Development of a Genetically Engineered *Escherichia coli* Lemo 21 with PETase Gene for Biodegradation of Polyethylene Terephthalate (PET) Plastics

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ABSTRACT

At present polyethylene terephthalate (PET) is a widely used plastic for being a strong, lightweight, semi-crystalline thermoplastic, excellently resistant to moisture and chemicals, high stiffness, commonly colorless and transparent with a density of ~ 1.38 g/cm³. Its high chemical stability contributes to its long-term environmental persistence and accumulation as microplastics and nanoplastics. This study targets to develop a genetically engineered strain of *Escherichia coli* overexpressing PETase for the biodegradation of PET plastics.

In this study, we selected *E. coli* Lemo21(DE3) for its strict regulations and high-level expression of recombinant protein under induced conditions. We were successful in transforming the strain with the plasmid pET-21b(+)-Is-PETase-W185A encoding PETase supplied by Addgene originated from *Ideonella sakaiensis*. The transformed cells were grown in presence of chloramphenicol and ampicillin for selection while the overexpression of PETase was induced with L-rhamnose (up to 2000 µm) followed by IPTG (100 mM) overnight. The cells were collected by centrifugation at 4°C and 2,000 g and got rid of the growth media, resuspended in NEB Express Lysis Reagent followed by adding T4 lysozyme and star-shaped PET plastic fragments (0.5 mm in thickness, 1/4-inch maximum in diameter) in separate vials along with a control. The vials were incubated overnight at 37°C for seven days followed by washing the PET plastic fragments in separate glass vials with distilled water three times with vigorous shaking. The pieces were dried for 20 minutes at 37°C.

The pieces of PET plastics were investigated individually using light microscopy (LM) followed by scanning electron microscopy (SEM) to determine the impact of the PETase extract from transformed *E. coli* Lemo21. The results showed significant degradation in PET plastic fragments by the extracts of the *E. coli* Lemo21 cells. The pieces of the PET plastics appeared cloudy with pronounced surface erosion and structural disruption compared to the controls. These findings demonstrate the extracted PETase from the PETase gene engineered *E. coli* Lemo21(DE3) is active in degrading the PET plastics. We are repeating the experiment to determine the concentration of L-rhamnose needed for optimal expression of PETase by the transformed *E. coli* Lemo21(DE3) and the effect of extended incubation time in PET plastic degradation.

INTRODUCTION

The post-World War II era marked a global transition toward the "Plastic Age," characterized by the mass production of synthetic polymers valued for their low cost, lightweight nature, and extreme durability (Andrady 2017; Pilapitiya and Ratnayake 2024). Since1950s, global plastic production has followed an almost exponential trajectory, reaching an estimated 400 million metric tonnes per year, with over 350 million tonnes entering the waste stream annually (OECD 2022; Tan, et al., 2025). Despite global efforts toward sustainability, approximately 79% of all plastic ever produced remains in landfills or natural ecosystems, while a mere 9% is recycled (OECD 2022). Central to this crisis is polyethylene terephthalate (PET), a semi-crystalline thermoplastic with a high density of ~1.38 g/cm³. Its exceptional resistance to moisture, sunlight, and microbial attack—qualities that make it ideal for the bottling and textile industries—simultaneously ensure its long-term environmental persistence (Andrady 2017).

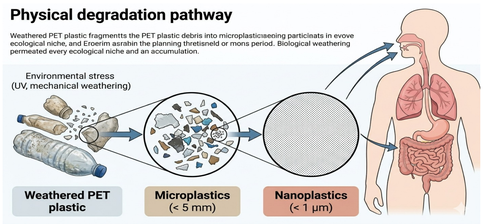

Figure 1. Physical degradation pathway of PET plastic and human exposure.(Diagram is generated by free access using Canva)

As PET resists complete degradation, it undergoes mechanical weathering into microplastics (< 5 mm) and nanoplastics (< 1 µm), which have now permeated every ecological niche from deep-ocean sediments to atmospheric snow (Xu et al. 2024). Recent clinical research indicates that this pollution has breached human biological barriers; a 2024 global review by Roslan et al., (2024) confirmed the presence of microplastics in major human organs, including the brain, liver, kidney, thyroid, and heart. Specifically, PET has been identified as a dominant polymer in human lung tissue samples, with studies identifying dozens of particles per sample across multiple patients (Wang et al., 2023; Ding et al., 2024). The accumulation of these particles is increasingly associated with severe cellular pathologies, including oxidative stress, chronic inflammation, DNA damage, and systemic tissue injury (Li et al., 2023; Kadac-Czapska et al., 2024; Nihart et al., 2025).

