Biodegradation and Depolymerization of HDPE Microplastics by Indigenous Marine-Derived Fungi from the Visakhapatnam Coast, India: Enzymatic Mechanisms and GC-MS Profiling

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ABSTRACT:

High-density polyethylene (HDPE) microplastics (MP) are pervasive marine pollutants resistant to conventional degradation. This study evaluates the bioremediation potential of indigenous marine-derived fungi isolated from the Visakhapatnam coast, India. Among twenty strains and their defined consortia, *Talaromyces calidominioluteus* (TC) showed the highest degradation, achieving 33.3±3.89% weight loss of HDPE MPs (500–2000 µm) within 30 days in Mineral Salt Media (MSM) without pre-treatment. Key extracellular enzymes, including laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP), were detected, with MnP identified as a significant predictor of degradation ($P = 0.0045$). Pearson correlation analysis demonstrated a direct positive relationship between increasing enzyme activity and HDPE weight reduction. Field Emission- Scanning Electron Microscope (FE-SEM) and Energy Dispersive Spectroscopy (EDX) confirmed physical deterioration and oxygen enrichment of 16.1% by TC, while Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR) and X-ray diffractometer (XRD) revealed a 7.5% reduction in crystallinity by TC and the emergence of new carbonyl (1710 cm^{-1}), hydroxyl (3200–3600 cm^{-1}), and ether (1100 cm^{-1}) functional groups. Gas Chromatography–Mass Spectrometry analysis (NIST14 library) identified fragmented short- and long-chain hydrocarbons and polar metabolites, including 3,4-hexanedione and 1-nonanol. Interestingly, fungal consortia exhibited lower efficiencies (5.03%–7.23%), suggesting antagonistic interactions. This study provides the first evidence that *T. calidominiluteus*, a marine-derived fungus, is a potent agent for HDPE mineralization, offering a sustainable, additive-free strategy to mitigate microplastic pollution in marine environments.

KEYWORDS: HDPE, Microplastics, Bioremediation, Marine-derived fungi, *Talaromyces calidominioluteus*, Extracellular enzymes, GC-MS, Visakhapatnam coast

1. INTRODUCTION:

Plastics are synthetic organic polymers made up of petrochemical molecules with high molecular mass and complex bonds that resist the natural process of degradation. Global plastic production has increased dramatically from approximately 2 million tons in 1950 to over 380 million tons annually by 2015, and is expected to exceed 460 million tons by 2025 (Saxena, 2025). Plastic usage and production are increasing daily because of their low cost, ease of use, water insolubility, corrosion resistance, electricity resistance, and lightweight in nature (Khatua et al., 2023). Among the various types of plastic polymers, the most popular and convenient ones include low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET). Abiotic exposure, like UV and physical abrasion, causes fragmentation of plastic debris into microplastics (MP) of size 1µm – 5mm, which is difficult to degrade (Ikhimalo & Ugbenyen, 2023). In the terrestrial and coastal environments, 25 million tons of synthetic plastics are accumulated annually. About 60–80% of all marine garbage in the aquatic environment is plastic (Derraik, 2002). Polythene makes up over 64% of the garbage generated by synthetic plastics and is thought to be the most prevalent solid waste that poses a serious risk to marine life (Lee et al., 1991). Polyethylene microplastics are accumulated in all the tissues of marine specimens (Keerthika et al., 2023). Traditional plastic waste treatment methods are the disposal of waste into landfills, incineration, and dumping into the

sea and free lands, but these methods release some contaminants into the environment, are slow in the process, and are not more effective.

To overcome this problem, microorganisms are used to biodegrade plastic more effectively because of their enzymatic action (Barrech, 2018). Filamentous fungi have shown promising results for plastic biodegradation due to their saprophytic lifestyle, extensive hyphal networks facilitating substrate colonization, and robust extracellular oxidative enzyme systems (Barrech, 2018; Tiago et al., 2023). Fungal cells secrete amphipathic hydrophobin proteins, characterized by eight conserved cysteines forming four disulfide bridges that act as biosurfactants to adhere to hydrophobic polymer surfaces and increase surface wettability for effective enzyme action (Wösten & Scholtmeijer, 2015; Roberts et al., 2024). Furthermore, the mycelial structures of fungi provide an added advantage of maximizing the wettability and surface contact area with plastic substrates, leading to enhanced enzymatic activity, which is often missing in bacterial systems. Among all the fungal species, Ascomycetes have more potential to degrade microplastics than Basidiomycetes, followed by Zygomycetes (Sánchez, 2020). Various research studies have shown that fungi like *Aspergillus niger*, *Aspergillus fumigatus*, and *Zalerion maritimum* possess the ability to break down MPs and use them as their exclusive carbon source (Das & Kumar, n.d.; Paço et al., 2017).

In general, plastic biodegradation takes place through four major steps of biodeterioration, bio-fragmentation, assimilation, and mineralization (Amobonye et al., 2021; Cowan et al., 2022). The bio-fragmentation step, a crucial phase in bioremediation where the recalcitrant macromolecules break down to simple oligomers, dimers, and monomer fragments, which takes place with the release of various extracellular oxidative enzymes like oxidases and hydrolases. Laccase, lignin peroxidase, and manganese peroxidase are three crucial enzymes produced by fungi that attack synthetic polymers and promote redox potential reactions to generate ROS, which attack the polymers (Okal et al., 2023). Some of the hydrolytic enzymes, like esterase, lipase, protease, and urease of fungi, are produced to enhance the mineralization process. Microbial degradation of plastics is assessed by monitoring fungal growth along with the physical and chemical variations of the polymer. Fungi that metabolize microplastics as their sole carbon source induce structural damage that is visible as microcracks, pits, or halos under light microscopy. In recent studies, accurately determining the chemical composition of unknown plastic debris has become a priority in microplastic research, with ATR-FTIR spectroscopy widely used for this purpose. (Campanale et al., 2023). A recent study reported in THE TIMES OF INDIA titled “Study Reveals Widespread Microplastic Presence in Seafood along Vizag Coast,” 2025, conducted along the Visakhapatnam coast, confirmed widespread microplastic contamination in marine organisms, emphasizing the severity of plastic pollution in these coastal waters. In response, the current study focuses on isolating indigenous marine-derived fungi from plastic debris collected at Visakhapatnam beaches and evaluating their capability to degrade high-density polyethylene microplastics. Using molecular identification, quantitative weight-loss assays, enzymatic assays, FE-SEM with EDX, XRD, and ATR-FTIR, we aim to elucidate the biodegradation potential and identify degradation products by GC-MS to study the underlying mechanisms of these isolates, thereby advancing sustainable bioremediation strategies to mitigate marine HDPE microplastic pollution.

2. MATERIALS AND METHODS:

2.1 Sample collection from the site: Plastic debris was collected from four coastal sites along the Visakhapatnam shoreline: Ramakrishna beach (17° 42' 51.4584" N, 83° 19' 25.2660" E); Yarada Beach (17° 41' 25.7064" N, 83° 13' 51.7764" E); Rushikonda beach (17°46'55.7"N " N, 83°23'06.3" E); Bheemili Beach (17° 53' 15.0648" N, 83° 26' 44.4444" E). The plastic pieces that floated on the seawater and that settled in marine sediments were carefully picked with sterile tweezers and placed in 50 mL Falcon tubes. The samples were immediately placed in an ice box, transported to the laboratory, and stored at 4 °C until further analysis.

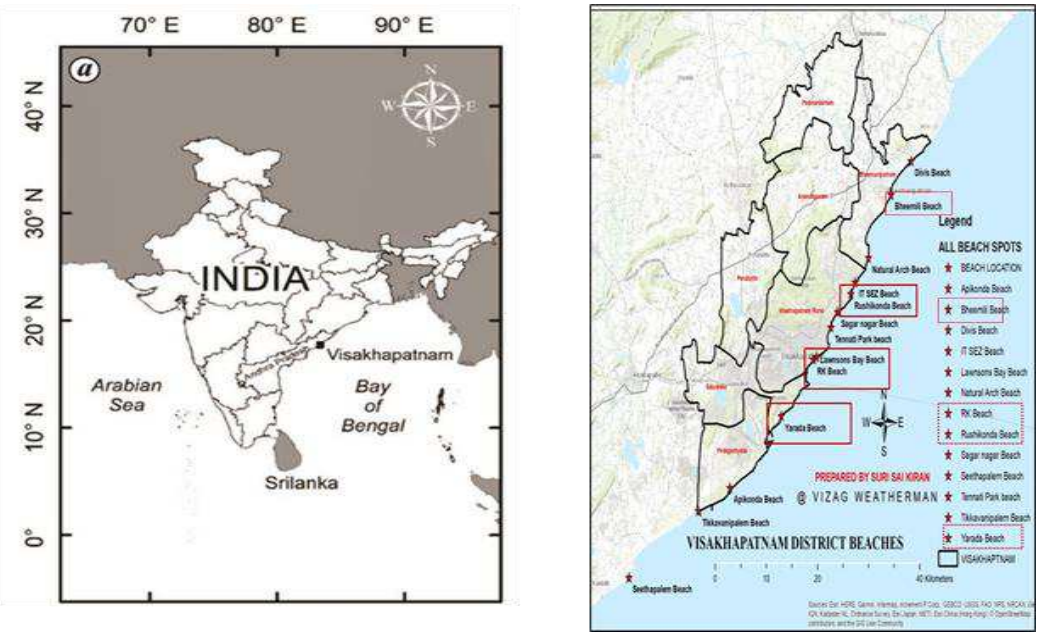


Fig. 1: Various sample collection sites of the Visakhapatnam coastal region

2.2 Isolation of marine-derived fungi from plastic debris: The collected plastic debris was cut into small pieces using sterile scissors and placed onto potato dextrose agar (PDA) medium prepared with a 1:1 mixture of seawater and distilled water, and pH adjusted to 8.0. The plates were incubated at 28ºC for 14 days. Individual colonies were picked and sub-cultured multiple times to obtain pure isolates. These pure fungal strains were maintained on PDA slants at 4ºC for long-term preservation and subsequent experimental analyses (Sangeetha Devi et al., 2015).

