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Microplastic Associated Microorganisms: Isolation, Identification and Assessment of Biofilm-Based Degradation Potential

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Abstract: Microplastics have become major environmental contaminants because of their persistence and extensive distribution in both terrestrial and aquatic ecosystems. The present study aimed to isolate and identify microorganisms associated with microplastics and to evaluate their potential for biofilm-mediated plastic degradation. Soil and water samples were collected from plastic-polluted sites, and microplastics were extracted using density separation followed by hydrogen peroxide digestion. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of polyolefin-based microplastics in the collected samples. Associated bacterial and fungal isolates were characterized through morphological and biochemical analyses. The biofilm-forming ability of the isolates was assessed using Congo Red Agar and tube assay methods. Furthermore, selected microbial isolates demonstrated noticeable plastic degradation potential based on weight loss measurements of treated plastic samples. The findings of this study emphasize the significant role of indigenous microorganisms in the sustainable bioremediation of microplastic pollution.

Keywords: *Microplastics; Bioremediation; Biofilm Formation; Plastic Degradation; FTIR Analysis*

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I. INTRODUCTION

Plastics have been widely used since 1907 due to their durability, lightweight nature, flexibility, and low cost. However, the continuous increase in plastic production and improper disposal have resulted in severe environmental pollution. Globally, more than 450 million tons of plastics are produced annually, and a significant portion accumulates in terrestrial and aquatic ecosystems because of inadequate recycling and waste management practices. Environmental factors such as ultraviolet radiation, temperature fluctuations, and mechanical abrasion fragment these plastics into particles smaller than 5 mm, known as microplastics (MPs) Andrady, (2011). Microplastics are now widely distributed in soil, water, and the atmosphere, where they persist for long periods and pose serious ecological and health risks by entering food chains and acting as carriers for toxic chemicals and pathogens Galloway, et al. (2017). Marine ecosystems are particularly affected, with millions of tons of plastic waste entering oceans every year Rani, et al. (2017). In recent years, biological approaches such as microbial bioremediation have gained attention as sustainable solutions for microplastic pollution. Several bacteria and fungi possess enzymes capable of degrading complex plastic polymers into simpler compounds Shah et al., (2008). Microorganisms such as *Bacillus*, *Pseudomonas*, *Acinetobacter*, and *Aspergillus* species have demonstrated significant plastic degradation potential, especially through biofilm formation, which enhances microbial attachment and enzymatic activity on plastic surfaces Urbanek, et al. (2017). Therefore, the present study focuses on the isolation and identification of microplastic-associated microorganisms from soil and water environments and evaluates their biofilm-mediated degradation potential as an eco-friendly strategy for microplastic remediation.

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II. MATERIALS AND METHODS

A. Soil and Water sample collection

Soil and water samples used in this study were collected from different plastic-contaminated locations in Coimbatore, India by following the method of Masura, et al. (2015). Soil samples were collected from KG Chavadi, Madukarai, and Walayar, designated as S1C and S2M, while water samples collected from different sites were designated as S1P, S2D, and S3C. Prior to sampling, stainless steel augers, shovels, and collection buckets were thoroughly rinsed with distilled water and ethanol to prevent contamination. Soil samples were collected up to a depth of 50 cm and transferred into sterile containers, whereas water samples were collected using stainless steel buckets and filtered through a 20 µm sieve. All samples were transported to the laboratory under sterile conditions. Soil samples were oven-dried at 60°C until constant weight, gently ground using a mortar, and sieved through a 5 mm stainless steel mesh. Water samples were stored in sterile glass bottles at refrigerated conditions until further analysis.

B. Isolation of Microplastics from Soil and Water Samples

Pretreatment of soil and water samples was carried out to remove debris and obtain uniform samples for microplastic isolation. Soil samples were homogenized using a 2 mm sieve, while water samples were filtered to eliminate larger particles. Density separation was performed using saturated sodium chloride solution to isolate low-density plastic particles Claessens, et al. (2013). The mixtures were stirred and allowed to settle, after which the supernatant was collected. Organic matter present in the samples was removed by digestion with 30% hydrogen peroxide at 60°C for 2–3 days Masura, et al. (2015). The digested samples were filtered through metal membranes and subjected to fractionated filtration methods to separate particles of different size ranges. Larger particles were manually isolated under a stereomicroscope and preserved for further analysis using FTIR.

