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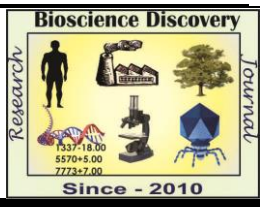

PHB production from bacteria isolated from oil contaminated soil

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Abstract

PHB is biodegradable plastics, a group of biopolymers synthesized by many bacteria and archaea. In present investigation PHB was prepared from the bacterial species isolated from oil contaminated soil. Five efficient producers were proceeded for PHB production. Two crude carbon sources, groundnut oil cake powder and molasses were tested versus pure sugar glucose as carbon source for production. This was done to establish the capabilities of local strains to utilize renewable and locally available substrates in polyhydroxybutyrate production. The isolates were biochemically characterized by Bergey's Mannual of Systematic bacteriology. The produced PHB was confirmed by Spectrophotometric method and FTIR. Better production was observed with groundnut oil cake powder as compared with pure glucose. Optimum pH, temperature and incubation time for PHB production was also determined.

INTRODUCTION

Plastic materials that have been universally used in our daily lives but now they are causing serious environmental problems. Millions of tons of these nondegradable plastics accumulate in the environment per year. For efficient management of used-plastic materials, recycling is one solution. But as the speed of utilization is much higher than speed of recycling, it cannot be used as a practical method that could be used on commercial scale. Another solution to reduce plastic residue is the use of biodegradable plastics and among them polyhydroxybutyric acids (PHB) are drawing much attention. They are common intracellular compounds found in bacteria, archaea, and in few eukaryotes such as yeasts and fungi. PHAs are carbon and energy reserve polymers produced in some microorganisms when carbon source is in plentiful and other nutrients such as nitrogen, phosphorus, oxygen or sulfur are limited. PHB is found to accumulate in varieties of microorganisms as reserve food material. They are the most common biodegradable polymer that can be used as promising alternative to synthetic nondegradable plastics. These polymers are accumulated intracellular membrane enclosed inclusion up to 90% of the cell dry weight under conditions of nutrient stress and act as energy reserve material. It has similar mechanical properties as those of the oil-derived conventional plastics like polypropylene or polyethylene which can be molded, made into films, spun into monofilaments, and used to make heteropolymers with other synthetic polymers and many more applications in agriculture, packaging, and medical field being biodegradable and also immunologically compatible with human tissues (Anish Kumari *et al.*, 2013).

Cellulose is always been the most abundantly available waste in the world. It can be in the form of agricultural waste, food industry waste, sugar industry waste, or paper waste. The waste paper is generally recycled in paper or cardboard industry. The paper pulp of waste paper of this industry can be used as an efficient source of carbon for PHB production (Muthu Kumar A, 2017).

The present study attempts to use paper pulp as crude carbon source for PHB production and the efficiency will be compared with use of pure glucose sugar.

Isolation of Bacteria

1g of paper pulp sample was taken from cardboard industry located in Nanded region. The sample was serially diluted with sterile saline and inoculated on Sterile nutrient agar plates. The plates were incubated for 24hrs at 37°C. After incubation, selected colonies were grown on minimal media supplemented with 1% of sterile paper pulp as carbon source. The plates were incubated for 24hrs at 37°C. After incubation, the appeared colonies were subjected for morphological and biochemical analysis. The obtained results were compared with Bergey's manual of systematic Bacteriology for strain identification (Bhairavi *et al.*, 2011).

Screening of PHB producers

For isolating PHB producing bacteria, Sudan black B and Nile blue A staining were performed and only the colonies showing positive results were carried forward for production (Anish Kumari *et al.*, 2014)

PHB Production

Modified Mineral Salt medium without glucose was used as production medium supplemented with 1% paper pulp which is separately autoclaved and then mixed with the medium (Anteneh and Fantahun, 2016).

A comparative set with mineral salt medium with its proper composition was also set with same selected strains. The medium is incubated at 37°C for 24hrs. Effects of pH, temperature and incubation time on PHB production were also determined. (Obruca *et al.*, 2015)

- **Extraction and Confirmation of PHB**

The extraction procedure was performed as described by Anteneh and Fantahun in 2016.

FTIR spectrophotometer analysis of PHB

About 1 mg extracted sample of PHB was dissolved in 5 ml chloroform. After pellet was formed by adding KBr, spectra were recorded at 4000–400 cm^{-1} range by Spectrum 65 FT-IR (Anteneh and Fantahun, 2016).

RESULTS AND DISCUSSION

PHB was produced from five bacterial isolates that are isolated from oil contaminated soil. By performing various biochemical and morphological tests and comparing their results with Bergey's Mannual of Systematic Bacteriology, the isolates were assumed to be isolate-1: *Pseudomonas*, isolate-2: *Klebsiella*, isolate-3: *Bacillus*, isolate-4: *Enterobacter*, isolate-5: *Staphylococcus*.

