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Original Article

Phenol degradation and synthetic dye decolorization by marine plastisphere-associated bacteria: Implications for mitigating environmental pollutants

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Abstract: The plastisphere is a unique microhabitat that supports diverse microbial communities, including bacteria with potential bioremediation capabilities. This study aims to isolate and characterize phenol- and synthetic dye-degrading bacteria inhabiting the marine plastisphere along the Cochin coast, Kerala, India. It also investigates how these bacterial communities interact with plastic substrates. We collected plastic debris bearing pre-formed biofilms from marine sites and isolated and characterized the associated bacterial strains using standard biochemical methods. Of the 17 isolates, 8 showed potential for phenol degradation. Of the 8 isolates that showed positive phenol-degradation results, 3 showed significant exopolysaccharide production and robust biofilm formation. The molecular identity of these isolates was *Bacillus altitudinis* (F1), *Vibrio alginolyticus* (T1), and *Shouchella clausii* (O1). Preliminary screening for biofilm-forming ability was conducted employing the Congo red agar method. The pollutant degradation efficiency of the isolates was quantitatively assessed in Minimal Salt Medium supplemented with the respective contaminants and analysed via spectrophotometry. Comparative evaluation of degradation efficiencies between individual planktonic cultures and their corresponding biofilm-associated forms revealed significantly enhanced pollutant degradation by biofilm-associated bacteria. Scanning electron microscopy of the plastisphere showed distinct morphological alterations on the plastic substrata, indicating early-stage plastic degradation associated with microbial activity.

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Introduction

Marine ecosystems furnish habitat for diverse organisms, from microorganisms to macrofauna. These ecosystems also play a significant role in regulating Earth's climate and sustaining biodiversity and human life. Coastal waters worldwide are experiencing deterioration in quality due to anthropogenic pressure. Human activities, including industries and mining, have contaminated coastal ecosystems (Cebe and Balas, 2018). Pollutant accumulation in oceans affects the ocean's overall functioning and carrying capacity. Plastic debris accumulation in the marine environment is at an unprecedented rate. In 2019, only 9% of the 370 million tons produced were recycled, 12% were incinerated, and the rest was disposed of in landfills or directly into the environment (Kumar et al., 2021). This pollution occurs across a spectrum of sizes, including megaplastic, macroplastic, mesoplastic, and microplastic (Zhang et al., 2023). Effluents from municipal and industrial sources introduce substantial loads of organic pollutants, making them a primary source of aquatic pollution (Akhtar et al., 2021).

Phenol and phenolic derivatives are recognized as hazardous aromatic compounds because of their inherent water solubility and persistence in aquatic environments and biological cycles (Samimi and Moghadam, 2020). Preliminary sources of phenolic contamination in water systems include a broad spectrum of sources, including petrochemical, pharmaceutical, textile, and iron and steel production (Gucbilmez, 2022). Phenolic compounds, notably nonylphenol and bisphenol, are endocrine-disrupting compounds. In recent decades, these have raised significant concern because of their adverse effects on endocrine and reproductive functions in various

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marine organisms (Zhang et al., 2021).

Plastic debris in marine environments poses severe threats to aquatic species' survival and well-being. Micro- and macroplastics proliferating in aquatic ecosystems seriously threaten marine organisms by directly impairing health or critically disrupting habitat (Muneer et al., 2021). Plastic debris impacts organisms in three major ways: entanglement, ingestion, and interactions (Awuchi, 2019). Global plastic waste consists of carbon-based polymers such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) (Du et al., 2022). In addition, plastic surfaces provide a favorable microhabitat for colonizing microbiota, including bacteria, fungi, viruses, archaea, algae, and protozoans, creating the microhabitat known as the "plastisphere" (Amaral-Zetller et al., 2013; Du et al., 2022). This artificial ecosystem also facilitates the long-distance dispersal of microorganisms (Dey et al., 2022). The microbial community on plastic substrata includes pathogens and microbes with high bioremediation potential. Plastic substrata offer physical support, nutrients, and habitats that resist environmental conditions (Jacquin et al., 2019). Quorum sensing increases collective adaptability, promoting survival in stressful environments and even improving antibiotic resistance and toxic chemical degradation capacity.

Biodegradation is the biologically mediated breakdown of complex organic chemicals into simpler, less-toxic products. Microbial biodegradation is an environmentally favorable method of pollutant elimination that works through the metabolic pathways, mechanisms, and genetic determinants of biodegradation (Mbachu et al., 2020). Microbial communities adapt to contaminated environments by developing enzymes and pathways that metabolize contaminants. Microbial strains have evolved specific metabolic pathways and enzymes—such as phenol hydroxylase, laccase, azoreductase, and peroxidase—that enable them to utilize phenol and dyes as carbon and energy sources (Dwivedi, 2018; Wang et al., 2019, Ghobadi Nejad et al., 2019). The bacterial community colonizing plastic debris can contribute to plastic biodegradation (Miao et al., 2023). Bacterial-mediated plastic degradation mainly occurs through a four-step process: biodeterioration, depolymerization, assimilation, and mineralization. This process relies on enzyme-mediated metabolic pathways that utilize the polymers as a primary energy source (Heris, 2024). Although various microbial strains can degrade pollutants such as phenol and synthetic dyes, the effectiveness of marine plastisphere biofilms as bioremediation agents in natural settings remains poorly understood. This study investigates the ecological role of these plastisphere-associated bacteria in degrading key pollutants, including phenol and synthetic dyes.

