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Isolation of Polyhydroxybutyrate (PHB) Producing Soil Microorganisms

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Abstract: ***Polyhydroxybutyrates (PHBs) are macromolecules synthesized by bacteria. They are inclusion bodies accumulated as reserve materials when the bacteria grow under different stress conditions. Because of their fast degradability under natural environmental conditions, PHBs are selected as alternatives for production of biodegradable plastics. The aim of this work was to isolate potential PHB producing soil bacteria and to evaluate PHB production***

Keywords: Polyhydroxybutyrate, soil bacteria

1. Introduction

Polyhydroxybutyrate (PHB) is a polymer having a place with the polyesters class that are of enthusiasm as bio-inferred and biodegradable plastics. The poly-3-hydroxybutyrate (P3HB) type of PHB is likely the most widely recognized kind of polyhydroxyalkanoate (Lichenthaler *et al*., 2010)

Biodegradable polymers are viewed as the potential answer for oversee issues concerning worldwide natural and strong waste organization. These biodegradable plastic materials can hold the coveted material properties of ordinary manufactured plastics, and can be totally debased without leaving any unwanted deposit. With the point of substituting the usefulness of plastics of petrochemical beginning, and growing the scope of utilization of bioplastics to an across the board range, polyhydroxyalkanoates (PHAs) are seen as the most reasonable materials in view of their flexibility as far as physical properties and synthetic qualities. Polyhydroxybutyrate (PHB) is the most broadly examined individual from the PHA family and the first that has been delivered at modern scale. It has been utilized, among different applications, to deliver containers, movies, and filaments for biodegradable bundling materials. Utilizations of PHB are not limited to these territories, and they have been stretched out to osteosynthetic materials, bone plates, surgical sutures, bolts, tacks, and numerous different materials in medication. PHB has a decent degree over control of air contamination and metropolitan wastewater administration by delivering PHB in biomass and it can likewise utilized by the businesses.

2. Materials and Methods

Collection of Soil Samples
Four soil samples collected
Zone I: Soil samples collected from rice field Tiruvallur district.
Zone II: Soil samples collected from peas field Tiruvallur district.
Zone III: Soil samples collected from lady's finger field Tiruvallur district.
Zone IV: Soil samples collected from sewage Ambattur, chennai

Soil sample were collected in clean sterile bags and transported to the laboratory.

Isolation of PHB Producing Organisms from Soil Samples (Yilmaz et al., 2005)
One gram of soil sample was suspended in 99 ml sterile distilled water and shaken vigorously for 2 min. This was serially diluted by mixing 1ml with 9ml sterile distilled water to get a dilution of $10^{-1.}$ This serial dilution was repeated to get dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10-7 and 10^{-8} for the isolation of organisms. 0.1ml of 10^{-7} and 10^{-8} dilutions were plated on to minimal salt medium by spread plate method for the propagation of microbial growth. Triplicates were maintained. The plates were incubated at 30°C for 24hrs.

Colonies with distinct features were picked and purified by repeated streaking on similar agar plates. The purified colonies were preserved on nutrientagars lants at4 °C.

Qualitative Screening for the Production of PHB using Sudan Black Staining Technique (Williamson and Wilkinson, 1958)
The isolated bacterial strains were screened for PHB production. As a preliminary step, screening of PHB producers was carried out using viable colony staining technique. The cultures were grown on minimal media supplemented with glucose (2%) as a sole carbon source, incubated at 40ºC for 48hrs. After incubation, the plates were flooded with Sudan black B solution for the detection of microbial intracellular lipid granules and kept undisturbed for 20 minutes. The excess of Sudan black solution was drained off. The isolated colonies were screened for PHA production by Sudan black staining and ranked based on the magnitude of their staining

Morphological and Biochemical Screening of the PHB positive isolates
The PHB producing bacterial isolates were subjected to a set of morphological, physiological and biochemical tests and identified to the Genus level based on Bergey's Manual of Determinative Bacteriology.

Extraction and Quantification of PHB
The bacterial isolates were inoculated in the nutrient broth and placed in a rotary shaker for 24 hours.After 24hours

broth samples were transferred to the centrifuge tube. It was centrifuged at 5000 rpm at 4℃ for 10 minutes. Then the pellet was re-suspended in sodium hypochlorite and left for 1 hour in the room temperature. Then it was centrifuged at 5000 rpm for 10 min after that pellet was washed with water, acetone and methanol. 10 ml of chloroform was added and placed in a boiling water bath for 10 min at 65ºC .1ml was dried and kept in water bath and allowed to dry completely. After drying 10 ml of concentrated was added and placed in boiling water bath for 20 min at 95 ºC. The solution was cooled and reading was taken at 235nm in uv-vis spectrophotometer with concentrated sulphuric acid as blank

3. Results

Bacterial isolates from soil sample
A total of 13 bacterial isolates were isolated from bacterial sample

