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Microbial transformation of plastics: A possible pathway to sustainable waste reduction

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ABSTRACT

Plastic pollution represents a major global environmental challenge due to the persistence of plastic materials and their widespread use across various sectors. The degradation of plastics can result in the release of microplastics into the environment, leading to food chain disruption, adverse effects on human and animal health, as well as contributing to environmental deterioration and climate change. Therefore, the excessive accumulation of plastic waste poses a significant threat to ecosystems and sustainable development. In this study, six bacterial species and four fungal species were isolated from plastic waste samples to evaluate their potential role in plastic biodegradation. The bacterial isolates were identified as *Bacillus altitudinis*, *Bacillus subtilis*, *Bacillus velezensis*, *Micrococcus luteus*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. Identification was performed using conventional microbiological methods, including colony morphology, Gram staining, cultural characteristics, hemolytic activity, and biochemical tests. The fungal isolates were identified as *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus terreus*. Notably, Aspergillus species were the only fungal isolates recovered, reflecting their ability to tolerate harsh environmental conditions and utilise complex polymeric substrates. Molecular characterisation confirmed the morphological identification of the fungal isolates. Enzymatic analysis revealed that the isolated microorganisms produced manganese peroxidase and lipase enzymes, indicating their potential involvement in plastic degradation. Comparative evaluation demonstrated that the fungal isolates exhibited greater plastic-degrading activity than the bacterial isolates. Among the fungi, *A. flavus*, *A. niger*, and *A. fumigatus* showed the highest biodegradation potential. In contrast, bacterial species displayed relatively lower degradation efficiency. These findings highlight the promising role of naturally occurring microorganisms, particularly *Aspergillus* species, in the biodegradation of plastic waste and their potential application in sustainable waste management strategies.

Keywords: Aspergillus, bacteria, plastic, biodegradation, peroxidase, lipase.

INTRODUCTION

There have been huge changes in the Earth in recent years due to plastics (Pilapitiya and Ratnayake, 2024). Plastic may incorporate the additives that improve characteristics and reduce cost (Gazal and Gheewala, 2020). Plastic pollution poses a major danger to world ecology due to its resilience to degradation and extensive application in numerous industries (Lamichhane et al., 2023). Plastic degradation can leach into the environment, resulting in microplastic contamination that causes disruption of the food chain, affecting the health of humans and other creatures as well as global warming (Shakir et al., 2023).

The most commonly utilised plastic kind is polypropylene (PP) plastic, and polyethylene terephthalate which is stable, clear, and low in weight

(Mohajan, 2025). High density polyethylene is employed in biomedical applications due to its flexibility, and resistance to chemicals (Mahmoud et al., 2025). Polyvinyl chloride is the third most produced polymer each year (Elgharbawy, 2022).

Many plastic-degrading microbes have been identified from the open environment, including the soil of a plastic-dumping site (Ru et al., 2020), waste of mulch films (Kansara et al., 2025), marine water and soil contaminated by crude oil (Zeng et al., 2025).

Fungi can be used as an alternative to old plastic management approaches in plastic breakdown (West et al., 2026). Fungi are more likely to damage hydrophobic surfaces than bacteria (Sun et al., 2024; Munhoz et al. 2026). Insoluble substrates can be colonised by *Aspergillus* sp., which can accelerate oxidation and decrease carbonyl index (Khruengsai et al., 2021; El-Sayed et al., 2021).

Plastic degraders, such as *Bacillus* and *Pseudomonas* sp. have a large number of enzyme systems (Wilkes and Aristilde, 2017; Elahi et al., 2021; Liu et al., 2022; Yadav et al., 2025). The decomposition of plastics by microbes involves several enzymes that degrade the plastic into monomers, CO_2, and water (Gao et al., 2024). There are many elements affecting plastic decomposition, including microbial species plastic characteristics and environmental parameters (Nabi et al., 2023). Various enzymes involved in plastic degradation include laccase, manganese peroxidase, urease and lipase (Suresh et al., 2025).

The present work deals with the research of breakdown of plastics by bacteria and fungi isolated from plastic waste.

MATERIAL AND METHODS

Sample collection

During the summer of 2024, numerous plastic sources were collected from different sites of Giza and Cairo governorates, Egypt.

Isolation of microorganisms

Bacterial species were isolated on nutrient agar medium, while fungal species were isolated on the Sabouraud dextrose agar media. Three isolates were performed for three months. One gram plastic was suspended in 9 mL sterile dist. water. For isolation of the plastic-degrading bacteria, one mL was applied to nutrient agar in Petri plates and for fungi; one mL was added to the Sabouraud dextrose agar.

