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"Poly-Hydroxy butyrate from bacteria" - An alternate for the commercial petrochemical plastics

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Abstract

The present study was undertaken to prove that the extracted product from different microorganisms from various sources is a biopolymer (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 Poly-hydroxy Butyrate. Acetone: Alcohol, boiling chloroform methods were employed to extract Poly-hydroxy Butyrate. Powdered form of PHB was obtained from *Pseudomonas* spp and where as *Streptococcus* spp produced phb in the form of sheet.Different analytical experiments were performed to confirm the presence of phb in the extracted polymer. They were, Thin Layer Chromatography, colorimetric analysis and ^{1}HNMR analysis.

Keywords: PHB, Brewery waste, Textile effluents, PHB medium, PHB staining, *Pseudomonas* spp and *Streptococcus* spp.

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 byproducts 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.

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. The dried sample was then used for further process. Textile effluents were collected in bottles and filtered to remove the solid materials and unwanted dusts.

Identification and isolation: Microbial strains like *Bacillus sp and Salmonella sp* were isolated from the above mentioned raw materials. The bacteria were subjected to Gram staining and Motility for morphological identification. The bacterium was identified by the Morphological, Cultural and Biochemical characteristics.

PHB Staining: PHB staining was done using sudan B black stain (**T. Prasanna et.al; 2011)**

PHB Extraction: After 48 hrs of incubation at 37˚C in the PHB medium, 30 ml of culture was taken and centrifuged at 10,000 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 hrs. After incubation, the mixture was centrifuged at 5000 rpm for 15 min and then washed with distilled water, acetone, methanol respectively for washing and extraction. After washing, the pellet was evaporated by pouring the solution on sterile glass plate kept at 4˚C. After evaporation, the powder was collected for analysis (**Kannahi and Rajalakshmi, 2012).**

After 48 hrs of incubation at 37˚C, 30 ml of 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 hr. After incubation, the mixture was centrifuged at 10000 rpm for 10 min and then washed with distilled water, acetone: methanol (1:1) respectively for extraction (**Prasanna et.al; 011), Yuji Jiang et.al; 2007; Zehranury. Ksekdaú et.al; 2002)**

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 (**DarshanMarjadi and Nishith Dharaiya, 2014)**

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

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

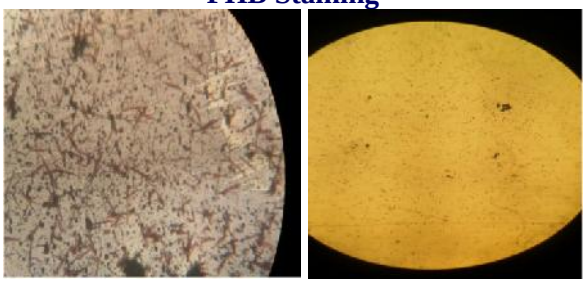
NMR (Nuclear Magnetic Resonance) : The nuclear magnetic resonance spectrum was recorded at on a BRUAK- 400 spectrometer, using a 5mm 1H probe, and deuterated chloroform, DMSO, Methanol was used as solvent. The ^{1}HNMR spectrum, which was utilized to identify the structure of PHB, was recorded at 500.13 MHz. For analysis, 5 mg of sample and 1ml of solvent were employed (**Labuzek et.al; 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 *Pseudomonas* spp and *Streptococcus* spp. 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-hydroxy butyrate 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* sp and *Salmonella* sp for 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 Poly-hydroxy Butyrate (PHB).

