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EXPLORING PETASE: A PROMISING ENZYME FOR PLASTIC WASTE MANAGEMENT

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ABSTRACT

Plastic pollution is a pressing global challenge owing to the pervasive, near-unmanageable threat it poses to living and non-living systems and the environmental stress it causes. The widespread use of plastic materials, their slow degradation rates, and their ability to travel vast distances through air and water currents have made plastic waste a significant environmental challenge. Plastics are classified as degradable and non-degradable ones based on their extent of degradation in natural environments. Conventional plastics, have an extremely slow degradation rate in the environments. Degradation of plastics by environmental microbes are fast, eco-friendly and minimises pollution. This study focuses on role of PETase in PET plastic degradation. Conventional methods such as photochemical, thermal are employed. Biological method using microbes provide greener solutions. Mutagenesis of marine hydrocarbonoclastic bacterium Pseudomonas aestusnigri showed PET degrading potential. The Ideonella sakaiensis mutant showed 3-fold increase in PET degradation compared to wild type. Two strains C. reinhardtii CC-124 and CC-503 degraded PET completely to terephthalic acid detected using HPLC. The in vitro technique of plastic degradation does not fit industrial applications. Thus, genetically engineered microorganisms combined with plastic-degrading enzymes would be a possible for practical application.

Keywords: Plastic, Petase, Biodegradation, Eco-Friendly, Microbes

1. INTRODUCTION

Plastic pollution is a pressing global challenge owing to the pervasive, near-unmanageable threat it poses to living and non-living systems and the environmental stress it causes. Herein, we define plastic pollution [encompassing macro-, micro-, and nano plastic debris] as the intrusion or invasion by plastic materials [i.e., polymeric systems], either through direct introduction or degradation processes, of environments [to which they are not native] to negatively or undesirably impact such environments [1][3]. Like greenhouse gases, persistent pollutants, and other environmental contaminants, plastic pollution transcends territorial boundaries and legal restrictions. It spreads across water bodies, travels through the air, and reaches distant locations through human activities, making it a global environmental challenge. [4]. Across the world, the issue of plastic pollution has brought about a paradigm shift in discourses on climate change and ocean and environmental sustainability [2]. In almost every country in the world, multiple individuals and groups have become environmental activists against plastic pollution [6][7]. In addition, governments, world leaders, and various stakeholders participate in discussions, conventions, and resolutions in concerted efforts to find a holistic solution to plastic pollution.[5]

Plastics are polymer- based system for example, polyethylene, polyacrylamides, polyesters, and polypropylene. Although plastics are generally polymers, not all polymers are plastics, such as natural cellulose, carbohydrates, proteins [e.g., leather], lignin, and natural rubber [Hevea brasiliensis] [14]. Plastics can be divided into degradable and non-degradable ones based on their degradability in natural environments [8[[9]. Conventional plastics, including polyethylene [PE],