To address this existential threat, research has shifted toward biological upcycling and enzymatic degradation. In 2016, Yoshida et al., identified the bacterium *Ideonella sakaiensis* 201-F6, which secretes a specialized enzyme known as PETase capable of hydrolyzing PET ester bonds. To enhance the efficiency of this process, researchers have engineered variants such as Is-PETase-W185A, which exhibits superior thermostability and binding affinity to PET (Austin et al., 2018). However, the high-level expression of such recombinant proteins often imposes a significant metabolic burden on host cells, leading to "leaky" expression and cellular toxicity. This study utilizes the *E. coli* Lemo21(DE3) strain, which provides a "tunable" expression system regulated by L-rhamnose. While IPTG induces the T7 promoter and L-rhamnose tunes T7 RNA polymerase activity by inducing the production of T7 lysozyme (an inhibitor of T7 RNA polymerase), this system allows for the precise modulation of PETase synthesis, ensuring optimal protein folding and host cell viability (Wagner et al., 2008). Through this engineered approach, we aim to validate the functional degradation of PET fragments, providing a scalable pathway for biological plastic remediation.

MATERIALS & METHODS

Producing Competent Cells

Lemo21 *E. coli* cells were obtained from New England Biolabs (NEB) and grown in LB 5 ml until reaching OD OD_{600} to 0.5. The cells were pelleted at 2000 g, at 4°C for 5 minutes, discarded the supernatant, gently resuspend the cells in 5 ml of ice-cold 100 mM $CaCl_2$, again palleted the cells at 2000 g, at 42°C for 5 minutes, poured off the supernatant, resuspended the cells in 2 ml of ice-cold 100 mM $CaCl_2$, aliquoted 100 µl cells in 20% sterile glycerol and stored at - 20°C for future use.

Cell Transformation

Competent cells were mixed with PETase plasmid DNA obtained from Addgene (Fig 2) kept on ice for 30 min, given a heat-shock at 42°C for 10 seconds (sec), chilled on ice, added 250 µl SOC medium, incubated at 37°C with vigorous shaking using a rotor (200 rpm) for 1 h. 100 µl cells were plated on LB agar + ampicillin (100 µg/ml) and incubated overnight.

Fig 2. Structure of pET-21b(+)-Is-PETase-W185A plasmid

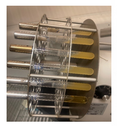

Fig 3. E coli DE3 (Lemo21)culture

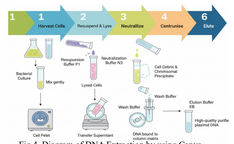

Fig 4. Diagram of DNA Extraction by using Canva

Plasmid DNA Extraction and Agarose Gel Electrophoresis

The pET-21b(+)-Is-PETase-W185A plasmid (Fig 2) was extracted from the transformed *E. coli* Lemo21 DE3 cells using Thermofisher Scientific Plasmid DNA Midi Prep Kit following the manufacturer's instructions. The transformed cells were grown in 4 tubes each containing 5ml LB 100 µg ampicillin/ml, cells were pelleted, supernatant discarded, cells were resuspended, lysed with SDS/NaOH, neutralized, and centrifuged to separate cell debris. The supernatant containing the plasmid DNA was run through a column containing +ve charged resin bound to the silica column, washed twice with a column wash solution containing 70% ethanol, dried out the ethanol, and the plasmid DNA was eluted by adding 100 µl nuclease-free water into the column, centrifuged at 12,000 g for 20 sec. The concentration and the purity of the plasmid DNA was determined using UV/VIS Spectrophotometer at 260nm/280nm (DNA/RNA absorption spectra) (Fig 3). The plasmid DNA size was confirmed using agarose gel electrophoresis in parallel to 1 kb ladder DNA.