PHB Staining



Pseudomonas spp *Streptococcus* spp

Table: 1 Biochemical tests

S.No	Content	*Pseudomonas sp*	*Streptococcus sp*
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	+	-

PHB Powder

Pseudomonas spp

Streptococcus spp

Table: 2 Thin Layer Chromatography

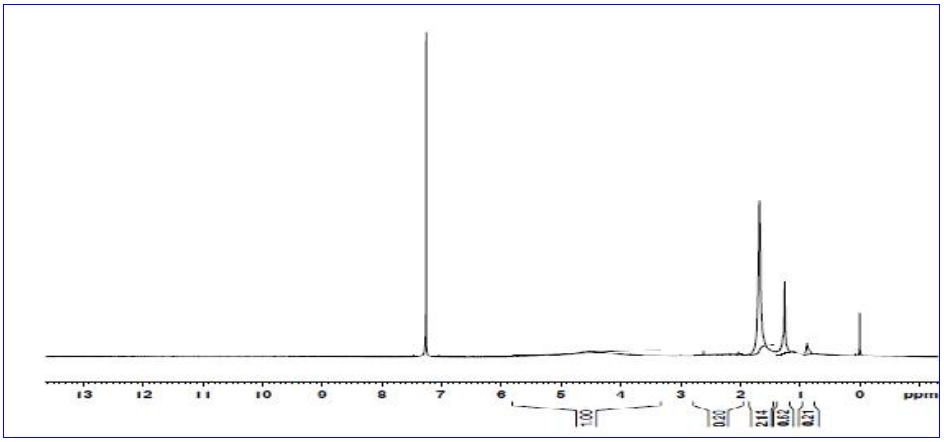
S.NO	ORGANISMS	R_f VALUE
1	*Pseudomonas* spp	0.54
3	*Streptococcus* spp	0.58

Table: 3 NMR

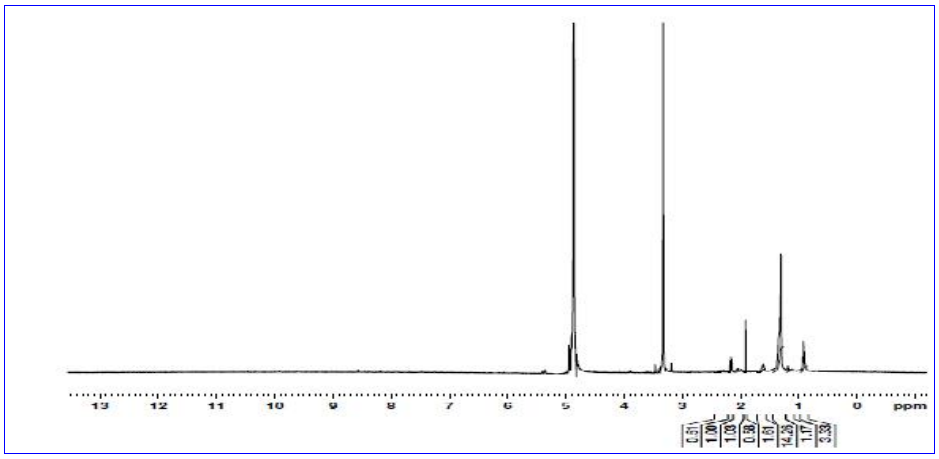
Type	Reference (ppm)	Sample	
		***Pseudomonas* spp**	***Streptococcus* spp**
A (- $CH_{3)}$	1.2	1.23	1.3
B (- CH)	5.0 - 5.5	NIL	4.8
C (- $CH_{2)}$	2.4 - 2.7	2.52	2.36

Table: 4 Colorimetric Analysis

S.No	Organisms	OD Value (nm)
1	*Pseudomonas* spp	0.9
2	*Streptococcus* spp	0.95



Graph: 1NMR spectrum of *Pseudomonas* spp (refer table:3)



Graph: 2NMR spectrum of *Streptococcus* spp (refer table: 3)

Conclusion

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 *Pseudomonas* sp and *Streptococcus*. 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 ebility 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 *Pseudomonas* sp and *Streptococcus* sp.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. High value was observed in *Streptococcus sp*. NMR studies showed a positive result for all the four microbial extract (polymer) by determing their peaks. This confirmed that the extracted polymer was a Poly-hydroxy butyrate (PHB). The values were represented in the form of tables. From this study it is concluded that the extracted polymer (Poly-hydroxybutyrate) 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.

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