Materials and Methods

Sampling: Sampling was done across three distinct sites along the coast of Kochi in the Ernakulam district of Kerala. Vypin, with the geographical coordinates of 10.009011°N; 76.230957°E, is characterized by high harbor traffic and intense fishing activity. Munambam (10.112063°N, 76.200593°E) is one of Kerala's major commercial fishing harbors and receives terrestrial and agricultural runoff via the Periyar River basin, as well as hydrocarbons and synthetic debris from vessel repair and harbor operations. Cherai (10.137633°N; 76.194624°E) is situated adjacent to backwater aquaculture systems and exposed to open marine wave action and heavy seasonal tourism. Marine plastic debris with pre-developed biofilms were collected in sterile containers from the selected sites. Both floating and sediment-embedded samples were collected from the sampling sites.

Isolation, subculturing and characterization: The collected plastic debris was sequentially washed with sterile phosphate-buffered saline (PBS) to remove loosely adherent particles. Aseptically scraped biofilm was suspended in PBS and vortexed. The resultant suspension was serially diluted (10^{-1} to 10^{-6}) in sterile saline. The 10^{-4} to 10^{-6} dilutions were spread-plated onto Zobell Marine Agar by taking a 0.5 mL aliquot and incubated at 37°C for 24-48 hours. Pure isolates with morphologically distinct colonies were maintained on Zobell Marine Agar slants. Both

morphological and biochemical characterization of pure isolates were done using standard procedures.

Screening for phenol degraders (planktonic forms): Phenol degraders can utilize phenol for energy production in the absence of other primary carbon sources. The screening for isolates with phenol-degrading ability was carried out by inoculating them in Minimal Salt Medium (MSM) with phenol as the sole carbon source. The Minimal Salt Medium composition included NH_4Cl (1.0 g/L), NaH_2PO_4 (0.5 g/L), K_2HPO_4 (0.5 g/L), KCl (0.5 g/L), $MgSO_4{\cdot}7H_2O$ (0.3g/L), $CaCl_2$ (0.07 g/L), and $FeSO_4{\cdot}7H_2O$ (2 mg/L). The minimal salt medium (MSM) was supplemented with phenol to a final concentration of 1000 mg/L. The cultures were kept under shaker-incubation at 150-180 rpm at 37°C for 7 days. The residual phenol concentration was determined every 24 hours using the 4-aminoantipyrine assay (APHA, 2005).

Screening for biofilm producers: The isolates included both biofilm formers and non-biofilm formers. To screen for biofilm formers, the Congo Red Agar method was performed. Congo Red Agar is a differential medium that distinguishes biofilm formers from non-biofilm formers. The medium comprised Tryptic Soy Broth (TSB) (30 g/L), Agar (15 g/L), Sucrose (36 g/L), and Congo Red dye (0.8 g/L). The isolates were streaked onto the prepared plates and incubated for 24-48 hours at 37°C.

Phenol degradation by biofilm-associated bacteria: Isolates that showed positive results on Congo Red Agar medium were inoculated into minimal salt medium containing Phenol (1000 mg/L) along with a sterile polyethylene substratum (3x3 cm). Initially, cultures were incubated under shaking conditions to provide aeration, and after three days, they were maintained under static conditions to promote biofilm formation. Samples were collected periodically at 24-hour intervals, and residual phenol concentration was quantified by the 4-aminoantipyrine assay.

Dye decolourization by planktonic bacteria: Quantitative analysis of dye decolourization by isolates was performed using minimal salt medium supplemented with synthetic dyes as the sole carbon source. Cultures were incubated under shaking conditions (150-180 rpm) at 37°C. Uninoculated negative controls were maintained for comparison. Absorbance was measured spectrophotometrically at 664 nm (Methylene Blue), 618 nm (Malachite Green), and 540 nm (Neutral Red). Percentage degradation is calculated as: Percentage Degradation (%) = $[(A_0 - A_t) / A_0] \times 100$, where A_0 = Initial absorbance (before incubation) and A_t = Final absorbance (after incubation at time t).

Dye decolourization by biofilm bacteria: To assess biofilm-mediated decolourization, the cultures that showed positive results in primary screening using Congo Red Agar were inoculated into MSM with the respective dye and sterile polyethylene substrata (3x3 cm) to facilitate biofilm development. Initial incubation was done in a rotary flask shaker, and the cultures were maintained under static conditions after three days. Dye degradation was quantified by measuring absorbance at the corresponding wavelengths using a UV-Visible Spectrophotometer.

Scanning electron microscopy (SEM) and molecular analysis: Scanning Electron Microscopy was employed to visualize the biofilm architecture and its dynamics on a polyethylene substratum. Surface morphology of the polyethylene film without biofilm (Control) was also analysed using SEM (JSM-6390, JEOL Ltd., Japan).

Molecular identification of the potential isolates was performed by 16S rRNA gene sequencing. We then used the EzBioCloud database to identify the sequences and performed phylogenetic analysis in MEGA7. To plot the phylogenetic tree, each identified bacterial sequence, with its 10 closest neighbor sequences, was aligned using the CLUSTAL W program in the MEGA software alignment tool. The aligned sequences were further exported in the MEGA format, and the evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987).

