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EXTRACTION OFPOLYHYDROXYBUTYRATE FROM BACTERIA PRESENT IN AGRICULTURAL SOIL TO PRODUCE BIO-DEGEADABLE PLASTIC

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ABSTRACT: Plastic usage plays a major role in various applications. Synthetic plastics are produced from nonrenewable resources like Petrochemicals and their intended use results in solid waste generation, global environmental pollution and deterioration of properties of substances stored in plastic containers. Therefore, there is a great need for replacement of conventional plastic. Bio- degradable plastic is the promising solution which is free from above disadvantages. Numerous sources and ways are available to synthesis bio-degradable plastics. The study focuses on production of Polyhydroxybutyrate (PHB), a type of bio-degradable plastic. PHB has physical and chemical properties similar to that of conventional plastics. PHB is a carbon energy source which is stored in bacterial walls of the species Staphylococcus sp. and Bacillus sp. under stressful growth conditions (low nutrient supply). The study focuses mainly to isolate these bacteria from rhizospheric soil and culture their growth to extract PHB from the bacteria. The work also involves the study of application of the produced biodegradable plastic in the medicinal field.

KEY WORDS: Biodegradable plastic, Polyhydroxybutyrate, soil bacteria.

I. INTRODUCTION

Conventional plastics including polyethylene, polypropylene and polystyrene are exclusively made from nonrenewable resources like petroleum. In this world a large amount of fossil carbons is used in Chemical industries and a part of it is used for production of plastics and polymers. These petroleum-based plastics are non-degradable and results in global menace. Hence there is a growing public demand for ecofriendly bio-degradable polymers made from renewable resources for the replacement of petroleum- based plastics.

Polyhydroxybutyrate (PHB) is a polymer belongs to the class of polyhydroxyalkanoates (PHA), which possess thermoplastic characteristics and resembles synthetic polymers to a great extent. PHA is bio-degradable in both aerobic as well as anaerobic environment. PHB is a type of polyester which can be synthesized by various microorganisms. It is stored as an energy reserve material by various microorganisms under stressed growth conditions i.e. either by limiting some of essential nutrients or by excessing the availability of carbon source. Microbes like Azotobacter, Bacillus, Pseudomonas, Rhizobium are able to produce PHB up to 30-80% of their dry cell weight (Lafferty et al., 1998).The rhizospheric soil i.e. soil nearer to the roots of plants are found to be colonized with number of bacteria which are capable to store PHB as carbon energy source, thus, making it a good source for PHB producers (Foster, 1985). By considering these points, the present study is focused on isolation of PHB producing bacteria from collected soil sample and to extract the carbon energy source which is stored under the bacterial cell wall as an energy reserve material for bacteria under culture conditions like carbon source, nitrogen source, and C/N ratio and incubation time.

II. MATERIALS AND METHODS

2.1 Materials Used

Soil samples, Agar agar, glucose, peptone, urea, incubator, autoclave, centrifuge.

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2.2 Methods

2.2.1 Sample collection

For the isolation of PHB producing bacteria, soil sample was collected from the rhizospheric area of groundnut crop, (Rhizospheric area is the area nearer to the roots of the plants). The soil samples are collected from different places randomly from the single farm. The randomly collected samples were mixed well and made into a single sample. The collected sample was dried under atmospheric condition to remove moisture. The dried sample was crushed gently by hands to removes lumps and the finely crushed soil sample is stored for further process.

2.2.2 Sample analysis

The collected soil sample were analyzed to identify the bacterial species present in the soil.

TABLE 1: Biochemical tests of soil sample

S. No.	Biochemical Test	Positive/Negative	
		CN1	CN2
1.	Gram Staining	+	+
2.	Catalase Test	+	+
3.	Mannitol Salt Agar Test	-	+
4.	Starch Hydrolysis Test	-	+
5.	Motility Test	-	+
6.	Coagulase Test	-	-
7.	Blood Agar Test (Gamma-Hemolysis)	+	+

1. Gram staining

Gram staining technique is used to characterize the bacterial species into either gram-positive or gram- negative based on the physical properties of the cell wall. Bacterial strains are coated on the sample placed in the plate, based on the color the bacteria in the sample are characterized as gram-positive bacteria.

