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BIODEGRADATION OF LOW-DENSITY POLYETHYLENE BY A NOVEL FUNGUS, *SAPROCHAETE PSYCHROPHILA*

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ABSTRACT
Polyethylene (PE) waste accumulation poses a significant threat to global ecosystems due to its extreme resistance to natural degradation. While microbial biodegradation offers a promising solution, most identified degraders operate optimally at mesophilic temperatures, limiting their application in cold environments [1-10]This study explores the potential of a psychrophilic fungus, *Saprochaete psychrophila*, to degrade low-density polyethylene (LDPE) at low temperatures. Cultivated in minimal salt medium with LDPE as the sole carbon source for 120 days, *S. psychrophila* demonstrated a weight reduction of 15.2%. Biodegradation was confirmed through scanning electron microscopy (SEM), which revealed significant surface erosion, pitting, and void formation. Furthermore, Fourier-transform infrared spectroscopy (FTIR) detected the appearance of new peaks corresponding to carbonyl (1715 cm-1) and hydroxyl (3300 cm-1) groups, indicating active oxidation of the polymer backbone. These findings suggest that S. psychrophila is a promising candidate for bioremediation.

KEYWORDS: polyethylene; low-density polyethylene; fungi; landfill soil; plastic biodegradation; microbial ecology

INTRODUCTION
Low-density polyethylene (LDPE) is a primary component of environmental plastic pollution, known for its longevity and resistance to biodegradation due to its high molecular weight and hydrophobic nature [MDPI, 2021]. Conventional cleanup methods are energy-intensive and environmentally costly, prompting intense research into microbial degradation pathways [11-13].
Most documented plastic-degrading microorganisms are mesophiles, yet plastic pollution is pervasive in polar and deep-sea environments. Therefore, the search for microbes capable of degrading polymers is critical [14-16]. The genus Saprochaete, particularly species like *Saprochaete psychrophila*, has shown remarkable resilience in extreme environments, including residential wastewater systems characterized by high organic loads and fluctuating temperatures [17-20]. The potent isolate A1 was identified as *Saprochaete psychrophila* by 18S rRNA gene sequencing Their ability to tolerate high hydrocarbon concentrations suggests a latent potential for breaking down synthetic polymers [21-23]. This study aims to demonstrate the capability of *Saprochaete psychrophila* to degrade LDPE and to identify the metabolites formed by this psychrophilic fungus.

MATERIALS AND METHODS
Study site and sample collection
Plastic-contaminated soil samples were collected from the Nashik Municipal Corporation (NMC) garbage dumping station in Nashik, Maharashtra, India, a municipal solid waste site characterized by high loads of mixed plastic waste including PE films. Soil adhering to visibly weathered plastic fragments and from surrounding plastic-rich surface layers (0–10 cm) was collected using sterile tools into clean containers, transported to the laboratory at ambient temperature and processed within 24 h.

Preparation of polyethylene films
Commercial low-density polyethylene (LDPE) films were cut into squares of 2 cm × 2 cm and cleaned to remove surface contaminants, then dried and weighed on an analytical balance to obtain initial weights. The prepared films were used as the sole or primary solid polymer substrate in mineral media for fungal colonization and degradation assays.

Culture media and mineral salt solutions
For enrichment and degradation experiments, mineral salt media were prepared using two stock solutions. Solution A contained (per 50 mL distilled water): KH2PO4 , 12.5 g; NH4Cl, 2 g; MgSO4, 0.5 g; and CaCl2, 0.25 g, while Solution B contained (per 100 mL distilled water): citric acid, 2.5 g; ZnSO4, 2.5 g; CuSO4, 0.01 g; MnSO4, 0.001 g; and Na2_22MoO4, 0.001 g. For some experiments, Vogel's medium was used as a basal medium, with or without the addition of sucrose, to examine the effect of an easily metabolizable carbon source on fungal growth and PE degradation.

Isolation and maintenance of fungal strains

Soil suspensions prepared from the landfill samples were plated on suitable agar media to obtain fungal colonies, which were purified by repeated sub-culturing and maintained on potato dextrose agar (PDA) slants at 4 °C. Individual isolates were coded as A1 and B2, and their colony morphology and microscopic characteristics were documented; a known fungus (e.g. Aspergillus niger) was used as a reference control in selected experiments.