Results

Screening for phenol degradation: A total of 17 isolates were obtained after spread plating on Zobell

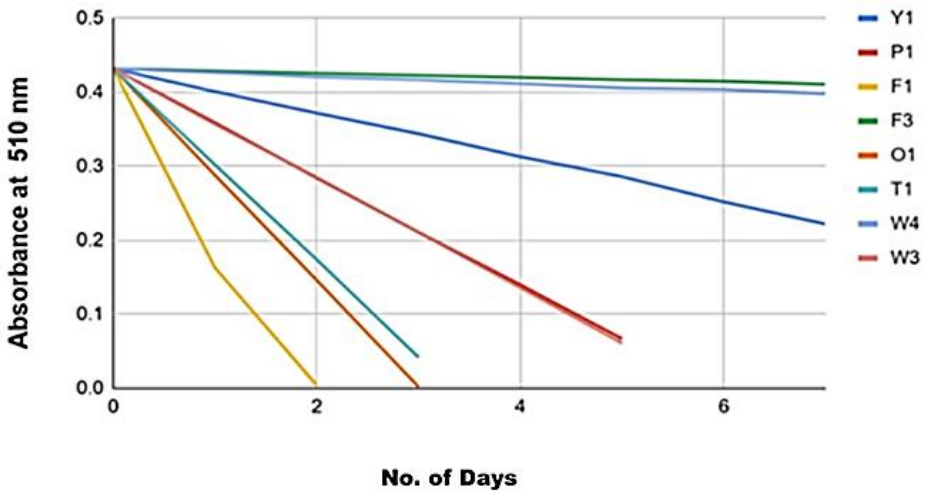


Figure 1. Screening of isolates in planktonic culture for phenol degradation over a 7-day incubation period.

Marine Agar medium and purification by quadrant streaking. The isolates were screened in minimal salt medium amended with phenol (1000 mg/L) to identify potential phenol degraders. Preliminary identification was based on turbidity and confirmed by the 4-aminoantipyrine assay at 510 nm. Of the 17 isolates, 8 showed potential phenol degradation in the 4-aminoantipyrine assay. Isolates F1, T1, and O1 were effective phenol degraders, with F1 yielding the most promising result (Fig. 1). Residual phenol was detectable up to day five with isolates W3 and P1, and up to day seven with isolates W4, Y1, and F3.

Phenol degradation potential of biofilm-associated bacteria: The primary screening to identify biofilm-producing bacteria revealed that, out of the 8 bacteria that showed positive results with phenol degradation, 3 isolates (F1, O1, and T1) displayed strong black, dry colonies indicative of significant exopolysaccharide production and robust biofilm formation (Fig. 2). The remaining 5 isolates produced red or pink colonies, suggesting limited or no biofilm-forming capacity. These results suggest that a subset of marine plastisphere-associated bacteria can form biofilms, potentially contributing to surface colonization. The three biofilm-producing isolates were further chosen for phenol degradation studies using a flask culture method with a polyethylene film to facilitate biofilm formation. Phenol degradation by biofilm-associated bacteria was assessed by a spectrophotometric method, and the efficiency of phenol degradation by these cultures was found to be in the order O1>T1>F1 (Fig. 3). The biofilm-associated cultures degraded phenol more effectively, and no residual phenol was detected after two days, in contrast to the planktonic forms, where residual phenol was detected up to day 3 in O1 and T1 cultures.

Evaluation of synthetic dye decolourization by planktonic isolates: The three isolates (F1, O1, and T1), which showed phenol degradation in both planktonic form and in biofilms, were selected for dye decolourization studies. The isolates were inoculated into minimal salt medium supplemented with synthetic dyes - Methylene Blue, Neutral Red, and Malachite Green separately, at a known concentration of 5 mg/100 mL. Spectrophotometric analysis was conducted at 24-hour intervals. The isolates exhibited significant dye removal ability within five days, and the analysis revealed a gradual decline in absorbance at the characteristic wavelength of the dye, confirming decolourisation by planktonic cells. Among the tested isolates, planktonic cultures of isolate F1 showed the highest percentage of dye decolourisation over an incubation period of 120 hours, as indicated by a marked reduction in colour intensity and corresponding decline in absorbance values (Fig. 4, 5 and 6). F1 yielded 71.62% decourization of methylene blue at 120 hours of incubation (Table 1), though the other two isolates, O1 and T1, yielded only 19.86% and 6.12% decolourization of methylene blue, respectively, even after five days of incubation.

Table 1. Percentage decolourization of methylene blue: Comparative efficiency of planktonic isolates over an incubation period of five days.

Percentage decolourization of methylene Blue – Planktonic form			
Day	**O1 (%)**	**T1 (%)**	**F1 (%)**
0	0	0	0
1	3.93	1.15	11.45
2	8.08	2.66	22.58
3	11.78	4.04	49.42
4	15.59	4.85	63.64
5	19.86	6.12	71.62