Identification of plastics-degrading bacteria

Polypropylene (PP) plastic was used as the substrate in the biodegradation test. The pladtic samples were cut into discs with a diameter of 6-10 mm. Plastic was sterilised within the egradation medium in auto clave. The incubation period was 30 days. Conventional microbiological methods were used to identify bacterial isolates. Primary isolation on nutrient agar plates was carried out using the quantitative streak plate method for isolation, incubation for 24 h at 37 °C. The colonies were tested for morphological characterisation of size, shape, colour and haemolytic activity. The isolated colonies were subcultured on blood agar and MacConkey agar after incubation (Bharti et al., 2019). The colonies obtained were identified using Gram staining, cultural morphology and biochemical characteristics which include IMVIC tests (indole production test, methyl red test, Voges-proskauer test and citrate utilisation test), TSI (Triple Sugar Iron) test, catalase test, oxidase test and urease test for Gram-negative bacterial isolates, whereas Gram-positive bacteria were subjected to catalase enzyme production and coagulase test (Cheesbrough 2009 and Bergey's Manual 2012).

Fungal identification

The fungi were identified based on the macroscopic colony shape and microscopic features (Larone, 2002; Samson et al., 2014). Molecular techniques then validated the identity.

Enzymatic activity assays

Manganese peroxidase MnP

Manganese peroxidase activity was determined by measuring the oxidation of 1.8 mM 2,2'-azino-di-[3-ethyl benzothiazoline-6-sulphonic acid] (ABTS) in 0.1 M Tris HCl buffer (pH 7) and 2.5 mM H_2O_2 (Dada and Tosin, 2023). The reaction was performed for five min at a wavelength of 414 nm. Six millilitres of a reaction mixture consisted of two millilitres of ABTS, two millilitres of culture filtrate and two millilitres of H_2O_2. Enzyme activity was expressed in units which were defined

as the amount of enzyme that oxidises one µmol ABTS per minute at 30 °C and pH 7.0.

Lipase activity

The quantitative determination of lipase activity was performed by utilising a UV spectrophotometer with p-nitrophenyl palmitate as a substrate in the work of Palazar et al. (2021). The enzyme activity was determined by the amount of p-nitrophenol emitted per minute.

Determination of protein content

The protein content was determined using bovine serum albumin (Olajuyigbe et al., 2015). In the experiment, 10 uL of sample solution was added to 200 times diluted dye reagent. The reaction mixture was mixed well and then incubated at room temperature for 15 min. to allow for adequate colour development. The absorbance was measured at 595 nm using a blank.

Molecular identification of fungi

DNA isolation

Genomic DNA was isolated from fungus using the QIAamp DNA Mini Kit according to the manufacturer's instructions. The fresh mycelia were harvested from the cultures and lysed in buffer and proteinase K. The lysates were processed for DNA purification on QIAamp spin columns. The columns were washed to remove any contaminants and the pure DNA was eluted with nuclease-free water. After determination of DNA concentration and purity, the samples were stored at -20 °C.

Gel electrophoresis on agarose

Agarose grade electrophoresis (1.5 g) was dissolved in 100 ml TBE buffer in a flask. The mixture was stirred and microwaved until all granules were dissolved. The mixture was allowed to cool and then 0.5 µg/ml of ethidium bromide was added and stirred well. The warm agarose was placed in the gel-casting apparatus and the required comb was placed on top and permitted to polymerise at room temperature. Carefully, the comb was removed before filling the electrophoresis tank with TBE buffer. Twenty microlitres of each sample of PCR product, negative control and positive control were placed onto the gel. The power supply was from one to five volts per centimetre of tank length. The run was stopped after about 30 minutes and the gel was placed in a UV cabinet. The gel was photographed with a gel documentation system and the data was analysed using software (Sambrook et al., 1989).

Statistical analysis

Statistical analysis was done using a statistical program for the social sciences (SPSS). A significant difference was determined by one way analysis of variance (ANOVA) at 95% confidence level (Ahuactzin-Pérez et al., 2018).