2.3 Polyethylene: High-Density Polyethylene (HDPE) of less than 50 microns was procured from local markets in Visakhapatnam. The plastic film was mechanically cut into pieces and sieved to isolate microplastics in the 500-2000 µm size range. The separated microplastics were sterilized with 70% ethanol and air-dried under sterile conditions (Indumathi & Kavitha, 2020).

2.4 Strain screening and isolation of HDPE degradation: Commercially obtained HDPE MPs were weighed and sterilized. Among all the fungal strains, plastic-degrading strains were isolated by using MSM media consisting of 0.7 g/L $K_2HPO_4.3H_2O$, 0.7 g/L K_2HPO_4, 0.7 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L NH_4Cl, 0.005 g/L NaCl, 0.002 g/L $FeSO_4 \cdot 7H_2O$, 0.002 g/L $ZnSO_4 \cdot 7H_2O$, and 0.001 g/L $MnSO_4 \cdot H_2O$. (Liu et al., 2022)

2.5 Molecular identification of MPs degrading fungi: All the isolated plastic-degrading marine fungal strains were identified based on nucleotide sequence analysis of the internal transcribed space (ITS) region. From the uncontaminated quality-checked agar plate, 1-2 fungal colonies were picked and suspended in 50 µL of molecular biology-grade water and mixed well. By thermal lysis process at 95 °C for 10 mins, the fungal cells were broken, and by quick centrifugation, the cell lysate was separated, and the supernatant was collected. The supernatant, which contains fungal DNA, was used as the template for the PCR (Romanelli et al., 2014). The ITS region was amplified by using universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3')(Martin & Rygiewicz, 2005). The conditions used for PCR amplification were as follows: initial denaturation stage of 95 °C for 5 min, followed by 35 cycles of 95 °C for 30s, annealing at 55 °C for 1min, extension at 72 °C for 1min, and a final extension stage at 72 °C for 6 min. The amplified PCR product was further purified by salt precipitation. The amplified products were analyzed through electrophoresis on 1.2% agarose gel (Sigma) and stained with 0.5 µg ml^{-1} ethidium bromide. The purified PCR product obtained was subjected to Sanger sequencing (Cycle sequencing reaction) by BigDye Terminator v3.1 chemistry developed by Applied Biosystems. These labelled sequences were loaded onto a Capillary electrophoresis sequencer (ABI 3500 XL Genetic analyzer), carried out at HiMedia Laboratories Pvt Ltd. (Maharashtra). The gene sequence obtained was subjected to a database search against the Unite 9.0 (Abarenkov et al., 2010) using the BLAST tool (Altschul et al., 1990). The multiple sequence alignment was used to produce a consensus phylogram using the maximum likelihood algorithm with 1000 iterations using MEGA11 (Molecular Evolutionary Genetic Analysis, version 11) software (Tamura et al., 2021).

2.6 Biodegradation assay of MP degrading marine fungi: Aseptically, 0.02 g of sterilized HDPE microplastics was added to 20 ml of Mineral Salt Media (MSM) (Aguiar et al., 2024) prepared in a 1:1 composition of seawater and distilled water (pH 8.0) and inoculated with individual highly potent plastic degradable fungal strains or defined consortia at weekly intervals (days 7, 14, and 21). Cultures along with uninoculated controls were incubated in an orbital shaker at 120 rpm and 28°C for 30 days in triplicate (Sangeetha Devi et al., 2015). Following incubation, microplastics were recovered by filtration and rinsed with 2% SDS to remove fungal colonies attached to them. They were then heated to 60°C for 2 hours to remove excess water for further secondary screening analysis. Degradation was further confirmed by FE-SEM combined with EDX, ATR-FTIR, and XRD analyses (Sowmya et al., 2014).

2.7 Enzymatic activity: After 30 days of incubation, the MSM broth was centrifuged at 6000 rpm for 5 minutes, and the cell-free supernatant was harvested for enzyme assays (Singh et al., 2024). Laccase activity was measured by monitoring ABTS (2,2 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) oxidation (0.5 mM ABTS, 1mL supernatant in 3 mL 0.1 M sodium acetate buffer, pH 4.5) at 420 nm. Manganese peroxidase activity was the same when using the same mixture supplemented with 1 mL H_2O_2. Lignin Peroxidase (LiP) activity was measured by veratryl alcohol oxidation (2 mM veratryl alcohol, 1 mL of 0.1 M sodium tartrate (pH 3) with 300 µL of 0.4 mM H_2O_2) at 310 nm (Kang et al., 2019; Sowmya et al., 2015). The reaction mixtures were incubated at 30°C for 15 minutes, and changes in color were measured using a UV spectrometer (Shimadzu UV-1780) at a specific wavelength. One unit (1U) of enzymatic activity was described as the enzyme quantity needed to oxidize 1micromole of substrate per minute (Papinutti & Martínez, 2006).

$$\text{Enzyme Activity (U/ml)} = \frac{\Delta A \times V\text{total}}{\varepsilon \times l \times V\text{enzyme} \times \text{time}}$$

2.8 Protein Estimation: Protein concentrations were measured by the Lowry method with standard as bovine serum albumin (Lowry et al., 1951), and specific enzyme activities were calculated employing the following formula:

$$\text{Specific Activity (U/mg)} = \frac{\text{Enzyme Activity (U/ml)}}{\text{Protein Concentration (mg/ml)}}$$

2.9. Methods of Analysis: The following describes the monitoring of the surface, elemental, chemical, and crystallinity changes in the polyethylene that took place during biodegradation.

2.9.1 Determination of dry weight of the residual MP: The recovered microplastics were collected on sterile filter paper and then air-dried overnight before final weighing. The weight loss was calculated using the formula:

$$\text{Percentage of weight loss} = \frac{\text{Initial weight} - \text{FInal weight}}{\text{Initial weight}} \times 100$$

2.9.2 FE-SEM & EDX Analysis: For surface morphology assessment of plastics post-biodegradation, HDPE films were fixed with a thin gold coating, desiccated in a vacuum chamber to eliminate moisture, and examined under a scanning electron microscope (TESCAN MIRA S6123) at 1-10 µm resolution. Along with FE-SEM to determine the atomic percentage of the carbon and oxygen content in the treated and untreated HDPE MPs film, Energy Dispersive Spectroscopy (EDX) was performed (Gupta et al., 2023).

2.9.3 ATR-FTIR Analysis: ATR-FTIR (Alpha II, Bruker model, Germany) was used to monitor the structural variations and the appearance or loss of functional groups in polyethylene during the

degradation process. Spectra were collected over the 4000–500 cm^{-1} range at 4 cm^{-1} and with 64 scans per sample (Yao et al., 2022).

2.9.4 XRD Analysis: The crystallinity of HDPE microplastics was analyzed by the X-ray diffractometer technique (Bruker, D8 Advance model) under Cu Kα radiation source (1.5418 A°) operated at 40 kV and 40 mA with θ/θ geometry. The divergence slit was fixed to a divergence slit size of 0.681°. The XRD patterns were recorded in between 5° and 60° at a scan rate of 0.5 s^{-1} with a step size of 0.02 at room temperature (Chaudhary & Vijayakumar, 2020).

2.10 Identification of depolymerization products by GC-MS: The culture broth was filtered to remove the HDPE MPs and centrifuged. The cell-free supernatant was collected and filtered with a 0.2 µm filter. An equal amount of chloroform and culture was taken in a separating funnel, shaken vigorously for 30 minutes, and then left at room temperature for 1 hour. The organic phase was collected into a flask and concentrated to 1 mL using a rotary evaporator. The samples were characterized using a GC-MS (Agilent Technologies) system, equipped with a DB-624 GC column and helium as the carrier gas. Samples were injected at 1 mL/min with a split of 10:1. The inlet temperature was 250 °C. The oven temperature was maintained at 40 ℃ for 3 minutes, then increased to 280 ℃ at a rate of 10 ℃ min^{-1}, and finally held at 280 ℃ for 4 minutes. The detector temperature was 280 ℃. The depolymerized products were mainly putative degradation products identified based on their molecular features and the relative abundance of the compounds, are determined by peak integration using Agilent MassHunter Qualitative Analysis version 10.0.368 in the NIST14 library database (Allemann et al., 2024; Liu et al., 2022).

2.11 Statistical analysis: All the experiments were performed in triplicate. One-way and Two- way ANOVA revealed a statistically significant difference among treatments, and Tukey's post hoc test was performed for pairwise comparison. The data obtained were expressed as mean ± standard deviation (SD). Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION:

3.1 Isolation of marine-derived fungal strains:

A total of 20 fungal strains were isolated from plastic debris across four environmental regions. Morphological and microscopic characterization identified the majority as *Aspergillus spp*. (12 strains) and *Penicillium spp.* (7 strains), with one strain representing *Talaromyces* (Fig.2). The prevalence of *Aspergillus* and *Penicillium* in this study is consistent with their known role as robust "decomposers, capable of producing various extracellular enzymes like oxidative and hydrolytic, which are essential for breaking down complex synthetic polymers (Nasrabadi et al., 2023). Their dominance indicates successful adaptation to the "plastisphere," where they use plastic as a potential carbon source in nutrient-limited environments. The identification of *Talaromyces*, related to *Penicillium* but often more thermotolerant or acid-tolerant, is especially remarkable (Pyrri et al., 2021). Subsequently, fungal strains capable of degrading plastics were isolated based on the weight loss of MPs after incubation.