PHB production was performed by using two crude carbon sources, groundnut oil cake powder and molasses against pure sugar glucose. Effect of different parameters like pH, temperature and incubation time was also performed. From the tables and their respective graphs it is clear that for all isolates, the most optimum pH is pH 7, the most optimum temperature is 35°C and most optimum incubation time is 48hrs. The results also declared that the crude carbon sources gave better production against pure sugar, which are showing similarity with the previous work.

Table-1: Morphological characters

Character	Isolate P1	Isolate P2	IsolateP3
Size	3mm	3mm	2mm
Shape	Rod	Rod	Rod
Colour	Cream	white	Cream
Margin	Irregular	entire	entire
Elevation	Concave	Concave	Convex
Consistancy	Smooth	Smooth	Mucoid
motility	motile	motile	motile
Opacity	Opaque	Opaque	Opaque
Gram's nature	Negative	positive	negative

Table-2: Biochemical Characters

Test	Isolate P1	Isolate P2	Isolate P3
Glucose Fermentation	Negative	Positive	Positive
Lactose Fermentation	Negative	Positive	Positive
Sucrose Fermentation	Negative	Positive	Positive
Mannitol Fermentation	Positive	Positive	Positive
Indole	Negative	Negative	Positive
MR	Negative	Negative	Positive
VP	Negative	Positive	Negative
Citrate	Positive	Positive	Positive
Oxidase	Positive	Negative	Positive
Catalase	Positive	Positive	Positive

Among the two crude sources, the results with groundnut oil cake powder were promising as compared with molasses. All isolates showed higher production on groundnut oil cake powder as compared with molasses. This was a purely innovative initiative taken by me, as in the references, this type of combination was not performed earlier.

Among the five isolates, the isolate-1 showed maximum production on groundnut oil cake powder, followed by glucose and least with molasses. The production was slightly higher when compared with the work of Reddy and Thirumala, the possible reason could be the presence of groundnut oil cake powder as carbon source in present study.

The isolate-2 also showed maximum production on groundnut oil cake powder, followed by glucose and least on molasses. Here also the production was slightly higher as compared with the work of Sushma Shenoy, where manitol was used as carbon source.

The isolate-3 showed maximum production on groundnut oil cake powder, followed by molasses and least with glucose. Here also the production was slightly higher as compared with the work of Sajida Munir and Nazia Jamil who used pure glucose as carbon source.

The isolate-4 showed maximum production on groundnut oil cake followed by glucose the least with molasses. The isolate-5 showed same production on groundnut oil cake powder and glucose and least with molasses. All results were approximately similar to the earlier studies.

The study finally concludes that crude carbon sources are better as compared to pure sugars for PHB production. Also the conditions for PHB production were also optimized that were pH 7, temperature 35°C and incubation time 48hrs.

Table-3: Effect of pH on PHB production

	Production on Groundnut oil cake powder 2%			Production on Molasses 2%			Production on Glucose 2%		
	pH 3	pH 7	pH 9	pH 3	pH 7	pH 9	pH 3	pH 7	pH 9
Isolate O1	0.3g	1g	0.5g	0.2g	0.8g	0.6g	0.2g	0.9g	0.7g
Isolate O2	0.22g	0.96g	0.4g	0.1g	0.92g	0.32g	0.11g	0.93g	0.34g
Isolate O3	0.42g	0.98g	0.7g	0.34g	0.97g	0.2g	0.4g	0.96g	0.33g
Isolate O4	0.2g	0.8g	0.3g	0.12g	0.7g	0.3g	0.2g	0.75g	0.32g
Isolate O5	0.12g	0.75g	0.45g	0.1g	0.73g	0.33g	0.1g	0.75g	0.22g

Table-4: Effect of Temp. on PHB production

	Production on Groundnut oil cake powder 2%			Production on Molasses 2%			Production on Glucose 2%		
	25°C	35°C	42°C	25°C	35°C	42°C	25°C	35°C	42°C
Isolate O1	0.55g	1g	0.75g	0.4g	0.8g	0.5g	0.5g	0.9g	0.84g
Isolate O2	0.32g	0.96g	0.5g	0.52g	0.92g	0.5g	0.45g	0.93g	0.85g
Isolate O3	0.22g	0.98g	0.6g	0.65g	0.97g	0.85g	0.6g	0.96g	0.8g
Isolate O4	0.12g	0.8g	0.58g	0.2g	0.7g	0.4g	0.48g	0.75g	0.65
Isolate O5	0.2g	0.75g	0.4g	0.5g	0.73g	0.62g	0.4g	0.75g	0.55g