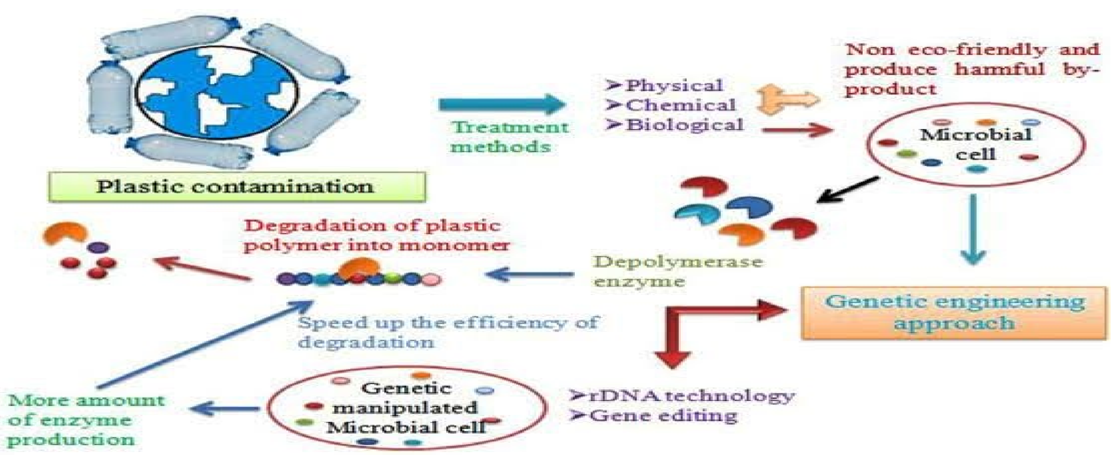
polystyrene [PS], polypropylene [PP], polyvinyl chloride [PVC], polyethylene terephthalate [PET], polyurethane [PUR], and other polymer compounds, have an extremely slow degradation rate in the environments [10]. These plastics are not biodegradable and get accumulated in the environment posing problems to all life forms.
Polyethylene terephthalate [PET] is a very common plastic. It is primarily utilized for manufacturing beverage bottles due to its durability against impact, lightweight nature, and exceptional transparency. [13]. The consumption of PET has significantly increased over the decades, primarily due to its outstanding mechanical properties.
PET wastes are incinerated, then a large amount of air pollutant such as CO_2 is emitted during this process [15-17]. Therefore, the biological treatments for plastic waste have been gaining attention. At present, scientists have confirmed 27 enzymes that can break down synthetic polymers or oligomers. PET hydrolase [PETase] and mono[2-hydroxyethyl] terephthalic acid hydrolase [MHETase], both of which demonstrate PET degrading
activity. PETase are a polyethylene terephthalate hydrolase, which can convert PET into mono[2-hydroxyethyl] terephthalic acid [MHET] and mono[2-hydroxyethyl] terephthalate hydrolase, responsible for the conversion of MHET to terephthalic acid [TPA] and ethylene glycol [EG][18-21]. Since then, numerous PET hydrolases, belonging to the esterase class [EC 3.1.1., carboxylic ester hydrolases] have been reported and characterized [22-28].
In the present study, PETase sources, mechanism of action is explored as a promising tool for plastic management and providing greener solution to reduce pollution.

