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Isolation and screening of bioplastic producing bacteria from polluted lake soil

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Abstract

Bioplastics are gaining attention as eco-friendly alternatives to conventional plastics due to their biodegradability and reduced environmental impact. This study focuses on the isolation and screening of bioplastics-producing bacteria from polluted lake soil, which may harbor unique microorganisms capable of synthesizing biopolymers. Soil samples were collected from polluted areas of Porur lake and Korattur lake and processed using serial dilution and culturing techniques on nutrient agar. A total of bacterial isolates were obtained and screened for bioplastics production using Sudan Black B staining. These methods detect intracellular lipid granules associated with polyhydroxyalkanoates (PHAs), a common type of bioplastics. This research demonstrates that polluted lake soil is a valuable source of bioplastics-producing bacteria. The identified isolates show promise for sustainable bioplastics production, offering a potential solution to plastic pollution. The results provide a foundation for future studies focused on optimizing production conditions and scaling up the process for industrial applications. To fully characterize the bioplastics-producing bacteria and confirm the chemical composition of the biopolymer, further research should include detailed biochemical tests, molecular identification, and spectroscopic analysis. This study contributes to the growing field of bioplastics by exploring natural microbial sources and promoting environmentally friendly alternatives to conventional plastics.

Keywords: Bioplastics, polyhydroxy alkanoates, pollution, screening techniques

Introduction

Plastics are materials that can be either synthetic or semisynthetic, and they are widely utilized in everyday life due to their exceptional qualities, including thermal and mechanical properties, as well as their durability and stability, resulting in extensive applications. This extensive use of plastics has led to numerous problems for both human health and the

environment. Almost 700 species, including those that are endangered, have been impacted by plastic pollution (Lokesh *et al.*, 2023). Plastic pollution is a major environmental issue, with millions of tons of plastic waste accumulating in landfills and aquatic ecosystems worldwide. Conventional plastics, derived from petroleum, are non-biodegradable and persist in the environment for hundreds of years, contributing to ecological imbalances and health hazards. As a sustainable alternative, bioplastics have emerged as eco-friendly materials due to their biodegradability and renewable origin (Zhou, W *et al.*, 2023). Among various types of bioplastics, polyhydroxyalkanoates (PHAs) are particularly promising because they are produced by microorganisms as intracellular storage materials and possess properties similar to conventional plastics (Borrelle S.B *et al.*, 2020). The commercial production of PHAs is currently limited by high production costs, primarily due to expensive raw materials and the need for specialized bacterial strains. Therefore, the search for cost-effective and efficient PHA-producing bacteria is crucial. Polluted lake soils, rich in organic pollutants and diverse microbial communities, provide a unique ecological niche that may harbor bacteria capable of synthesizing bioplastics (Geyer R *et al.*, 2017). These environments often contain microorganisms that have adapted to high levels of pollutants and nutrient stress, potentially enhancing their capacity for biopolymer production. Several studies have reported the isolation of PHA-producing bacteria from contaminated environments, including wastewater, sludge, and polluted soils (Kane A.I *et al*; Larue C. *et al.*, 2021). However, the potential of polluted lake soil as a source of bioplastics-producing bacteria remains underexplored. This study focuses on isolating and screening bacteria from polluted lake soil samples to identify efficient PHA producers. The objectives of this research are to collect soil samples from polluted lake areas, isolate bacteria using standard culturing techniques, and screen the isolates for PHA production using Sudan Black B and staining method. By exploring the bioplastic-producing potential of bacteria from polluted lake soils, this study contributes to the development of sustainable solutions to plastic pollution and promotes the use of eco-friendly bioplastics (Prabhu PP 2022; Jain R 2023).

Materials and Methods

Sample Collection

Soil samples were collected from polluted areas of Porur lake and Korattur lake known for high levels of organic pollutants and plastic waste. Samples were taken from the top 10 cm of soil at three different locations around the lake. Sterile gloves and tools were used to

avoid contamination. The collected samples were stored in sterile containers and transported to the laboratory under refrigerated conditions for further analysis.

Figure 1: Sample collection

Isolation of Bacteria

The soil samples were air-dried and ground to remove debris. Serial dilutions were prepared by suspending 1 g of soil in 9 mL of sterile distilled water, followed by vigorous shaking. Dilutions (10^{-2} to 10^{-6}) were spread on nutrient agar plates and incubated at 30°C for 24-48 hours. Distinct colonies were selected based on morphological characteristics (size, shape, color, and texture) and purified by repeated streaking on fresh nutrient agar plates. The purified isolates were preserved on agar slants at 4°C for further screening.

Serial Dilution and Plating on Nutrient Agar

Soil samples were subjected to serial dilution in sterile saline. After dilution, 100 µL of each dilution was plated onto nutrient agar plates. The plates were incubated at 30°C for 48 hours to allow microbial Colonies that appeared on the agar plates were isolated and sub-cultured onto fresh nutrient agar plates to obtain pure bacterial cultures. These cultures were stored at 4°C for further use.

