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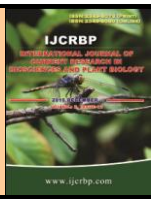


Original Research Article

PHB (Poly-Hydroxy Butyrate) Production Using Bacteria Isolated from Effluents

Priscilla Anitha and Priya Iyer*

P. G. and Research Department of Biotechnology, Women's Christian College, Chennai-600 006, Tamil Nadu, India

**Corresponding author.*

Abstract	Keywords
The present study was undertaken to prove that the extracted product from different microorganisms from various sources is a biopolymer, PHB (Poly-Hydroxy Butyrate). The major objective underlying this study was to ascertain whether the extract (polymer), can be used as a bioplastics in future to save our environment. Various microorganisms were isolated from the samples (brewery waste and textile effluent). Biochemical identification techniques and Gram staining were performed to characterize the isolated bacteria. This involved various biochemical tests. After the isolation, slants were prepared and then the microbes were stored in the refrigerator below 2°C for further use. A special medium called PHB medium was prepared and the microbes were inoculated and incubated. PHB staining technique was performed to confirm the production of PHB. Acetone : Alcohol, boiling chloroform methods were employed to extract PHB. Powdered form of PHB was obtained from *Bacillus species*, whereas *Salmonella species* produced PHB in the form of sheet. Different analytical experiments, Thin Layer Chromatography, colorimetric analysis and ^{1}HNMR analysis, were performed to confirm the presence of PHB in the extracted polymer.	*Bacillus* spp. Brewery waste Poly-Hydroxy Butyrate *Salmonella* spp. Textile effluents

Introduction

Plastics play a major role in our everyday lives. From water bottles to prosthetics, plastics can be seen everywhere. However, these oil-based polymers take many years to degrade, which poses an environmental problem in some areas; to overcome this, production of environmental-friendly plastics are been discovered. The amount of plastic waste increases every year and the exact time needed for its biodegradation is unknown. Nowadays plastics and synthetic polymers are mainly produced using petrochemical materials that cannot be decomposed. Therefore they contribute to environmental pollution and are a danger to many animals. During the last decade, much attention has been focused on the production of bacterial polyesters. Bioplastics are plastics derived from renewable biomass sources, such as vegetable fats and oils, corn starch, or microbiota. Bioplastic can be made from agricultural by-products and also from used plastic bottles and other containers using microorganisms. Common plastics, such as fossil-fuel plastics (also called petrobased polymers), are derived from petroleum or natural gas. Bacteria are potential sources of biopolymers and the studies clearly

evidenced the production of such polymers by *Bacillus* and many other species of bacteria (Prasanna et al., 2011; Kannahi and Rajalakshmi, 2012). The present study was aimed to find out the production of polyhydroxy butyrate by the bacterial species isolated from textile effluent.

Materials and methods

Sample preparation

Brewery waste (mixture of maize, wheat, barley) was used as the sample and the source for the growth of bacteria. The sample was air dried, then used for further processes. Textile effluents were collected in bottles and filtered to remove the solid materials and unwanted dusts.

Identification and isolation

The microbial isolates, *Bacillus species* and *Salmonella species* isolated from the textile effluents, were subjected to Gram staining and motility for morphological identification. The bacterial isolates were identified using the morphological, cultural and biochemical characteristics.

PHB staining

PHB staining was done using Sudan B black stain (Prasanna et al., 2011).

PHB extraction

After 48 h of incubation at 37˚C in the PHB medium, 30 ml of culture was taken and centrifuged at 10000 rpm for 15 min. The supernatant was discarded and resuspended in PBS. Again the supernatant was discarded and treated with 10ml of sodium hypochlorite and the mixture was incubated at 30˚C for 2 h. Then, the mixture was centrifuged at 5000 rpm for 15 min and washed with distilled water, acetone, methanol respectively for washing and extraction. The pellet obtained was evaporated by pouring the solution on sterile glass plate kept at 4˚C. Finally, the powder was collected for analysis (Kannahi and Rajalakshmi, 2012).

After 48 h of incubation at 37˚C, 30 ml of bacterial culture was taken and centrifuged at 10000 rpm for 15 min. The supernatant was discarded and treated with 10ml of sodium hypochlorite and the mixture was incubated at 30˚C for 1 h. After incubation, the mixture was centrifuged at 10000 rpm for 10 min and then washed with distilled water and acetone: methanol (1:1) respectively for extraction (Anderson and Dawes, 1990; Prasanna et al., 2011; Jiang et al., 2007).

