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# Biological Adsorption Method to Enhance the Degradation of PET Plastic in the Environment

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***Abstract:*** Plastics have provided many conveniences in our lives, but they also pose a serious threat to the ecological environment. Poly (ethylene terephthalate) (PET) is one of the most abundant synthetic polymers produced and is accumulating in the environment at an alarming rate as discarded packaging and textiles. Recently, a study found that the PET-degrading enzyme (PETase) contained in the bacterium Ideonella sakaiensis 201-F6 can hydrolyze PET, but the enzyme activity is relatively low. In the enzymatic degradation process of PET, the spatial distance between PET plastic and PETase plays a crucial role in the functioning of PETase. This study used the SpyTag-SpyCatcher system and enhanced biofilm to shorten the distance between PETase and PET plastic. Through p-NP analysis, we found that shortening the distance between the enzyme and the substrate can improve the degradation efficiency of PETase. The engineered bacteria achieved effective degradation of PET by optimizing the adsorption and degradation processes in the PETase enzymatic reaction.

***Keywords:*** PET Plastic; Biofilm; PETase

## 1. Introduction

Poly (ethylene terephthalate) (PET) is a kind of synthetic polyester that is currently produced as plastic polymers and is accumulating in the environment at a staggering rate as discarded packaging and textiles [1-2].

Being the most abundantly produced kind of plastic but also being hard to degenerate, it brings out the problem of how to make it both suitable for daily use and produces the least amount of pollution to the environment. PET can be degraded by physical, chemical, or biological methods, and the degradation products can be reused to produce new PET plastics, achieving resource recycling. Biological degradation is currently the most environmentally friendly method for disposing of PET waste [3-4].

Recently, researchers have discovered that a bacterial enzyme known as PETase can effectively degrade PET plastic film. It has been reported that enhancing the adsorption of plastic is crucial for improving the hydrolysis efficiency of high-crystallinity PET (hcPET) by PETase [5]. Biofilms, which possess adsorptive properties, are of particular interest. Escherichia coli produce biofilms through the curli operon, which is regulated by two proteins, OmpR and CsgD [6].

In this work, we have chosen to overexpress OmpR234 to increase the production of E. coli biofilms. Subsequently, we introduced the Spy system, which includes Spy-Tag and Spy-catcher labels, and these were recombined with parts of PETase and OmpR234, respectively, to create the PETase-SpyCatcher and OmpR234-SpyTag systems. These systems aim to facilitate the efficient degradation of PET through an enzymatic process that involves both adsorption and degradation steps. The p-NP analysis shows that the system has the potential to enhance the degradation of PET.

## 2. Materials and Methods

### Chemicals and reagents

The powder of LB substrate and the enzyme DNA polymerase for PCR is from Sangon Biotech. The recombinant expression vector E.coli Rosetta is from Beyotime Biotechnology.

### Genes acquisition and identification

The codon-optimized PETase-SpyCatcher and OmpR234-SpyTag gene sequences were synthesized by GenScript. Plasmids were transferred into E. coli Rosetta (DE3) competent cells. All primers used for identification, which were synthesized by Sangon Biotech.

### Protein Induced Expression

The bacteria were incubated at 37℃ for approximately 2 hours, until the OD600 reached a value of 0.4-0.6. Subsequently, the culture was supplemented with isopropyl β-D-1-thiogalactopyranoside (IPTG) at a concentration of 0.5 mmol/L and continued for an additional 16 hours at 16℃.

### p-NP assay

The enzyme activity of PETase was performed by p-NP assay. We selected p-Nitrophenyl Butyrate (p-NPB) as the substrate, which can be hydrolyzed to p-nitrophenol (p-NP). The concentration of p-NP can be detected by measuring the absorbance at a wavelength of 405 nm (A405) using a microplate reader (BioTek Syner gyH1). Bacteria that express PETase-SpyCatcher and PETase-SpyCatcher+OmpR-SpyTag proteins were separately mixed with 1 mM and 4 mM p-NPB substrates, respectively, and the absorbance was measured at multiple time points.

