

THE ROLE OF MARINE FUNGI IN DEGRADATION OF MICROPLASTIC AND PLASTICS – A REVIEW

Abstract

Plastic has become an essential and fundamental necessity worldwide. Currently, global plastic production exceeds 300 million tons per year. Plastics possess various attributes, such as cost-effectiveness, inertness, relatively low weight, and resistance to accumulating as waste in the environment. In the entirety of 2015, the world generated 6.3 billion tons of plastic waste, with 79% either disposed of in landfills or left in the natural environment. Furthermore, projections indicate that approximately 12 billion tons of plastic waste will accumulate on Earth by 2050. Consequently, there is a pressing need to develop an efficient plastic biodegradation process that can expedite the natural rate of plastic decomposition.

Researchers have identified over 400 species of microorganisms capable of breaking down plastic. The existing literature provides a comprehensive list of previously documented plastic-degrading fungi and highlights the most significant fungal groups involved in this process. Moreover, a phylogenetic analysis of plastic-degrading fungi was conducted using a dataset that included ITS, LSU, SSU, TEF, RPB1, and RPB2 sequences from 395 strains. The findings confirm that plastic-degrading fungi span 11 fungal classes, including Ascomycetes (Dothideomycetes, Eurotiomycetes, Leotiomyces, Saccharomycetes, and Sordariomycetes), Basidiomycetes (Agaricomycetes, Microbotryomycetes, Trimellomycetes, and Sordariomycetes), and Mucoromycetes.

Keywords: fungi, global plastic production, accumulation of plastic waste, synthetic polymers, Polygenic phylogeny

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I. INTRODUCTION

Plastic pollution is widely acknowledged as a critical menace to global ecosystems due to its stubborn persistence, resulting in the long-term accumulation of environmental issues that affect both non-living and living components. In 2018, global plastic production surpassed 360 million tons and is anticipated to triple by 2050. Asia notably leads as the largest producer and consumer of plastic products, as underscored by a Plastics Europe Plastics Recycling and Recovery Associations review. The onset of the COVID-19 pandemic in 2019 prompted a surge in the use of disposable plastic items like gloves, masks, and napkins, further exacerbating the plastic pollution predicament.

Ever since large-scale plastic production took off in the 1950s, plastic waste has been steadily amassing, even infiltrating ocean depths. The fate of plastics in marine environments has now become an urgent environmental concern, significantly impacting marine life. Microbial degradation has been proposed as a potential mechanism for breaking down marine plastic waste, drawing attention to microbial colonization and their role in plastic decomposition. However, the interaction between marine fungi and plastic remains enigmatic. Although marine fungi have often been underexplored, they have demonstrated the capability to metabolize various compounds, including specific types of plastics, hinting at their potential involvement in breaking down complex marine organic matter.

Recent research has highlighted that particular strains of marine fungi, such as *Zalerion maritimum*, possess the ability to degrade polyethylene. Microplastics (MPs), categorized as plastic particles smaller than 5 mm in any dimension, have been found in all ecosystems, including remote and pristine environments like Antarctica, high mountain regions, and seemingly untouched deep-sea floors. Another substantial source of MPs arises from the disintegration of larger plastic debris through mechanical, chemical, or natural processes, leading to the generation of secondary MPs. Furthermore, it has been observed that MPs can absorb organic pollutants from water, compounding their environmental repercussions.

This comprehensive overview furnishes foundational insights into the ramifications of microplastics, their origins, and their impact on marine life, encompassing microalgae, while also considering potential implications for humans. Additionally, it delves into the involvement of fungi in the degradation of plastic and microplastics.

1. Biodegradation of Various Plastics: The majority of synthetic materials originate from petrochemical origins, encompassing traditional plastics, which are typically considered non-biodegradable. Traditional plastics such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) are primarily characterized by a carbon-carbon structure. Conversely, specific plastics like polyethylene terephthalate (PET), polyurethane (PU), or polyamides (PA, Nylon) incorporate heteroatoms [3].