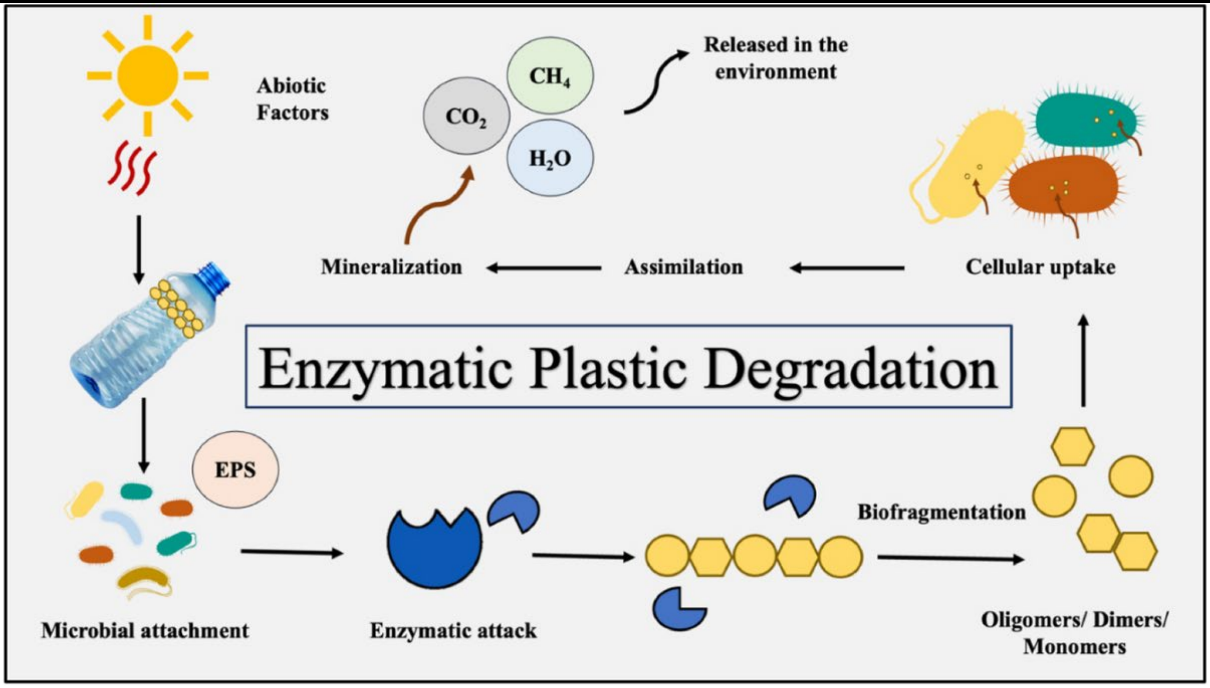
2. METHODOLOGY

Usually, the degradation mechanisms of plastics include the conventional methods such as

- photooxidation degradation
- catalytic degradation
- ozone induced degradation
- thermal degradation
- mechanical degradation

The most advanced method for degradation of plastics is biological method called as "Biodegradation". In biodegradation, the final products of biodegradation are CO_2 and water, which has the advantages of green environmental protection and low energy consumption [11].
The plastics in the environments can be degraded into microplastics [MPs] or nano-plastics [NPs] under the action of weathering, cracking, and decomposition, involved in the physical, chemical, and biological processes [13].





MECHANISM OF ACTION OF PETASE

The key factor influencing the biodegradability of plastic polymers is the nature of the bonds that connect the monomers. Among the six major types of synthetic plastics [PE, PP, PS, PVC, PUR, and PET], the backbone containing C–C bonds of PE, PP, PS, and PVC are highly resistant while PUR and PET with a hydrolysable backbone are more vulnerable to enzymatic degradation.

In the degradation of ester-linked PET, both PET and PET hydrolase can act on the terminal or ring structures of the polymer chains, facilitating enzymatic hydrolysis. This process enhances the hydrophilicity of PET, thereby improving the efficiency of subsequent enzymatic breakdown. [26].*Ideonella Sakaiensis 201-F6*, a bacterium from the genus *Ideonella*, was reported to degrade and assimilate PET [27] after the generation of PETase and MHETase that efficiently converts PET into environmentally friendly monomers, terephthalic acid, and ethylene glycol. [27]. Notably, this PET hydrolase has 45–53% homology with actinomycete keratinase [28] but can completely degrade PET, compared to other PET hydrolases.However, the low stability of PETase limits its wide application. After enzymes hydrolose the ester bond, PET is broken down into MHET. MHET can continue to be hydrolyzed into TPA and EG under the action of MHETase [29] and finally enter the tricarboxylic acid cycle [TCA cycle] [30].