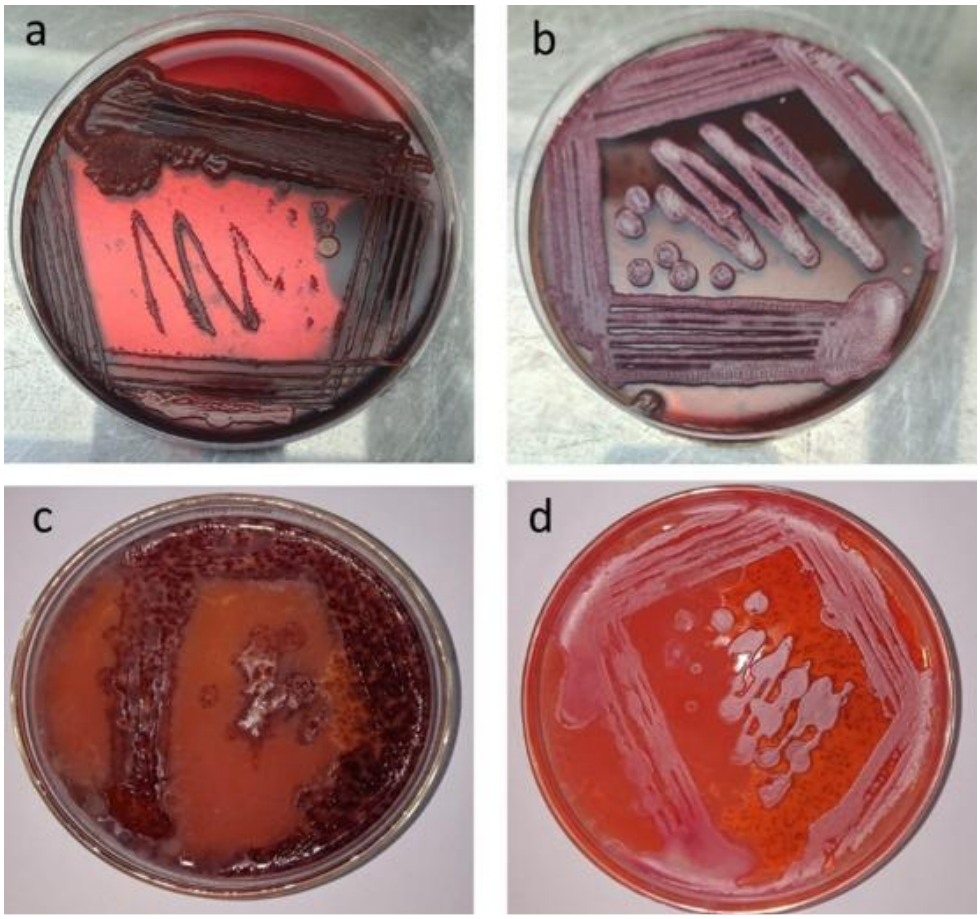


Figure 2. Congo red agar screening method for the identification of biofilm forming isolates. Isolates with black colonies indicate biofilm production. a) Isolate O1 b) Isolate T1 c) Isolate F1 d) Isolate F4 – negative for biofilm production.

Isolate F1 was also the most promising malachite green decolorizer. A substantial decolourization of 76.95% was attained in 120 hrs (Table 2). However, the overall decolourization pattern was different from that of methylene blue, as the other two isolates, O1 and T1, also exhibited more than 70% decolourization over an incubation period of 120 hrs. In the case of neutral red, the percentage decolourization was found to be 69.18, 52.67, and 79.83%, respectively, for isolates O1, T1, and F1 (Table 3).

Biofilm-mediated synthetic dye decolourization: Among the three isolates subjected to biofilm-mediated decolourization, the F1 isolate exhibited rapid decolourization, achieving high percentage removal of methylene blue (79.42%, Fig. 7), malachite green (87.53%, Fig. 8), and Neutral red (89.75%, Fig. 9). Both O1 and T1 yielded less than 30% decolourization of methylene blue even after five days of incubation (Table 4). The isolates O1 and T1 in biofilm form exhibited similar decolourization efficiency with malachite green (Table 5) and neutral red (Table 6).

Table 2. Percentage decolourization of Malachite green: Comparative efficiency of planktonic isolates over an incubation period of 5 days.

Percentage decolourization of malachite green – Planktonic form			
Day	**O1 (%)**	**T1 (%)**	**F1 (%)**
0	0	0	0
1	11.77	7.46	20.70
2	32.18	26.09	43.67
3	43.29	38.23	47.46
4	67.38	66.88	73.29
5	70.03	70.65	76.95

Table 3. Percentage decolourization of neutral red: Comparative efficiency of planktonic isolates over an incubation period of 5 days.

Percentage decolourization of neutral red – Planktonic form			
Day	**O1 (%)**	**T1 (%)**	**F1 (%)**
0	0	0	0
1	16.95	11.8	22.11
2	33.26	21.17	37.57
3	42.51	36.51	43.63
4	59.75	47.34	62.12
5	69.18	52.67	79.83

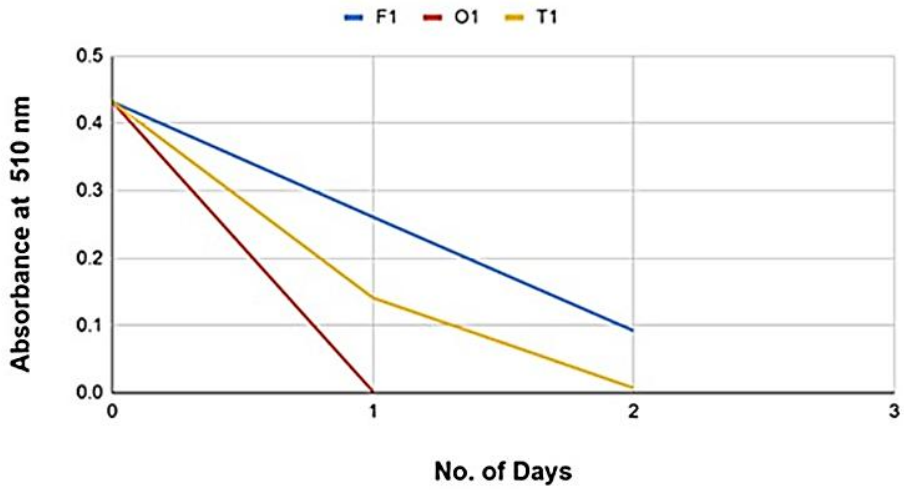


Figure 3. Phenol degradation by biofilm forming isolates over an incubation period of three days.