- **Polyethylene Terephthalate (PET):** Polyethylene terephthalate, abbreviated as PET, is a thermoplastic material distinguished by its semi-crystalline structure and well-known for its robustness, durability, chemical and thermal stability, low gas permeability, and straightforward production. PET serves a wide range of industries, including electronics, automotive components, household goods, lighting fixtures,

power tools, sports gear, photographic materials, X-ray sheets, textiles, and notably, food and beverage packaging, particularly in the production of soft drink and water bottles. In contrast, polyethylene and polypropylene, constituting approximately 92% of synthetic plastics, are primarily employed in the manufacture of plastic bags, disposable containers, bottles, packaging materials, and more. The enzymes responsible for PET degradation, such as PET hydrolase, tannase, and MHETase, fall within the serine hydrolase category, which includes cutinases, lipases, and carboxylesterases. Effective agents for PET breakdown have been identified in fungal cutinases from *Fusarium* and *Humicola*. Additionally, fungal enzymes from sources like *Aspergillus oryzae*, *Candida Antarctica*, and *Penicillium citrinum* have been researched for their potential in PET degradation [4].

- **Polypropylene (PP):** Polypropylene, or polypropene, is classified as a thermoplastic polymer known for its partially crystalline properties, making it a widely used material. PP serves various purposes in the production of items such as rugs, mats, carpets, ropes, chairs, and even laboratory equipment like wash bottles, centrifuge tubes, Eppendorf tubes, and pipette tips. The hydrophobic nature of polypropylene surfaces stems from the presence of CH and CH₂ groups within its molecular structure, as well as a pendant CH₃ group. To enhance the hydrophilicity of the polymer surface, various pretreatment methods such as UV radiation, gamma sterilization, or heat treatments are applied. Notably, specific fungi, including *Bjerkandera adusta* and *Lasiodiplodia theobromae*, have been associated with the degradation of polypropylene [5].
- **Polyvinylchloride (PVC):** Polyvinylchloride (PVC) is a resilient polymer composed of repetitive chloroethyl units, renowned for its cost-effectiveness and resistance to biological and chemical factors. PVC exists primarily in two forms: rigid and flexible. While pure PVC can dissolve in tetrahydrofuran, it remains insoluble in alcohols. Its structural similarity can be likened to chlorophenol compounds. In comparison to PET and PS, polyvinylchloride (PVC) is generally considered a challenging plastic to biodegrade. Several fungal species have showcased the capacity to break down PVC, including *Cochliobolus* sp., *Phanerochaete chrysosporium*, *Aspergillus niger*, *Penicillium funiculosum*, and *Trichoderma viride* [6].
- **Polystyrene (PS):** These materials can exist in either solid or foamed structures, with a chemical formula denoted as (C₈H₈)_n. Polystyrene (PS) is susceptible to degradation when exposed to substances like acetone, chlorinated solvents, and aromatic hydrocarbon solvents. Its applications span protective packaging, including food packaging and jewel cases, as well as the production of CD and DVD cases, lids, bottles, trays, and more. Recent research has highlighted that strains like *Gloeophyllum striatum* DSM 9592 and *Gloeophyllum trabeum* DSM 1398 can lead to an almost 50% reduction in the molecular weight of polystyrene. Fungi such as *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Trametes versicolor*, and the brown-rot fungus *Gloeophyllum trabeum* have demonstrated their capacity to depolymerize polystyrene when co-incubated with lignin [7].

- **Polyurethane (PUR):** Polyurethane is a polymer consisting of natural units interconnected by carbamate (urethane) links. It exists in rigid and flexible foam forms, varnishes, coatings, adhesives, electrical compounds, and fibers, such as spandex and polyurethane laminate (Gama et al. 2018). Polyurethanes, a type of plastic with extensive industrial applications, are produced from polyols and polyisocyanates..
- **Polycarbonate (PC):** Polycarbonate, a thermoplastic polymer featuring carbonate groups (-O-(C=O)), is a rigid and robust material. It is employed in the production of electronic components like TVs, monitors, PC monitors, compact discs, DVDs, automotive and aircraft parts, and certain safety elements like bulletproof sheets and eyeglass lenses. Polycarbonates, due to their rigid structure, typically degrade over many years during use. Notably, *Phanerochaete chrysosporium* NCIM 1170, a white-rot fungus, has demonstrated degradation capabilities towards polycarbonates. Other common fungal species displaying biodegradation of polycarbonates include *Fusarium*, *Ulocladium*, *Chrysosporium*, and *Penicillium* [8].

