ABSTRACT

Samples of bacteria from easily accessible sources, contaminated water, were isolated to determine the potential for producing biodegradable plastics (PHB). Among the bacteria, PHB was found to be produced by three bacterial isolates identified as C1, C2, and C3. These isolates were tested for their ability to produce PHB under various growth conditions, such as pH, time, and temperature. This study emphasizes the importance of isolating and characterizing PHB-producing bacteria and their potential for producing bio-degradable plastics.

INTRODUCTION

Plastic materials generated from petroleum-based sources are non-biodegradable and impose environmental hazards. Synthetic polymers are cheaper and more cost-effective than their petroleum-based counterparts, but their production is associated with environmental impacts. Therefore, there is a need to study and develop new biodegradable polymers with plastic-like properties. Polyhydroxyalkanoates (PHA), produced by microorganisms, have been identified as a promising alternative to petroleum-based plastics.

Biodegradable plastics, such as polyhydroxybutyrate (PHB), have properties similar to various synthetic thermoplastics like polypropylene. This makes them useful for a wide range of applications, including biodegradable plastics. Polyhydroxyalkanoates that accumulate as a carbon/energy reserve material in various microorganisms, such as PHB, are thus considered an alternative to petroleum-based plastics. Because of their unique properties and biocompatibility, these biodegradable plastics are expected to play a significant role in the future.

METHODS

Contaminated water samples were collected from Gopalpur beach, Bhubaneswar, and used as the source of bacteria. Three colonies were isolated from the water sample and named as C1, C2, and C3. The colonies were identified by colony morphology. The Sudan black screening test was used to screen for the production of PHB by the bacterial isolates.

RESULTS

The bacterial isolates C1 and C2 showed a positive result for the production of polyhydroxybutyrate (PHB). Presence of PHB granules in Cocobacillus and Rod shaped bacillus was confirmed.

CONCLUSION

Polyhydroxybutyrate (PHB), a kind of microbial polyester that accumulates as a carbon/energy reserve material in various microorganisms, was thus concluded to be a decent alternative for plastics. Because of their special characteristics and broad biological applications, biodegradable plastics are compounds with a promising future.

Keywords: Bacteria, Biochemical tests, Bioplastic, Biodegradable, Polyhydroxybutyrate

INTRODUCTION

Plastic materials generated from petrochemicals are non-biodegradable and impose environmental hazards. Synthetic polymers are cheaper and more cost-effective than their petroleum-based counterparts, but their production is associated with environmental impacts. Therefore, there is a need to study and develop new biodegradable polymers with plastic-like properties. Polyhydroxyalkanoates (PHA), produced by microorganisms, have been identified as a promising alternative to petroleum-based plastics.

Biodegradable plastics, such as polyhydroxybutyrate (PHB), have properties similar to various synthetic thermoplastics like polypropylene. This makes them useful for a wide range of applications, including biodegradable plastics. Polyhydroxyalkanoates that accumulate as a carbon/energy reserve material in various microorganisms, such as PHB, are thus considered an alternative to petroleum-based plastics. Because of their unique properties and biocompatibility, these biodegradable plastics are expected to play a significant role in the future.

At the beginning, the reversible condensation of two molecules of acetyl-CoA, by the action of the 3-ketoacyl-CoA thiolase, produces the Acetyl-CoA into R-(−)-3-hydroxybutyryl-CoA. The reductase enzyme is NADPH-linked and finally, R-(−)-3-hydroxybutyryl-CoA is polymerized to form PHB under the action of PHA synthase. Many beneficial properties of PHB have been found through studies done by researchers. They have: Good oxygen permeability, good ultra-violet resistance but poor resistance to acids and bases, soluble in chloroform and other chlorinated hydrocarbons, biocompatible and hence is suitable for medical applications, melting point 175 °C and glass transition temperature 2 °C, tensile strength 40 MPa close to that of polypropylene, sinks in water (while polypropylene floats), facilitating its anaerobic
biodegradation in sediments. Nontoxic, less 'sticky' when melted, water-insoluble and relatively resistant to hydrolytic degradation. This property distinguishes PHB from most of the presently available biodegradable plastics, which are either water-soluble or moisture-sensitive.

