Establishing Kinetic Baselines for Evaluating Engineered Fast-PETase Variants Expressed in *Escherichia coli*

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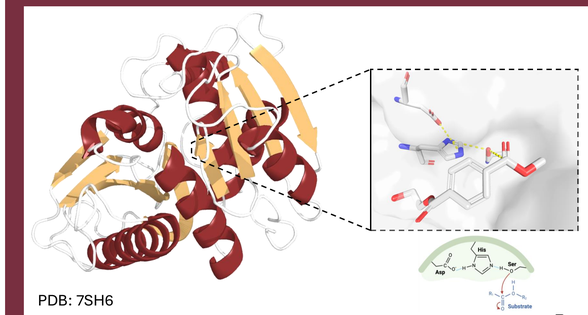
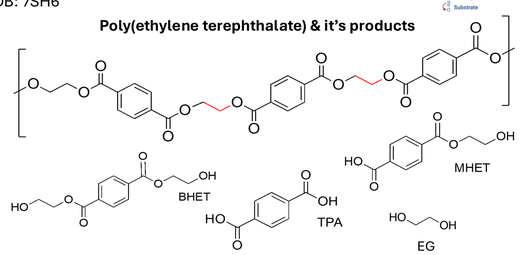
ABSTRACT

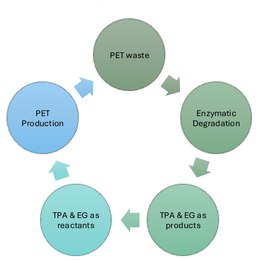
Polyethylene terephthalate (PET) plastic pollution and waste accumulation continues to pose significant environmental concerns, driving the engineering of PET hydrolases with enhanced catalytic performance to better address industrial recycling efforts. Fast-PETase, a PET hydrolase variant, has been shown to exhibit increased thermostability and catalytic efficiency when compared to the wildtype *Is*-PETase, making it one of the most promising engineered enzymatic variations. Despite ongoing research into the specific activity, inconsistent assay conditions and the absence of standard kinetic benchmarks limit quantitative comparisons between engineered variants. In this study, we establish kinetic baselines for Fast-PETase to serve as a foundation for future comparative analyses. Fast-PETase was expressed, purified, and kinetically characterized under systematically varied temperature and pH. Activity was quantified by measuring soluble hydrolysis products using ultraviolet-visible spectroscopy. Establishing standardized temperature, pH, and temporal benchmarks strengthens reproducibility and enables quantitative evaluation of catalytic enhancements in subsequent protein engineering efforts.

INTRODUCTION



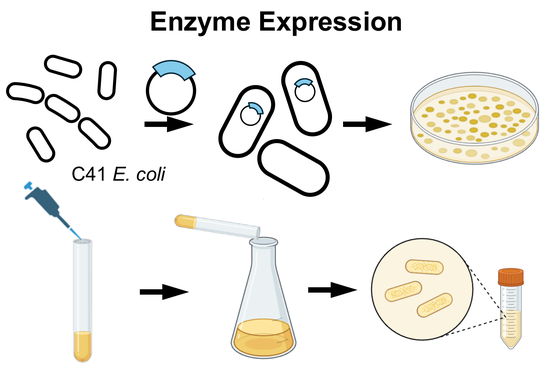
Poly(ethylene terephthalate) & it's products