## 3. Results and Discussion

### 3.1 Bacterial Strains Preparation

The sequences of PETase-SpyCatcher and OmpR-SpyTag were synthesized and cloned into the pET-21a (+) vector by GenScript. The recombinant plasmids were transformed into E. coli Rosetta (DE3) competent recipient cells and used as the subsequent protein expression (Figure 1). The products of PCR amplification were detected by 1% agarose gel electrophoresis, which showed a specific fragment at about respectively 1200 bp and 900 bp and was consistent with the expected result of the full length of the PETase-SpyCatcher and OmpR-SpyTag (Figure 2).

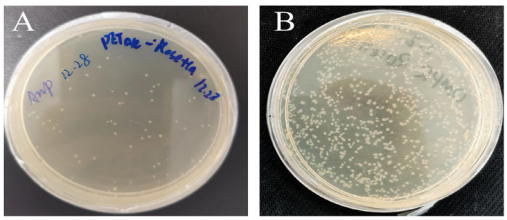


Figure 1 Colonies of PETase-SpyCatcher_pET-21a (+) (A) and OmpR-SpyTag_pET-21a (+) (B).

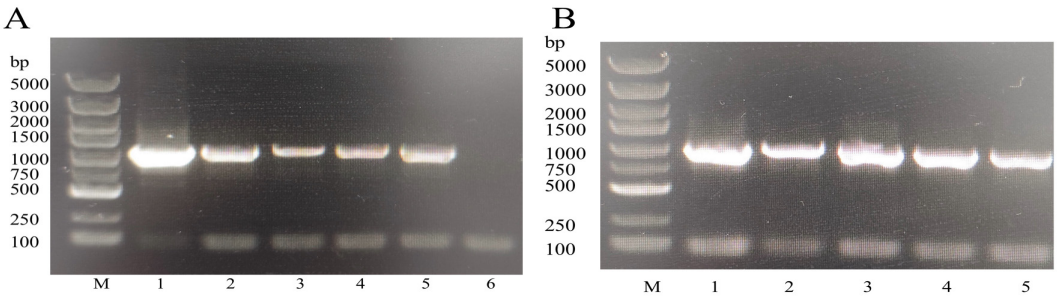


Figure 2 Colony PCR identification. (A) Validation of PETase-SpyCatcher_pET-21a (+). M: DNA Marker; 1: positive control (PETase-SpyCatcher_pET-21a (+) plasmid); 2 to 5: Colonies transformed with PETase-SpyCatcher_pET-21a; 6: negative control. (B) Validation of OmpR-SpyTag_pET-21a (+). M: DNA Marker; 1: positive control (OmpR-SpyTag_pET-21a (+) plasmid); 2 to 5: Colonies transformed with OmpR-SpyTag_pET-21a (+).

### 3.2 Protein Expression

SDS-PAGE electrophoresis was conducted to assess the expression of PETase-SpyCatcher and OmpR-SpyTag proteins. As shown in Figure 3, the presence of a band in the lane corresponding to PETase-SpyCatcher (40 KDa) and OmpR-SpyTag (27 KDa) suggested success-

ful expression of these two proteins, in comparison to the controls without IPTG induction.

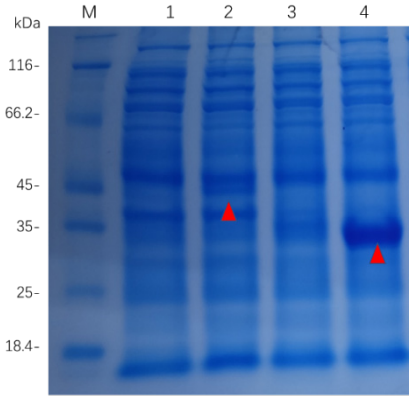


Figure 3 Expression of PETase-SpyCatcher and OmpR-SpyTag. M: Protein marker; 1: Expression of PETase-SpyCatcher without IPTG induction; 2: Expression of PETase-SpyCatcher with IPTG induction for 16 h at 16 ℃; 3: Expression of OmpR-SpyTag without induction; 4: Expression of OmpR-SpyTag with IPTG induction for 16 h at 16 ℃.