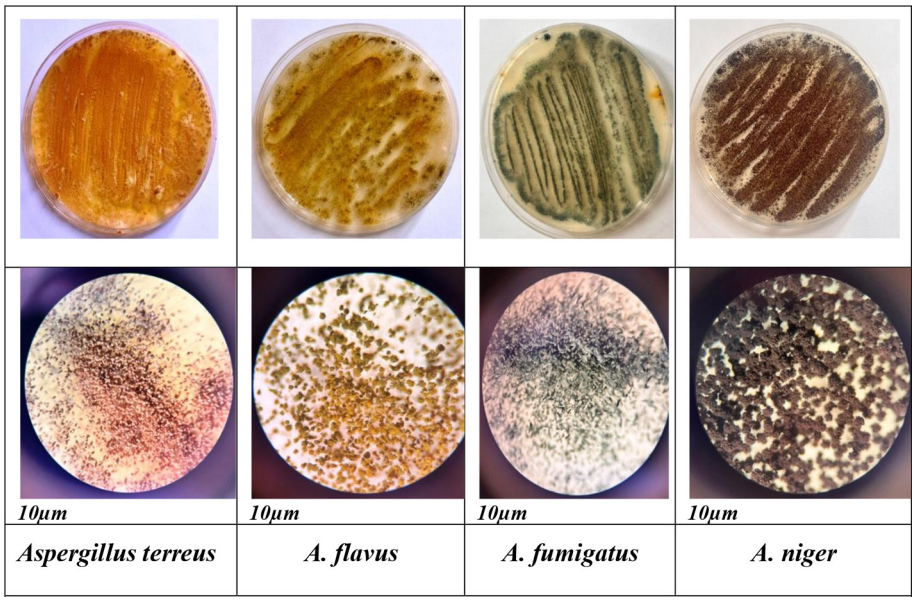

Figure 1. Morphological characterization characterisation of fungal species isolated from plastic waste

Table 1. Isolated microorganisms and their colony count

Table 1: Isolated microorganisms and their colony count

Microorganisms	Colony count			Total count
	1st isolate	2nd isolate	3rd isolate	
Aspergillus flavus	4	10	6	20
A. fumigatus	2	4	3	9
A. niger	6	5	10	21
A. terreus	2	1	1	4
Bacillus altitudinis	2	1	2	5
B. subtilis	13	8	4	25
B. velezensis	15	8	14	37
Micrococcus luteus	20	22	12	54
Moraxella catarrhalis	12	8	6	26
Staphylococcus aureus	14	10	15	39
Total	90	77	73	240

Note: the microbial yield was reasonably similar throughout the three isolated experimental arms, with the first isolate having the maximum overall recovery (n=90) followed by the second isolate (n=77) and the third isolate (n=73).

RESULTS

Bacteria and fungi regularly colonise plastic and may be able to break it down. Four fungal species were isolated; they were *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *A. terreus* (Table 1 and Figure 1).

Six bacterial species were recovered from various plastic wastes (High-density, low-density polyethylene and polyethylene terephthalate) which were *Bacillus altitudinis*, *B. subtilis*, *B. velezensis*, *Micrococcus luteus*, *Moraxella catarrhalis* and *Staphylococcus aureus* (Table 1).

The microbial yield was reasonably similar throughout the three isolated experimental arms, with the first isolate having the maximum overall recovery (n=90) followed by the second isolate (n=77) and the third isolate (n=73).

The maximum microbial burden was detected in *M. luteus*, with a total of 54 colonies (the second isolate had a peak of 22 colonies). High density was also seen in *S. aureus* (n=39) and *B. velezensis* (n=37). The top row depicts the macroscopic colony morphology of the respective *Aspergillus* spp. grown on the Sabouraud Dextrose Agar plates after incubation for 7 days at 37 °C, while the bottom row demonstrates the equivalent microscopic features. The isolated fungi were morphologically identified according to (Moubasher, 1993). Then molecular identification was done to corroborate the identity (Figures 1 and 2).

To examine the evolutionary relationships of the isolated species, the phylogenetic tree was constructed for sequencing analysis (Figure 2).

The isolation and identification of bacteria were carried out using Gram staining, cultural morphology and biochemical tests, like IMVIC tests (indole production test, methyl red test, Voges-Proskauer test and citrate haracteriz test), TSI (Triple Sugar Iron) test, catalase test, oxidase test and urease test for Gram-negative bacterial isolates and Gram-positive bacteria were tested for catalase enzyme production, coagulase test, novobiocin, bacitracin sensitivity and bile-esculin hydrolysis (Table 2).