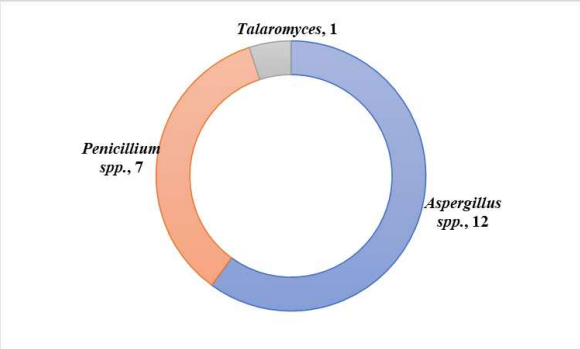
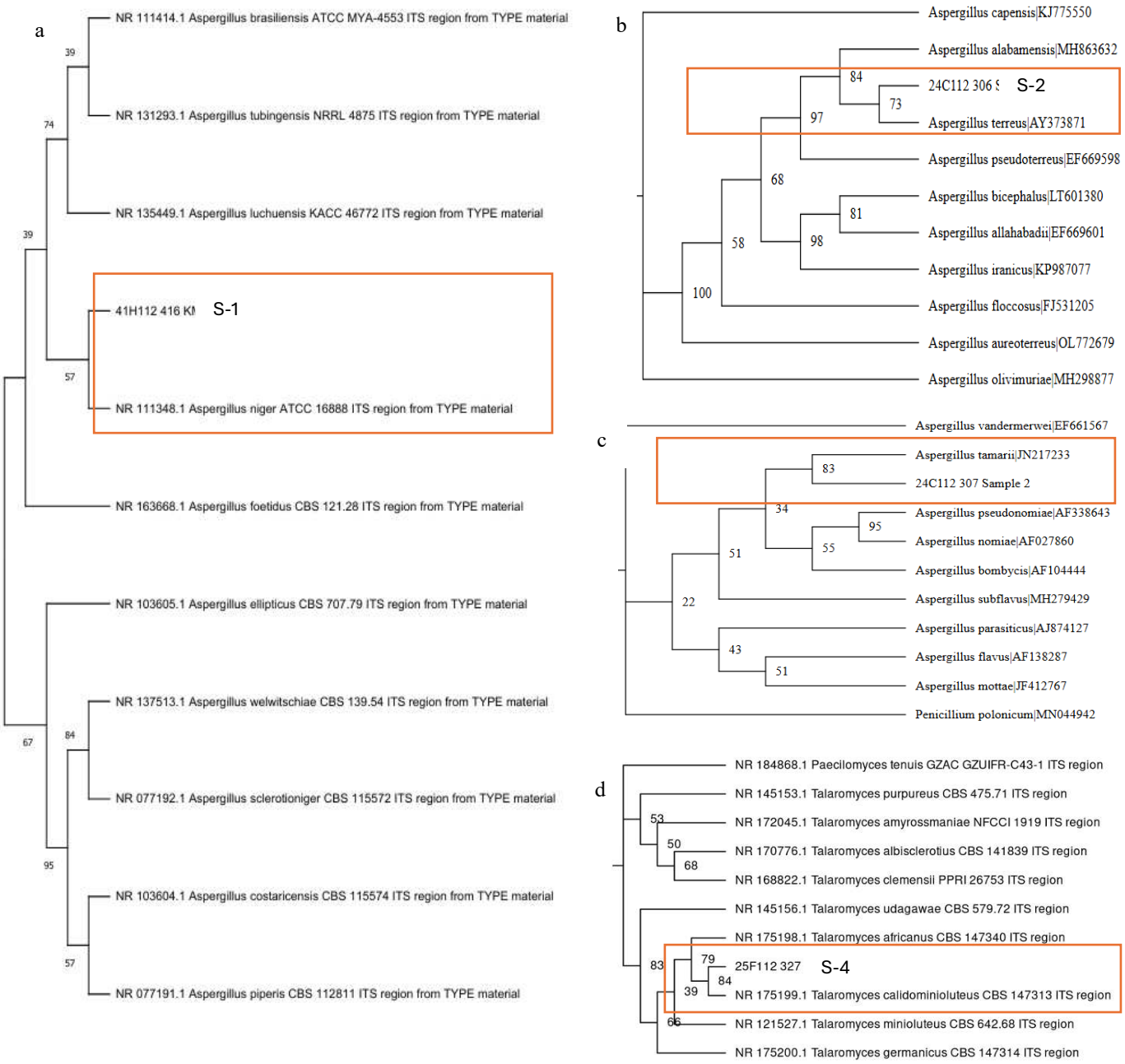


Fig. 2: Taxonomic distribution of the isolated marine-derived fungal strains based on microscopy

3.2. Molecular identification of biodegradable fungal strains:



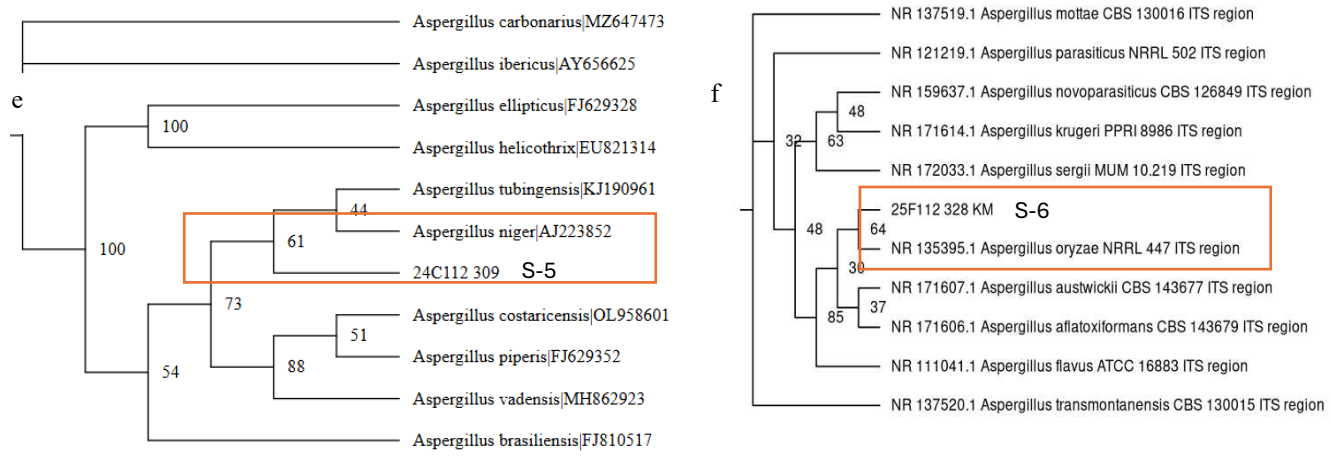


Fig. 3 (a, b, c, d, e, f)- Molecular phylogenetic analysis of isolated marine fungal strains by ITS sequencing. The phylogenetic tree was obtained by the Maximum Likelihood method. Evolutionary analysis was conducted in MEGA 11.

The evolutionary history was obtained by the maximum likelihood method described in the Kimura parameters model (Kimura, 1980). In fig 3 (a, b, c, d, e, f) phylogenetic analysis displayed 100% homology of isolated strains S-1, S-2, S-3, S-4, S-5, S-6, with *Aspergillus niger* (AN-1), *Aspergillus terreus* (ATE), *Aspergillus tamarii* (ATA), *Talaromyces calidominioluteus* (TC), *Aspergillus niger* (AN-2), and *Aspergillus oryzae* (AO) respectively. The sequence of marine fungal isolates *Aspergillus niger* (Accession no. PV300375), *A. terreus* (Accession no. PV951569), *A. tamarii* (Accession no. PV951570), *T. calidominioluteus* (Accession no. PV936923), *A. niger* (Accession no. PV942218), *and A. oryzae* (Accession no. PV936927) were deposited in the GenBank sequence repository.

3.3. Weight loss of MP residues after biodegradation:

After the biodegradation assay, the MPs are filtered and rinsed with water to eliminate the fungal residues and weighed. Then, the values are calculated using the (% of weight loss) formula. All the values of MP residual weight loss are mentioned in Fig. 4. Among all the plastic-degradable fungal strains and consortia, AN-1, ATE, and TC showed the highest biodegradation of MPs, with 24.6±2.58%, 20.7±3.07%, and 33.3±3.89% weight loss, respectively. Statistical significance was indicated by the compact display letter. Kumar & Das, 2014 studied the ability of *Aspergillus* and *Fusarium* species to degrade LDPE (low-density polyethylene), with a decrease in LDPE weight of 6-8% and 9%, respectively. Sarkhel et al., 2019 showed the degradation of plastic bottle waste polymers, with 35% degradation employing bacteria and 22% with fungal strains. DSouza et al., 2021 suggested using potato dextrose broth, 26.15% of LDPE weight loss occurred. (Şimşek Uygun & Malkoç, 2024) reported that *Aspergillus flavus* and *Aspergillus versicolor* degraded polyethylene MP with removal efficiencies of 18.8% and 6.7%. In this work, without prior treatment or the addition of pro-oxidants, the fungal strains *Aspergillus niger-1*, *Aspergillus terreus*, and *Talaromyces calidominioluteus* can degrade HDPE MP more efficiently.