C. Microbiological Analysis and Biofilm Formation Assays

Microorganisms associated with microplastic-contaminated samples were isolated using serial dilution and spread plate techniques. Bacterial isolates were cultured on Nutrient Agar plates and incubated at 37°C for 48 hours, while fungal isolates were cultured on Potato Dextrose Agar plates at room temperature. Pure cultures were obtained through repeated subculturing. Morphological and biochemical characterization of bacterial isolates was carried out using Gram staining, catalase test, and mannitol fermentation test. Fungal isolates were characterized using Lactophenol Cotton Blue staining. Biofilm formation ability of the isolates was evaluated using Congo Red Agar and tube assay methods Christensen, et al. (1985). Formation of black crystalline colonies on Congo Red Agar and visible films on tube walls indicated positive biofilm formation.

D. Microbial Degradation of Plastic and Weight Loss Analysis

Pretreated plastic films of uniform size (2 × 2 cm) were used to evaluate microbial degradation potential. Plastic samples were washed, chemically pretreated using 30% hydrogen peroxide, sterilized under UV light, and weighed before inoculation. Fungal degradation studies were conducted using Sabouraud Dextrose broth inoculated with fungal spores, whereas bacterial degradation assays were performed using Nutrient Broth inoculated with selected bacterial isolates. Pre-weighed plastic samples were incubated under shaking conditions to facilitate microbial interaction with the polymer surface. After incubation, the plastic films were washed, dried, and reweighed to determine degradation efficiency Shah, et al. (2008). The percentage of weight loss was calculated using the formula:

$$WL\%=W1-W2/\ W1\times100$$

where W_1 represents the initial weight of the plastic sample and W_2 represents the final weight after microbial treatment.

III. RESULTS

Density separation of soil samples using saturated sodium chloride solution resulted in the formation of a clear supernatant layer containing floating particulate matter suspected to be microplastics. Repeated extraction improved the recovery of floating particles, although the quantity decreased slightly during the final extraction shown in Figure 1. Organic digestion with 30% hydrogen peroxide effectively removed organic matter, as indicated by visible effervescence, reduction in turbidity, and lightening of sample color. In water samples, density separation also resulted in the isolation of suspended particles; however, no visible microplastics were recovered from the analyzed water samples, possibly due to the collection of samples from flowing water bodies.

A. Identification of Microplastics by FTIR Analysis

FTIR analysis confirmed the presence of polyolefin-based microplastics in the isolated samples. The first microplastic sample (MP1) exhibited characteristic absorption peaks corresponding to Ethylene–Propylene Copolymer shown in Figure 2, while the second sample (MP2) showed spectral similarity with isotactic polypropylene as seen in Figure 3. The observed absorption bands represented aliphatic hydrocarbon functional groups, confirming that the recovered particles belonged predominantly to the polyolefin class of polymers commonly found in environmental samples.

B. Isolation and Characterization of Microbial Isolates

Bacterial and fungal isolates associated with microplastic contaminated soil and water samples were successfully isolated using serial dilution and spread plate techniques. Two bacterial isolates, designated as BO1 and BY2, formed circular, smooth, opaque colonies on Nutrient Agar. Gram staining revealed that both isolates were Gram-positive cocci. Mannitol fermentation and catalase tests indicated that the isolates belonged to the genus *Staphylococcus*. Similarly, two fungal isolates, FN1 and FF2, were isolated on Potato Dextrose Agar. LPCB staining of FN1 revealed septate hyphae and radiating conidial heads characteristic of *Aspergillus* species, while FF2 exhibited septate hyphae with conidial clusters

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C. Qualitative Assessment of Biofilm Formation

Biofilm formation was evaluated using tube assay methods. Among the bacterial isolates, BO1 demonstrated positive biofilm formation through the development of a visible ring along the wall of the test tube. In contrast, fungal isolates did not show visible film formation under the tested conditions, indicating negative biofilm production. These findings suggest that bacterial isolates possessed greater biofilm-forming ability compared to fungal isolates.

D. Microbial Degradation of Plastic and Weight Loss Analysis

Pretreated plastic samples exposed to fungal and bacterial inoculated broths showed visible surface alterations and partial degradation after two weeks of incubation. Weight loss analysis demonstrated varying degradation efficiencies among the treatments. The fungal inoculated broth exhibited higher degradation efficiency, with maximum weight loss observed in sample P2a (65.52%), followed by P1a (44.88%). In comparison, bacterial inoculated broth showed lower degradation rates, with weight losses of 30.77% for P1b and 11.11% for P2b. These findings indicate that fungal isolates possessed greater plastic degradation potential than bacterial isolates under the experimental conditions.