2. Brief Description about Fungal Kingdom: The fungal kingdom constitutes a significant lineage within the eukaryotic domain, having diverged from the common ancestor of animals more than 800 million years ago. Fungi display remarkable adaptability and can thrive in a wide range of redox conditions. While many fungi favor aerobic environments, certain species excel in extremely low-oxygen settings, even under anaerobic conditions. Morphologically, fungi can take on unicellular forms, such as yeasts and flagellate cells like zoospores, or filamentous structures, including molds. They can exist as free-living organisms, engage in symbiotic relationships like mycorrhizae and lichens, contribute to the gut microbiome, or function as parasitic pathogens.

From a taxonomic standpoint, there are nineteen major fungal phyla officially recognized, which are: Aphelidiomycota, Ascomycota, Basidiobolomycota, Basidiomycota, Blastocladiomycota, Calcarisporiellomycota, Caulochytriomycota, Chytridiomycota, Entomophthoromycota, Entorrhizomycota, Glomeromycota, Kickxellomycota, Monoblepharomycota, Mortierellomycota, Mucoromycota, Neocallimastigomycota, Olpidiomycota, Rozellomycota, and Zoopagomycota. Some of these fungal phyla receive limited attention, and our knowledge of deep clade relationships remains restricted. Recent advancements in genomics have significantly increased the availability of fungal genomes, uncovering intricate interspecific relationships, including introgression and hybridization, which challenge traditional species definitions. Although it is estimated that there could be anywhere from 2 to 4 million fungal species, the descriptions available to date are meager, with only about 100 formally recognized, and a mere 1,100 identified in marine environments [9].

Numerous fungal species inhabit both terrestrial and marine environments. The earliest diverging fungi, specifically Chytridiomycota and Rozellomycota, are believed to have originated in aquatic environments, with representatives of these phyla still thriving in today's oceans. Conversely, fungi from the Ascomycota and Basidiomycota phyla, commonly found in marine settings, are thought to have evolved from terrestrial ancestors. This dichotomy in their origins, terrestrial versus aquatic, poses challenges in precisely defining "marine fungi." Marine fungi exhibit a wide distribution across various

marine ecosystems, including coastal regions, mangroves, the water column, and sediments. Metabolically, fungi actively engage in diverse biogeochemical processes and occupy a range of ecological niches. They play a crucial role in breaking down recalcitrant substrates, thus making significant contributions to carbon cycling and nutrient regeneration, especially in terrestrial environments. For instance, saprophytic fungi accelerate the turnover of carbon and nitrogen, while symbiotic fungi like Mycorrhizal networks enhance primary production in both their symbionts and host organisms. In aquatic environments, much like in terrestrial systems, parasitic fungi exert substantial impacts on pelagic food webs and consequently influence biogeochemical cycles. Furthermore, several symbiotic interactions have been observed in marine settings, such as those involving phytoplankton algae and sponges. Additionally, akin to their counterparts on land, fungi in coastal and surface marine environments have been recognized as key contributors to wood degradation [10].

In addition located that complex algal additives of marine fungi which includes agar they are able to carry out the role of saprophytic microorganism .Fungi in Marine fungi mitigate metallic toxicity by secreting siderophores to mobilize metals and act as biosorbents for some metals. Those methods have an effect on the cycling of numerous of those elements: Fe, Mn, Hg, Ni, Zn, Ag, Cu, Cd and Pb. Sooner or later, some fungi destroy down rocks and minerals for nutrients.