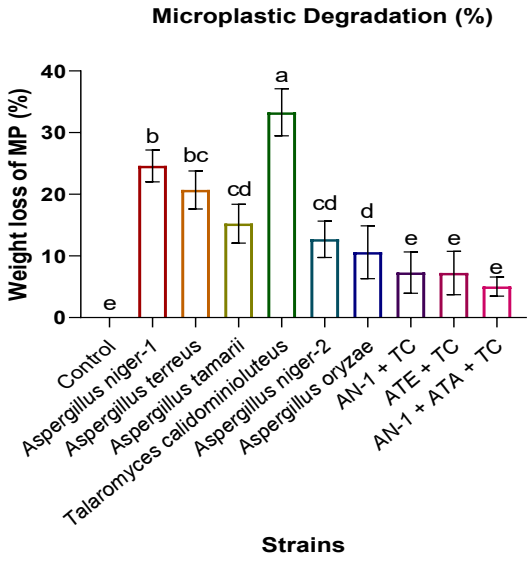


Fig. 4: Comparative weight loss (%) of HDPE MPs after a 30-day incubation period with various plastic-degrading fungal strains and their consortia, with mean ±SD (n=3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Different lowercase letters above the bars indicate a statistically significant difference ($p < 0.05$). Among all the strains, AN-1, ATE, and TC reduced MPs by 24.6±2.58%, 20.7±3.07%, and 33.3±3.89%, respectively.

3.4. Extracellular oxidative enzymatic activity:

Extracellular oxidative enzymes, including laccases and peroxidases, are crucial for breaking down plastics by attacking chemically stable carbon-carbon bonds via free-radical mechanisms. They also increase the surface hydrophilicity of hydrophobic polymers, making it easier for microbes to colonize and for enzymes to further degrade the plastics. Understanding these oxidative processes is key to developing effective bioremediation approaches to address the environmental problem posed by synthetic plastics (Ekanayaka et al., 2025).

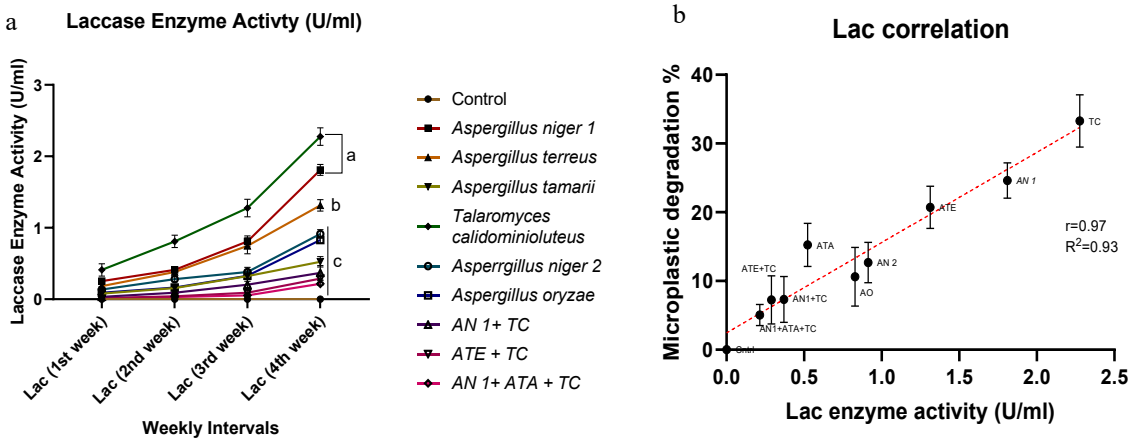


Fig. 5.1: (a) Time course profiling of enzymatic activity (U/ml) of extracellular oxidative enzyme Laccase produced by fungal strains during degradation, with mean ±SD (n=3). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Different lowercase letters (a, b, c) at the 4th-week interval denote statistically significant differences between the fungal treatments. (b) Pearson correlation between Laccase and weight loss of HDPE MPs (%) at week 4. Data points include mean values ± SD (n=3) with the red dashed line representing the linear regression fit.

In (Fig. 5 .1. a), the temporal profile of laccase activity showed a significant, time-dependent increase across all fungal treatments, with peaks at week four and a clear statistical hierarchy (groups a–c). Strain TC was the most effective producer, achieving higher titers (2.27±0.122 U/ml, $p < 0.05$) compared to moderate performers like AN 1 and the lower-activity groups in 'c'. In (Fig. 5.1. b), correlation analysis confirmed a strong, positive linear relationship between enzymatic peaks and HDPE weight loss ($r = 0.97$, $R^2 = 0.93$), indicating that laccase secretion primarily drives degradation. Notably, strain TC's higher enzyme output directly led to the maximum 33.3% degradation, while strains with lower laccase levels exhibited proportionally less weight loss. These results statistically support laccase activity as a reliable marker for fungal biodegradation effectiveness, with strain TC standing out as the most promising for degradation.

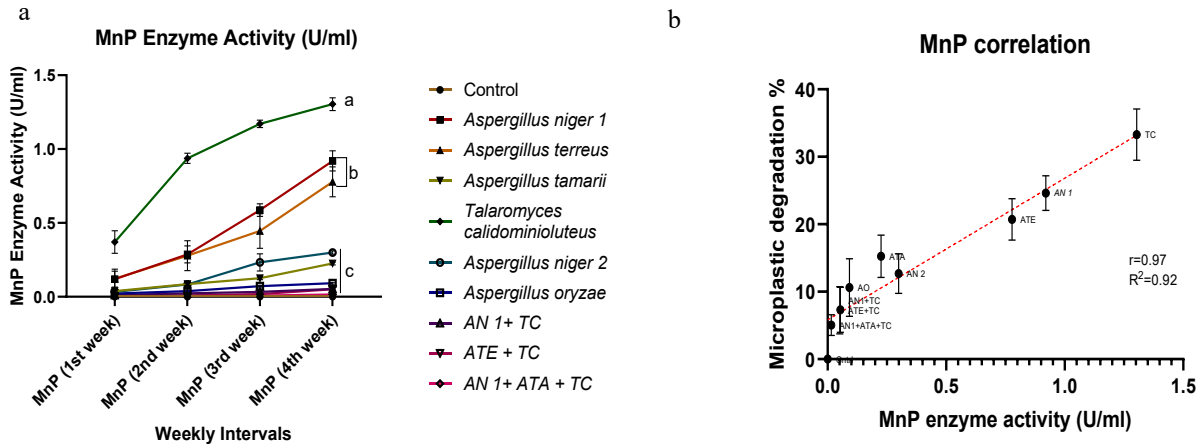


Fig. 5.2: (a) Time course profiling of enzymatic activity (U/ml) of extracellular oxidative enzyme MnP produced by fungal strains during degradation, with mean ±SD (n=3). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Different lowercase letters (a, b, c) at the 4th-week interval denote statistically significant differences between the fungal treatments. (b) Pearson correlation between MnP and weight loss of HDPE MPs (%) at week 4. Data points include mean values ± SD (n=3) with the red dashed line representing the linear regression fit.

In (Fig. 5.2.a), Manganese peroxidase (MnP) activity showed a progressive increase, with strain TC (group a) achieving a significantly higher 4th-week peak of 1.3±0.043 U/ml ($p < 0.05$) than moderate (group b: AN 1, ATE) and low producers (group c). In (Fig. 5 .2. b), correlation analysis revealed a strong positive linear relationship between this enzymatic peak and HDPE weight loss ($r = 0.97$, $R^2 = 0.92$), indicating that MnP secretion is a primary driver of

degradation. Specifically, the superior titer of strain TC directly facilitated the maximum observed weight loss of 33.3%, whereas strains with lower activity showed proportionally less degradation. These results statistically validate MnP as a critical oxidative component in the breakdown of the HDPE backbone and confirm that its activity levels serve as a robust indicator of fungal remediation efficiency.

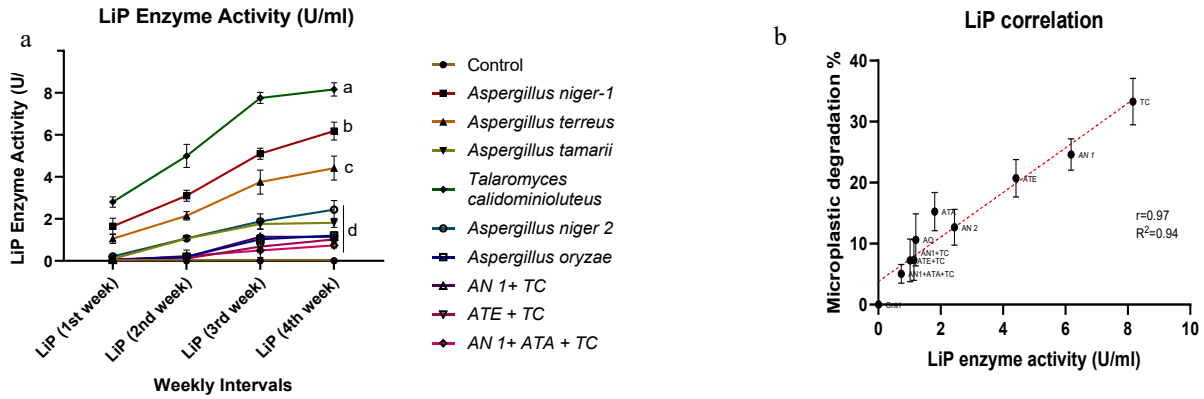


Fig. 5.3: (a) Time course profiling of enzymatic activity (U/ml) of extracellular oxidative enzyme LiP produced by fungal strains during degradation, with mean ±SD (n=3). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Different lowercase letters (a, b, c, d) at the 4th-week interval denote statistically significant differences between the fungal treatments. (b) Pearson correlation between LiP and weight loss of HDPE MPs (%) at week 4. Data points include mean values ± SD (n=3) with the red dashed line representing the linear regression fit.