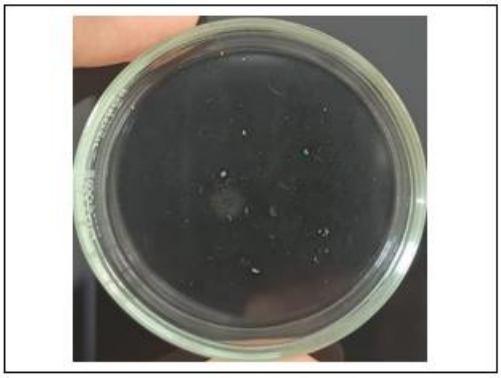

Fig 1 Isolated Microplastics from Soil

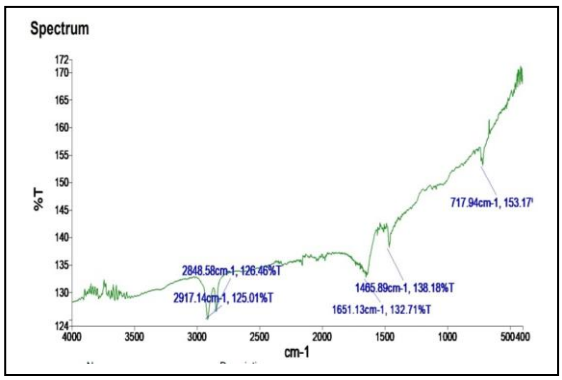

Fig 2 FTIR Analysis of MP1 Isolate

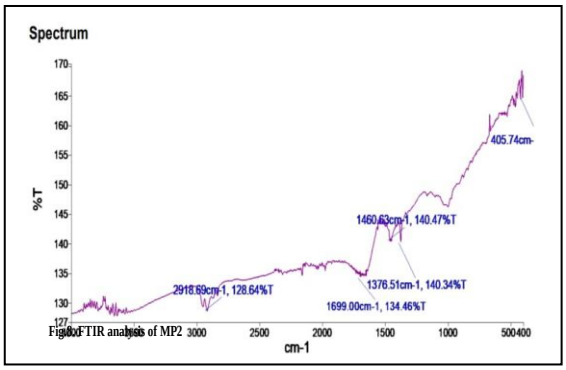

Fig 2 FTIR analysis of MP2

Fig 2 FTIR Analysis of MP2 Isolate

Fig 3 Biofilm Formation by BO1 Isolate

Fig 4 Plastic Incorporated Bacteria Inoculate Nutrient Broth

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IV. DISCUSSION

The present study investigated the occurrence of microplastics in environmental samples and evaluated the role of associated microorganisms in their biodegradation. Microplastics were successfully isolated from soil and water samples using density separation with saturated sodium chloride followed by hydrogen peroxide digestion, confirming the effectiveness of these methods for microplastic extraction. FTIR analysis identified the recovered particles as polyolefin polymers, mainly ethylene–propylene copolymer and polypropylene, which are widely reported as dominant environmental microplastics due to their persistence and extensive use (Andrady, (2011); Hartmann et al. (2019). Microbial analysis revealed the presence of bacterial isolates belonging to *Staphylococcus* species and fungal isolates identified as *Aspergillus* species. The isolated microorganisms demonstrated biofilm-forming ability, which enhances microbial attachment and enzymatic activity on plastic surfaces. Similar microbial colonization of plastics, known as the "plastisphere," has been previously reported by Zettler et al. (2013). Partial degradation of plastic materials was observed through measurable weight loss after incubation with bacterial and fungal isolates, with fungal strains showing comparatively higher degradation efficiency. These findings support earlier studies indicating that indigenous microorganisms associated with microplastics possess significant potential for biofilm-mediated biodegradation and may provide an eco-friendly approach for reducing environmental microplastic pollution.

V. SUMMARY AND CONCLUSION

Microplastics were successfully isolated from soil and water samples using density separation and hydrogen peroxide digestion, and FTIR analysis confirmed the presence of polyolefin polymers such as polypropylene and ethylene–propylene copolymer. Microbiological analysis revealed the presence of bacterial (*Staphylococcus* sp.) and fungal (*Aspergillus* sp.) isolates associated with microplastic particles, which also exhibited biofilm-forming ability. Laboratory incubation studies showed microbial colonization on plastic surfaces along with slight weight reduction, indicating the potential involvement of these microorganisms in the early stages of plastic biodegradation. Overall, the study highlights the occurrence of microplastics in environmental samples and suggests that microplastic-associated microorganisms may contribute to sustainable bioremediation processes.

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