Identification of microorganisms and biochemical tests

The six bacterial isolates were phenotypically and biochemically characterized using a broad panel of 21 diagnostic assays (Table 2). The isolates were identified as *Bacillus altitudinis*, *B. subtilis*, *B. velezensis*, *Micrococcus luteus*, *Moraxella catarrhalis* and *Staphylococcus aureus* based on morphological and biochemical reactivities.

All the six isolates were catalase positive. Among these only *S. aureus* was differentiated by a positive coagulase reaction. Many enzymes are involved in plastic degradation; two such enzymes, i.e. manganese peroxidase and lipase, were studied. One way ANOVA was performed to compare the differences between the experimental groups. No statistically significant difference in manganese peroxidase activity between groups was found ($p>0.05$) (Table 3).

The manganese peroxidase profiles of the isolated microorganisms showed substantial

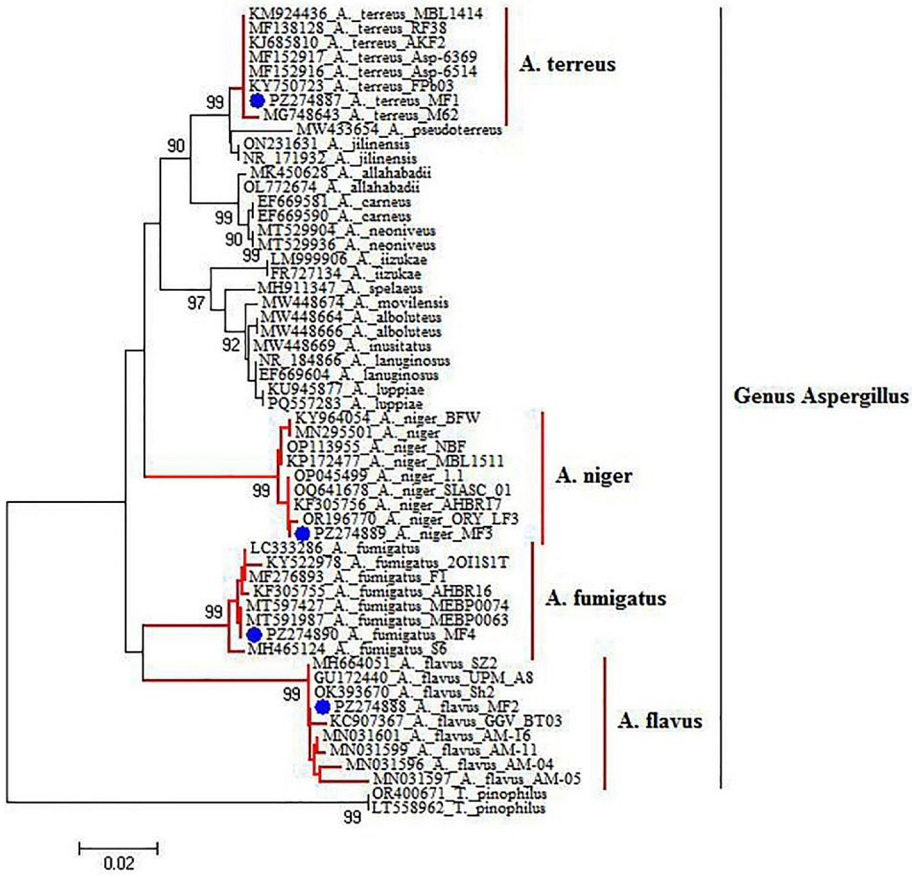

Figure 2. Phylogenetic relatedness of fungal *18S rRNA* gene. Maximum-likelihood tree generated after 500 bootstraps indicated clustering of MF1, MF2, MF3 and MF4 strains with *A. terreus*, *A. flavus*, *A. niger* and *A. fumigatus* strains, respectively apart from other *Aspergillus* types

Table 2. Biochemical tests used for identification of isolated bacteria

Bacterial species	Catalase	Coagulase	Indole	Methyl red	Oxidase	Voges-Proskauer	Citrate utilisation	H_2S production	Urease	Motility	Lysine decarboxylase	Gelatin liquefaction	Glucose	Lactose	Maltose	Mannitol	Sucrose	Salicin	L-arabinose	D-Sorbitol	Blood hemolysis
Bacillus altitudinis	+	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-
B. subtilis	+	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	+
B. velezensis	+	-	-	-	+	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-
Micrococcus luteus	+	-	-	-	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	-	-
Moraxella catarrhalis	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	+	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+

Note: + Positive reaction/growth; - Negative reaction/no growth.

variability. *Aspergillus flavus* and *A. niger* had the highest enzyme activity (19.1 and 18 U/mL; respectively) and specific activity (27.6 and 12 U/mg; respectively) which were substantially greater than all other strains tested. High enzyme synthesis did not necessarily correspond to high specific activity. For instance, A. flavus and *A. niger* showed a high enzyme activity, but the protein concentration was low (1.6 and 1.5 mg/mL; respectively), which led to a higher specific activity (27.6 and 12 U/mg; respectively).