II. PLASTIC BIODEGRADATION POTENTIAL OF FUNGI

Fungi display the ability to break down synthetic compounds, including persistent organic pollutants (POPs), and polycyclic aromatic hydrocarbons (PAHs) such as benzene, toluene, ethylbenzene, xylenes, and various pesticides. Many of the fungi capable of degrading plastics belong to the Ascomycete phylum, which includes well-known genera like *Aspergillus*, *Fusarium*, and *Penicillium*.

Within the *Penicillium* genus, multiple strains have been identified as potential plastic decomposers. Notable examples encompass *P. chrysogenum*, *P. oxalicum*, and *P. simplicissimum*, all of which were originally isolated from soil. Furthermore, *Penicillium* species isolated from seawater have demonstrated their ability to break down polyethylene (PE). Similarly, various species within the *Aspergillus* genus have also exhibited their potential as plastic degraders.

Fungi are recognized for their capacity to generate a wide range of enzymes capable of breaking down the chemical bonds present in plastic polymers. Among these enzymes, manganese peroxidase (MnP) and lignin peroxidase (LiP) are commonly linked to the decomposition of lignin. These enzymes facilitate oxidation-reduction reactions involving free radicals, resulting in the conversion of various substances into oxidized or polymerized products.

Peroxidases, including MnP and LiP, are utilized in various industrial processes to degrade challenging organic contaminants like polycyclic aromatic hydrocarbons (PAHs), industrial dyes, and chlorophenols. Lignin peroxidase, in particular, stands out due to its high redox potential, which allows it to oxidize non-phenolic aromatic compounds. Importantly, an increase in the activities of laccase, manganese peroxidase, and lignin peroxidase was

observed during the degradation of polyethylene (PE) by a fungal consortium in a mangrove environment. It is reasonable to assume that these enzymes also play a significant role in the potential degradation of plastics in marine ecosystems.

Fungi originating from marine environments demonstrate the ability to produce enzymes that facilitate their development in liquid cultures using single carbon sources like agar, alginate, carrageenan, laminarians, and ulvans. These polymeric substances are commonly encountered in marine settings. The flexibility of marine fungi, which allows them to adjust their metabolic processes and flourish in various environments with different substrates, implies that the number of marine fungi capable of plastic degradation may be underestimated.

Impact of Micro Plastics on Marine Organism: Microplastics can exert a substantial influence on aquatic plant and animal life by serving as a carrier for the transportation of absorbed heavy metals, multidrug-resistant *E. coli*, bacterial pathogens affecting fish, and persistent organic pollutants. As a result, microplastics give rise to a novel environment conducive to the formation of microbial biofilms comprising algae, bacteria, and fungi, and they possess the capacity to facilitate the transmission of microbial infections and antibiotic resistance [12].

Microplastics have an effect on living creatures-

- Microplastics can infiltrate the aquatic fauna's food chain, leading to intestinal blockage, alterations in nutritional absorption, disruptions in the endocrine system, immune and neurological repercussions, as well as reproductive dysfunction.
- The presence of microplastics and the release of toxic leachate can result in harm to microalgal cell walls, dysfunction in metabolic processes, and impairment of photosynthesis due to shading effects.
- Human exposure to microplastics occurs through three primary routes: ingestion, inhalation, and skin contact, all of which provoke inflammatory and immunological responses.

III.MICROPLASTIC DEGRADATION

The fungal kingdom comprises a diverse array of organisms, primarily consisting of saprotrophs, opportunistic parasites, or obligate parasites. They possess remarkable adaptability and can thrive across a wide range of aquatic and terrestrial ecosystems, as well as various climatic conditions. Notably, genera such as *Zalerion maritimum*, *Aspergillus niger*, *Cladosporium*, and *Penicillium simplicissimum* are prominent contributors to the degradation of various polymer types, including polyethylene, polypropylene, and polyethylene terephthalate. These fungi utilize microplastics as their sole carbon source after breaking them down through extracellular enzymes, reducing their hydrophobicity and promoting the formation of various chemical bonds, including carboxyl, carbonyl, and ester functional groups.