MATERIALS AND METHODS
This project was done in St. Francis College for Women, Hyderabad in collaboration with Nizta Biologicals Institute in the month of May, 2019 as an undergraduate project. Water sample was collected from the contaminated water of Gopalpur beach, Bhubaneswar, because of the approachability and requirement of seawater. Samples were serially diluted and inoculated/spread plated on a sterile nutrient agar plate (peptone-5 gm, sodium chloride-5 gm, beef extract-3 gm, agar-18 gm, distilled water-1000 ml). The plated were then kept for incubation for 24 h at 37 °C. Colonies showing specific features were picked and purified by recurrent streaking on similar agar plates. The purified colonies were conserved on nutrient agar slants.

Qualitative testing was done for all the bacterial isolates to check for PHB production following the viable colony method of screening using Qualitative testing was done for all the bacterial isolates to check for PHB production following the viable colony method of screening using

PHAs exhibit dark granules. The bacteria that exhibit colonies were PHB production following the viable colony method of screening using Qualitative testing was done for all the bacterial isolates to check for PHB production following the viable colony method of screening using

Production of polyhydroxy-butrate
Two production media were prepared for the production of PHB. First media had modified nutrient broth (peptone-5 gm, sodium was chlorides-5 gm, beef extract-3 gm, distilled water-1000 ml) with 1% glucose and 0.2% sodium chloride. The second media was a Minimal Salt Medium (MSM) (KH2PO4-1.5 gm, Na2HPO4-2H2O-1.5 gm, NaCl-1.0 gm, (NH4)2SO4-2.0 gm, MgSO4-0.2 gm, CaCl2-2H2O-0.02 gm, Na2FeIIIcitrate-0.05 gm, trace element solution-1 ml/l, glucose-10 gm/l) (Trace element solution-ZnSO4-7H2O-100 mg/l H3PO4-300 mg/l, CaCl2-200 mg/l, CuSO4-6 mg/l, NCl2-20 mg/l, Na2MoO4-30 mg/l, MgCl2-25 mg/l, the trace element solutions was prepared separately, autoclaved and mixed with the media[1])

The bacterial isolates showing positive in the screening for the polyhydroxybutyrate were then incubated into the production media and were then kept for 72 h at 37°C.

Extraction of polyhydroxy butyrate
The culture was then collected and centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was discarded and the pellet was treated with 10 ml sodium hydrochlorite and incubated at 30 °C for 2 h. After incubation, the mixture was centrifuged at 8000rpm for 10 min at 4 °C and washed sequentially with distilled water, acetone and alcohol. Finally the residue was extracted with boiling chloroform and poured in a sterile glass petri plate for evaporation of chloroform. After all the chloroform is evaporated, the extract is stored in 20 °C for further analysis.

Estimation of PHB produced-colorimetric estimation
The UV absorption spectrum of PHB was analysed after its conversion to crotonic acid by treating it with conc. H2SO4. The development of brown colour in the solution due to crotonic acid formation was significantly used to determine the concentration counting on the intensity of the colour [11]. Sample was moved into a clean test tube and 10 ml of concentrated H2SO4 was added to test tubes and capped. The capped test tubes were heated for 10 min at 100 °C in a water bath. After that, the solution was allowed to cool and mixed thoroughly. Sample was transferred to silica cuvette to read the absorbance at 445 nm. The absorbance was measured against sulphuric acid as blank [11].