Screening for Bioplastics Production Sudan Black B Staining

Bacterial isolates were cultured in nutrient broth and incubated at 30°C for 48 hours. Smears of bacterial cells were prepared on glass slides, heat-fixed, and stained with 0.3% Sudan Black B solution for 10 minutes. The slides were rinsed with ethanol and

counterstained with safranin. The presence of dark blue-black granules within the cells indicated the accumulation of lipid inclusions, suggesting bioplastic production.

Microscopic Examinations

To confirm the purity and morphology of the bacterial isolates, a small sample of each bacterial culture was stained using the Gram stain method. The stained samples were then observed under a light microscope to determine the bacterial type and shape.

Results

Isolation of microorganisms

Soil samples collected from Porur Lake and Korattur Lake, known for high levels of organic pollutants, were processed using serial dilution and plated on nutrient agar. After 24-48 hours of incubation at 30°C, a total of number bacterial colonies was observed. The colonies were obtained using the spread plate method**,** which evenly distributed the diluted soil samples across the nutrient agar surface. The colonies exhibited diverse morphological characteristics, including variations in color, shape, and texture. Based on distinct morphological features, unique isolates were selected and purified by repeated streaking on nutrient agar plates. The purified isolates were preserved on agar slants at 4°C for further screening.

Subculture Technique

The suspected colonies obtained from the spread plate method were selected based on their distinct morphological characteristics and were streaked onto fresh nutrient agar plates for further purification. This technique was used to isolate pure bacterial cultures by obtaining well-separated colonies from mixed populations. The streaking was performed using a sterile inoculating loop under aseptic conditions to prevent contamination. After streaking, the plates were incubated at 30°C for 24 hour**s** to promote bacterial growth. Following incubation, well-isolated colonies were observed on the nutrient agar plates. These colonies were then carefully examined for consistency in color, shape, size, and texture to ensure purity. Colonies showing uniform morphology were considered pure isolates.

Microscopic observation

The organisms were confirmed by morphological characterization as gram stain method. The organisms confirmed as gram positive bacteria - *Bacillus*.

Figure 2: Isolation of microorganisms

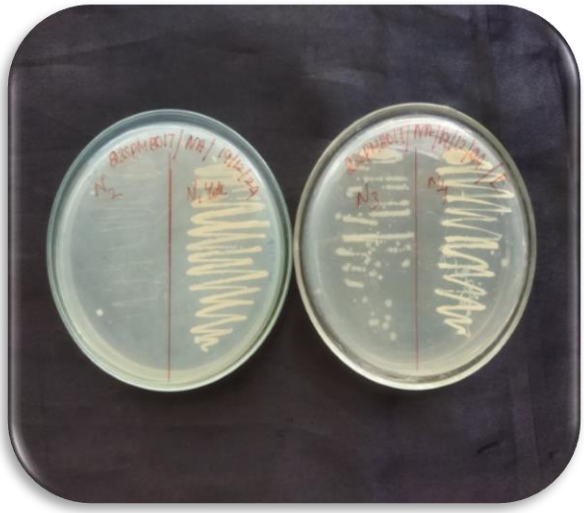

Figure 3: Streaking on nutrient agar plate

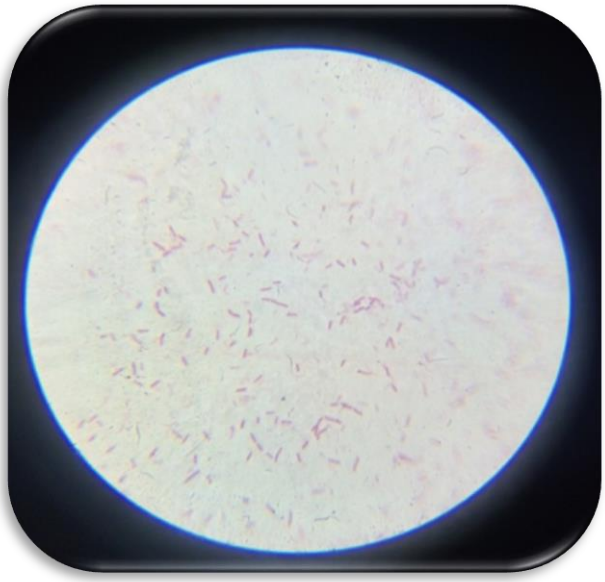

Figure 4: Sudan Black staining

Discussion

This study successfully isolated and screened bioplastic-producing bacteria from polluted lake soil, demonstrating the potential of contaminated environments as sources of eco-friendly bioplastic producers. The results revealed that several bacterial isolates were capable of producing polyhydroxyalkanoates (PHAs), a type of biodegradable bioplastic. This finding aligns with previous studies that have identified PHA-producing bacteria in nutrient-rich and polluted habitats, where microorganisms adapt to environmental stress by