To assess the difference between the experimental groups, a one way ANOVA was performed. No statistically significant difference among the groups in lipase activity between groups ($p>0.05$) (Table 4).

The enzymatic profiles of the isolated microorganisms showed substantial variability. *Aspergillus flavus* had the highest enzyme activity (23.4 U/

Table 3. Manganese peroxidase activity of the isolated microorganisms

Bacterial species	Enzyme activity (U/mL)	Protein Conc. (mg/mL)	Specific activity (U/mg)
Control	00.0±0.00^{d}	0.0±0.00^{b}	00.0±0.00^{f}
Aspergillus flavus	19.1±0.37^{a}	1.6±0.15^{a}	27.6±0.90^{a}
A. fumigatus	11.3±0.69^{b}	2.0±0.20^{a}	09.8±0.46^{bc}
A. niger	18.0±0.58^{a}	1.5±0.30^{a}	12.0±0.53^{b}
A. terreus	10.4±0.78^{b}	2.2±0.36^{a}	04.7±0.46^{de}
Bacillus altitudinis	08.1±1.00^{c}	1.4±0.30^{a}	05.8±2.10^{cd}
B. subtilis	06.0±1.00^{d}	2.2±0.25^{a}	02.7±0.64^{e}
B. velezensis	06.2±0.47^{c}	1.3±0.12^{a}	04.8±0.46^{de}
Micrococcus luteus	08.5±0.62^{d}	2.0±0.15^{a}	04.3±0.70^{e}
Moraxella catarrhalis	05.2±0.47^{d}	1.9±0.22^{a}	02.7±0.46e
Staphylococcus aureus	07.9±0.15^{c}	2.1±0.21^{a}	03.8±0.29^{e}
df	32	32	32
F value	0.10	0.14	0.0002
P value	0.90	0.87	0.99

Note: Each value represents the mean ± SD (n=3). Values within the same column sharing a common superscript letter are not significantly different (p>0.05) according to Tukey's post-hoc test.

Table 4. Lipase activity of the isolated microorganisms

Bacterial species	Enzyme activity (U/mL)	Protein content (mg/mL)	Specific activity (U/mg)
Control	00.0±0.00^{g}	0.0±0.00^{e}	0.0±0.00^{f}
Aspergillus flavus	23.4±0.29^{a}	3.3±0.81^{ab}	7.1±0.66^{a}
A. fumigatus	20.4±0.76^{b}	4.1±0.97^{a}	5.0±0.93^{b}
A. niger	21.6±0.64^{ab}	4.6±0.53^{a}	4.7±0.46^{bc}
A. terreus	14.1±0.53^{c}	3.2±0.64^{ab}	4.4±0.53^{bcd}
Bacillus altitudinis	08.4±0.23^{d}	2.5±0.70^{bcd}	1.9±0.64^{e}
B. subtilis	06.2±0.81^{e}	3.1±0.50^{abc}	2.0±0.72^{e}
B. velezensis	04.0±0.35^{f}	1.2±0.35^{de}	3.3±0.35^{cde}
Micrococcus luteus	05.3±0.50^{ef}	2.2±0.40^{bcd}	2.4±0.21^{e}
Moraxella catarrhalis	04.1±1.30^{f}	1.8±0.36^{bcd}	2.3±0.26^{e}
Staphylococcus aureus	04.8±0.36^{ef}	1.6±0.80^{cd}	3.0±0.32^{de}
df	32	32	32
F value	0.00	3.0	0.02
P value	1.00	0.06	0.98

Note: each value represents the mean ± SD (n=3). Values within the same column sharing a common superscript letter are not significantly different (p>0.05) according to Tukey's post-hoc test.

mL) and specific activity (7.1 U/mg) which were substantially greater than all other strains tested. High enzyme synthesis did not necessarily correspond to high specific activity. For instance, *A. niger* showed a high enzyme activity of 21.6 U/mL, but its high protein concentration (4.6 mg/mL) led to a much lower specific activity (4.7 U/mg).