In (Fig. 5.3.a), Lignin peroxidase (LiP) activity showed a significant, progressive increase, with strain TC (group a) achieving a peak of 8.16±0.317 U/ml ($p < 0.05$), significantly outperforming AN 1 (group b) and ATE (group c). In (Fig. 5.3.b), correlation analysis revealed a robust, positive linear correlation between 4th-week LiP titers and HDPE weight loss ($r = 0.97$, $R^2 = 0.94$), identifying LiP as a primary enzymatic driver of the degradation process. The superior output of strain TC directly facilitated the maximum observed weight loss of 33.3%, whereas strains with lower activity (groups c and d) exhibited minimal degradation. These results statistically validate LiP as a critical oxidative agent and a reliable biochemical indicator for the efficacy of fungal-mediated microplastic remediation. A prior study of an HDPE-degrading fungal consortium reported by Sowmya et al. (2015) showed that, after 12 weeks, the degradation of HDPE by the fungal consortium showed laccase and MnP activity of only 0.00441 ± 0.0002 and 0.00451 ± 0.0003, respectively. However, in our study, laccase, MnP, and LiP activities were substantially higher after 4 weeks when compared to 12 weeks, indicating efficient degradation.

3.5.1. Total Protein Estimation:

Total protein estimation serves as a critical indicator of whether the isolated strains are actively growing and secreting enzymes in response to the plastic substrate, thereby facilitating degradation (Esmaeili et al., 2013).

a

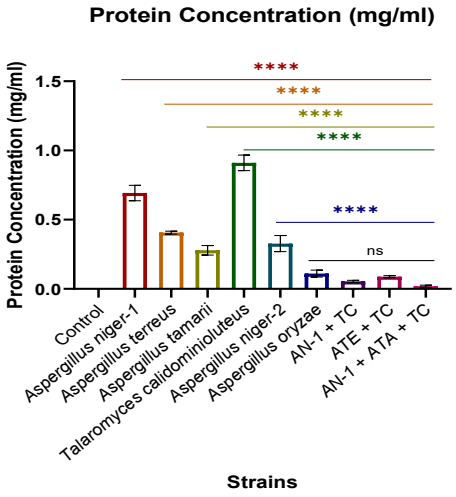


Fig.6. a: Extracellular protein concentration (mg/ml) produced by marine-derived fungal isolates during HDPE MPs degradation, mean values ± SD (n=3). Statistical significance was determined using a one-way ANOVA followed by Tukey's HSD post-hoc test. Results indicate that *Talaromyces calidominioluteus* produced significantly higher protein concentrations ($p < 0.0001$, ****) than the consortia groups.

From Fig. 6.a, the protein concentration of *A. niger*-1, *A. terreus*, and *T. calidominioluteus* was 0.692±0.055 mg/ml, 0.406±0.0105 mg/ml, and 0.911±0.056 mg/ml after 30 days of incubation. The *Talaromyces calidominioluteus* emerged as the most effective treatment for HDPE microplastic degradation, significantly outperforming the consortia ($p < 0.0001$). Total extracellular protein estimation via the Lowry method revealed that this strain achieved a peak protein profile, which directly correlated with a maximum weight loss of 33.3% and the highest recorded activities of extracellular oxidative enzymes (laccase, manganese peroxidase, and lignin peroxidase). This indicates that the metabolism within the individual TC creates a superior oxidative environment, where the high concentration of secreted enzymes facilitates the depolymerization of the HDPE MPs backbone. The superior performance of this specific strain compared to complex multi-strain consortia suggests that metabolic compatibility is the key driver of efficient plastic mineralization.

3.5.2. Multiple Linear regression analysis of Specific activity with weight loss of MPs:

Calculating specific activity is fundamental because it identifies which specific enzyme system is being prioritized for polymer breakdown. When this data is integrated with gravimetric results, which measure the plastic's physical weight loss, regression analysis can determine the statistical correlation between the multiple variables. A high R^2 value in a linear regression model effectively confirms that a specific enzyme, such as a lac, MnP, or LiP, is the primary driver of the observed degradation (Giacomucci et al., 2019).

b

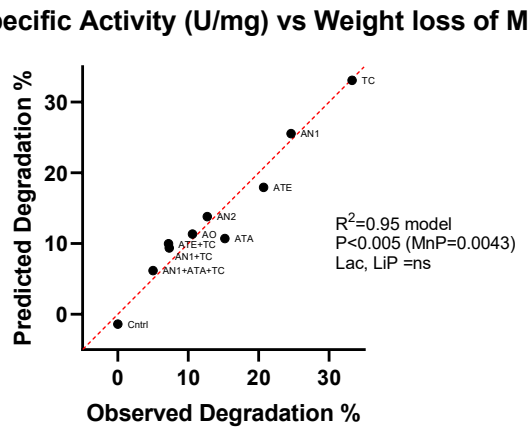


Fig. 6. b: Multiple linear regression analysis of enzyme-specific activities (U/mg) vs. HDPE MPs degradation (%). Statistical analysis shows that MnP (P=0.0043) is the primary significant contributor to the model, while Lac and LiP showed non-significant individual effects in this combined multivariable context.

Specific activities of laccase in *A. niger*-1, *A. terreus*, and *T. calidominioluteus* were 1.53±0.106 U/mg, 1.45±0.236 U/mg, and 4.08±0.261 U/mg, respectively; while MnP specific activity was 1.98±0.252 U/mg, 1.434±0.095 U/mg, and 2.361±0.091 U/mg; and LiP specific activity was 8.91±0.616 U/mg, 10.86±1.401 U/mg, and 8.959±0.348 U/mg after 30 days of HDPE degradation. In fig. 6. b, the multiple regression analysis showed a highly accurate prediction of microplastic weight loss based on enzyme-specific activities ($R^2 = 0.95$, $p < 0.005$). Although individual enzyme correlations were strong, the combined model identified manganese peroxidase (MnP) as the sole statistically significant factor in degradation (0.0043), while laccase and LiP were not significant. Strain TC had the highest specific activity, which correlated with the maximum degradation of about 33.3%. Notably, this strain significantly outperformed all tested consortia, such as AN1+ATA+TC and ATE+TC, which showed degradation below 10%. This indicates that the high, specialized MnP activity of strain TC is more effective for HDPE breakdown than the enzymatic profiles of the consortia, which may have been suppressed by microbial antagonism or enzymatic dilution. (Sowmya et al., 2015) reported that laccase and MnP specific activities were 0.0490 ± 0.002 and 0.0499 ± 0.001, respectively, by the fungal consortium in the HDPE biodegradation process. (Sowmya et al., 2014) showed that the specific activity of manganese peroxidase (0.0540± 0.001 µmol/ml/mg/min) was higher compared to laccase (0.051±0.004 µmol/ml/mg/min) after the degradation of UV-treated polyethylene by the *Trichoderma harzianum*. But in our study, without UV treatment or compared to the fungal consortia, individual fungal strains showed the highest specific activity, favoring the degradation of MPs.

3.6. Methods of Analysis:

3.6.1 SEM & EDX Analysis:

SEM analysis was carried out to investigate the surface changes in the microplastic post-degradation. SEM images of before degradation and control (Fig. 7 .1a, b, c) displayed smooth, intact films, while the images after degradation

with isolates AN-1 (Fig. 7 .2a, b), ATE (Fig. 7 .3a, b), and TC (Fig. 7 .4a, b) revealed extensive mycelial colonization, spore adherence, and the appearance of cracks and pits, which are clear indicators of biodegradation. The results are in compliance with studies carried out by Sangeetha Devi et al. (2015), where surface changes are clearly observed on the HDPE surface after treatment with *Aspergillus* spp. with the addition of mineral oil. Other studies carried out by DSouza et al. (2021) exhibited surface erosion and folded LDPE surfaces treated with *Aspergillus* spp. consortia in PDB for 55 days. (Das & Kumar, n.d.) also reported the presence of microbial attachment and holes on the LDPE surface after 60 days of degradation with *Aspergillus* spp. and *Fusarium* spp. A noteworthy finding that can be observed in our study is the ability of the fungi to depolymerize microplastics without any prolonged treatment or addition of lubricants, indicating better remediation ability of our marine fungal isolates. In addition, EDX analysis was also performed to determine the elemental changes in HDPE MPs following interactions with different fungal strains. EDX analysis (Fig. 8 a, b, c, d, e) has shown 98.55% of carbon in HDPE MPs control films, but treated samples have displayed 89.1%, 93.14%, and 83.9% carbon, indicating a significant decrease after a 30-day incubation period with the AN-1, ATE, and TC, respectively. The drastic change in the carbon content of films confirms the biodegradation process. Interestingly, a decrease in the atomic percentage of carbon was compensated by an increase in the oxygen percentage, i.e., 10.9%, 6.86%, and 16.1% for the AN-1, ATE, and TC fungal strains, respectively (Table 1), confirming that an oxidation reaction occurred on the MPs during biodegradation. In the study by Gupta et al. (2023), a 2.4% increase in oxygen was observed after treatment with the *B. parabrevis* CGK45 strain. However, in our findings, a greater oxygen shift was observed following the degradation process, due to a decrease in carbon.