accumulating intracellular storage materials such as PHAs. The use of Sudan Black B and Nile Blue A staining methods proved effective in screening for bioplastic producers. Sudan Black B staining identified lipid inclusions within bacterial cells, while Nile Blue A staining specifically confirmed the presence of PHAs through fluorescence microscopy. These methods provided a rapid and reliable approach for preliminary screening, which is crucial for selecting potential bioplastic-producing strains. However, the accuracy of these methods could be enhanced by complementing them with more advanced analytical techniques, such as gas chromatography or nuclear magnetic resonance (NMR) spectroscopy, for precise characterization of biopolymer composition. The quantitative analysis showed that the most efficient isolate produced mg of bioplastic per gram of dry cell weight, demonstrating its potential for bioplastic synthesis. This yield is comparable to those reported in other studies of PHA-producing bacteria isolated from contaminated environments, highlighting the productivity of native strains adapted to polluted conditions. Polluted lake soil, with its high organic content and diverse microbial communities, serves as a promising reservoir for isolating bioplastic producers. This study contributes to the growing field of sustainable bioplastic production by exploring an underutilized environmental niche. However, further research is needed to complete the biochemical characterization and molecular identification of the isolates to understand their metabolic pathways and optimize. It similarly related to Mathiyazhagan Narayanan, Sabariswaran Kandasamy and Suresh Kumarasamy *et al.*, (2020).

Conclusion

This study successfully isolated and screened bioplastic-producing bacteria from polluted lake soil, demonstrating the potential of contaminated environments as valuable sources of eco-friendly bioplastic producers. Using Sudan Black B staining method, several bacterial isolates were identified as capable of producing polyhydroxyalkanoates (PHAs), a biodegradable bioplastic with properties similar to conventional plastics. The most promising isolate showed significant bioplastic production, highlighting its potential for sustainable plastic alternatives. These findings indicate that polluted lake soils, enriched with organic pollutants and diverse microbial communities, provide a unique ecological niche for discovering efficient bioplastic producers. By utilizing naturally occurring bacteria from polluted environments, this research offers a sustainable approach to bioplastic production, contributing to the reduction of plastic pollution and supporting circular bio-economy practices. However, further studies are needed to complete the biochemical characterization and molecular identification of the isolates to understand their metabolic pathways fully.

Additionally, optimizing production conditions and scaling up the process are essential for assessing the commercial viability of the bioplastics produced. This study lays the groundwork for future research in microbial bioplastic production and highlights the importance of exploring environmental sources for sustainable solutions to plastic pollution.

References

Zhou W, Bergsma S, Colpa, DI, Euverink GW and Krooneman J. (2023). Polyhydroxyalkanoates (PHAs) synthesis and degradation by microbes and applications towards a circular economy. *Journal of Environmental Management*, *341*, 118033.

Borrelle, SB, Ringma J, Law KL, Monnahan CC, Lebreton L, McGivern A, Murphy E, Jambeck, J, Leonard GH, Hilleary MA, Eriksen M, Possingham HP, De Frond H, Gerber LR, Polidoro B, Tahir A, Bernard M, Mallos Z, Barnes M and Rochman CM. (2020). Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science (New York, N.Y.)*, *369*(6510), 1515-1518.

Geyer R, Jambeck JR and Law KL. (2017) Production, use, and fate of all plastics ever made. *Science advances*, *3*(7), e1700782.

Kane AI, Clare M Dispersion. (2019) Accumulation, and the ultimate fate of microplastics in deep-marine environments: a review and future directions, *Front. Earth Sci. 7(2019*).

Larue C, Sarret G, Castillo-Michel H, Pradas Del Real AE. (2021) A Critical Review on the Impacts of Nanoplastics and Microplastics on Aquatic and Terrestrial Photosynthetic Organisms. *Small*. 17(20), e2005834.

Lokesh P, Shobika R, SoghraNashath Omer, Madhavi Reddy, Panchamoorthy Saravanan, Rajeshkannan R, Saravanan V, Venkatkumar S. (2023) Bioremediation of plastics by the help of microbial tool: A way for control of plastic pollution. *Sustainable Chemistry for the Environment* 3 (100027).

Prabhu PP, Pan K, Krishnan NJ. (2022) Microplastics: global occurrence, impact, characteristics and sorting. *Front. Mar. Sci.*, 9 (893641), 10.3389/fmars.2022.893641.

Jain R, Gaur A, Suravajhala R, Chauhan U, Pant M, Tripathi V, Pant, G. (2023). Microplastic pollution: Understanding microbial degradation and strategies for pollutant reduction. *The Science of the total environment, 905* (167098).

Wang P, Liu J, Han S, Wang Y, Duan Y, Liu T, Hou L, Zhang Z, Li L and Lin Y. (2023) Polyethylene mulching film degrading bacteria within the plastisphere: Co-culture of plastic degrading strains screened by bacterial community succession. *Journal of hazardous materials*, 442 (130045).