Serine hydrolase is typically instrumental in the degradation of polyurethane. Two specific fungi, *Aspergillus tubingensis* VRKPT1 and *Aspergillus flavus* VRKPT2, were identified as capable of degrading high-density polyethylene from marine coastal areas, with

degradation rates of $6.02 \pm 0.2\%$ and $8.51 \pm 0.1\%$, respectively. Additionally, recent research by Kunlere et al. [13, 14] reported promising degradation of low-density polyethylene by *Mucor circinelloides* and *Aspergillus flavus*.

IV. FUNGAL ENZYMES ASSOCIATED WITH THE DEGRADATION OF MICROPLASTICS

Fungi possess a diverse array of intracellular and extracellular enzymes capable of catalyzing various reactions, enabling them to break down petroleum-based polymers. Enzyme systems like epoxidases and transferases associated with the cytochrome P450 family play a pivotal role in oxidation and conjugation reactions, supporting the metabolism of aliphatic, alicyclic, and aromatic molecules. These enzymes execute a multitude of chemical processes, including epoxidation, sulfoxidation, desulfuration, dehalogenation, and deamination. The cytochrome P450 enzyme families utilize cofactors such as heme, NADPH + H⁺, and FAD to form the spore wall and maintain the integrity of the hyphal wall.

In contrast, hydrolases are extracellular enzymes that aid in the breakdown of complex polymers, rendering pollutants more soluble and thereby preventing bioaccumulation. Enzymes belonging to the class II peroxidases, capable of oxidizing various substrates, serve as effective environmental cleaning agents. Notable examples encompass manganese peroxidase, lignin peroxidase, laccases, and dye-decolorizing peroxidases. The thermostability of these enzymes encourages their potential use in large-scale reactors, facilitating the breakdown of polypropylene at elevated temperatures and with high kinetic reactions. Overall, a wide array of fungal strains exhibit the ability to convert plastics into environmentally friendlier substances [15, 16].

V. ANALYTICAL TOOLS FOR PLASTIC DEGRADATION

1. Gravimetric Measurements and Growth of Biomass: The term "gravimetric measurements and biomass growth" is utilized to describe the assessment of weight/mass reduction in a polymer during a specified period while it is exposed to an environmental context or cultured microorganisms.

In various tests, plastic has exhibited weight reduction when exposed to environments such as soil, the marine water column, and sediments. Likewise, research focused on the capability of specific fungal strains to degrade particular plastic polymers has also indicated weight loss in plastics. Some of the fungal isolates discovered in municipal solid waste, such as *Aspergillus fumigatus*, *Lasiodiplodia crassispora*, *Trichoderma harzianum*, *Aspergillus Niger*, and *Fusarium oxysporum* sp., have demonstrated the ability to reduce the weight of polyethylene (PE) and polyurethane (PU).

In addition to tracking the weight loss of the polymer, certain studies have observed fungal biomass growth as an indicator of fungal activity. It has been hypothesized that the carbon derived from plastics is displaced by the growth of fungi [17].

2. **Scanning Electron Microscopy:** A quick approach that makes it possible to see surface attachment and morphological microstructures is scanning electron microscopy. By using FIBSEM, SEM observations without chemical fixation can be made. The production and adhesion of biofilms is not always a sign of biodegradation, and SEM does not provide phylogenetic identification of the bacteria. Moreover, despite being a useful tool for visualising plastic surface flaws, SEM does not allow for scaling in the Z-direction. Instead, atomic force microscopy could be used to do this.
3. **Fourier-Transform Infrared Spectroscopy:** Fourier transform infrared spectroscopy (FTIR) is a technique employed to obtain an infrared spectrum that elucidates a material's absorption, emission, and photoconductivity characteristics, enabling the chemical identification of most polymers. The calculation of the carbonyl index serves as a means to quantitatively assess the extent of carbonylation. Carbonyl indices have found utility in the evaluation of numerous polymers, including polyurethane (PU), polyethylene (PE), polystyrene (PS), and polypropylene (PP), for degradation assessment.