a b c

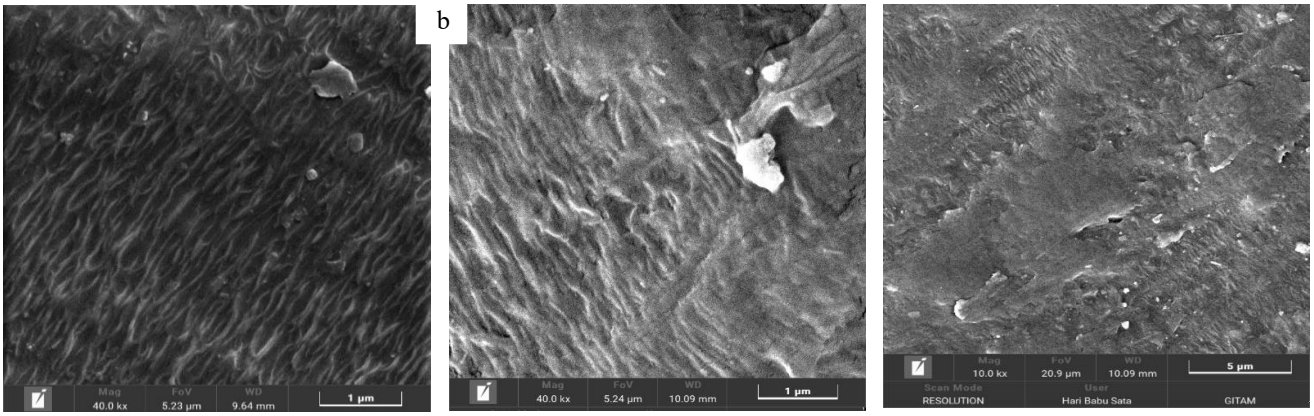


Fig. 7 .1 (a, b, c) SEM images of the MPs before the biodegradation process and control at 10.0 kx magnification and 1µm and 5µm resolution, indicating a plain surface without any visibility of cracks or pores.

a b

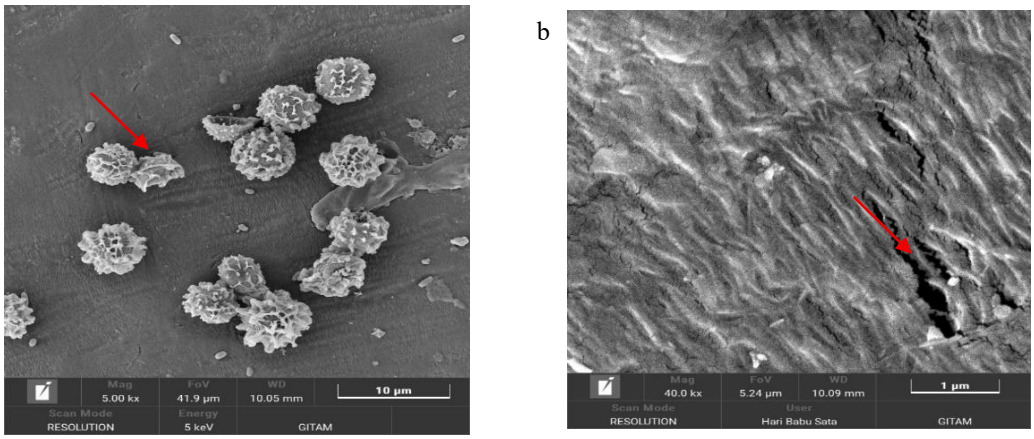
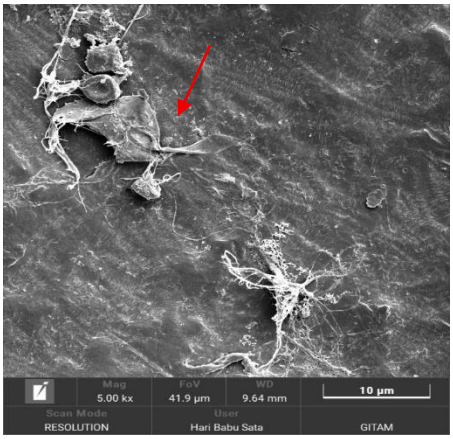


Fig. 7.2 (a,b): SEM images of the MPs after the biodegradation process with AN-1 at 10.0 kx magnification and 1µm and 10 µm resolution, (a) showing the adhesion of spores on the plastic film surface, (b) formation of cracks on the film due to depolymerization of plastic.

a



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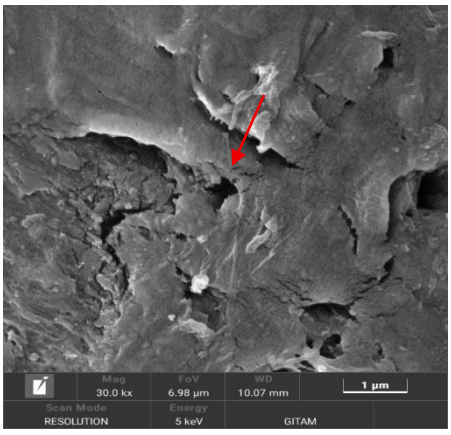
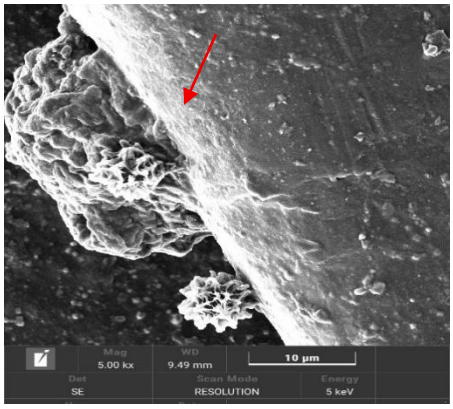


Fig. 7.3 (a,b): SEM images of the MPs after treatment with ATE at 10.0 kx magnification and 1µm and 10 µm resolution, (a) showing deep cracks and (b) hollow formation due to breakdown of plastic films.

a



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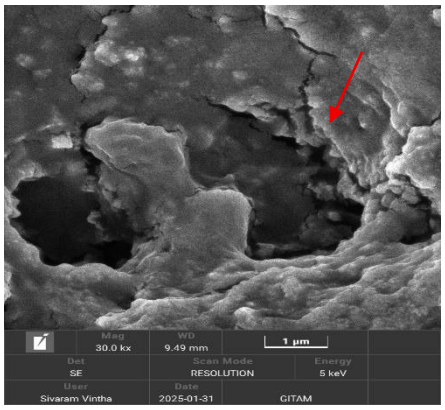
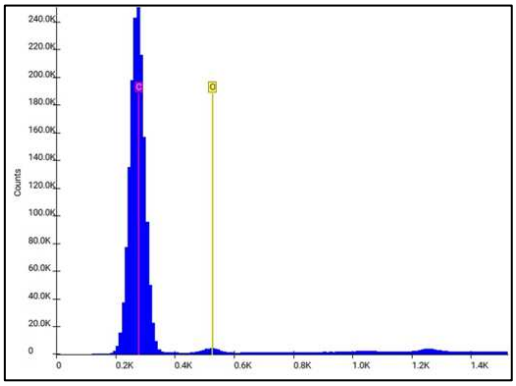


Fig. 7 .4 (a, b): SEM images of the MPs after the biodegradation process with TC at 10.0 kx magnification and 1µm and 10 µm resolution, (a) showing the spreading of mycelial mat on the microplastic film, (b) showing the formation of deep grooves due to enzymatic activity of fungi.

a



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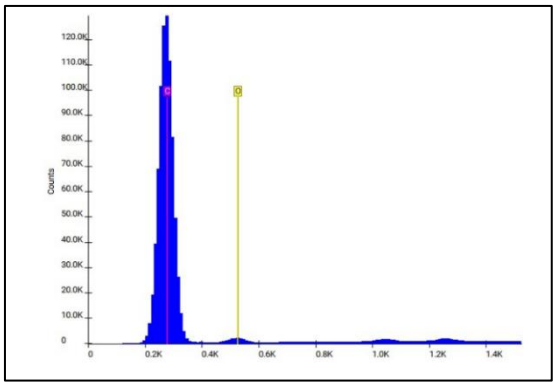

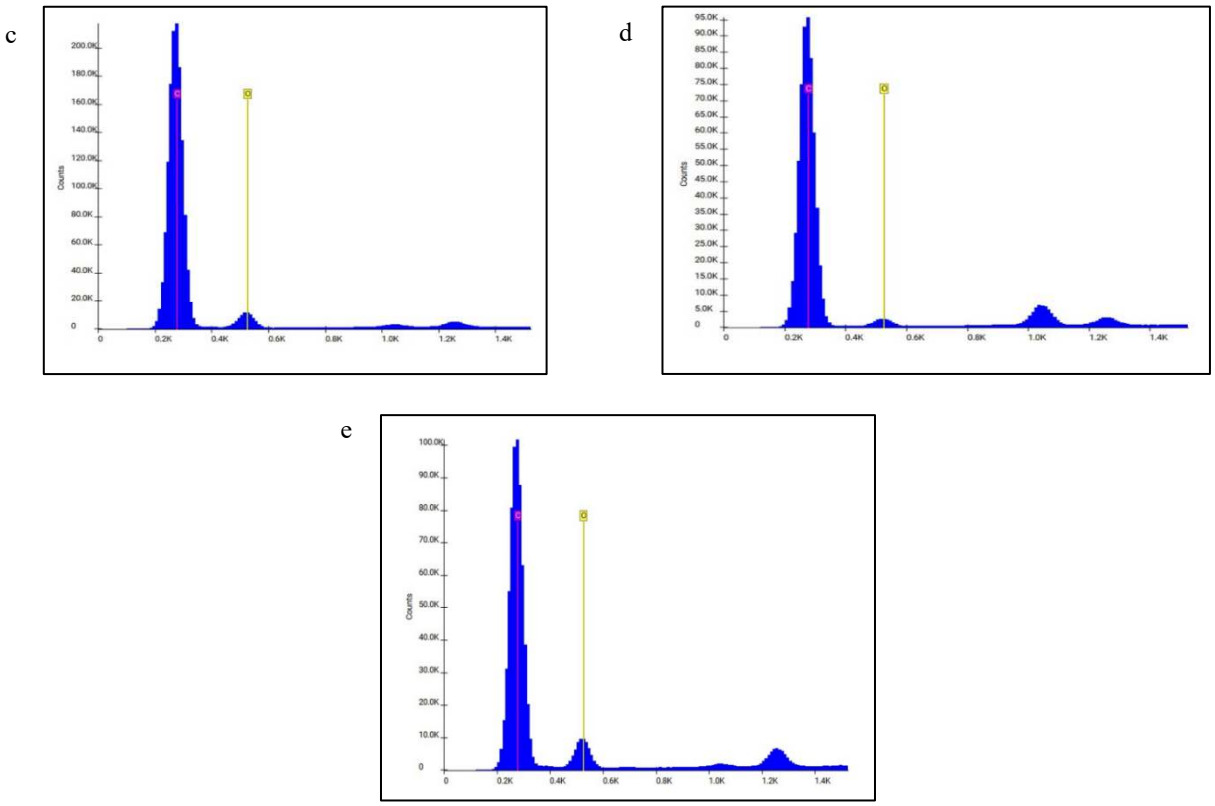

Fig. 8 (a, b, c, d, e): EDX images of the MPs (a) before and after the biodegradation process with (b) control, (c) AN-1, (d) ATE, and (e) TC, respectively.