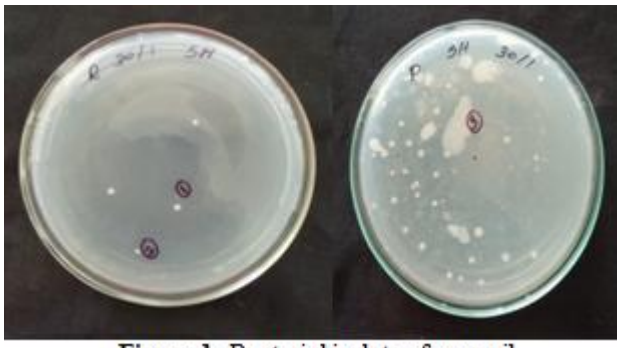


Figure 1: Bacterial isolates from soil

Sudan Black B Staining
Three isolates showed positive with sudan black b staining method. The black granules present inside the cells of bacteria results as PHB producing bacteria.

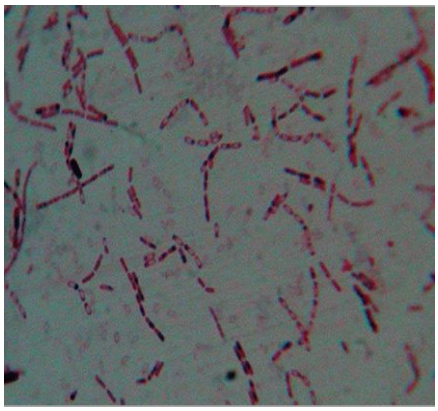


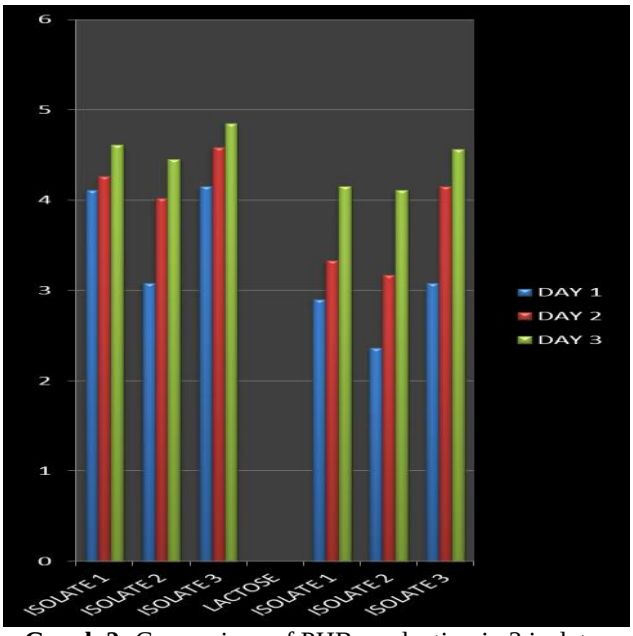
Figure 2: PHB producing bacterias howing black granules with Sudan Black B Staining

Identification of the isolates based on biochemical tests
Based on the biochemical tests as shown in table 1, the three isolates were identified as ***Citrobacter sp, Protease sp and Pseudomonas sp.***

Table 1: Biochemical tests to identify bacterial isolates

Test	Isolate- 1	Isolate- 2	Isolate- 3
Motility	Motile rods	Motile rods	Motile rods
Catalase	+ ve	+ve	+ve
Oxidase	+ ve	+ ve	+ve
VP	-ve	-ve	-ve
Urease	- ve	+ve	+ ve
Citrate	-ve	+ve	+ ve
Nitrate	-ve	+ve	-ve
Indole	- ve	-ve	-ve
MR	+ ve	- ve	+ve
Glucose Gas/color	+ve /+ve	+ve /+ve	-ve /-ve
Sucrose Gas/color	+ve /+ve	-ve /-ve	+ve /-ve
Lactose Gas/color	-ve /-v`e	+ve /-ve	-ve /-ve

Production of PHB from Various Carbon Sources Such as Glucose and Lactose
Glucose and lactose is used as the carbon source all the positive isolates shows more PHB production in glucose comparing with lactose.



Graph 2: Comparison of PHB production in 3 isolates between glucose and lactose

4. Discussion

The aim of this study was to screen and select the suitable organism for PHB production. 3 isolates was found to be more effective for PHB production in the media containing various carbon sources like orange peel and pineapple peel and without any additional substrate. In this orange peel shows more amount of PHB production in isolate 1. Other additional carbon sources like glucose and lactose is supplemented as the carbon source. In that glucose shows more amount of PHB production comparing to lactose and isolate 3 shows more production of PHB.

The focus of the future research would be to reduce the cost of production as well as improve the quality of the polymers to make it suitable for high cost products. A balance

between operating cost, product yield and quality is necessary to make it more economical.

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