IJPSR (2024), Volume 15, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 June 2024; received in revised form, 08 July 2024; accepted, 19 July 2024; published 01 December 2024

PRODUCTION OF BIODEGRADABLE PLASTIC FROM SOIL MICROORGANISMS

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Keywords:

Polyhydroxybutyrate, Soil microorganisms, Biodegradable plastic, Degradation

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ABSTRACT: The present investigation aimed to design a biodegradable plastic from soil microorganisms that produce PHB (Polyhydroxy-butarate). The microorganism was isolated using a specifically designed medium as per the requirement and identified the organism by Sudan black and gram staining methods. Their biochemical identification was done by the Indole test. Then extraction of PHB was done through the centrifugation method where a pellet was used to make biodegradable plastic. Four different types of microorganisms were isolated, from which pseudomonas species were selected and produced in large quantities for the production of PHB, which is produced by bacteria under stressed conditions. The indole test was positive, the gram nature of the bacteria is negative and the shape is rod. The formation of plastic film was successfully done. The results obtained in this research work confirm that biodegradable plastic can be obtained from agricultural microorganisms also. Thus, the plastic film is easily degraded within a short period.

INTRODUCTION: Plastic accouterments began from petrochemicals beget serious environmental problems due to their non-degradable nature. Similar synthetically produced polymers are generally affordable, but their continuity has a significant environmental impact. With the imminent reactionary energy extremity, the intimidating rate of petroleum prices, and the environmental impact associated with the products, the hunt for druthers is essential in reducing humanity's dependence on non-renewable coffers. Biodegradable plastics offer the stylish result of covering the terrain from hazards caused by conventional petroleum-grounded plastics as they are eco-friendly.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(12).3611-16

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(12).3611-16

There are numerous types of biodegradable plastics with different degrees of biodegradability. Among them, polyhydroxy butyrate (PHB) is the only 100 biodegradable [1]. Polyhydroxybutyrate are macro-molecules synthesized by bacteria and are inclusion bodies accumulated as reserve material when the bacteria grow under different stress conditions. They are polymers possessing properties like various synthetic thermoplastics like polypropylene. This makes them useful for extensive applications and future commercial mass production of biodegradable plastics that can replace plastic materials currently obtained from petroleum bases [2].

Thermoplastics produced from renewable agricultural and forest resources (biomass), also called bioplastics, are gaining importance. For this study, we are investigating the opportunity for bio-based thermoplastics in the disposable plastics market segments as opposed to engineering thermoplastics or thermoset plastics resins. For a bioplastic to be considered compostable it must satisfy three criteria:

Biodegradation: Breaks down into carbon dioxide, water, and biomass at the same rate as cellulose.

Disintegration: The plastic is indistinguishable in the compost from other biomass material after a fixed schedule of time.

Non-toxic: The residual biomass material must not be harmful to animals or plants in final form.

They are polyesters obtained by the polymerization of monomers prepared by the fermentation process (semi-synthetic polymers) or produced by a range of microorganisms, cultured under different nutrient and environmental conditions (microbial polymers). These materials accumulate in microorganisms as storage materials [3].

Poly (3-hydroxybutyrate) [P (3HB)] and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) [P (3HB-co-3HV] are the most studied polyesters in the PHA family. These polymers share the physical and mechanical properties like petroleum-derived thermoplastics polypropylene (PP) and polyethylene (PET). The polymer typically constitutes up to 30% of the dry mass of the cell, but under controlled conditions, involving excess carbon and a limited supply of nutrients like nitrogen, the yield can be increased to as high as 80% of dry weight. It is insoluble in water, but has poor resistance to acids and bases and dissolves in chlorinated solvents. The unfavorable characteristics of Poly (3HB) such as high crystalline, stiffness, brittleness, and low extension to break limit its range of application [4, 5].

Another challenge it possesses as it is difficult to process polyhydroxy butyrate in a molten state because it starts to degrade at a temperature of 175℃ which is not much higher than the melting temperature of plastic. To overcome some of these problems, the bacterial production process can be modified to produce polyhydroxy butyrate that is copolymerized with PHV. Copolymerization reduces the melting temperature to as low as 75 °C depending upon the composition. The degradation time depends on the composition, as well as the environment. It can be as little as a few months where there are a lot of bacteria or it may take a few years when there are few bacteria. Much attention has been spent on optimizing the PHA production process, recovery, and blending with other polymers to reduce the PHA production cost.

The production cost of polyhydroxyalkanoates (PHA) is between $4,000 and $15,000 per metric ton. Which is higher than the price of $1,000 to $5,000 per metric ton for polymers derived from fossil fuels. The price of PHAs can be up to 16 times higher than the price of major petroleum-derived polymers [6].