Strains	Carbon (%)	Oxygen (%)
B	98.9%	1.1%
C	98.55%	1.45%
AN	89.1%	10.9%
ATE	93.14%	6.86%
TC	83.9%	16.1%

Table-1: Atomic percentage of carbon and oxygen

3.6.2. ATR-FTIR Analysis:

ATR-FTIR spectral analysis was performed to evaluate the structural variations in the functional group profiles of HDPE films following fungal incubation (Fig. 9. a). The pristine HDPE displayed characteristic peaks at 2914 and 2847 cm^{-1} for CH_2 stretching vibrations (Sangeetha Devi et al., 2015), CH_2 bending at 1464 cm^{-1} (Campanale et al., 2023; Kim & Lee, 2023), and rocking deformation at 872 and 717 cm^{-1} (Sathiyabama et al., 2024), characteristic of polyethylene. Post-treatment, all the fungal strains induced the CH_2 stretching bands shifted to 2918 and 2848 cm^{-1} with reduced intensity, indicating cleavage in the hydrocarbon chain, with new bands appearing at 3288 cm^{-1} (hydroxyl groups) and 1638 cm^{-1} (carbonyl groups), which is consistent with oxidative degradation (Karim et al., 2021; Yang et al., 2005), and extensive changes in the fingerprint region (1500–500 cm^{-1}), reflecting diverse degradation products (Almond et al., 2020; Kim & Lee, 2023; Krimm et al., 1956). The most significant changes were seen in the TC-

treated samples. A broad, intense band appeared at 3290 cm^{-1} (O-H stretch), indicating a marked increase in surface hydrophilicity. This was accompanied by a prominent peak at 1033 cm^{-1} (C–O stretch) and a sharp increase in the C=O (carbonyl) region. Quantitatively, the Carbonyl Index (CI) (Fig. 9. b) for strain TC reached 0.27 (Keto CI) and 0.21 (Ester CI) ($p<0.0005$), both significantly higher than those for AN-1 and ATE. This increase in CI values confirms that TC has a greater oxidative capacity, likely due to the high levels of LiP and MnP previously observed. The incorporation of oxygen, as demonstrated by EDX analysis (which shows increased oxygen and reduced carbon content), promotes the conversion of inert alkanes into oxygenated intermediates, such as ketones and carboxylic acids. These groups containing carbonyls then undergo β-oxidation, enabling the breakdown fragments to enter the citric acid cycle for complete mineralization into CO_2 and H_2O (Albertsson et al., 1987). Therefore, the FTIR and CI data provide conclusive molecular evidence that strain TC is the most effective for the oxidative degradation and metabolic assimilation of HDPE microplastics.

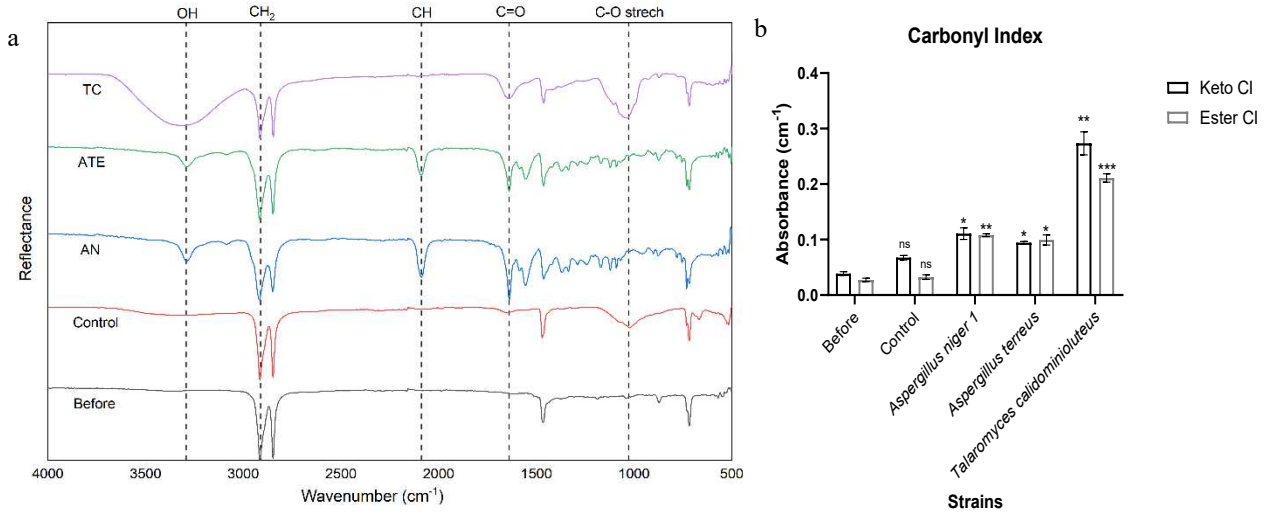

Fig. 9 (a) ATR-FTIR spectra of MPs before and after treatment with control, AN-1, ATE, and TC. (b) Carbonyl index (CI) analysis of HDPE MPs following the fungal treatment, mean values ± SD (n=3) $p<0.0005$ is ***, $p<0.005$ is **, $p<0.05$ is *.

3.6.3. XRD Analysis:

X-ray diffraction of the samples shows several peaks, among which two distinct peaks at 2θ = 21–22, which correspond to the (110) reflection, and another at 2θ = 23.5–24, which corresponds to the (200) reflection (Musuc et al., 2013). These reflections correspond to the orthorhombic crystal structure of HDPE (Morancho et al., 2006). Several weaker reflections were observed at higher diffraction angles, corresponding to higher-order lattice planes of crystalline polyethylene, indicating a high degree of structural ordering and crystallite perfection (Chouit et al., 2014). The greater decrease in peak intensity was observed in HDPE MPs treated with TC, i.e., from 96.71% to 89.21%, followed by AN-1 with 92.08% and ATE with 94.04% (Fig 10). Ultimately, the impressive ability of strain TC to transform the polymer from a highly crystalline to a more amorphous state explains its maximum 33.3% weight loss. The 7.5% decrease in crystallinity without any prior treatment shows that the fungal oxidative machinery was able to

penetrate deep into the tightly packed crystalline lattice. These results are confirmed by FTIR and CI data, which reveal that adding polar oxygenated groups disturbed the forces holding the crystalline structure together. This clearly indicates that this strain promotes thorough mineralization rather than just surface change. Similar reduction in the crystallinity after incubation with bacterial strains to the extent of 7% has been reported by (Balasubramanian et al., 2010). In a study by Chaudhary & Vijayakumar (2020), the combined effect of chemical treatment and fungal attachment resulted in a 6.61 ± 0.05% decrease in HDPE crystallinity.

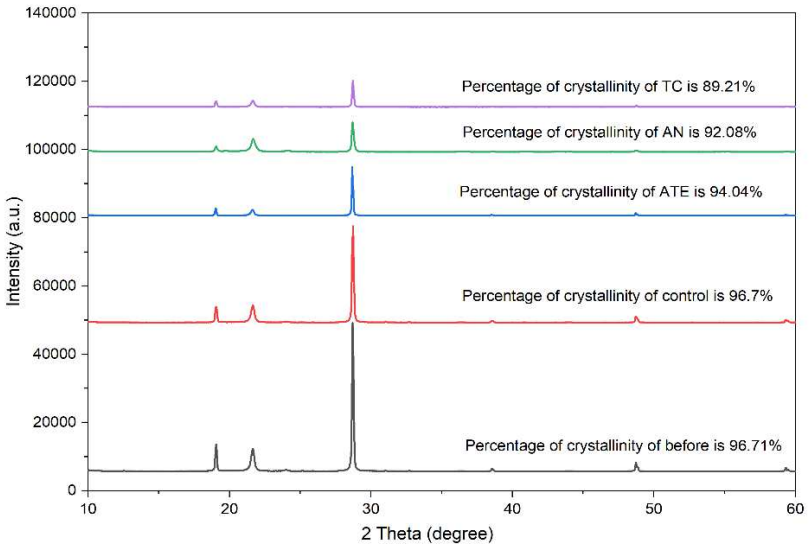

Fig. 10 XRD spectra of MPs before and after treatment with control, AN-1, ATE, and TC.