### 3.1 Activity Test

The enzymatic activity of PETase was evaluated using p-NP assay. Solutions of bacteria expressing PETase-SpyCatcher and PETase-SpyCatcher+OmpR-SpyTag proteins were respectively mixed with different concentrations of p-NPB substrates, followed by measurement of absorbance at a wavelength of 405 nm. Figure 4 shows that over time, the OD405 value increases for both the single PETase-SpyCatcher system and the system containing both PETase-SpyCatcher and OmpR-SpyTag proteins. The OD405 values for the co-expression system are significantly higher than those for the single PETase-SpyCatcher. In other words, with the help of the enhanced biofilm, the degradation activity of PETase can be increased. It is possible that the proximity effect between the substrate and the enzyme is one of the reasons for the improved degradation efficiency.

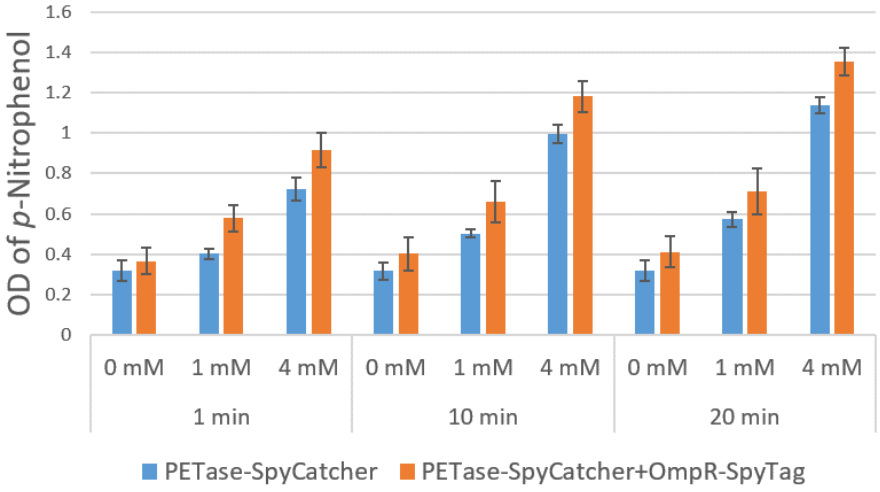


Figure 4 Activity Test of PETase-SpyCatcher and PETase-SpyCatcher+OmpR-SpyTag

## 4. Conclusion

This study investigates on the potential influence of spatial distance on the degradation rate of PETase during the degradation of PET plastics. We aim to reduce the distance between PETase and PET plastics by utilizing the SpyTag-SpyCatcher system and enhanced biofilm technology. Through p-NP analysis, we found that the modified bacteria achieved effective degradation of PET by optimizing the adsorption and degradation processes in the PETase enzymatic reaction. This discovery offers a novel approach to addressing the issue of PET plastic accumulation in the environment.

Future research can continue to explore other factors affecting PETase activity, such as temperature and pH, to achieve higher efficiency in PET degradation. Additionally, studying the relationship between the structure and function of PETase at the molecular level and optimizing its activity through genetic engineering techniques could be pursued. Furthermore, exploring other microorganisms and enzymes with potential PET degradation capabilities could enrich degradation strategies and provide more possibilities for solving the problem of PET plastic pollution.

In summary, this study provides an effective method for the biodegradation of PET plastics and has certain application prospects. Using SpyCatcher and SpyTag to attach OmpR to combine with PETase can increase the rate of enzyme degradation, and to also promote plastic conservation.

## References

[1] Santos, R. G., Machovsky-Capuska, G. E. & Andrades, R. (2021). Plastic ingestion as an evolutionary trap: toward a holistic understanding. Science 373, 56–60.

[2] Brandon, C. Knot et al. (2020). Characterization and engineering of a two-enzyme system for plastics depolymerization. Proc. Natl. Acad. Sci. U.S.A. 117 (41), 25476–25485.

[3] Kawai, F., Kawabata, T. & 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.

[4] Ellis, L. D. et al. (2021). Chemical and biological catalysis for plastics recycling and upcycling. Nat. Catal. 4, 539–556.

[5] Z., Chen et al. (2022). Biodegradation of highly crystallized poly (ethylene terephthalate) through cell surface codisplay of bacterial PETase and hydrophobin. Nat. Commun. 13 (1): 7138.

[6] Michelle, M. Barnhart & Matthew, R. Chapman (2006). Curli biogenesis and function. Annu. Rev. Microbiol. 60, 131-47.