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Isolation of Polyhydroxybutyrate Microbial Producer from Local Egyptian Soil

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Abstract

Synthetic plastics' slow breakdown poses a serious risk to the environment; hence it is imperative that eco-friendly alternatives be used in their place. Because of their characteristics as biodegradable thermoplastics, biodegradable polymers like polyhydroxyalkanoate (PHAs) have lately been identified as polyesters. The biodegradable biopolymer polyhydroxybutyrate (PHB) has a number of uses in industry, agriculture, and medicine. This study's primary goal was to identify and classify an effective producer of PHB from soil samples taken from several locations in Egypt. The viable colony method of screening with Nile red dye was used to qualitatively assess all of the bacterial isolates for PHB synthesis. An effective PHB-producing bacterium was isolated from soil in the current investigation. Based on the viable colony staining method of screening using Nile red dye, 19 of the 52 distinct species of bacteria that were extracted were determined to be PHB positive. The 17 pinkish colonies that tested positive for Nile red staining under a microscope were bacilli-shaped, Gram-positive bacteria. The other two colonies were yeast as well. The findings showed that the majority of PHB in soil is produced by bacteria (89.5%), followed by yeast (10.5%).

Keywords: *Polyhydroxybutyrate, Nile red Dye, Bacillus sp., Egyptian Soil* .

1. Introduction

Petroleum, the primary energy source, is used to make plastics including polypropylene, polyethylene, and polystyrene (1). The production of plastics uses about 270 million metric tons of fossil fuels annually (2). Petroleum will run out in the next 60 to 80 years if consumption continues at present rate (3). However, the buildup of plastics in the environment has become a global issue (4). As a result, biodegradable polymers which are seen as environmentally beneficial substitutes for plastics replaced petroleum-based plastics (5).

The typical form of polyhydroxy alkonoates (PHA) used to make bioplastics is poly-3-hydroxy butyric acid (PHB) (6). PHB is employed in medicine because of its special qualities, which include being resistant to UV light, insoluble in water, oxygen permeable, very resistant to hydrolytic destruction, poorly resistant to acids and bases, and soluble in chloroform and other chlorinated hydrocarbons (7).

PHBs are regarded as sources of polymeric materials that are both biodegradable and biocompatible (8). As a result, the current issues brought on by a reduction in nonrenewable energy resources and environmental degradation from plastic waste is lessened. Agriculture, the food industry, and material science all use PHB (9). When some vital nutrients, including phosphate, magnesium, and nitrogen, are few and there are too many carbon sources available, many bacteria can produce PHB as an internal energy reserve material (10). PHB can be produced by a wide variety of microorganisms, including *Bacillus sp.* (11), *Pseudomonas spp.*(12), *Azotobacter vinelandii* (13), *Sinorhizobium meliloti*, and *Escherichia coli* (13, 14).

PHB is used in a wide range of industries, including the food, paint, packaging, cosmetics, and pharmaceutical sectors (15). Because PHB is more expensive to produce than plastics generated from petroleum, its commercialization is currently restricted (16). Economic substrates such methanol, ethanol, starch, whey, and beet molasses, as well as wheat hydrolysate, fungal extract, soy cake, and cane molasses, have been used in a number of earlier studies to lower the cost of producing PHB (17, 18). Additionally, it was stated that the primary expense is attributed to the fermentation process and product recovery (20).

Finding and isolating a local bacterial strain that produces PHB, followed by phenotypic identification and

classification analysis were the aim of this work.

2. Materials and Methods

2.1. Isolation of microorganisms from Local Egyptian Soil

Eight diverse soil samples from two separate locations in Egypt (Giza and Banha) yielded microbial isolates. To obtain a dilution of 10^{-1}, one gram of soil sample is dispersed in 10 milliliters of sterile distilled water, violently mixed, and then 1 milliliter is collected and added to a second tube with 9 milliliters of sterile distilled water. To obtain dilutions for the isolation of organisms, this serial dilution is repeated; 0.1 ml of each dilution was plated using the spread plate method onto a nutrient agar medium to promote microbial growth. For 48 hours, the plates were incubated at 30 ºC. On nutrient agar slants, colonies with various unique features were kept as pure cultures and kept at 4 °C (17).

2.2. Detection of PHB producer isolates

The living colony method of examination with Nile red dye was used to qualitatively assess all of the bacterial isolates for PHB synthesis. In order to get a final concentration of 0.5 µg Nile red/ml medium, 20 µl was placed onto sterilized pre-made (minimum salt agar media) plates for this screening of PHB producers. Following inoculation, the plates were then incubated at 30 ^{o}C for the entire night. The prepared clay soil samples were spread out over the surface of minimal salt agar media using a sterile glass rod after being sub-cultured by 0.1 ml samples. For 48 hours, the plates were incubated at 30 ^{o}C. In order to determine the buildup of PHB based on the lit plates, isolates from colonies with pinkish pigment that suggested PHB production were subjected to UV light (312 nm) and were given affirmative indicators. Sub-culturing these isolates on the same media allowed for their subsequent collection and purification (17).