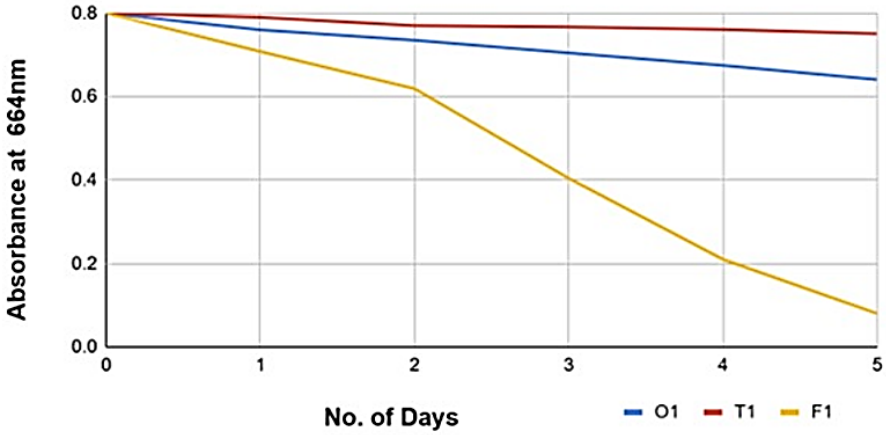


Figure 4. Time dependent decolourization of methylene blue by selected isolates in planktonic form.

Impact of biofilm on polyethylene substratum: Scanning electron micrographs of the control polyethylene film and the one used as a substratum for biofilm formation showed significant changes. Scanning electron micrographs showed extensive bacterial colonization of the polyethylene film,

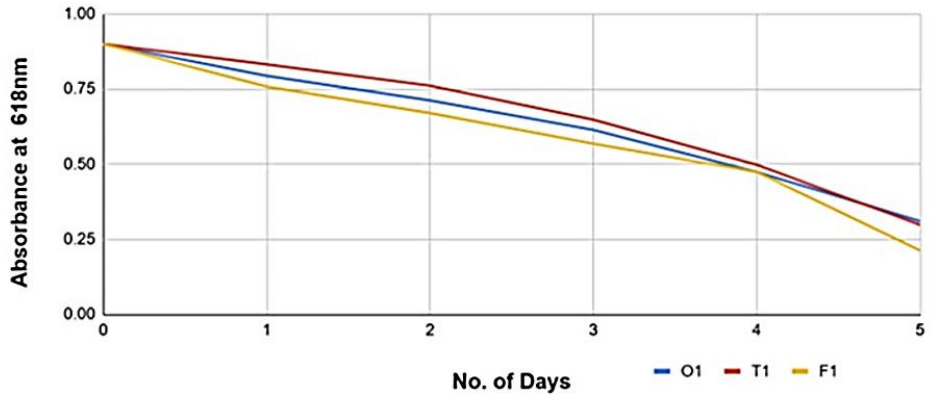


Figure 5. Time-dependent decolourization of malachite green by selected isolates in planktonic form.

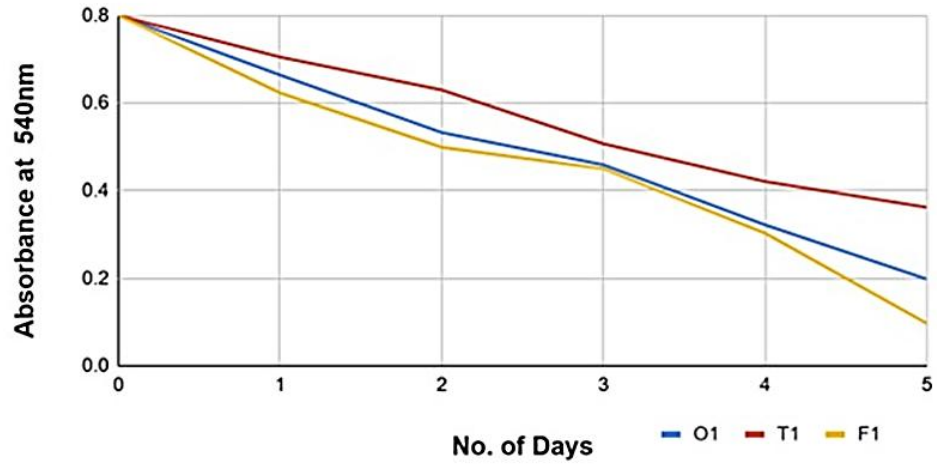


Figure 6. Time-dependent decolorization of neutral red by selected isolates in planktonic form.

