed in a fluorescent microscope at 460 nm. The appearance of yellow coloured granules inside the cell indicates PHB production..The bacteria positive for PHA production was selected by observing the granules under fluorescence microscope.

TLC:

About 10 ml of sample was loaded on the TLC plate and allowed to run in the solvent system consisting of ethylacetate and benzene (1:1) mixture for 40 min. 50 ml of iodine solution was vapourized in boiling water for staining. TLC plate was kept over the beaker containing iodine solution for 5-10 min in order to get saturated with iodine vapour. After 10 min black colour spots appeared indicating the presence of PHB. The RF values were measured.

Determination of amount of PHB:

Centrifugation was done with bacterial cells containing the polymer at 10,000 rpm for 10 min. The pellet was washed with acetone and ethanol to remove the unwanted materials and resuspended in equal volume of 4% sodium hypochlorite and incubated at room temperature for 30 min. The entire mixture was again centrifuged and the supernatant discarded. The cell pellet containing PHB was again washed with acetone and ethanol. Finally, the polymer granules were dissolved in hot chloroform, allowed evaporating, and PHB weight was noted. Then concentrated 10 ml hot H2SO4 was added to the polymer granules. The addition of sulfuric acid converts the polymer into crotonic acid which is brown colored. The solution was cooled and the absorbance read at 235 nm against a sulfuric acid blank. By referring to the standard curve, the quantity of PHB produced was determined.

Results and discussion:

Culture showing substantial fluorescence was selected in this study.The isolates were tested for PHB production following screening method based on the intensity of staining. The cultural conditions

including pH, temperature and carbon source showed an important role in the production rate of PHB. The results obtained are showing maximum growth and highest PHB production by Azotobacter vinelandii on nutrient agar medium supplemented with 1 %. The yield of PHB in these cells observed as 1.84g /100m l in 11.094 of cell dry weight. Black colour bands were observed in TLC and RF value was measured and calculated 1. 20 which indicated the presence of PHB in the medium. Spectroscopy study was carried out with standard PHB and bacterial cells. The standard curve was referred to determine the quantity of PHB produced.

Conclusion:

Biomaterials produced by different microorganisms are getting very much importance both in agriculture, economics and health sciences. Among the biomaterials that are industrially important are bioplastics. As they can be produced from renewable resources, they are recyclable and there is a need to optimize the conditions for high production of biopolymers. In this present study, the marine soil sample collected from the suryalanka beach, Guntur District, has been investigated to isolate and indentify new strains in order to enhance the production of PHB.

References:

1. Purification of Poly-3-Hydroxybutyrate by Density Gradient Centrifugation in Sodium Bromide kenneth W. Nickerson, Applied and Environmental Microbiology, May 1982, p. 1208-1209 Vol. 43, No. 5 0099-2240/82/051208-02$02.00/0
2. Hardman, R. 1981. Eastbourne site of U.K. Biotech crisis. Genet. Eng. News 1(3):1-13.
3. Hirs, C. H. W. 1967. Glycopeptides. Methods Enzymol. 11:411-413.
4. Kominek, L. A., and H. 0. Halvorson. 1965. Metabolism of poly-,-hydroxybutyrate and acetoin in Bacillus cereus.J. Bacteriol. 90:1251-1259.
5. Law, J. H., and R. A. Slepecky. 1961. Assay of poly-phydroxybutyric acid. J. Bacteriol. 2:33-36.
6. Lundgren, D. G., R. M. Pfister, and J. M. Merrick. 1964. Structure of poly-3-hydroxybutyric acid granules. J. Gen.Microbiol. 34:441-446.
7. Nickerson, K. W., G. St. Julian, and L. A. Bulla, Jr. 1974. Physiology of sporeforming bacteria associated with insects: radiorespirometric survey of carbohydrate metabolism In the 12 serotypes of Bacillus thuringiensis. Appl. Microbiol. 28:129-132.