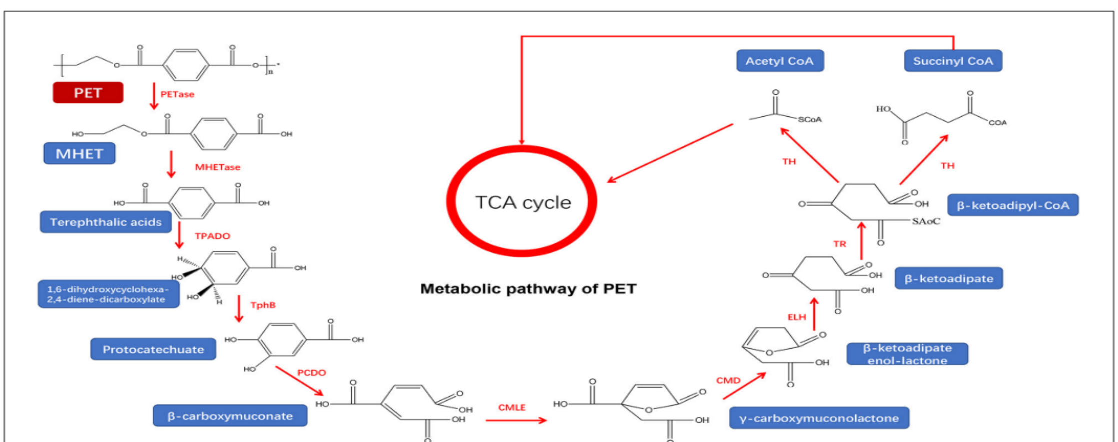


FIGURE 4
Metabolic pathway of PET (adapted from previous studies Yoshida et al., 2016; Ru et al., 2020).

3. RESULTS AND DISCUSSION

Degradation of plastics by environmental microbes has drawn more attention. Recent studies have explored various microorganisms, including actinomycetes, algae, bacteria, and fungi, for their ability to biodegrade different plastic polymers. So far, over 56 bacterial and fungal species from 25 genera have been identified as capable of degrading polyethylene, with soil and landfill environments being their primary sources. [31]. The polyester degrading ability of a novel carboxylic ester hydrolase identified in the genome of the marine hydrocarbonoclastic bacterium *Pseudomonas aestusnigri was studied. The* crystal structure was solved at 1.09 Å resolution. A rational mutagenesis study to improve the PET degrading potential showed improved activity. The crystal structure of this variant was solved at 1.35 Å [32].

Three mutations in *Ideonella sakaiensis* PETase active site for enhancing its PET-degrading activity was studied. Notably, the S238Y mutant, positioned near the catalytic triad, exhibited a 3.3-fold increase in degradation activity compared to the wild-type enzyme. This structural modification significantly enhanced the enzyme's ability to break down highly crystallized PET (~31%), commonly used in commercial soft drink bottles. Furthermore, microscopic analysis of PET samples treated with the enzyme revealed that IsPETase performs more effectively when mechanical stress alters the smooth surface of highly crystalline PET.

A green alga, *Chlamydomonas reinhardtii*, which produces PETase, was developed. Two representative strains, *Chlamydomonas reinhardtii* CC-124 and CC-503, were analyzed, revealing that CC-124 effectively expressed PETase. To confirm the catalytic activity of PETase produced by *C. reinhardtii*, the transformant's cell lysate was incubated with PET samples at 30°C for up to four weeks. Following incubation, high-performance liquid chromatography analysis detected terephthalic acid (TPA), the fully degraded product of PET. Additionally, morphological changes, such as holes and dents on the surface of PET film, were observed using scanning electron microscopy [34].

4. CONCLUSION

The degradation of plastic by microorganisms occurs at a relatively slow rate, limiting its industrial applicability. At present, no in vitro plastic degradation method is suitable for large-scale industrial use. Therefore, the development of genetically engineered microorganisms incorporating highly efficient plastic-degrading enzymes presents a promising solution for practical implementation.

CONFLICT OF INTERESTS

None.

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REFERENCES

Thushari, G. G. N.; Senevirathna, J. D. M. Plastic Pollution in the Marine Environment. Heliyon 2020, 6, No. e04709.

Borrelle, S. B.; Ringma, J.; Law, K. L.; Monnahan, C. C.;Lebreton, L.;McGivern, A.; Murphy, E.; Jambeck, J.; Leonard, G. H.;Hilleary, M. A.; Eriksen, M.; Possingham, H. P.; De Frond, H.;Gerber, L. R.; Polidoro, B.; Tahir, A.; Bernard, M.; Mallos, N.; Barnes,M.; Rochman, C. M. Predicted Growth in Plastic Waste Exceeds Efforts to Mitigate Plastic Pollution. Science 2020, 369, 1515−1518.