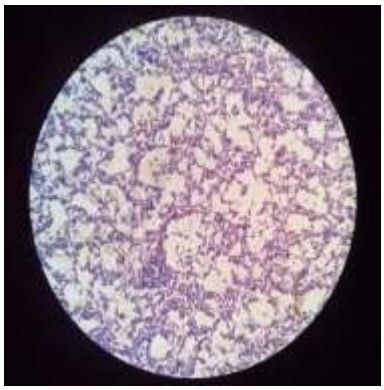

Fig.1.1 Gram Positive- Cocci

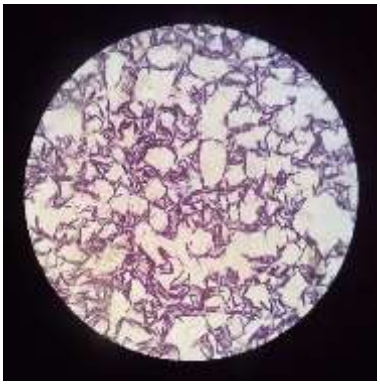

Fig.1.2 Gram Positive- Rod

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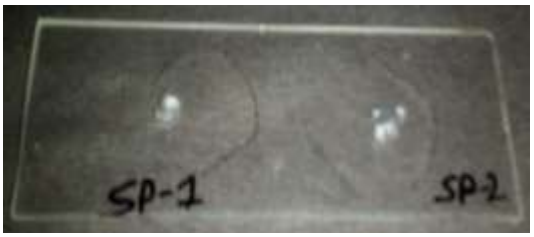
2. Catalase Test

Catalase test are done to identify whether the bacteria in the sample are catalase positive or catalase negative. This test mainly used to differentiate among gram-positive cocci. If an bacterial organism produce catalyse enzyme they are termed as catalase positive which protects them from toxic and harmful substance. Presence of catalase enzyme can be confirmed if they forms air bubble when reacted with hydrogen peroxide.

Fig.2.1Catalase Test of CN1 and CN2



3. Mannitol Salt Agar Test

Mannitol Salt Agar test is recommended for isolating pathogenic staphylococci from samples. The test boost the growth of certain bacteria and inhibiting the growth of others. When bacteria grows under high salt condition, they ferment mannitol and produce acid, hencethe pH gets Varied.

Fig.3.1 Mannitol-Not Fermented in CN

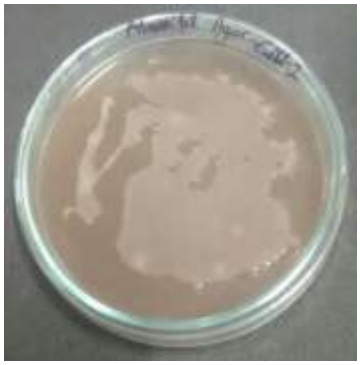


Fig 3.2 Mannitol - Fermented in CN2

4. Starch Hydrolysis Test

This test is carried out to identify whether the bacteria can hydrolyses the starch or not. This can be done by adding iodine to the bacteria. Iodine turns blue, purple, or black in the presence of **starch**. A clearing around the bacterial growth indicates that the organism has **hydrolyzed starch**.

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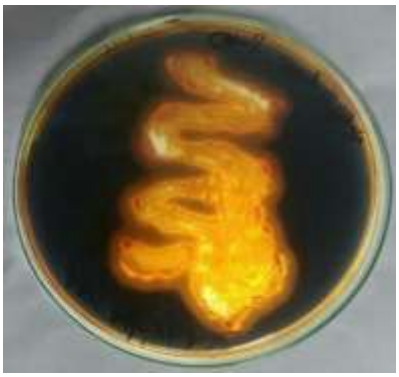

Fig 4.1 Starch-No Hydrolysis in CN1 **Fig 4.2 Starch - Hydrolysis in CN2**

5. Motility Test

Motility allow the assessment and identification of abnormal patterns and physiology.

Fig 5.1 Motility-No Turbidity in CN1 **Fig 5.2 Motility -Turbidity Formed in CN2**

6. Coagulase Test

Coagulase test is done to identify the presence of enzyme coagulase in the bacteria.