In the context of PE degradation within bacterial and fungal cocultures, featuring *Lysinibacillus xylanilyticus* and *A. Niger* isolated from soil, FTIR has been employed for analysis. For instance, the *Penicillium variable* CCF3219 strain, following a 16-week incubation period and ozonation treatment, demonstrated a decrease in the carbonyl peaks of pre-oxidized ¹⁴C-labeled polystyrene (¹⁴C-PS) [18].

Assays with ¹³C or ¹⁴C Labeled Polymer: The use of isotopically labeled plastics provides a precise and highly sensitive method for tracking their conversion into biodegradation products. In the initial investigations involving isotopically labeled plastics, the radioisotope ¹⁴C was the primary choice. For instance, the fungus *Fusarium redolens* was observed to release ¹⁴C-CO₂ as a result of the breakdown of ¹⁴C-labeled high-density polyethylene (HDPE). Another study employed ¹⁴C-labeled polystyrene to evaluate the polystyrene degradation capabilities of 17 different fungal species over a 14-day incubation period, resulting in the degradation of 0 to 0.24 percent of the ¹⁴C-labeled polystyrene (¹⁴C-PS). Radioisotope probing enables the detection of extremely low plastic degradation rates due to its exceptional sensitivity in measuring and quantifying radioactivity. Interestingly, no research has yet explored the interactions between marine fungi and conventional plastics (such as PE, PP, PET, PS, PU, and nylon) labeled with ¹³C or ²H isotopes [19].

VI. LIMITATIONS OF STUDYING FUNGAL COMMUNITIES

Marine fungi have received significantly less attention and research compared to bacteria, resulting in a lack of understanding about their potential roles as saprotrophs and their ability to degrade plastics. Further investigation is necessary to establish the relevance of fungi in these contexts. However, studying (marine) fungi poses unique challenges in terms of taxonomic and physiological characterization. Unlike bacterial communities, there are no widely accepted standardized approaches for studying fungi. Moreover, molecular studies on fungi are still affected by traditional challenges and biases, such as biases in nucleic acid extraction methods, marker genes like 18S rRNA or ITS spacer, and protocols. Additionally, culture-based methods are complicated, morphological identification is

challenging, defining growth conditions is delicate, and fungi exhibit complex life cycles. These factors collectively present significant hurdles in molecular studies of fungi [20].

VII. CONCLUSION

Plastic has become an integral part of the global landscape, but it also presents a significant challenge to the environment. With the current rapid annual production of 300 million tons of plastic and its inherently slow natural degradation, plastics accumulate in natural environments, causing serious harm to biodiversity and natural ecosystems. Researchers and scientists are currently exploring whether microorganisms can accelerate the breakdown of plastic. In this study, we investigated the role of fungi in plastic degradation. Fungi capable of breaking down plastic are taxonomically classified. Basidiomycota, including Agaricomycetes, Microbotryomycetes, Tremellomycetes, Tritirachiomycetes, and Ustilaginomycetes, are known to degrade plastic. Within the Mucoromycetes, fungi belonging to the Mucoromycota were identified as plastic degraders. Eurotiomycetes within the fungal kingdom have the most associations with plastic degradation, but various fungal taxa capable of breaking down plastic were also found in the Sordariomycetes class. Future research on related topics is urgently needed to address the widespread issue of plastic accumulation in nature. However, there are still significant knowledge gaps that must be filled to make an informed assessment of marine fungi and their role as plastic decomposers. First, a more comprehensive and basic understanding of fungi in the marine environment needs to be achieved by examining fungal diversity and abundance in the ocean. Expanding the available sequence databases for previously unclassified fungi detected on plastic polymers and employing new molecular markers could help classify these fungi. Similar discovery approaches should be used to enable comparisons between different strains, types of polymers, and studies, ultimately shedding light on the biodegradation potential of fungi.

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