MATERIAL AND METHODOLOGY:

Isolation of the Bacteria:

- 5 soil samples were collected from different agricultural fields.
- All glassware was sterilized using moist heat in an autoclave.
- 1 gram of sample was weighed on pan balance and was diluted by serial dilution method.
- Nutrient agar medium and PHB medium were prepared and autoclaved.
- In laminar airflow medium was poured into the Petriplates and was allowed to solidify.
- Using the spread plate method, the samples were streaked on the plate.
- Plates were incubated for 24-48 hours [7].

Characterization of Polyhydroxy Butyrate (PHB) Producing Bacteria:

Sudan Black Staining:

- The culture was spread on a clean, sterile, grease-free slide, with the use of a nichrome wire loop. The smear should cover an area of about 10 mm x 30 mm.
- The smear was allowed to air dry.
- The smear was fixed by holding the slide with forceps, and it was passed horizontally through a small Bunsen flame 2–3 times.
- The slide was heat-fixed.

- A few drops of Sudan Black solution were placed on the fixed prepared slide. After 5–10 minutes, the ethanol in the stain should be evaporated. Any excess liquid was carefully drawn off using the edge of a piece of filter paper.
- The slide was immersed in ethanol till it was completely decolorized (this takes about 10 seconds), and the slide was allowed to dry.
- The slide was flooded with the Safranin solution for 10 seconds, then the slide was gently rinsed with running water.
- The slide was allowed to air dry and was observed under oil immersion (100x) [8].

Identification using Gram Staining:

- Bacterial smear was prepared on a microscope slide and was allowed to air dry.
- Heat-fixing was done to the smear on the slide.
- The slide was flooded with a crystal violet stain for 1 minute, and was rinsed with water.
- Gram's iodine solution was added for 1 minute.
- The slide was decolorized with ethanol until the runoff was colorless.
- The slide was rinse with water and Counterstain with safranin for 1-2 minutes.
- The slide was allowed to air dry and will be observed under a microscope lens at 10x, 40x, and 100x.
- Gram-positive bacteria appear purple and Gram-negative bacteria appear pink or red.

Extraction of Polyhydroxy Butyrate [PHB]:

- For the assay of polymer in small quantities of cells, the organisms were centrifuged in polypropylene centrifuge tubes (Eppendorf).
- The cell paste was resuspended in a volume of commercial NaCl and NaOH solution (Clorox or equivalent) equal to the original volume of the medium.
- The lipid granules (pellet) were dissolved in solution and again centrifuged at 10,000rpm for 10 minutes, then washed with water followed by washing with distilled water and absolute alcohol.
- Then the pellet was dissolved in the chloroform[9].

Biochemical Characterization:
IMVIC Test / Indole Test:

- Tryptone broth was prepared
- Inoculated the microorganism into the broth.
- Broth was incubated for 24-48 hrs.
- The addition of the Kovacs reagent showed the result (i.e. red or brown color shows the test positive and yellow color shows the test negative).

RESULTS: Isolation of bacteria was successfully done by a specific media (PHB) and bacterial colonies were isolated by the incubation of 48 hours at 37°C. Specific colonies were selected and subculturing was done, pseudomonas culture was observed in plate 1 **Fig. 2**. Ralstonia eutrophes colonies were observed in serial dilution plates 2 and 7 **Fig. 3** and **4**.