Fig 6.1 Coagulase Test of CN1 and CN2

7. Blood Agar-Hemolysis Test

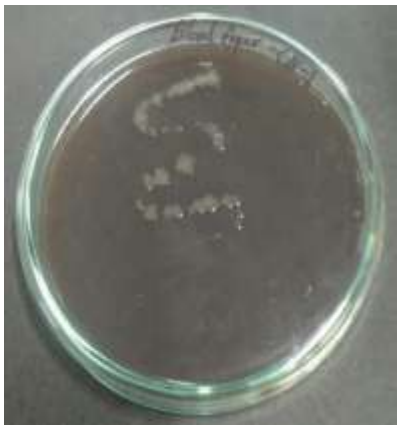

Fig 7.1 Gamma-Hemolysis in CN1

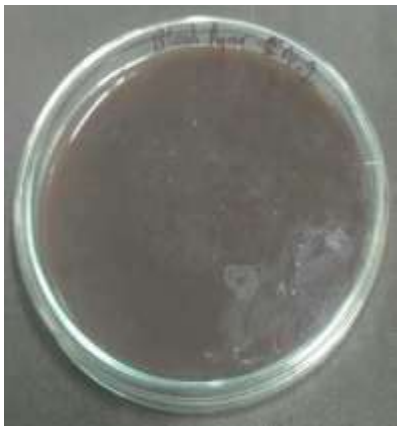

Fig 7.2 Gamma-Hemolysis in CN2

From the above biochemical analysis tests which concludes the bacteria and its genus.

i) The CN1 named bacteria can be identified at a genome of ***Staphylococcus sp.***

ii) The CN2 named bacteria can be identified at a genome of ***Bacillus sp.***

2.2.3 Extraction of PHB

The finely crushed soil sample was plated in the petri plate which is autoclaved with agar agar and agar medium. The bacterial growth was observed in the petri plate which is incubated for 24 hours at room temperature. Staining technique is used to identify the gram positive and gram-negative bacteria. Gram positive bacteria are mostly PHB producing than gramnegative.

All PHB producing bacterial isolates (gram positive bacteria) were suspended in the nutrient broth. The nutrient broth consists of both carbon source (glucose) and nitrogen sources (peptone) in specific C/N ratio as 7:3. The bacterial isolates are incubated in nutrient broth for 48 hours under room temperature. PHB positive bacteria were raised in the broth and cell growth isolates containing polymer was pelleted in centrifuge at 10,000 rpm for 10 min. The supernatant was discarded and cell pellets were washed with acetone and ethanol to remove unwanted materials. The washed pellets were dried in hot air oven at a temperature of 45^0c for 2 hours to get granules of PHB.

2.2.4 Optimization of culture medium parameter

Parameters influencing PHB production:

Nutrient sources (carbon and nitrogen source) Temperature
Carbon- Nitrogen ratio (C/N ratio) Incubation time

Parameters such as nutrient sources, C/N ratios, temperature and incubation time influences the PHB production. The present study mainly concerned with different nutrient sources and different C/N ratio and its influence over PHB production. Other parameters like temperature as room temperature and incubation time as 48 hours are maintained throughout the process.

TABLE 2: The various influencing parameters concerned in this study

Carbon source	Glucose	Fructose
Nitrogen source	Urea	Peptone
C/N ratio	4:1	7:3

Growth of bacterial isolates were studied by using various carbon and nitrogen sources as a nutrient broth under different C/N ratio. The bacteria is also subjected to grow under stressed condition i.e. by limiting the nitrogen

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source and supplying excess amount of carbon source. Glucose and fructose were used as a carbon source and peptone and urea were used as a nitrogen source. C/N ratios such as 4:1, 19:1 and 7:3.