DISCUSSION

The quantitative assessment of microbial colonisation reveals distinct variations in colony counts across the individual isolates examined. This indicates a robust microbial presence. However, the distribution of these microorganisms varied among the successive isolation stages.

Various microorganisms, including *Alcanivorax*, *Pseudomonas*, *Vibrio*, and Tunicata-associated symbiotic bacteria, have the potential to degrade polyethylene (Kosiorowska et al., 2026; Lubis et al., 2026; Malik et al., 2026).

There is an immediate need for sustainable, environmentally friendly bioremediation solutions due to the growing problem of synthetic plastic waste. From samples of plastic waste, four different types of fungi were detected and isolated in this study: *Aspergillus terreus*, *A. flavus*, *A. fumigatus*, and *A. niger* (Valenzuela et al., 2025; Wechselberger et al., 2026)

The survival and proliferation of these specific *Aspergillus* species on plastic substrates strongly indicate their metabolic adaptation and

potential capacity to utilise complex plastic polymers as carbon sources (Ekanayaka et al., 2025; Ullah et al., 2025).

The genus *Aspergillus* is renowned for its robust enzymatic machinery and environmental resilience (Rosas-Vega et al., 2025.). The isolation of *A. niger* and *A. flavus* from plastic debris aligns with previous literature demonstrating their capability to colonise and partially degrade various polymer types, including polyethylene (PE) and polyurethane (PU) (Philippe et al., 2024).

These fungi typically initiate degradation through the secretion of extracellular enzymes, such as esterases, endoglucanases, and laccases, which introduce oxygen into the hydrophobic polymer backbone, reducing its molecular weight (El-Gendi et al., 2021). Furthermore, *A. fumigatus* and *A. terreus* have increasingly gained attention in mycoremediation studies due to their high thermotolerance and rapid growth rates (Paulussen et al., 2017).

The successful isolation of these four diverse species from a heavily urbanised or localised plastic pool suggests that indigenous microbial communities naturally evolve to exploit persistent synthetic materials. This localised adaptation holds significant promise for screening hyperdegrading strains. Future work should focus on quantifying the weight loss of specific plastic types when exposed to individual and consortia treatments of these isolates, alongside evaluating their specific enzymatic activities.

The molecular identification of the fungal isolates was confirmed through a comprehensive phylogenetic analysis based on sequence alignment. The constructed phylogenetic tree clustered the isolated strains into four distinctly supported clades belonging to the genus *Aspergillus*, establishing high sequence homology with reference strains retrieved from official repositories.

The phenotypic and biochemical characterisation of the bacterial isolates provided critical baseline identification, matching established profile standards for each respective genus and species.

The genus *Bacillus* was prominently represented among the isolates, sharing core characteristics such as being catalase-positive, motile, and capable of gelatin liquefaction and carbohydrate fermentation (glucose, maltose, mannitol, sucrose, and salicin) (Fahim et al., 2022). However, key metabolic variations allowed for distinct species-level differentiation.

The quantitative evaluation of activity, concentration of protein, as well as specific activity provides critical insights into the efficiency and crude characteristics of the targeted enzymatic systems.

A critical observation from the data is that a high total protein concentration does not inherently correlate with elevated catalytic output (Niazi, 2025) For instance, while certain groups demonstrated maximum protein accumulation, their corresponding enzyme activities remained modest. Conversely, the highest crude enzyme activity was achieved under a condition yielding a relatively moderate protein concentration (Pham et al., 2026).

Plastic degradation is the process by which polymer chains are broken down into smaller molecules through physical, chemical or biological processe (Cai et al., 2023) The general mechanism included the following steps: biodeterioration where microorganisms attach to the plastic surface and form a biofilm (Dungani et al., 2019). Environmental factors such as UV radiation, heat, and moisture create surface cracks and increase roughness, making the plastic more accessible to microbes (Rostampour et al., 2025) Depolymerisation by microbial enzymes cleave the long polymer chains into shorter oligoomers, dimers, and monomers (Mohanan et al., 2020). Common enzymes include esterases, lipases, cutinases, laccases and peroxidase (Suresh et al., 2025). Assimilation: the resulting low-molecular-weight compounds are transported into microbial cells and used as carbon and energy source (Morono et al., 2011). Mineralisation: the absorbed compounds are completely metabolised into CO_2, water, and biomass (Wilcox et al., 2025).

CONCLUSION

Fungi are more active in plastic degradation than bcteria. *A. flavus* and *A. niger* exhibited the highest manganese peroxidase and lipase activity, suggesting their potential involvement in degradation.

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