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Biofilm From Polyhydroxybutyrate Granules.

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ABSTRACT

Poly-β-hydroxybutyrate is biodegradable and biocompatible polymer produced by bacteria which has many industrial applications. The work here focuses on isolation and characterization of poly-β-hydroxybutyrate producing bacteria from the soil samples of garden and dumping yard. The screening for poly-β-hydroxybutyrate producing bacteria was done by Sudan Black B staining. The extraction of poly-β-hydroxybutyrate granules was done from the isolates which were found to be positive by Sudan Black B staining. The characterization and identification showed that the isolates were *Alcaligenes* and *Sphaerobacter* sp. A poly-β-hydroxybutyrate based film was developed, demonstrating its potential in biodegradable plastic production. This poly-β-hydroxybutyrate can be used as eco-friendly alternative to conventional plastics which is important.

Keywords: Biological, Economical, Environment, Plastics, Sustainable.

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INTRODUCTION

Polyhyroxybutyrate (PHB) is biopolymer which belong polyhdyroxyalkonate family. PHB is produced by bacteria as intracellular energy reserve under nutrient limitation with excess carbon source. PHB has immense industrial applications and has gained attention [1]. PHB-producing bacteria are *Bacillus megaterium*, *Alcaligenes eutrophus* and *Azotobacter vinelandii*. One of the application of PHB is in biodegradable plastics and packaging materials, which is eco-friendly alternative to conventional plastics. Due to its ability to degrade naturally in soil and aquatic environments, PHB-based plastics are used in packaging of food products, disposable cutlery, and agricultural films [2].

MATERIALS AND METHODS

Sample collection

The soil samples were collected from Pimpri and Moshi dumping yard, Pune, Maharashtra in clean zip-lock bag and labelled properly.

Isolation of PHB producing bacteria

For the isolation of PHB producing bacteria, the soil samples (1 g) were diluted up to 10^{-4} dilution. 0.1 ml of $^{-4}$ dilution was spread plate on Nutrient Agar (NA), Modified Nutrient and Mineral Salt Medium (MSM) agar media. The plates were incubated in the incubator at 37 °C for three days [3].

Screening to determine PHB positive isolates

Polyhydroxybutyrate (PHB) production was assessed using Sudan Black B [4], a lipophilic stain that selectively binds to intracellular lipid-like inclusions.

Characterization and identification of PHB producing bacteria

The PHB producing isolates were identified based on morphological characteristics, motility, oxidase, and catalase production and biochemical tests such as sugar fermentation.

Extraction of PHB

The extraction of PHB was done as per the method of [5]. For the extraction of PHB, the bacterial isolates (S1, S2, S3, and S4) were inoculated in 100 ml NB supplemented with glucose as a carbon source in 250 ml conical flasks. The cultures were incubated at 37 °C for 72 hr. After incubation, 50 ml bacterial culture was collected and transferred into sterile centrifuge tubes. The cultures were centrifuged at 8000 rpm for 15 min to separate the bacterial cells. The supernatant was discarded and the pellet was treated with 10 ml of sodium hypochlorite (4% w/v). After incubation, the lysed cell suspension was centrifuged at 10,000 rpm for 20 min. The pellet containing PHB was collected and washed sequentially with acetone to remove lipid and non-PHB cellular components and 95% ethanol to further purify the PHB and remove residual impurities. Each washing step involved centrifugation at 10,000 rpm for 10 min, followed by discarding the supernatant and retaining the pellet. Further, 5-10 ml of chloroform was added to the pellet and chloroform extract was filtered through pre-wetted Whatman No. 1 filter paper to remove any residual debris. The filtrate was transferred to a clean glass petri dish and allowed to evaporate at room temperature until complete drying. The remaining solid was purified PHB.

Applications

Method for producing PHB film

PHB film was prepared by dissolving 200 mg of PHB powder in 30 ml of chloroform with stirring at room temperature until a homogeneous solution was obtained. To improve the flexibility and binding properties of the film, glycerine 5 ml was added to the solution and mixed properly. Once the solution was well-blended, it was poured into a clean petri dish to ensure uniform thickness. The solvent was then allowed to evaporate at room temperature. After complete drying, the formed PHB film was carefully peeled off and packed in clean petri dish [6].

