Isolation and Screening of Poly β-Hydroxybutyrate Producing Microbes

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ABSTRACT: : *Poly-3-hydroxybutyrate (PHB) is a natural biopolymer produce by wide range of microbes which is a storage granule used by microbes as an energy as well as carbon source. PHB exhibit stiffness, brittle to rubber like, biodegradable, environment friendly properties. In present work we were isolated 34 bacteria and 10 fungi at 37°C, 30°C for 3-5 and 5-7 days incubation respectively by using nutrient agar medium and potato dextrose agar medium from cardboard industry waste water and sugarcane farm soil. Among which 25 bacteria and 4 fungi shown PHB accumulation which was confirmed by Sudan Black B staining.*

Key Words: *PHB, Sudan Black B, Nutrient agar medium, Potato dextrose agar medium, Biodegradable plastic*

Introduction:

Polyhydroxybutyrate (PHB) is member of polyhydroxyalkanoates (PHAs) family that is found in varieties of microorganisms as reserve food material. PHB are accumulated by different microorganisms such as bacteria, fungi, Achaea, yeast under aerobic condition when carbon source is in excess amount but one or several nutrients such as nitrogen, sulfur, phosphorus are limited [1]. There are more than 300 species which produce PHB such as *Alcaligenes latus, Ralstonia eutropha, Alcaligens eutrophus, Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Azotobacter, Pseudomonas oleovorans,* etc. Shape, size, structure, physical properties of these granules are differing from organism to organism [2]. PHB degrade naturally and completely to carbon dioxide and water under natural environment by different microorganisms [3].

French microbiologist Maurice Lemoigne in 1923 first time isolated and characterized PHB [4]. PHB has various applications such as in agriculture, bioimplants, bioplastic production, biofuel production, drugs and chemicals, food and feeds, etc. There are many applications of bioplastic as they were used for the fabrication of bottles, fibres, latex and several products of agriculture, commercial or packaging interest. Nowadays, these polymers have been used for medical application such as sutures, implants urological stents, neural and cardiovascular tissue engineering, fracture fixation, treatment of narcolepsy and alcohol addiction, drug delivery vehicles, cell microencapsulation, support of hypophyseal cells etc. [5].The present study focus on the isolation and screening of most prominent PHB producing bacteria.

Materials and method:

Chemicals:

Nutrient broth, Potato Dextrose Agar, Sudan Black B Stain, Sodium Hypochlorite, Chloroform, etc. All chemicals are purchased from Hi media and of analytical grade.

Collection of Sample:

Soil samples and waste water sample were collected from sugarcane farm and cardboard industry respectively in sterile container and labeled them [1].

Isolation of PHB producing microbes:

Isolation of PHB producing microbes were proceed by using serial dilution method, One gram of soil sample and 1ml of waste water sample was suspended in 10ml of sterile D/W and dilutions were prepared from 10^{-1} to 10^{-7} . 0.1ml of each dilution was spreaded on nutrient agar medium and potato dextrose medium which is incubated at 37°C for 24 hours & 30°C for 3-5 days respectively [6] .

Screening of PHB producing microbes:

Isolates were further screened by Sudan Black B staining where PHB producers appears bluish black color while non PHB producers remain white. 0.02% Sudan Black B solution applied to the petriplate containing microbial growth and plate were kept undisturbed for 30 min to stain. Excess dye was decanted and plates were rinsed with 100% ethanol then observed color changed [1] . The most proficient bacteria were proceed for production.

PHB production and extraction:

The isolates with highest PHB producing capacity were proceed for the PHB production Triplicates sets of 250 ml flask containing 200 ml modified LB medium and Nitrogen deficient medium was inoculated by PHB

producing bacteria and fungi, incubated at 37˚C and 30˚C for 3-5,5-7 days respectively[7], further proceed for the extraction using sodium hypochlorite method (Gupta Shraddhaet.al., 2011). Extraction of bacterial PHB was comparatively easy than fungi and more capable to produce bioplastic sheet.

Identification of PHB producing bacteria:

Most proficient PHB producing bacterial isolates were promote for identification as per Bergey's manual of systemic bacteriology.

Result and Discussion:

Isolation of PHB producing microbes:

In the present study, 34 bacteria & 10 fungi were isolated from collected soil samples & waste water sample on nutrient agar medium & potato dextrose medium respectively. In previous study, isolation of PHB producing fungi were not reported as per literature cited. Hoseinabadi[4], in their research isolated 61 bacteria from oil sludge sample. 10 isolates were obtained by Gupta Shraddha[6], from cardboard industry waste water and Rhizospheric soil respectively. In present study, isolates were more as compare to literature cited.

Fig. No.1 Fungal isolates from sugarcane farm soil

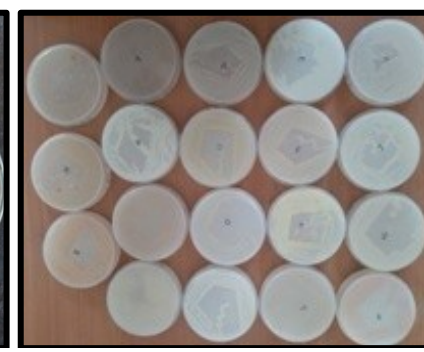

Fig.No.2 Bacterial isolates from sugarcane farm soil

Fig. No.3 Bacterial isolates from Cardboard industry waste water

Screening of PHB producing microbes:

Among 34 bacteria & 10 fungi, 25 bacteria & 4 fungi shows the PHB accumulation. Hoseinabadi[4], in their research only got 21 PHB producing bacteria. Gupta Shraddha[6] obtained only 6 PHB producer. In present study, as compare to literature cited PHB isolates were more.