Wilke, C. Plastics Are Showing up in the World's Most Remote Places, Including Mount Everest; Science News: Washington, DC, 2020.

Teles, M.; Balasch, J. C.; Oliveira, M.; Sardans, J.; Peñuelas, J. Insights into Nanoplastics Effects on Human Health. Sci. Bull. 2020,65, 1966−1969.

Shen, M.; Huang, W.; Chen, M.; Song, B.; Zeng, G.; Zhang, Y.[Micro]Plastic Crisis: Un-Ignorable Contribution to Global Greenhouse Gas Emissions and Climate Change. J. Cleaner Prod. 2020, 254, No. 120138.

Marlin, D.; Ribbink, A. J. The African Marine Waste Network and Its Aim to Achieve 'Zero Plastics to the Seas of Africa. S. Afr. J.Sci. 2020, 116, No. 8104.

Eriksen, M. Junk Raft: An Ocean Voyage and a Rising Tide of Activism to Fight Plastic Pollution; Beacon Press Books: Boston, MA,2017.

Brodhagen, M., Peyron, M., Miles, C., and Inglis, D. A. [2015]. Biodegradable plastic agricultural mulches and key features of microbial degradation. Appl. Microbiol. Biotechnol. 99, 1039–1056. doi: 10.1007/s00253-014-6267-5

Major, I., Fuenmayor, E., and Mcconville, C. [2016]. The production of solid dosage forms from non-degradable polymers. Curr. Pharm. Des. 22, 2738–2760.doi: 10.2174/1381612822666160217141049

Wang, J., Tan, Z., Peng, J., Qiu, Q., and Li, M. [2016]. The behaviors of microplastics in the marine environment. Mar. Environ. Res. 113, 7–17.doi: 10.1016/j.marenvres.2015.10.014

Lu, C., Liu, L., Li, J., Du, G., and Chen J. [2013]. Isolation and characterization of a microorganism degrading starch/polyethylene blends. Chin. J. Appl. Environ.Biol. 19, 683–687

Adrados, A., deMarco, I., Caballero, B. M., López, A., Laresgoiti, M. F., and Torres, A. [2012]. Pyrolysis of plastic packaging waste: a comparison of plastic residuals from material recovery facilities with simulated plastic waste. WasteManag. 32,826–832. doi: 10.1016/j.wasman.2011.06.016

Luo, Y., Zhou, Q., Zhang, H., Pan, X., Chen, T. U., Lianzhen, L. I., et al. [2018]. Pay attention to research on microplastic pollution in soil for prevention of ecological and food chain risks*. Bull. Chin. Acad. Sci.* 33, 1021–1030.

Austine Ofondu Chinomso Iroegbu,⊥ Suprakas Sinha Ray, Vuyelwa Mbarane, João Carlos Bordado,and José Paulo Sardinha Plastic Pollution: A Perspective on Matters Arising: Challenges and Opportunities

15 Taniguchi I, Yoshida S, Hiraga K, Miyamoto K, Kimura Y, Oda K. Biodegradation of PET: current status and application aspects. ACS Catal. 2019;9:4089–105.

Lin Y-H, Yang M-H. Catalytic conversion of commingled polymer waste into chemicals and fuels over spent FCC commercial catalyst in a fluidised-bed reactor. Appl Catal B. 2007;69:145–53.

Chen Z, Wang Y, Cheng Y, Wang X, Tong S, Yang H, Wang Z. Efficient biodegradation of highly crystallized polyethylene terephthalate through cell surface display of bacterial PETase. Sci Total Environ. 2020;709:136138.

Yang, Y., Yang, J. & Jiang, L. Comment on "a bacterium that degrades and assimilates poly[ethylene terephthalate] ". *Science [80-].*353, 759 [2016].

Austin, H. P. *et al.* Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proc. Natl. Acad. Sci. U. S. A.* 115, E4350–E4357 [2018].