Application of PHB film in food Industry

For application testing, chocolate got the market was wrapped in PHB biofilm to see its potential as a biodegradable packaging material. The ability of the film to encapsulate and protect the chocolate was observed considering its flexibility and strength.

RESULTS AND DISCUSSION

Isolation of PHB producing bacteria

Total five isolates were obtained from the soil samples which were grown on NA, modified NA and MSM agar media. Single colony was streaked on NA media. The purified isolates were further maintained on NA slants.

Screening for positive PHB producing bacteria

The PHB producing granules were seen as black colour within pink colour vegetative cells. The PHB producing bacterial isolates were named as S2 and S3.

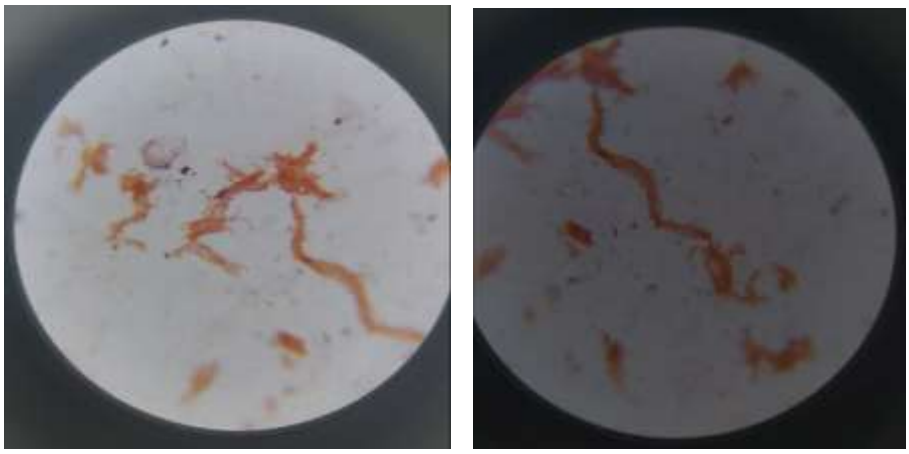


Figure 1: Staining for PHB granules by Sudan black for isolates S2 and S3

Extraction of PHB granules

The PHB granules were extracted from the isolates S2 and S3.

Figure 2: PHB granules extracted from the isolates S2 and S3

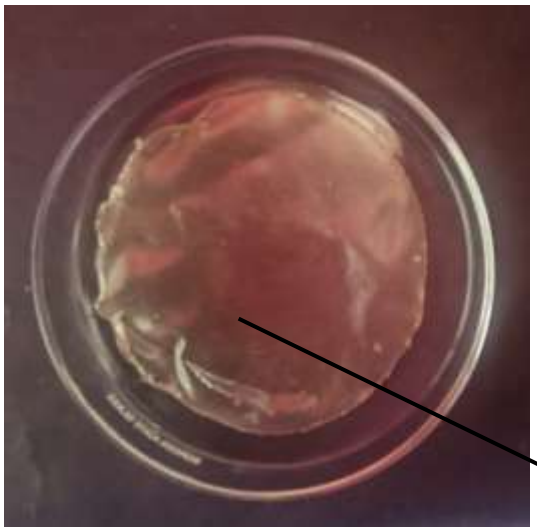
Characterization and identification of the isolates

The isolate S2 was Gram negative short rod and motile. The isolate S3 was Gram positive rod and non-motile. Both the isolates were catalase positive and oxidase negative and showed glucose

fermentation. From the Bergey's Manual of Determinative Bacteriology [7], the isolates S2 and S3 were found to be *Alcaligenes* and *Sphaerobacter* sp. respectively.