2.3. Phenotypic identification and classification

Phenotypic identification and classification were performed according to Gram's stain. involves

3. Results and Discussion

Numerous bacteria have been detected to produce PHA particles under nitrogen and phosphorus constraints with an excess of carbon supply. Numerous studies indicate that the depletion of essential nutrients including magnesium, phosphate, and nitrogen combined with an excess carbon supply which facilitates the metabolism's transition from growth to PHB production causes high-density PHB

creation (21, 22). Because PHB polymerase allows various microbes to readily ingest it in the environment and produce harmless compounds, PHB is the most prevalent component in the PHA group (23).

Using serial dilution, microorganisms were extracted from eight clay soil samples for this investigation. 52 bacterial colonies according to various morphological characteristics were chosen and assigned numbers. To be examined, bacterial colonies were maintained on enriched nutrition agar medium. Figure 1 shows that 19 out of 52 colonies had a positive pinkish colony for Nile red staining, with a production percentage of 36.5%. The submerged fermentation technique was used for preliminary screening in order to assess the bacterial strains' capacity to produce PHB; spectrophotometric quantification was performed.

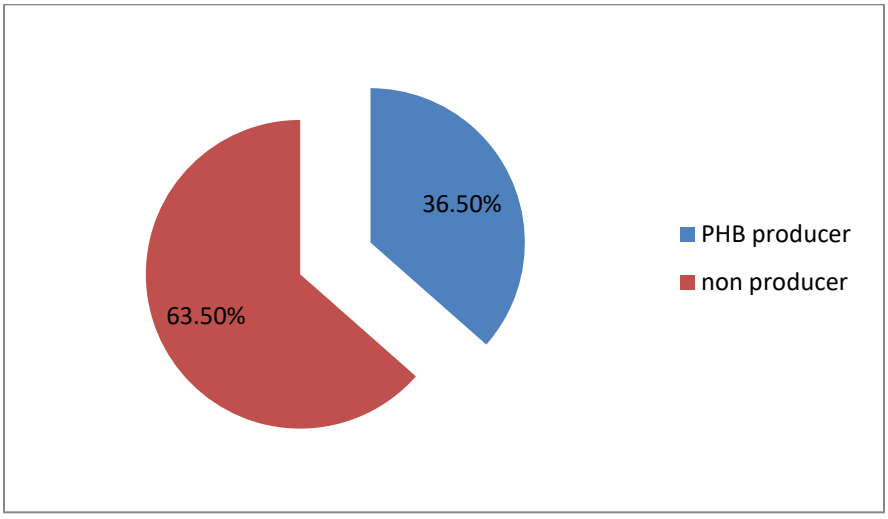

Figure 1: Percentage of PHB producer microorganisms for 52 isolates form 8 soil samples

The 17 pinkish colonies that tested positive for Nile red staining under a microscope were bacilli-shaped, Gram-positive bacteria. The other two colonies were yeast as well. According to the results, the majority of PHB in soil is produced by bacteria (89.5%), whereas yeast (Figure 2) accounts for 10.5%.

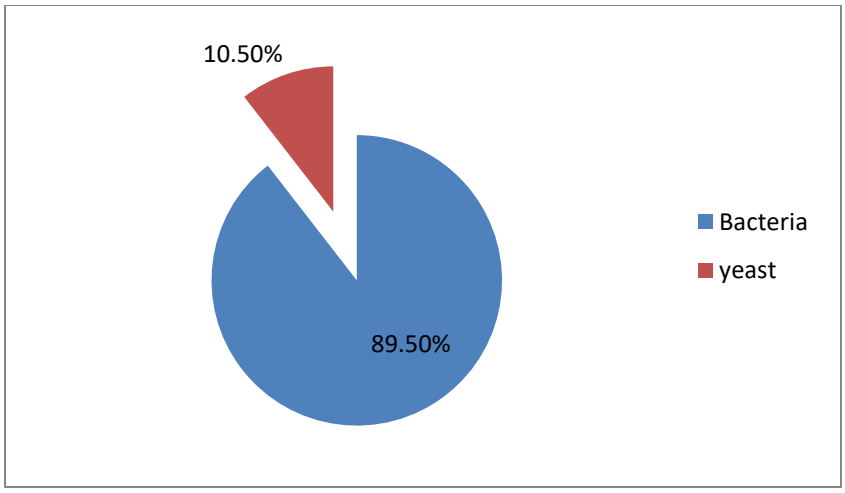

Figure 2: Percentage of bacteria to yeast in 19 positive pinkish colonies

An effective bacterium that produces polyhydroxybutyrate was isolated from soil, according to another funded investigation (17). The viable colony staining method of testing using Nile red dye revealed that 15 of the 38 distinct species of bacteria that were extracted were PHB positive. NMR was used to confirm that the isolate (6N) produced a maximum of 0.17 g/L of PHB. 16S rRNA was used to identify the most potent isolate (6N-NRC), and phylogenetic analysis made it abundantly evident that the strain belongs to the genus Bacillus and is known as *Bacillus aryabhattai*. Three of the nine distinct isolates detected by other study (24) were determined to be PHB positive based on the color utilizing Nile Red stain. The potent strain was identified as *Bacillus safensis.*

4. Conclusion

Using a microscope, a bacterial isolate that could produce PHB was isolated from clay soil in Egypt. The findings showed that the majority of PHB in soil is produced by bacteria (89.5%), followed by yeast (10.5%). As a result, the current study offered valuable information for the safe local isolate used in PHB production in industry.

5. References

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