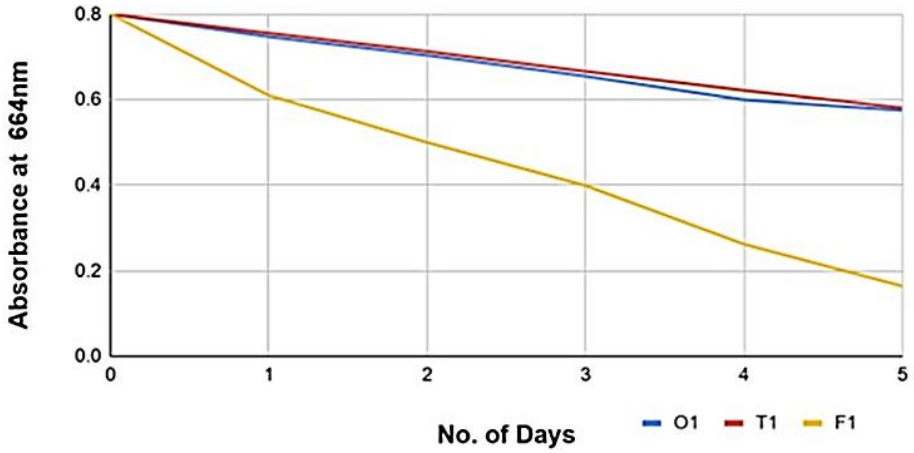


Figure 7. Time-dependent decolorization of methylene blue by biofilm-associated bacteria.

forming a strong biofilm (Fig. 10). Early degradation features, such as micro-cracks on the polyethylene surface, indicated that the biofilm-associated bacteria can initiate plastic degradation.

Molecular identification of isolates: Molecular identification of the bacterial isolates was performed by 16S rRNA gene sequencing followed by BLAST analysis using the NCBI database. We compared the obtained nucleotide sequences with available sequences in the GenBank database to determine the

Table 4. Percentage decolourization of methylene blue: Comparative efficiency of biofilm-associated bacteria over an incubation period of five days.

Percentage decolourization of Methylene blue - Biofilm			
Day	**O1 (%)**	**T1 (%)**	**F1 (%)**
0	0	0	0
1	6.58	5.43	23.67
2	11.89	10.85	37.42
3	18.01	16.63	50.02
4	24.98	22.17	67.22
5	29.06	27.37	79.42

Table 5. Percentage decolourization of malachite green: Comparative efficiency of biofilm-associated bacteria over an incubation period of five days.

Percentage decolourization of Malachite green - Biofilm			
Day	**O1 (%)**	**T1 (%)**	**F1 (%)**
0	0	0	0
1	20.68	12.23	24.57
2	36.21	28.77	39.62
3	49.25	39.23	52.81
4	66.36	52.59	71.88
5	82.59	72.04	87.53

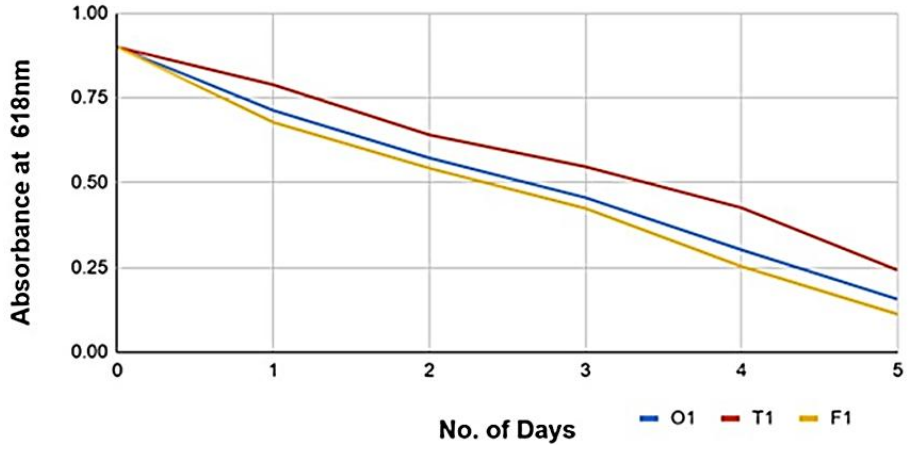


Figure 8. Time-dependent decolorization of malachite green by biofilm-associated bacteria.

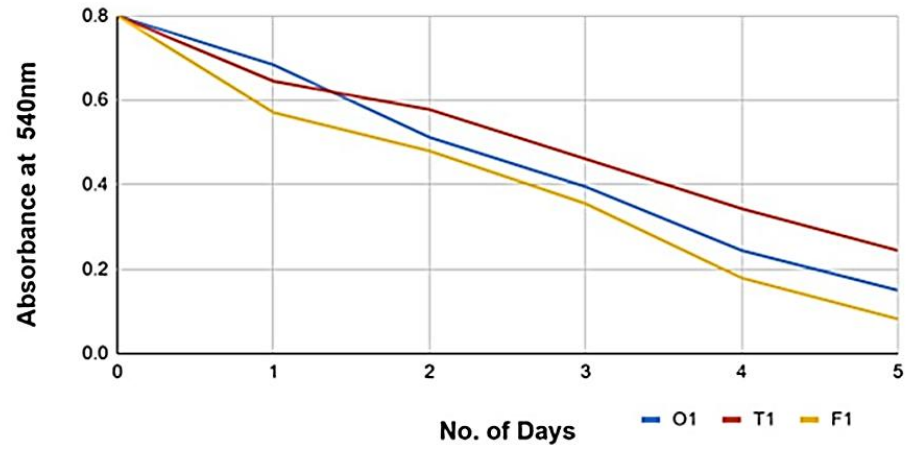


Figure 9. Time-dependent decolourization of neutral red by biofilm associated bacteria.

Table 6. Percentage decolourization of neutral red: Comparative efficiency of biofilm-associated bacteria over an incubation period of five days.