Knott, B. C. *et al.* Characterization and engineering of a two-enzyme system for plastics depolymerization. *Proc. Natl. Acad. Sci.U. S. A.* 117, 25476–25485 [2020].

Meyer-Cifuentes, I. E. & Ozturk, B. Mle046 is a marine mesophilic MHETase-like enzyme. *Front. Microbiol.* 12, 1–9 [2021]. Tournier, V. *et al.* Enzymes' power for plastics degradation. *Chem. Rev.* https:// doi. org/ 10. 1021/ acs. chemr ev. 2c006 44 [2023].

Tournier, V. *et al.* An engineered PET depolymerase to break down and recycle plastic bottles. *Nature* 580, 216–219 [2020].

Buchholz, P. C. F. *et al.* Plastics degradation by hydrolytic enzymes: The plastics-active enzymes database—PAZy. *Proteins Struct.Funct. Bioinform.* 90, 1443–1456 [2022].

Puspitasari, N., Tsai, S. L. & Lee, C. K. Class I hydrophobins pretreatment stimulates PETase for monomers recycling of waste PETs. *Int. J. Biol. Macromol.* 176, 157–164 [2021].

Ronkvist, A. M., Xie, W., Lu, W. & Gross, R. A. Cutinase-catalyzed hydrolysis of poly[ethylene terephthalate]. *Macromolecules* 42,5128–5138 [2009].

Pirillo, V., Orlando, M., Tessaro, D., Pollegioni, L. & Molla, G. An efficient protein evolution workflow for the improvement of bacterial PET hydrolyzing enzymes. *Int. J. Mol. Sci.* 23, 264 [2022].

Wei, R. *et al.* Mechanism-based design of efficient PET hydrolases. *ACS Catal.* 12, 3382–3396 [2022].

Kawai, F., Kawabata, T., and Oda, M. [2019]. Current knowledge on enzymatic PET degradation and its possible application to waste stream management and other fields. *Appl. Microbiol. Biotechnol.* 103, 4253–4268.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., et al. [2016]. A bacterium that degrades and assimilates poly [ethylene terephthalate]. *Science* 351, 1196–1199. doi: 10.1126/science. aad6359

Wei, R., and Zimmermann, W. [2017]. Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? *Microb. Biotechnol.* 10,1308–1322. doi: 10.1111/1751-7915.12710

Peng, B. Y., Su, Y., Chen, Z., Chen, J., Zhou, X., Benbow, M. E., et al. [2019]. Biodegradation of Polystyrene by Dark [Tenebrio obscurus] and Yellow [Tenebriomolitor] Mealworms [Coleoptera: Tenebrionidae]. *Environ. Sci. Technol.* 53, 5256–5265. doi: 10.1021/acs.est.8b06963

Ronkvist, Å. M., Xie, W., Lu, W., and Gross, R. A. [2009]. Cutinase-Catalyzed Hydrolysis of Poly [ethylene terephthalate]. *Macromolecules* 42, 5128–5138.

Alexander Bollinger1, Stephan Thies, Esther Knieps-Grünhagen, Christoph Gertzen, Stefanie Kobus, Astrid Höppner, Manuel Ferrer, Holger Gohlke Sander H. J. Smits and Karl-Erich Jaeger. A Novel Polyester Hydrolase from the Marine Bacterium Pseudomonas aestusnigri – Structural and Functional Insights.

Maria Eduarda Sevilla , Mario D. Garcia , Yunierkis Perez-Castillo, Vinicio Armijos-Jaramillo, Santiago Casado , Karla Vizuete , Alexis Debut and Liliana Cerda-Mejía. Degradation of PET Bottles by an Engineered Ideonella sakaiensis PETase.

Kim *et al. [2020].* Functional expression of polyethylene terephthalate-degrading enzyme [PETase] in green microalgae. *Microb Cell Fact 19:97.* https://doi.org/10.1186/s12934-020-01355-8