Thin Layer Chromatography

To detect the presence of PHB by TLC method, the solvent ethyl acetate : benzene (1:1) was used. The Retention factor (R_f) of the compound was calculated using the formula (Marjadi and Dharaiya, 2014).

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

PHB colorimetric method

Few grams of the sample was taken in a test tube and dissolved in concentrated sulphuric acid of 2.5 ml and heated for 10 min at 100%. After cooling, the solution was measured colorimetrically at 450 nm against sulphuric acid blank.

NMR (Nuclear Magnetic Resonance)

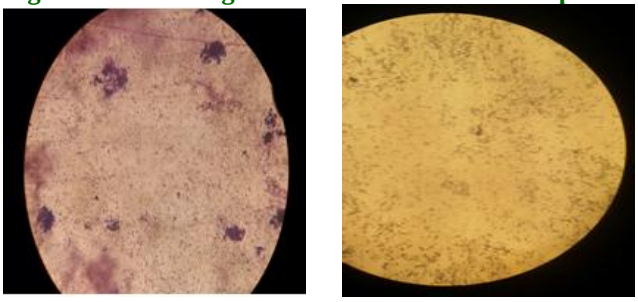
The nuclear magnetic resonance spectrum was recorded at on a BRUAK- 400 spectrometer, using a 5mm 1H probe, and deuterated chloroform, DMSO, Methanol was used as solvent. The ^{1}HNMR spectrum, which was utilized to identify the structure of PHB, was recorded at 500.13 MHz. For analysis, 5 mg of sample and 1ml of solvent were employed (Labuzek and Radecka, 2001).

Results and discussion

Different types of micro organisms were isolated from the raw materials (brewery waste and textile effluent) used. Biochemical identification methods (Table 1) and Gram staining techniques were performed to confirm the isolated bacteria. The isolated organisms were found be *Bacillus species* and *Salmonella species*. PHB medium were prepared using glucose as the carbon source and the microbes were inoculated and incubated. PHB staining were performed in order to confirm that the isolated microorganisms have the ability to produce PHB and that confirmed the presence of Poly-hydroxybutyrate in the form of black granules when viewed under the microscope. After the confirmation of the presence of PHB, the cultures were subjected to centrifugation and extraction (acetone: alcohol method). When boiling, chloroform was added and allowed to evaporate, the product was obtained in the form of

powder for *Bacillus* species and for *Salmonella* species it appeared in the form of a sheet. The resultant product was analysed chromatographically. Thin Layer Chromatographic technique was done. Colorimetric analysis was also done using Sulphuric acid as the blank at 450 nm. The OD values were tabulated.NMR studies showed a positive result for all the four microbial extract (polymer) by determining their peaks. This confirmed that the extracted polymer was a PHB (Tables 2-4; Figs. 2-4).

Fig. 1: PHB staining of *Bacillus* and *Salmonella* species.



Bacillus species *Salmonella species*

Table 1. Biochemical characterization of *Bacillus* and *Salmonella* species.

S.No.	Biochemical tests	*Bacillus* species	*Salmonella* species
1.	Indole	+	+
2.	Methyl red	—	+
3.	Voges Proskauer test	+	—
4.	Citrate test	+	+
5.	Oxidase test	—	—
6.	Catalase test	+	+
7.	Triple sugar iron agar test	+	+
8.	Sugar	+	+
9.	Dehydrgenase test	+	+
10.	Nitrogen reduction test	+	+
11.	Urease test	+	+
12.	Gelatinase test	—	+
+ = positive; — = negative.			

Fig. 2: PHB powder obtained from *Bacillus* and *Salmonella* species.

Bacillus species *Salmonella species*

Table 2. R_f values obtained for PHB of *Bacillus* and *Salmonella* species using Thin Layer Chromatography.

S. No.	PHB produced by	R_f value
1	*Bacillus species*	0.57
2	*Salmonella species*	0.40

Table 3. NMR results for PHB of *Bacillus* and *Salmonella* species.

Type	Reference (ppm)	PHB sample	
		Bacillus species	*Salmonella species*
A (- CH_3)	1.2	1.23	1.3
B (- CH)	5.0 - 5.5	NIL	4.8
C (- CH_2)	2.4 - 2.7	2.52	2.36

Table 4. Colorimetric analysis results for PHB of *Bacillus* and *Salmonella* species.