Percentage decolourization of Neutral red – Biofilm			
Day	**O1 (%)**	**T1 (%)**	**F1 (%)**
0	0	0	0
1	23.95	19.3	28.59
2	35.90	27.67	39.92
3	50.52	42.26	55.59
4	69.49	57.02	77.59
5	82.23	71.39	89.75

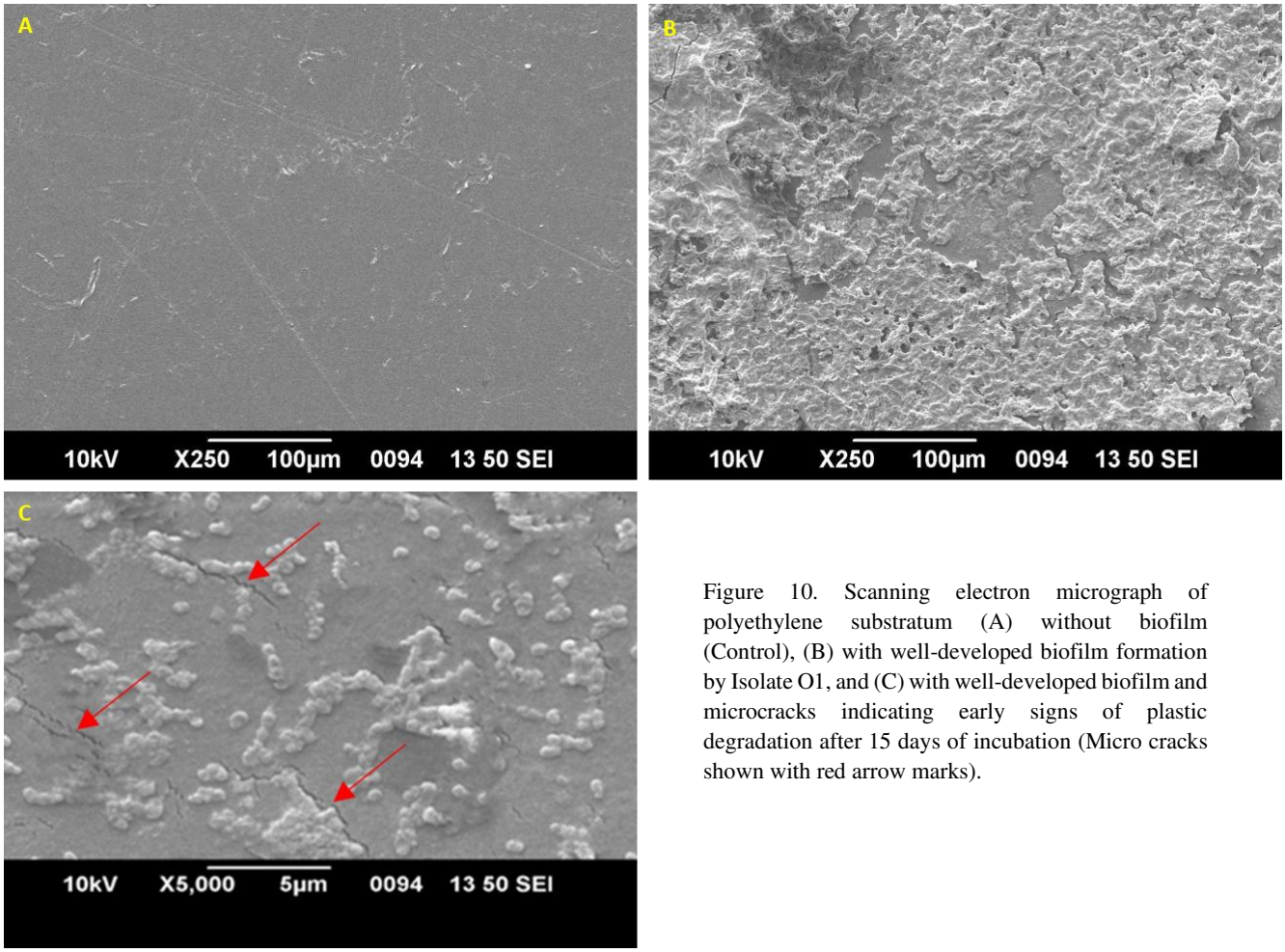


Figure 10. Scanning electron micrograph of polyethylene substratum (A) without biofilm (Control), (B) with well-developed biofilm formation by Isolate O1, and (C) with well-developed biofilm and microcracks indicating early signs of plastic degradation after 15 days of incubation (Micro cracks shown with red arrow marks).

closest phylogenetic relatives. The results showed that the 16S rRNA gene sequence of isolate F1 exhibited high sequence similarity to *Bacillus altitudinis*, indicating a close taxonomic relationship with this species. Similarly, isolate T1 showed the highest sequence homology with *Vibrio alginolyticus*, while isolate O1 showed high similarity with *Shouchella clausii*. The high percentage identity values and low E-scores obtained from the BLAST analysis confirmed the accurate affiliation of these isolates with their respective species. These findings conclusively established the molecular identity of the isolates as *Bacillus altitudinis* (F1), *Vibrio alginolyticus* (T1), and *Shouchella clausii* (O1).

Discussions

This study aimed to investigate the biodegradation potential of marine plastisphere-associated bacteria against two major classes of marine pollutants: phenol and synthetic dyes. Given the increasing stress of plastic-associated pollution in coastal waters, this research also examined how these bacterial isolates interacted with low-density polyethylene (LDPE). The results revealed the plastisphere-associated microbial

capacity to initiate plastic degradation under laboratory conditions. A comparative approach was employed to determine the effectiveness of biofilm-forming versus free-living bacterial forms, focusing on their potential to degrade common environmental pollutants.