3.7.1. Peak Integration of depolymerized products by GC-MS:

The chemical transformation of the HDPE films was definitively characterized through GC-MS analysis, with the resulting compositional shift illustrated in Fig. 11 and detailed in Table 2. While the Control, AN-1, and ATE treatments remained overwhelmingly dominated by long-chain hydrocarbons, strain TC exhibited a profound metabolic divergence, achieving a 7–8% conversion of the polymer backbone into oxygenated derivatives, specifically alcohols and ketones. Peak-by-peak integration analysis reveals that strain TC facilitated a more aggressive chain-scission mechanism than its counterparts. This is evidenced by the high relative abundance of short-chain alkanes such as Heptane (61.78%) and the unique liberation of Dodecane (9.91%) (Wafaa et al., 2025). Crucially, the detection of oxygenated fragments, such as Propan-1-ol (8.09%) and 1-Nonanol (1.66%), in the TC and AN-1 samples provides molecular evidence for the oxidative pathway. The presence of the complex ketone 7,9 Di-tert-butyl-1-oxaspiro deca-6,9-diene-2,8-dione (9.01%) exclusively in the TC treatment further underscores its advanced enzymatic capacity to break down recalcitrant structures. Based on these findings, we propose a synergistic degradation mechanism driven by the high activity of LiP, MnP, and laccase in strain TC. The process begins with extracellular peroxidase-mediated random chain scission, converting the high-molecular-weight polyethylene into long-chain alkenes (e.g., 1-Docosene and 5-Eicosene). These intermediates are subsequently targeted by laccases and peroxidases, which convert them into alcohols and ketones, thereby significantly increasing the polymer hydrophilicity. This chemical shift from

hydrophobic hydrocarbons to polar oxygenates is statistically unique to the TC strain and lowers the energy barrier for microbial assimilation, directly resulting in the maximum observed 33.3% weight loss and confirming *Talaromyces calidominiluteus* as a highly efficient candidate for microplastic mineralization. In the study by (Gupta et al., 2023) degradation products such as octane, nonane, dodecane, hentriacontane, pentanoic acid, and hexanoic acid might be utilized by *B. parabrevis* CGK45 for their growth and metabolism via β-oxidation.

S.No	Compounds	RT (min)	Relative abundance				Class
			C	AN-1	ATE	TC	
1	Heptane	5.757	1.76	56.41	34.04	61.78	Hydrocarbons
2	Hexane	15.73				1.75	
3	Dodecane	22.817				9.91	
4	Decane	15.729			2.34		
5	Tetradecane	18.723		2.43	5.01	5.04	
6	Octadecane-1	30.45		11.98			
7	3-Tetradecene	18.677		6.05			
8	7-Hexadecene	21.331	1.46	28.19	35.06		
9	3-Octadecene	23.709	1.87	36.13	38.81		
10	1-Docosene	27.88		24.5	27.37	39.18	
11	5-Eicosene	25.857	1.63		34.68	48.94	
12	Undecane	20.116			3.3		
13	Hexadecane	20.693			2.61		
14	Cyclopropane	18.663			2.97	6.39	
15	1-Nanonol	15.66		1.15		1.66	Aldehydes
16	Propan-1-ol	20.785				8.09	
17	7,9 Di tert-butyl-1-oxaspiro deca-6,9 diene 2,8 dione	26.818				9.01	Ketones

Table-2: Relative abundance of HDPE degradation products by marine-derived fungal isolates by peak integration analysis.

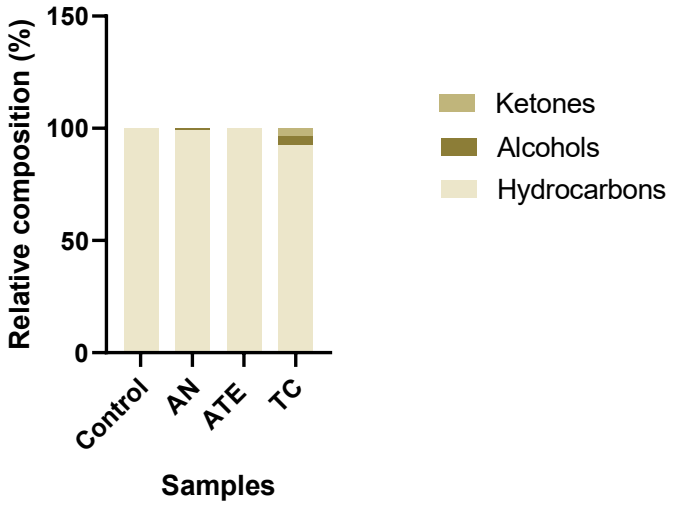

Fig. 11: GC-MS relative abundance of hydrocarbons, alcohols, and ketones across different fungal treatments.

3.7.2. Putative degradation products based on molecular features by GC-MS:

The heatmap of putative degradation products (Fig. 12) reveals a clear metabolic divergence between the fungal treatments and the pristine control. While the control remained chemically inert, fungal incubation triggered the emergence of a diverse array of oxygenated and short-chain intermediates. Strain TC exhibited the most advanced metabolic fingerprint, uniquely producing highly oxidized compounds such as 3,4-Hexanedione, Ethyl-4-Methyl Pentanol, and 1-Tridecene (Sanniyasi et al., 2021). These specific molecular features correlate with the high Carbonyl Index and superior weight loss recorded for TC, suggesting a deeper mineralization of the HDPE backbone. Furthermore, the shared detection of Oxalic acid across all strains (AN, ATE, and TC) indicates a common acidogenic strategy to facilitate enzymatic attack, while the unique presence of Undec-10-ynoic acid and various alkanols across treatments confirms that each strain employs distinct oxidative pathways to transform recalcitrant hydrocarbons into bioavailable metabolic intermediates. Earlier researchers have documented the occurrence of alkanes and carboxylic acid compounds as polyethylene breakdown by-products by *Ralstonia* sp. strain SKM2 and *Bacillus* sp. strain SM1 isolated from a landfill soil site (Biki et al., 2021).

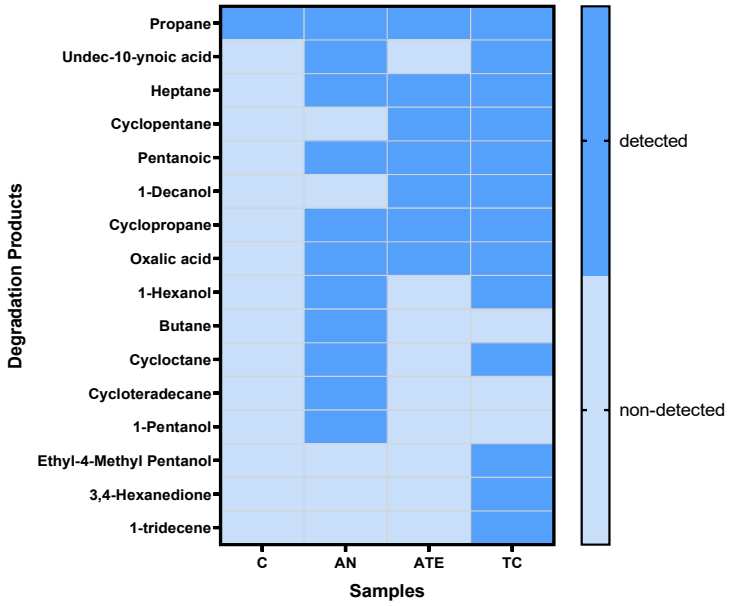

Fig. 12: Binary matrix heatmap of putative degradation products identified via GC-MS molecular features across various HDPE-degrading fungal strains.

4. CONCLUSION:

HDPE is the most widely used plastic, and MPs are major environmental pollutants affecting marine ecosystems and human health. In the current study, indigenous marine fungi isolated from the Visakhapatnam coast exhibit substantial potential for the biodegradation of HDPE microplastics under laboratory conditions. Among the tested six strains and their consortia, *Talaromyces calidominioluteus* achieved the highest degradation, reducing HDPE weight by 33.3±3.89% over 30 days in MSM broth (pH 8, 28 °C, 120 rpm) without any pre-treatment or pro-oxidant additives. Enzymatic assays revealed convincing extracellular activities of laccase, manganese peroxidase, and lignin peroxidase in all three leading strains, and, by Pearson correlation, it is justified that, with an increase in the weight loss of HDPE MPs, there is a simultaneous increase in enzyme activity. Among all the extracellular oxidative enzymes studied, only MnP specific activity was a significant predictor (P=0.0045) of microplastic weight loss, suggesting a critical functional role independent of its low activity levels. FE-SEM revealed prominent surface erosion, pits, and cracks on degraded MPs, and EDX showed a simultaneous decrease in carbon and an increase in oxygen, confirming physical biodeterioration. ATR-FTIR analysis detected new carbonyl (~1710 cm^{-1}), hydroxyl (3200–3600 cm^{-1}), and ether (~1100 cm^{-1}) functional groups, indicating oxidative breakdown of polyethylene chains. The 7.5% reduction in crystallinity achieved by TC demonstrates the superior capacity of its enzymatic machinery to penetrate the tightly packed crystalline lattice, thereby transitioning HDPE from an ordered to a more amorphous state. GC-MS analysis shows that strain TC superior HDPE breakdown produces highly oxidized intermediates, such as 3,4-hexanedione and oxygenates. This chemical shift from hydrocarbons to polar metabolites confirms *that Talaromyces calidominioluteus* exhibits high efficiency in microplastic bioremediation, as evidenced by substantial weight loss and enzymatic activity.

Mixed-strain consortia (AN-1+TC, TA+TC, AN-1+TA+ATA) displayed lower degradation efficiencies (5.033%–7.23%) than the individual strains, suggesting that competitive or inhibitory effects among the strains might negate the synergistic advantages under these conditions. These findings emphasize the importance of optimizing species combinations and culture conditions for performing consortium-based studies. The exceptional performance of *T. calidominioluteus*, coupled with significant degradation of MPs by *Aspergillus* species, underscores the need to delve into marine ecosystems for potent plastic-degrading fungi. The inbuilt capability of these marine fungi to sustain, colonize, and oxidize polyethylene without the addition of chemicals offers a sustainable strategy for mitigating microplastic pollution. This study represents the first documentation of *T. calidominioluteus'* ability to degrade plastic in marine environments, opening new avenues for research into its degradation mechanisms and the potential to discover novel enzymes or metabolites.

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