S. No.	PHB produced by	OD value (nm)
1	*Bacillus species*	0.93
2	*Salmonella species*	0.82

Fig. 3: NMR spectrum of *Bacillus species* (refer Table 3).

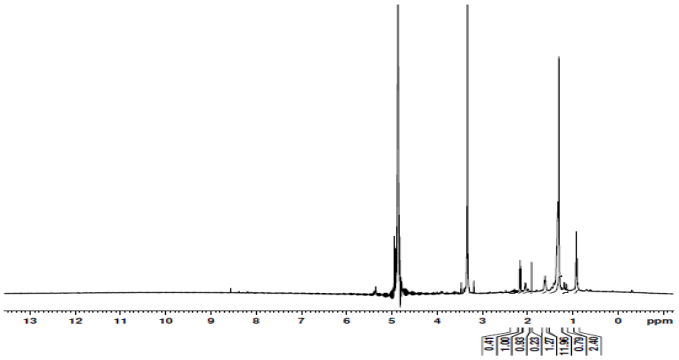
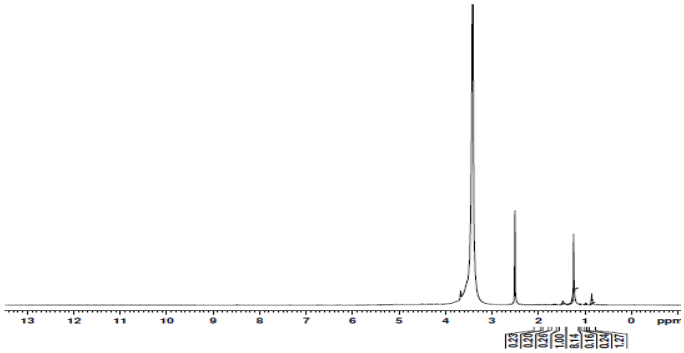


Fig. 4: NMR spectrum of *Salmonella species* (refer Table 3).



This study evidently shows that the polymer extracted from different microorganisms can be used as an alternate for the petro-chemically derived plastics in future. Different types of micro organisms were isolated from the raw materials (brewery waste and textile effluent) used. Biochemical identification methods and

Gram staining techniques were performed to confirm the isolated bacteria. The isolated organisms were found be *Bacillus species* and *Salmonella species.*

PHB medium were prepared using glucose as the carbon source and the microbes were inoculated and incubated. PHB staining were performed in order to confirm that the isolated microorganisms have the ability to produce PHB and that confirmed the presence of Poly-hydroxybutyrate in the form of black granules when viewed under the microscope. After the confirmation of the presence of PHB, the cultures were subjected to centrifugation and extraction (Acetone : alcohol method). When boiling chloroform was added and allowed to evaporate, the product was obtained in the form of powder for *Bacillus species* and sheet like for *Salmonellas species.* The resultant product was analysed chromatographically and a high value was observed for *Bacillus species*. NMR studies showed a positive result for all the four microbial extract (polymer) by determining their peaks. This confirmed that the extracted polymer was a Poly-hydroxy butyrate (PHB). The values were represented in the form of tables.

Conclusion

From this study it is concluded that the extracted polymer (Poly-hydroxy butyrate) can be used as an alternate for commercially available petrochemical plastics in future since it had the features of common plastics which are non-degradable. The plastic sheet that was obtained from the extract can be used in packaging of materials and also in wrapping.

References

Anderson, A.J., Dawes, E.A., 1990. Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxy alkanoates. Microbiol. Rev. 54, 450-472.

Jiang, Y., 2008. High poly (-hydroxybutyrate) production by *Pseudomonas fluorescens*A2a5 from inexpensive substrates. Enzyme Microb. Technol. 42, 167–172.

Kannahi, M., Rajalakshmi, M., 2012. Production and optimization of PHB by *Bacillus megaterium* and *Azospirillum spp*. Int. J. Chem. Pharmaceut. Sci. 3(3), 15-18.

Labuzek, S., Radecka, I., 2001. Biosynthesis of PHB tercopolymer by *Bacillus cereus* UW85. J. Appl. Microbiol. 90, 353-357.

Marjadi, D., Dharaiya, N., 2014. Recovery and characterization of poly (3-hydroxybutyric acid) synthesized in *Staphylococcus epidermidis*. Afr. J. Env. Sci. Tech. 8(6), 319-329.

Prasanna, T., 2011. Production of poly (3-hydroxybutyrates) by *Bacillus* species isolated form soil. J. Pharma Res. Rev. 1, 15-18.