RESULTS
The plates were incubated for 24 h after spread plating of water sample. Three colonies were isolated from the water sample. The samples were named as C1, C2 and C3. The colonies were first identified by colony morphology. The Sudan black screening test is done to screen for the production of (PHB) poly hydroxyl butyrate by bacterial isolates. The bacterial isolates C1 and C2 has shown a positive result for the biochemical tests (table 1 and fig. 2), gram staining and endospore staining (table 2 and fig. 3) and production of poly hydroxybutyrate (PHB) fig. 4.

| Biochemical tests | Objective                                                                 | Observations |
|-------------------|---------------------------------------------------------------------------|--------------|
| Starch hydrolysis | To differentiate organism based on their α-amylase enzyme activity (hydrolysis of starch). | +ve +ve       |
| Catalase test     | For differentiating aerobic and obligate anaerobic bacter ia.               | +ve +ve       |
| Simmons test      | It is used for the differentiation of Gram-negative bacteria on the basis of citrate utilization. | +ve -ve       |
| Voges proskauer test | The Voges-Proskauer (VP) test is used to determine if an organism produces acetyl/methyl carboid from glucose fermentation. | +ve -ve       |
| Mannitol fermentation test | The purpose is to see if the microbe can ferment the carbohydrate (sugar) mannitol as a carbon source. | +ve +ve       |

| Sample | Gram staining | Morphology       | Endospore staining |
|--------|---------------|------------------|-------------------|
| Colony 1 | +ve | Cocobacillus    | +ve               |
| Colony 2 | +ve | Rod shaped chined bacillus | +ve               |

| Sample | Colony | Absorbance |
|--------|--------|------------|
| Water (marine) sample | C1   | 0.20       |
| Water (marine) sample | C2   | 0.22       |
Fig. 2: Observations of biochemical tests

Fig. 3: Observations of gram staining and endospore staining

Fig. 4: Extracted PHB molecules
DISCUSSION

Polyhydroxybutyrate (PHB) accumulates as a carbon/energy reserve material in various microorganisms and is a type of microbial polyester that can prove to be a decent alternative to plastics derived from petroleum [10]. In the present study, 3 bacterial strains were screened for PHB production. Three colonies were isolated from the water sample. The samples were named as C1, C2 and C3. The colonies were identified for colony morphology and biochemical tests along with gram staining and endospore staining were done. The results were positive for biochemical tests (table 2 and fig. 2) in C1 and C2. These two samples i.e; C1 and C2 showed positive results for gram staining and endospore staining as well (table 3 and fig. 3). Based on these observations it was concluded that C1 and C2 samples were of Cocobacillus and Rod shaped bacillus respectively. These conclusions were derived in accordance with the studies done earlier [7, 8].

Two production media-modified nutrient broth media and minimal salt media was prepared for production of PHB. C1 and C2 samples showed positive results in the screening for the polyhydroxybutyrate and were therefore incubated into the production media for PHB production (fig. 4). PHB thus produced was extracted and processed for colorimetric estimation at 445 nm and absorbance was read (table 4). Presence of PHB granules in Cocobacillus (Colony C1) and Rod shaped bacillus (Colony C2) was confirmed with bacillus showing more concentration of PHB formation (absorbance 0.22).

Based on the observations and results of our study it was understood that many other bioplastics with different structures, properties and applications could be obtained if the appropriate organism were selected and genetically manipulated [1, 6]. However, the primary problem for large-scale production and commercialization of PHB is their high assembly cost as compared to plastics derived from petrochemicals. Lately, many attempts has been committed to scale back the assembly cost of PHB by using techniques such as; developing efficient bacterial strains, enhancing fermentation and recovery processes. Most reports regarding the manufacture of PHB suggested that the main contributor to the general PHB production cost was due to carbon substrate cost [1]. As such, the selection of efficient carbon substrate is a key aspect, which defines the total cost of the final product. The alternative approach is to look for renewable, economically achievable and easily available carbon substrates for microbial growths and therefore efficient PHB production [1]. Because of their special characteristics and broad biotechnological applications, bioplastics are compounds with promising future [12].

CONCLUSION

The studied bacillus sps. Rod shaped bacillus were found to be more efficient in production of PHB in comparison to Cocobacillus. Bioplastics which are biodegradable and have many beneficial properties compared to petroleum derived plastics are the need of time due to environmental concerns. Further studies can be done with more resources for better results. Bacteria from different sources and different species can be isolated and studied for their capabilities to produce more amount of PHB for commercial benefits.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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