Table-5: Effect of incubation time on PHB production

	Production on Groundnut oil cake powder 2%			Production on Molasses 2%			Production on Glucose 2%		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Isolate O1	0.8g	1g	0.87g	0.7g	0.8g	0.75g	0.85g	0.9g	0.85g
Isolate O2	0.85g	0.96g	0.9g	0.85g	0.92g	0.89g	0.89g	0.93g	0.89g
Isolate O3	0.9g	0.98g	0.92g	0.88g	0.97g	0.9g	0.9g	0.96g	0.91g
Isolate O4	0.68g	0.8g	0.75g	0.65g	0.7g	0.68g	0.7g	0.75g	0.68g
Isolate O5	0.7g	0.75g	0.72g	0.68g	0.73g	0.7g	0.7g	0.75g	0.7g

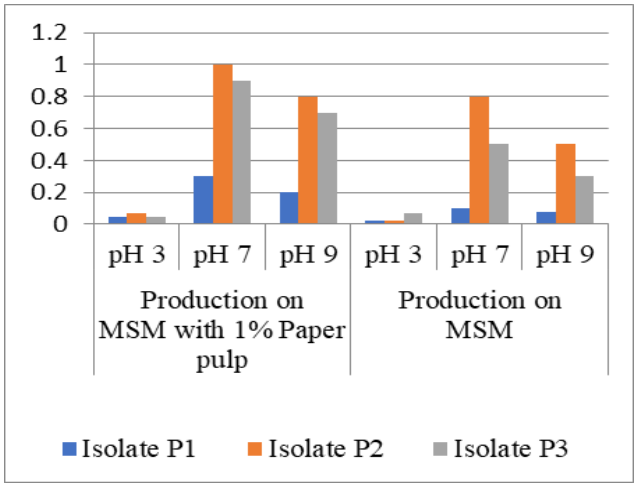

Fig. 1: Effect of pH on PHB production

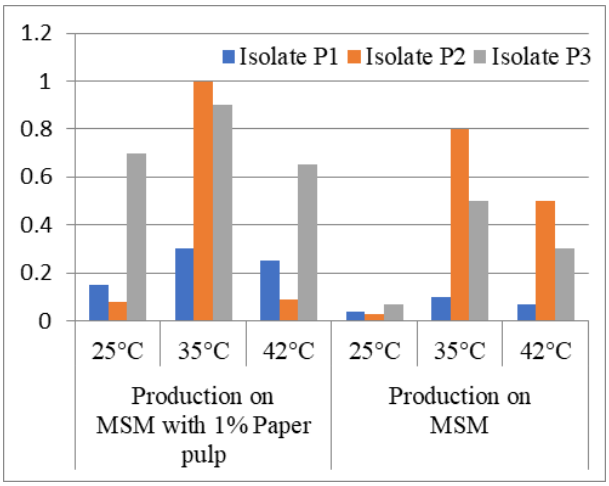

Fig. 2: Effect of temp on phb production

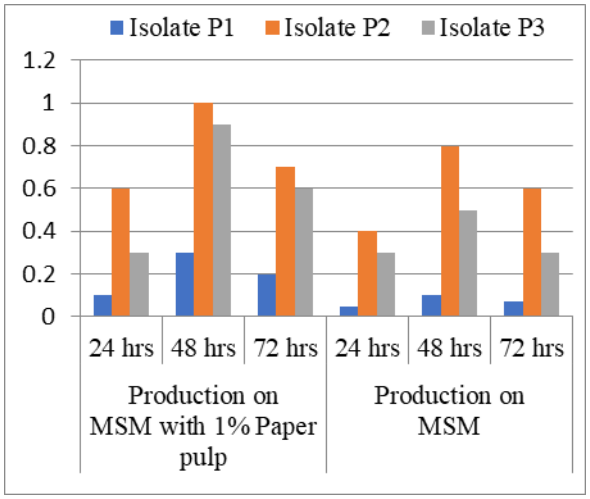

Fig. 3: Effect of incubation time on PHB production

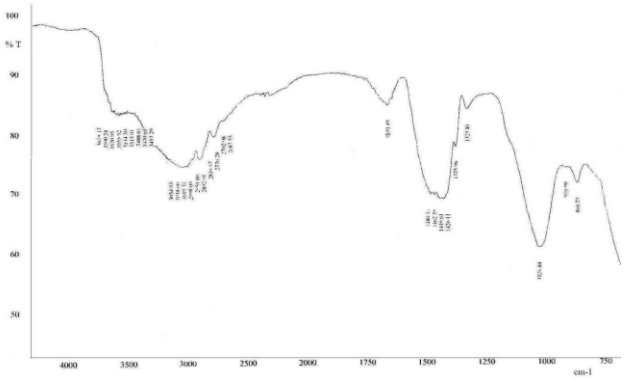

Fig. 4: FTIR Spectrum of produced PHB

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