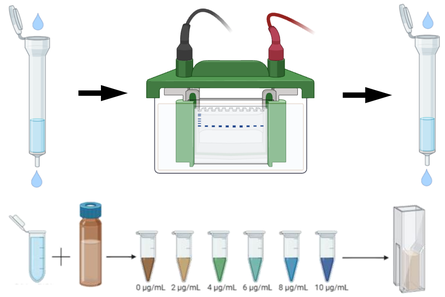


METHODS

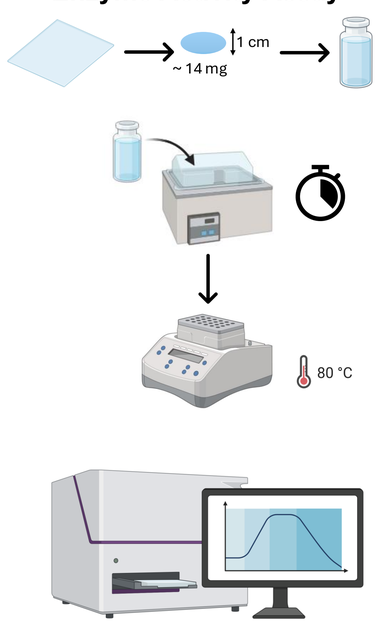
Enzyme Expression



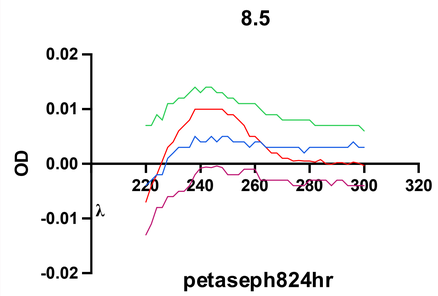
Enzyme Purification & Quality Control

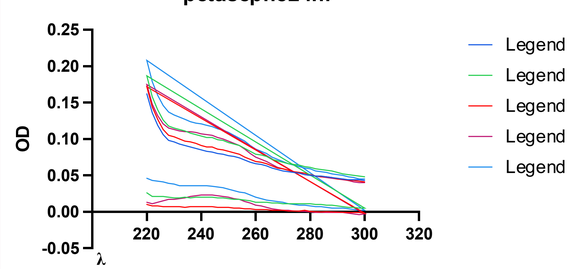


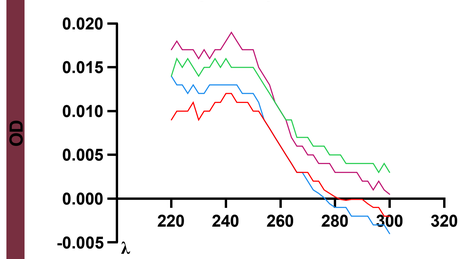
Enzyme Activity Assay

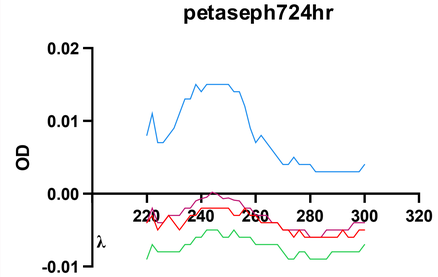


RESULTS

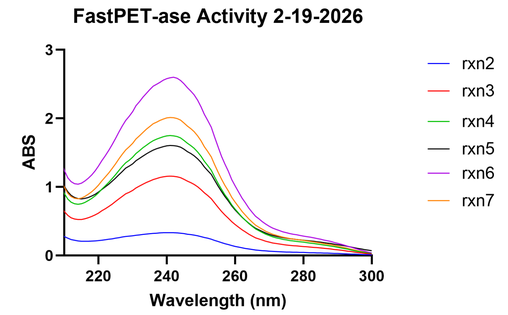


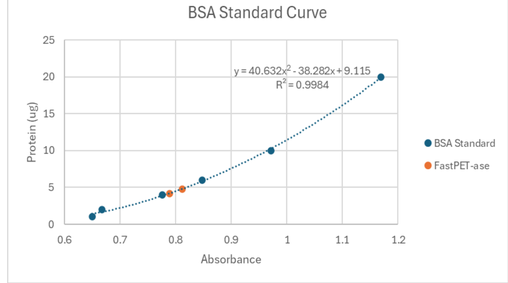






RESULTS





CONCLUSIONS

The data collected suggests that the FastPET-ase enzyme was only able to minimally degrade the PET plastic used in this experiment. One possible explanation for this is that the PET film may have undergone surface modification, such as corona treatment or other chemical treatments during manufacturing. These treatments can oxidize or alter the outer molecular layers of the polymer, potentially reducing enzyme binding and limiting access to ester bonds that PETase normally hydrolyzes. As a result, the enzyme's ability to break down the plastic may have been inhibited. Although the UV–Vis spectra show only a small signal at approximately 240 nm, a detectable peak is still present. This indicates that a small amount of degradation product was formed during the reaction. Therefore, while the extent of degradation was limited, the presence of the peak suggests that PETase was still able to catalyze some hydrolysis of the PET substrate.

REFERENCES

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