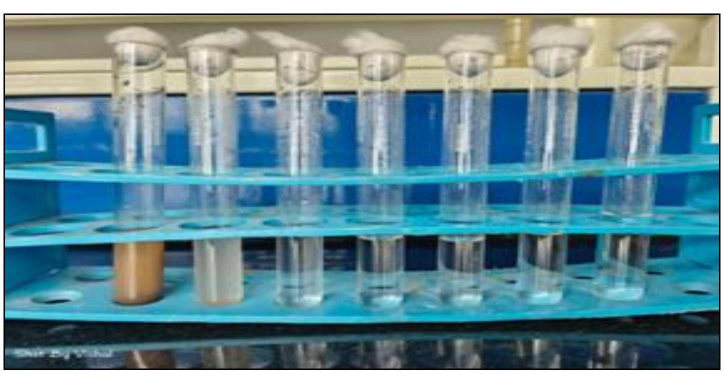

FIG. 1: SERIAL DILUTION OF SOIL SAMPLE

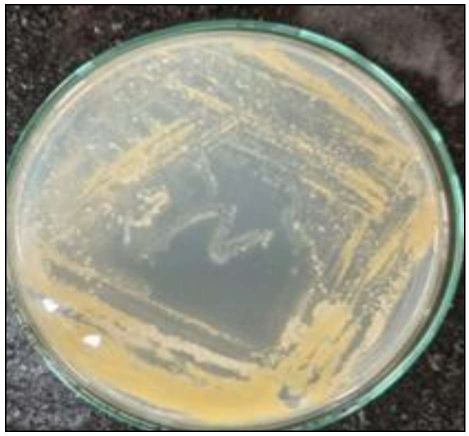

FIG. 2: PESUDOMONAS CULTURE 1

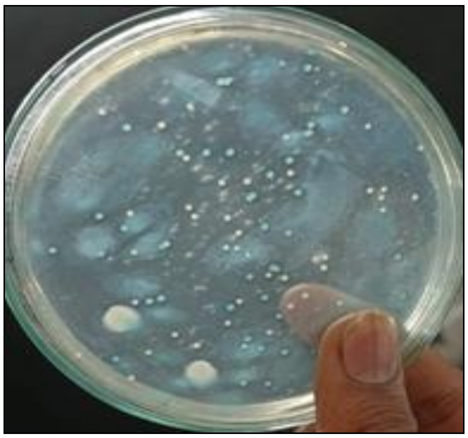

FIG. 3: SERIAL DILUTION 2

FIG. 4: SERIAL DILUTION 7

Characterization of the bacteria was successfully done using the Indole test **Fig. 6**. The Indole test has given a cherry red color which is a positive result indicating the presence of Ralstonia eutrophes and Sudan Black staining was used to confirm the bacteria **Fig. 5**. The bacteria were rod-shaped.

FIG. 5: BACTERIAL OBSERVATION UNDER

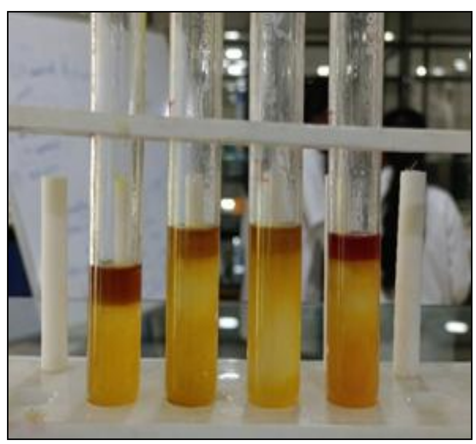

FIG. 6: INDOLE TEST

Sudan Black Staining: The polymer extraction from the culture (broth) was successfully done and the culture was incubated for over a month and then centrifuged and washed with the Solution (NaCl and NaOH), ethanol, chloroform, and Distilled water **Fig. 7.**

By using soil bacteria such as Ralstonia eutrophes, pseudomonas, bacillus, and Rhodococcus species, we successfully produced Biodegradable plastic which formed its film after washing with tap water **Fig. 8.**

FIG. 7: PELLET FORMATION

FIG. 8: PLASTIC FILM MADE BY SOIL BACTERIA

DISCUSSION: This research has led to the creation of various biodegradable and compostable bioplastic polymers, including polylactic acid (PLA), polyhydroxyalkanoates (PHA), polyhydroxy butyrate (PHB), and others. These biodegradable plastics have evolved significantly over the years, with improved materials and expanded applications. We isolated different types of colonies according to their appearance: white yellow and pinkish, isolated within 24 hr. 23 distinct colonies were chosen based on their shapes and colors. After a 24-48 hours culture period, Sudan Black B staining was done to confirm the presence of PHB granules [10-15].

Many prior works have analyzed PHB just as a concern it can produce plastic [16]. Environmental concerns continue to grow regulations become more stringent, and the development and adoption of biodegradable plastics are expected to continue to evolve. However, it's important to strike a balance between the advantages of biodegradable plastics and addressing their challenges to ensure a more sustainable future. Increased environmental awareness has driven consumers and businesses to seek more sustainable options. This has encouraged the development and adoption of biodegradable plastics. Public and private sector collaboration is driving innovation. Companies are investing in research and development to create biodegradable alternatives to traditional plastics. The global market for biodegradable plastic bags is growing rapidly, fueled by regulations and a ban on traditional plastic bags. The market was valued at $7.9 billion in 2023 and is projected to reach $20.9 billion by 2028. Biodegradable plastic bags are used in many industries, including textile, food packaging, shopping, garbage collection, and toys [16].

CONCLUSION: The results obtained in this research work conclude that biodegradable plastic can be obtained from agricultural microorganisms such as Pseudomonas, *Ralstonia eutrophes*, and *Rhodococcus* sps. The easily degradable plastic that might be produced in the industries by using such microorganisms will be helpful to society in decreasing earth pollution.

ACKNOWLEDGMENT: I would like to express my sincere gratitude to the Institute of Biosciences and Technology, MGM University for their invaluable support throughout this project.

The institute's resources, facilities, and collaborative environment were instrumental in bringing this work to fruition.

CONFLICT OF INTEREST: NIL

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How to cite this article:
Parabathina RK, Lolge V and Aghadte G: Production of biodegradable plastic from soil microorganisms. Int J Pharm Sci & Res 2024; 15(12): 3611-16. doi: 10.13040/IJPSR.0975-8232.15(12).3611-16.

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