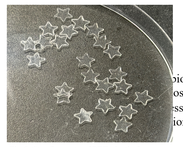

Fig 5. Star figments of PET plastic for biodegradation experiment using PETase transformed Lemo21 (DE3) cells.

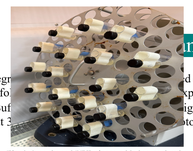

Fig 6. Star shaped PET pieces added to each tube for biodegradation incubated at 37°C in a rotor. .

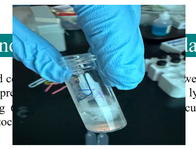

Fig 7. Biodegraded PET star pieces were being washed using distilled water.

Table 1. Protocol for polyethylene terephthalate plastic degradation with engineered *E. coli*.

DAY		A	B	C	D
Day 1	LB Media Free from glucose	N/A	1ml	1ml	1ml
	E. coli (Lemo21)		100 µl	N/A	N/A
	E. coli (Lemo21) contain PETase		N/A	100 µl	100 µl
	Chloramphenicol (30 µg/ml)		0.9 µl	0.9 µl	0.9 µl
	Ampicillin (100µl/ml)		0.5 µl	0.5 µl	0.5 µl
	L- rhamnose (NEB)		0.0 µl	0.0 µl	2 µl, 1,000 µM final concentration
			Culture at 37°C until OD_{600} to 0.4		
	Add 100mM IPTG	N/A	0.4 µl	0.4 µl	0.4 µl
	Culture at 37°C for overnight	☆	YES ☆	YES ☆	YES ☆
Day 2	Added 5 mm diameter PET plastic star pieces.				
		N/A	Centrifuge 12,000 g for 20 sec at 4°C and removed the supernatant		
	Add NEB Express Lysis Reagent and vortex		25 µl	25 µl	25 µl
	Add T4 Lysozyme		1 µl	1µl	1µl
			Incubated at 37°C for 12 hours		
After 7 Days	Wash the star plastic pieces 3x with distilled water.		Washed	Washed	Washed
	Dry at 37°C oven for 20 min.		Dried	Dried	Dried

RESULTS

The results of biodegradation of the PET plastics by PETase expressed by the bioengineered Lemo21 DE3 *Escherichia coli* were determined by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The results and presented below:

Raw PET Negative Control (Untreated), Fig 8

LM and SEM pictures of the untreated PET plastic sample, Table 1, Column A.

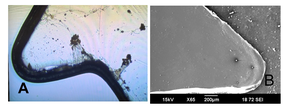

Fig 8. LM and SEM pictures of PET plastic as controls. A, Light Microscopy (LM) magnification to be determined (TBD), and B, Scanning electron microscopy (SEM). A and B do not represent the same arm of the star.

Positive Control – No PETase Expression, Fig 9

The PET plastic sample treated with original Lemo21 DE3 cells as listed in Table 1, Column B. The PET plastic sample retained a uniform, smooth surface similar to the control (Fig 8) indicating that the cells did not inherently express any PETase.

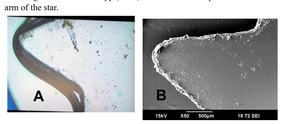

Fig 9. LM (A), magnification TBD and SEM (B) pictures of PET plastics exposed to PETase transformed cell grown in absence of L-rhamnose. A and B do not represent the same arm of the star.

PETase Present Induction with Fig 10.

PET plastic sample treated as in Table 1, Column C. displayed irregularities and localized roughness, suggesting expression of PETase and degradation of PET plastic.

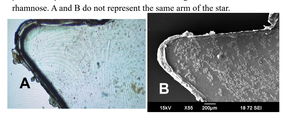

Fig 10. LM (A) mag TBD and SEM (B) pictures of PET plastics exposed to PETase transformed cell grown in absence of L-rhamnose. A and B do not represent the same arm of the star.

PETase Induced with 1,000 µM L-rhamnose, Fig 11.