Screening for polyethylene degradation in mixed cultures

To assess PE degradation by mixed fungal communities, an approximate amount of mixed culture inoculum derived from landfill soil was introduced into flasks containing 50 mL of Vogel's medium and a pre-weighed PE film. Flasks were incubated under static conditions at room temperature (approximately 27 ± 2 °C) for 15–30 days, after which PE films were recovered, washed with 70% ethanol, rinsed, dried at 80 °C to constant weight and weighed to determine final weight.

Measurement of fungal biomass and hyphal growth

Fungal mycelial growth in PE-containing cultures was evaluated by filtering the culture through muslin cloth to collect hyphal biomass, which was then dried and weighed. Growth measurements were compared between treatments (e.g. with and without sucrose) and among strains to examine whether increased biomass correlated with greater PE weight loss.

RESULTS

Fungal isolates from plastic-contaminated landfill soil

Multiple fungal isolates were obtained from plastic-contaminated soil from the NMC dumping station and designated as A1 and B2. The isolates displayed distinct colony colours on growth media, with A1, showing black growth and B2 appearing yellow indicating morphological diversity within the landfill-derived fungal community. Strain A1 was identified as a novel Fungus, Saprochaete psychrophile and was selected for further biodegradation studies.

Polyethylene degradation by individual strain

Experiments using separate culture of Saprochaete psychrophile with PE films as the solid substrate, confirmed that strain *Saprochaete psychrophila* is capable of reproducible PE weight reduction compared with uninoculated controls. For instance, replicate assays for A1 showed small but consistent decreases in PE film weight, whereas control films maintained their original weight, indicating that weight loss was associated with fungal activity rather than handling. These results identify *Saprochaete psychrophila* as promising candidate PE-degrading strains from the landfill fungal community.

Fungal biomass production and relation to PE degradation

Measurement of hyphal biomass in PE-containing cultures revealed increased mycelial dry weights for several strains over the incubation period. In particular, *Saprochaete psychrophila* showed pronounced increases in hyphal mass compared with controls, reflecting their active growth on or in the presence of PE films (Fig. 4). While greater biomass often coincided with higher PE weight loss, some treatments with considerable growth showed limited PE degradation, suggesting that enhanced biomass alone is not a sufficient indicator of effective polymer breakdown.

Gas Chromatography Coupled with Mass Spectroscopy (GC–MS) Analysis of PE degraded by *Saprochaete psychrophila* strain.

The degraded products can be identified by GC–MS analysis of the samples. In oxidized PE intermediate profiles, peaks around m/z approx 146 -147 often correspond to low-molecular-weight oxygenated products such as dicarboxylic acid fragments, ester derivatives, or aromatic intermediates (e.g., phthalic acid derivatives/esters) [Fig. 5A and 5B].

DISCUSSION

This study demonstrates that fungal communities associated with municipal plastic-contaminated landfill soil harbour strains capable of degrading PE films under laboratory conditions. The identification of strain *Saprochaete psychrophila* which consistently reduced PE weight and produced abundant hyphae in PE-containing media, supports the view that landfill environments select for fungi with traits that enable colonization and partial degradation of recalcitrant polymers. The observation that sucrose enhanced both fungal growth and, for several isolates, PE degradation is consistent with co-metabolic models of plastic biodegradation, in which an easily metabolizable carbon source supports energy generation while oxidative enzymes act on the polymer. Similar stimulation of PE degradation by added carbon sources has been reported for various fungal and bacterial systems, highlighting the importance of nutrient context when evaluating plastic biodegradation potential [24-26]. However, the fact that some strains grew vigorously with sucrose yet showed limited PE degradation indicates that sucrose may also favour opportunistic colonizers without strong polymer-degrading capacity [27-29].

The heterogeneous performance of isolates (e.g. effective degradation by A1 and B1 versus reflects functional differentiation within the landfill fungal community. Such differentiation may arise from variation in enzyme repertoires, surface adhesion properties and tolerance to oligomeric degradation products, which together shape ecological roles on plastic surfaces. Detailed taxonomic and enzymatic characterization of the isolates, coupled with surface analyses of PE films (e.g. FTIR, SEM), will be essential in future work to elucidate mechanisms of PE modification and mineralization.