The findings showed that biofilm-forming bacteria not only had high potential to degrade phenol and synthetic dye but also showed early signs of plastic weathering, such as micro-cracks. In the planktonic form, effective phenol degradation occurred within 72 hours, and synthetic dye decolorization reached more than 70% in 120 hours, specifically by F1 isolate (*Bacillus altitudinis*). Various bacteria, including *Bacillus* and *Pseudomonas* spp., have been reported to degrade high concentrations (1500 ppm) of phenol, and the authors claim tolerance to even higher concentrations (2000 ppm) (Salem et al., 2021). Both phenol degradation and synthetic dye decolourization were remarkably enhanced under biofilm-associated conditions. These findings support the emerging view of the plastisphere as an active biocatalytic niche that promotes pollutant bioremediation, and they align closely with Gu et al. (2017), who reported faster phenol degradation under biofilm conditions, although they reported high biodegradation rates at low phenol concentrations. It has also been suggested that plastic-associated biofilms have specific metabolic adaptations, including producing enzymes required to oxidize phenol and derivative aromatic ring structures (Oberbeckmann et al., 2016). The consistently high efficiency across studies supports the conclusion that plastisphere-associated bacteria possess specialized adaptations for phenol metabolism, likely developed through prolonged exposure to hydrocarbon-rich microenvironments on plastic surfaces (Zettler et al., 2013).

A core finding was the high performance of the most efficient biofilm-forming isolate, O1 (*S. clausii*), which degraded phenol much faster than the planktonic forms. This enhancement mirrors observations by Irankhah et al. (2018), attributing similar biofilm advantages to the combined effects of extracellular polymeric substance (EPS)-mediated substrate concentration, enzymatic synergy, and cellular proximity within the biofilm matrix. Our results further substantiated the ecological relevance of biofilms in marine pollutant remediation, as their structural organization appears enhanced for the cooperative breakdown of persistent aromatic compounds like phenol. The plastisphere forms a microbial community that colonizes coastal plastic debris, leading to adsorptive accumulation of pollutants on the plastic surface and promoting the growth of a unique microbiome that respire organic pollutants (Dodhia et al., 2023; Liu et al., 2024). This implies that plastisphere communities may use shared enzymatic pathways for both phenol and plastic hydrocarbon degradation, and the current study's finding of effective phenol degradation by plastisphere-associated bacteria aligns with previous reports (Irankhah et al., 2018; Liu et al., 2024). Together, these findings position plastisphere-associated biofilms as promising candidates for bioremediation in contaminated marine systems.

The study also revealed that isolates from the marine plastisphere decolorized the selected synthetic dyes within 120 hours under aerobic conditions. Isolate F1, or *B. altitudinis*, consistently exhibited high decolorization efficiency for all three azo dyes under study. The other two strains, O1 and T1, showed differences in their ability to decolorize the three dyes, though both showed the least decolorization with methylene blue. Methylene blue is a recalcitrant heterocyclic aromatic dye with a complex fused phenothiazine ring system, which makes it highly resistant to bio-decolorization by most bacterial isolates (Ariffin and Anuar, 2022), and the present study clearly aligns with the previous reports. This study observed that biofilm cultures showed greater potential for dye decolorization than their planktonic forms. The isolate F1 (*B. altitudinis*), however, showed great potential in decolourizing all three dyes, including methylene blue, both in the planktonic form and biofilm. These results complement the work of Tahir et al. (2021), who reported similar efficiency by marine *Bacillus* sp. The potential of bacterial consortia, including biofilm-forming *Bacillus* spp.,

has also been discussed in the decolurization of reactive dyes (Barathi et al., 2022). Together, these findings demonstrate the remarkable adaptive capacity of marine microorganisms to degrade complex synthetic dyes. Similar studies that illustrate biofilm cultures of *Bacillus* sp. as a strong decolourizing agent have also been reported (Zafar et al., 2022). In the present study, isolate O1, identified as *S. clausii* (formerly *Bacillus clausii*), showed the most efficient biofilm production and phenol degradation potential. *Shouchella clausii* has been reported to produce biofilms and enzymes like multi-copper oxidases and specific hydroxylases capable of mediating aromatic ring cleavage of phenolic compounds (Ghelardi et al., 2022), which supports the findings of the current study. SEM was employed to analyse the surface morphology of the plastisphere that served as the substratum for biofilm formation by *S. clausii*, which was found to yield the best biofilm production based on the primary screening. The micro-cracks observed on the surface of polyethylene film strongly indicate the biofilm's plastic-degrading potential, consistent with Erni-Cassola et al. (2020). In the present study, micro-crack formation on low-density polyethylene film was observed within two weeks, which was evidently faster than the previously established view of Delacuvellerie et al. (2019), who documented initial signs of plastic degradation within 2 months. This difference in degradation rate may reflect differences in the bacterial strains involved.

Overall, this study underscores the potential of plastisphere-associated bacteria to address some persistent pollutants in marine ecosystems. Phenol degradation and dye decolourization, coupled with early signs of plastic deterioration, highlight the multi-functional role of microbial biofilms on plastic debris. These findings collectively highlight the promise of harnessing plastisphere-associated microbial communities as natural bioengineering tools for mitigating marine pollution.

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