Applications of PHB granules

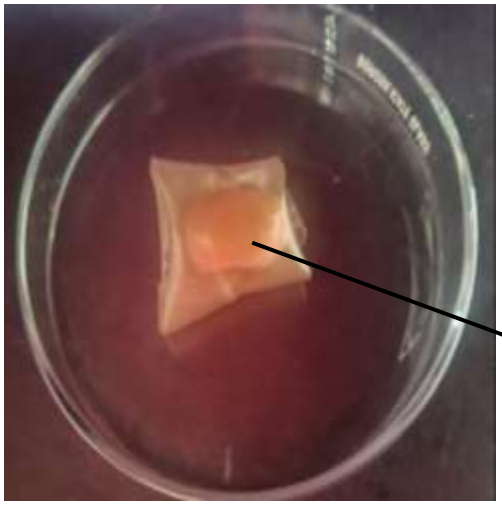
The biofilm was prepared from the PHB granules extracted from *Alcaligenes* and *Sphaerobacter* sp.



Film made of PHB powder

Figure 3: PHB biofilm

PHB can be a better alternative to the conventional plastics in market used for food wrapping, hence PHB film can be used in future in large quantity for food products wrapping.



Market available chocolate wrapped in PHB film

Figure 4: PHB biofilm used for food product wrapping

There are reports on PHB producing bacteria by *Bacillus* sp. [8], *Pseudomonas* sp., etc. There is a report on screening of PHB by *Lysinibacillus fusiformis* from environmental sources [9]. Study is done on bioplastic PHB from microbes isolated from paddy and sugarcane fields and fabrication of biodegradable film [10].

CONCLUSION

The bacterial cultures isolated from soil samples were *Alcaligenes* and *Sphaerobacter* sp. The PHB produced has important application in food industry. This can be used for packaging and wrapping of

other food products like fruits. This PHB produced by the bacterial isolates will be eco-friendly, economical and alternative to conventional plastics.

REFERENCES

[1] Anjum A, Zuber M, Zia K, Noreen A, Anjum M, Tabasum S. Microbial production of polyhydroxyalkanoates (PHAs) and its applications in biomedicine and food industry. Int J Biol Macromolecules 2016; 89:161-174.

[2] Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V. Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing, and potential applications in packaging. Express Polymer Letters 2014; 8:791-808.

[3] Sharma P, Bajaj B, Thakur R. Enhanced production of polyhydroxybutyrate (PHB) from *Bacillus cereus* PS10 under submerged fermentation using response surface methodology. Bioresour Technol 2017; 240:183-190.

[4] Kodali V, Vohra A, Makkar R. A simple method for screening and quantification of poly-β-hydroxybutyrate granules in microorganisms. Methods X 2021; 8, 101506.

[5] Koller M, Hesse M. Isolation and characterization of polyhydroxyalkanoates (PHA) from microbial biomass. Curr Microbiol 2015; 70:778-786.

[6] Rawia F, Gamal M, Abdelhady A, Khodair S, El-Tayeb E, Khadiga A. Semi-scale production of PHBs from waste frying oil by *Pseudomonas fluorescens* S48. Braz J Microbiol 2013; 44:539-49.

[7] Holt J. Bergey's Manual of Determinative Bacteriology. Walters Kluwer Publisher 1993; 9th edn.

[8] Hamdy M, Danial W, F Gad El-Rab, Shoreit A, Hesham A. Production and optimization of bioplastic (Polyhydroxybutyrate) from *Bacillus cereus* strain SH-02 using response surface methodology. *BMC Microbiol* 2022; 22, 183.

[9] Adebajo S, Bankole P, Ojo A, Akintokun P. Screening and optimization of polyhydroxybutyrate (PHB) by *Lysinibacillus fusiformis* from diverse environmental sources. The Microbe 2024; 2, 100043.

[10] Balasubramanian V, Chellapandi R, Balakrishnan M, Murugan K, Kennedy J, Murugan S, Khumalo M, Sarangi P, Jui-Yu Chou, Muthuramalingam J. Biosynthesis of bioplastic polyhydroxybutyrate (PHB) from microbes isolated from paddy/sugarcane fields and fabrication of biodegradable thin film. Process Safety Environ Protect 2024; 187:1178-1188.