III. RESULTS AND DISCUSSION

Highest yield of polymer was observed for glucose and peptone as carbon and nitrogen source under the C/N ratio7:3 (7 parts of glucose and 3 parts of nitrogen)

TABLE 3: Effect of Nitrogen limitation on PHB production by Staphylococcus sp. and Bacillus sp. after 48 hours of incubation**.**

Organisms	**Carbon Source**	**itrogen Source**	**C/N**	**PHB mg/ L**
Staphylococcus sp. and Bacillus sp.	Glucose	peptone	4:1	82
	Glucose	peptone	7:3	120
	Glucose	Urea	4:1	91
	Glucose	Urea	19:3	54

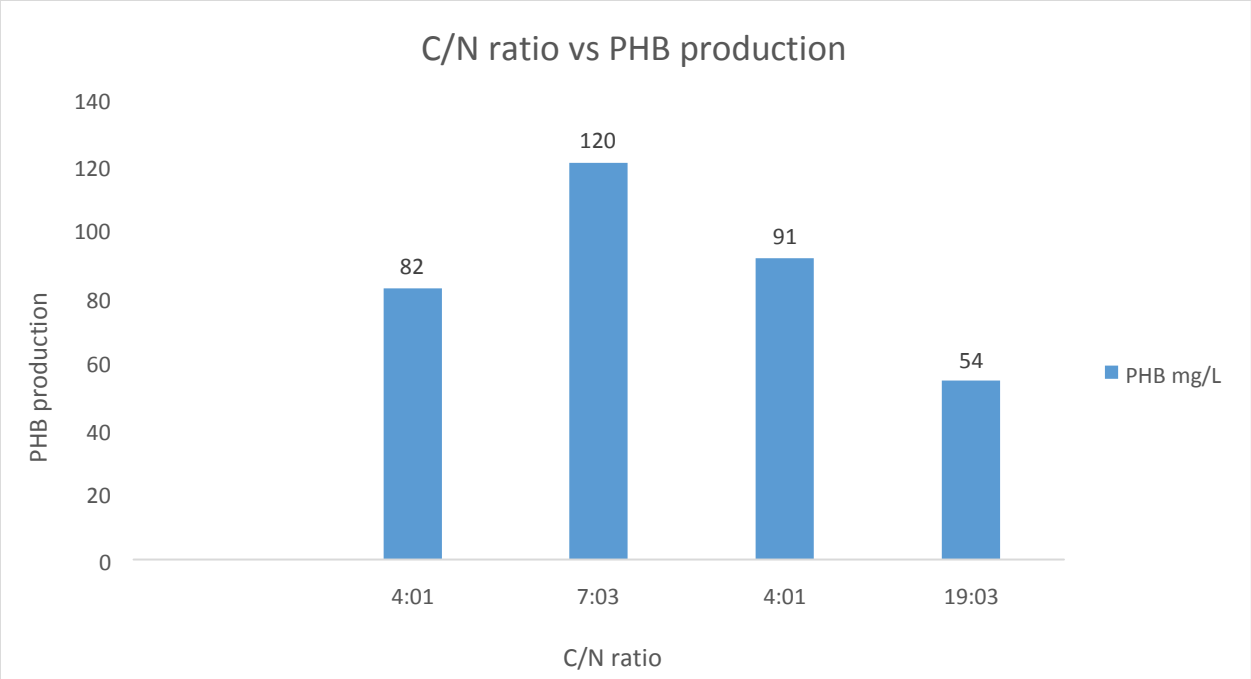

Fig 3 Graph between C/N ratio and PHB production

The above table shows the PHB production under different conditions and nutrient sources. From the table and graph it is observed that highest yield of Polyhydroxybutyrate is achieved by using glucose and peptone as carbon and nitrogen source with C/N ratio 7:3.
Bacteria are cultivated in both liquid broth and plate type growth, which is shown is fig 1 and fig 2

Fig 1: Bacteria in Nutrient Medium (liquid broth) Fig 2: Bacteria in Nutrient Medium (plate)

IV. CONCLUSION

The main objective of this study is to isolate PHB producing strains and to optimize the culture conditions to obtain the maximum PHB yield. From the results obtained it is observed that the optimum culture conditions for maximum PHB production from groundnut soil sample include supplementation of culture medium with glucose as carbon source and peptone as nitrogen source under the C/N ratio 7:3 and incubation for 48 hours at room temperature. Thus the present study provides useful data for PHB production that can be utilized for industrial production of PHB.

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