PET sample treated as in Table 1, Column D demonstrated pronounced surface disruption, including pitting, erosion, and heterogeneous texture formation. SEM imaging confirmed extensive microstructural damage, consistent with active enzymatic hydrolysis of PET polymer chains.

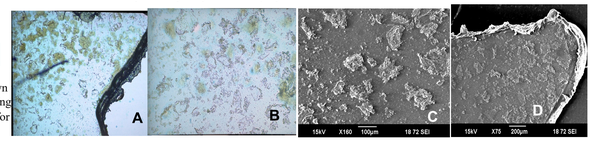

Fig 11. Pictures of the same PET plastic star piece exposed to PETase transformed Lemo 21 in presence of 1,000 µM L-rhamnose. A and B, Light Microscopy (LM). B is 2.5X magnified than A. Magnification undetermined as it was shot from a projection screen, and C and D, Scanning electron microscopy (SEM) with magnification bar. None of the area represent the same arm or location of the star. Both in LM and SEM represents clear degradation of PET plastics greater than or comparable to PET plastic exposed to 100 µM L- rhamnose.

DISCUSSION

This study clearly demonstrated the successful transformation of Lemo 21 DE3 *E. coli* cells as indicated by their resistance to ampicillin and the size of the plasmid determined by agarose gel electrophoresis. These results also demonstrated the L-rhamnose induced the expression of T7 lysozyme expression and thus controlling the T7 RNA polymerase activity followed by induction by IPTG (Isopropyl β-D-1-thiogalactopyranoside) for the expression of the target gene by activating the T7 RNA polymerase, Fig 11.

L-rhamnose in Lemo21(DE3) *E. coli* cells acts as a titratable inducer to modulate T7 lysozyme expression, which in turn tunes the activity of T7 RNA polymerase to optimize protein production. It controls the expression of T7 lysozyme, the natural inhibitor of T7 RNA polymerase, via the *rhaBAD* promoter (Fig 12).

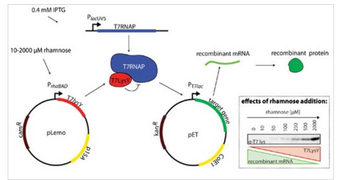

Fig 12. The role of L-rhamnose and IPTG in regulating the expression of the target protein, the PETase, in this experiment (Victrom et al., 2013).

The protein extracts contained a significant amount of PETase that degraded the PET plastics (Fig 10 and Fig 11). The extraction procedure containing NEB Express Buffer and T4 lysozyme were effective in lysing the transformed Lemo 21 cells without affecting the function of the PETase enzyme protein. The PET plastic degradation by engineered bacteria with PETase gene and their mutants obtained from *Idonella sakaiensis* is fully supports our findings (Sevilla et al., 2023).

FUTURE WORK

We are planning to repeat the experiment with varying concentrations for L-rhamnose to determine its optimal and effective concentration. Furthermore, we would like to determine the effect of time in the degradation process. In addition, we plan to find out the effect of transformed intact cells without lysis in PET degradation. We will also examine the combined effect of PETase and MHETase (mono 2-hydroxyethylene terephthalate) in this biodegradation process. Furthermore, PETase and MHETase in combination will produce terephthalic acid (TPA) and ethylene glycol (EG) monomers that can be used to regenerate PET plastics and thus drastically reducing its environmental impacts (Fig 13).

The future experiments will underscore the key principles in genetic engineering and synthetic biology. Our findings does not simply demonstrate PET degradation, but rather reveals the dynamic interplay between gene regulation, enzyme activity, and material properties. The engineered microbial system is a promising and evolving tool for plastic biodegradation, requiring further refinement to overcome inherent biological and material constraints. (Fig 13).
While the current findings establish proof-of-concept for biologically mediated PET degradation, advancing this system toward real-world application requires addressing both biological efficiency and material resistance.

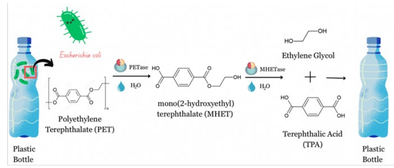

Fig 13: Degradation of PET plastics using PETase and MHETase by Engineered *E. coli* (Jaren Tasmin)

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