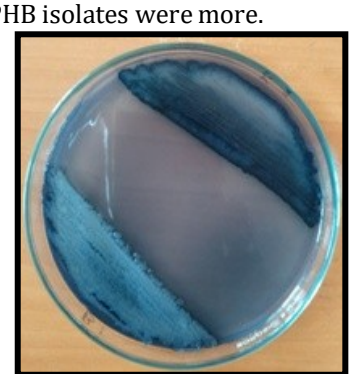

Fig.No.4 Sudan Black Staining

Production and Extraction of PHB :

Maximum PHB production was obtained in modified LB medium as compared to nitrogen deficient medium. Extraction was done by sodium hypochlorite method in which the bacterial isolates produced 2.10 g/L PHB whereas fungal isolates produced 0.07 g/L. It indicates that bacteria was more efficient for PHB production than fungi. 74mg/L of PHB was produced by bacillus spp. Isolated from organic waste and soil sample[9].

Identification by Morphological and Biochemical Characteristics:

Bacteria was Gram positive, motile, rod shape. As per Bergey's Mannual of Determinative Bacteriology the most prominent PHB producer i.e. B1 III identified as ***Bacillus macquariensis*** (Table no. 1) while Hawas[3] identified their proficient bacteria as *Pseudomonas boreopolis.* Probabilistic identification of bacteria identification system was used by Gupta Shraddha[6] for the identification of selected isolates.

Table No.1 Identification of bacteria by Morphological and Biochemical Characteristics

Tests	R	S	Q	B1 III
Gram's nature	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve
Oxidase	+ve	-ve	-ve	+ve
Gelatinase	+ve	+ve	+ve	+ve
Casein	+ve	+ve	+ve	+ve
Starch	+ve	-ve	+ve	+ve
Indol	+ve	+ve	+ve	+ve
MR	+ve	+ve	+ve	+ve
VP	-ve	+ve	-ve	-ve
Citrate	-ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	+ve
Glucose	A= +ve G= -ve	A= +ve G= -ve	A= +ve G= -ve	A= +ve G= -ve
Sucrose	A= +ve G= -ve	A= +ve G= -ve	A= +ve G= -ve	A= +ve G= -ve
Lactose	A= -ve G= -ve	A= -ve G= -ve	A= -ve G= -ve	A= -ve G= -ve
Mannitol	A= +ve G= -ve	A= +ve G= -ve	A= +ve G= -ve	A= -ve G= -ve
Arabinose	A= -ve G= -ve	A= -ve G= -ve	A= -ve G= -ve	A= -ve G= -ve

Conclusion:

34 bacteria and 10 fungi were isolated from different sugarcane farm soil and cardboard industry waste water among which 25 bacteria and 4 fungi produce PHB. PHB isolates were screened by Sudan Black B staining. As per the Bergey's Mannual of Determinative Bacteriology the most prominent PHB producer may

be identified as ***Bacillus macquariensis***. Bacterial isolates were more proficient for PHB production than fungal isolates.

References:

1. Anish Kumari Bhuwal,[1]Gulab Singh, Neeraj Kumar Aggarwal, Varsha Goyal & Anita Yadav, " Isolation and Screening of Polyhyroxyalkanoates Producing Bacteria From Pulp, Paper, and Cardboard Industry Waste". Hindawi Publishing Corporation International Journal of Biomaterials, volume 2013.
2. Abdullah A. Alarfaj, Mohammed Arshad, Essam N. Sholkamy, Murugam A. Munusamy, "Extraction and Characterization of Polyhydroxybutyrate (PHB) from Bacillus thuringiensis KSADL127 Isolated from Mangrove Environments of Saudi Arabia." Brazilian Archives of Biology and Technology, volume 58, number 5, Sept/Oct 2015.
3. Jehan Mohammed El- Mohamedy Hawas, Tarek El-Said El- Banna, Elsayed Belal Abdelmonteleb Belal, et.al., "Production of Bioplastic from some Selected Bacterial Strains." International Journal of Current Microbiology and Applied Sciences, volume 5, number 1 (2016) pp. 10-22.
4. Akram Hoceinabadi[1], Iraj Rasooli, Mojtaba Taran, "Isolation and Identification of Poly β - Hydroxybutyrate Over-Producing Bacteria and Optimization of Producing Medium". Jundishapur J Microbiol 2015 July; 8(7).
5. Neelam K. Naranje, Bharat J. Wadher, Hemant J. Purohit, Manohar G. Muddeshwar, "Bacillus megaterium as potential producer for Polyhydroxybutyrate." IOSR Journal of Environmental Science, Toxicology and Food Technology, volume 1, issue 6, pp. 07-10, Sept- 2015.
6. Gupta Shraddha, Rajput Yogita, Shit Simanta, Shukla Aparna, Shukla Kamlesh, "Screening and Production of bioplastic (PHAs) from Sugarcane Rhizospheric Bacteria." International Multidisciplinary Research Journal 2011, 1(9): 30-33.
7. B. Senthil Kumar and G Prabhakarn, "Production of PHB (bioplastic) using bio-effluents substrate by Alcaligens eutrophus." Indian Journal of Biotechnology volume 5, Jan 2006, pp. 76-79.
8. S. Kumaravel, R. Hema& R. Lakshmi, "Production of Polyhydroxybutyrate (bioplastic) and ite Biodegradation by Pseudomonas lemoignei and Aspergillusniger." E Journal of Chemistry 2010, 7(S1), S536-S542.
9. Wala'a Shawkat Ali, Neihaya Heikmat Zaki and Somaya Yassen Najim Obiad "Production of bioplastic by bacteria isolated from local soil and organic wastes." Current Research in Microbiology and Biotechnology Vol. 5, No. 2 (2017) : 1012-1017.