From an applied perspective, the identification of landfill-derived fungal strains with reproducible PE-degrading activity is an important first step towards developing fungal consortia or enzyme preparations for biotechnological plastic waste treatment. Nevertheless, the modest weight losses observed over relatively short incubation times underscore the need for process optimization, including longer incubation, pre-treatment of PE, and controlled co-substrate supply, to achieve practical levels of degradation.

CONCLUSIONS

Fungal isolates obtained from PE-contaminated soil at a municipal garbage dumping station in Nashik exhibited variable but, in some cases, significant capacity to degrade LDPE films in vitro. Strain *Saprochaete psychrophila* emerged as promising PE degraders, particularly under conditions where sucrose was available as a co-substrate, highlighting the role of carbon availability in modulating fungal plastic degradation. These findings expand the ecological evidence for landfill-associated fungi as potential agents of PE biodegradation and provide a basis for further taxonomic, enzymatic and process-development studies aimed at mitigating plastic pollution.

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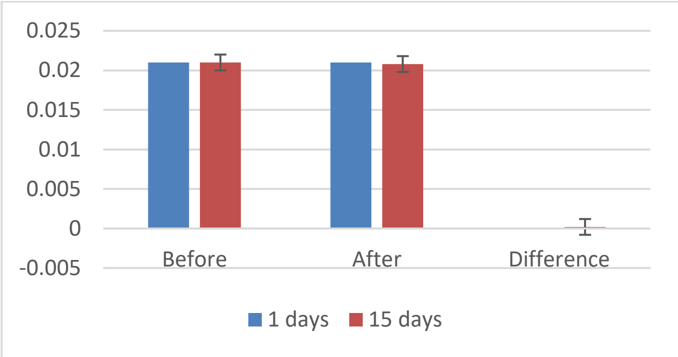

Fig. 1

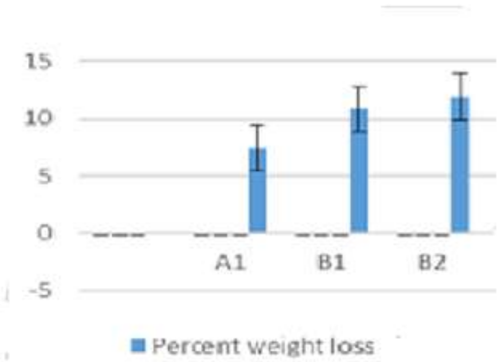

Fig. 2

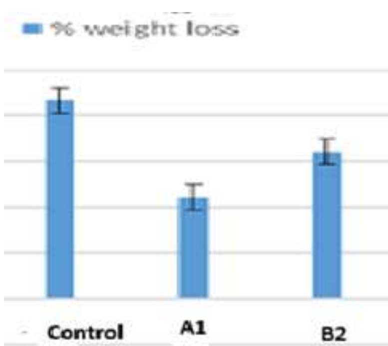

Fig. 3

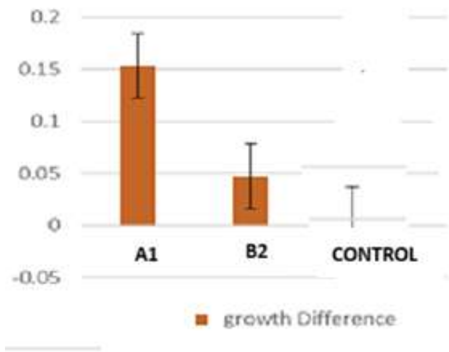

Fig. 4

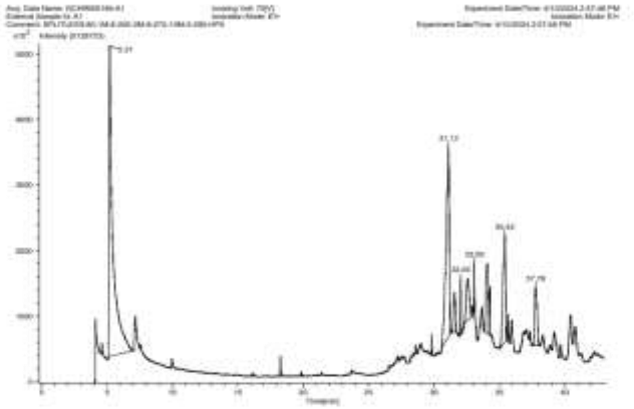

Fig. 5A and Fig. 5B