Computational Modeling and Experimental Analysis of DuraPETase, a Thermostable Variant of PETase Plastic Degrading Enzyme

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ABSTRACT

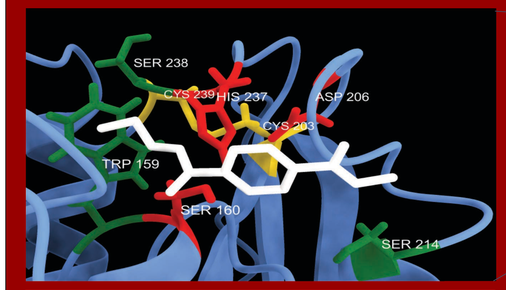
Waste created by fossil-fuel-based single-use plastics grows tremendously each year. Global statistics show production has reached 367 million metric tonnes as of 2020. The effects of the global pandemic, including facial masks, testing kits, packaging etc., have caused the production of single-use plastic items to skyrocket. While necessity has stimulated the creation of new companies for plastic production technologies, it is noted that the creation of recycling/degradation technologies of used plastics is lagging behind. Plastic ends up either in landfills or seeping into nature because plastic waste outpaces the capacity that exists to control it. While recycling may provide a solution to reuse some plastics, another solution to eliminate this environmental pollutant is microbial biodegradation.

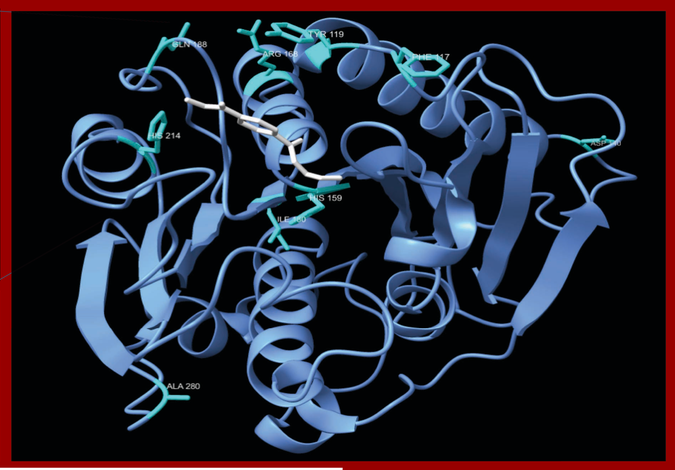
Through microbial biodegradation, plastics are degraded into simple monomers by microbes so that they may be naturally utilized by such organisms and be reincorporated into nature's carbon cycle. Studying the most commonly used plastics, it is noted that Polyethylene terephthalate (PET), used most commonly in water bottles, can be degraded by the PETase enzyme from the bacteria, *Ideonella sakaiensis*. Mutagenesis studies have produced a thermostable PETase called DuraPETase, which offers optimized degradation potential in organisms that produce it[1].

Our project attempts to understand DuraPETase's properties through bioinformatics tools and through wet-lab techniques of expressing DuraPETase using a pET expression vector, isolating and purifying the enzyme, and testing its ability to degrade PET and other common plastics. Research into this field is vital to discover new mitigation methods of plastic pollution.

BIOINFORMATICS DATA COLLECTION AND ANALYSIS

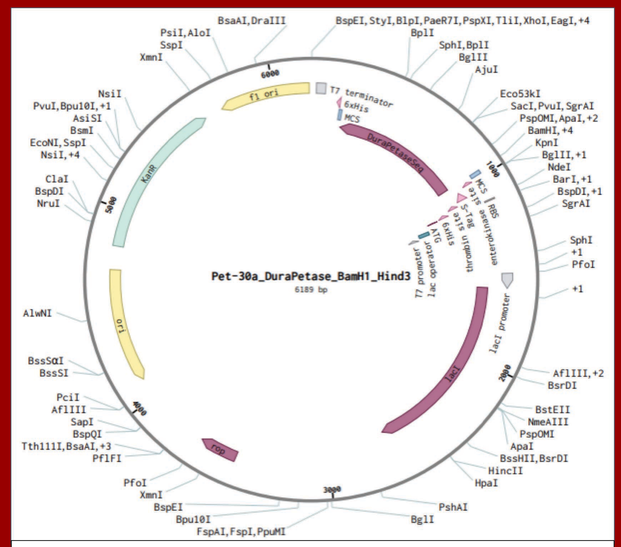
Based on results from NCBI E-utilities, automated literature searches expand upon a previously curated list of known hydrolases, esterases, lipases, etc. with the goal of identifying new enzymes and organisms (mainly fungi) with different plastic degrading potential. Beyond data collection from literature, BLAST searches based on PETase and other plastic degrading enzymes were performed to expand potential enzymes for studies. Research supported that while PET, HDPE, and LDPE plastics had been well-documented to be targeted by several different enzymes and microorganisms, other heavily used plastic such as PVC, PP, and PS have some uncharacterized enzymes associated with degradation. White-rot fungi, *Aspergillus niger* fungi, and *Pseudomonas aeruginosa* bacteria were identified as high potential multi-plastic degraders during the collection, with enzymes yet to be characterized through any experimental procedure. Peer-reviewed literature also revealed a novel thermostable variant of PETase, named DuraPETase, which was created with predicted advantageous substitutions from the GRAPE strategy and maintained higher degradation potential due to its temperature stability.[1] Consensus information produced from MSA alignments of other PETase variants, supplemented with PDB information, assisted in noting important structural components of DuraPETase.





Above: Close-up of PETase Binding Pocket
Green: Binding Site, **Red**: Active Site, **Yellow**: Disulfide Bridge, **White**: 2-Hydroxyethyl methyl terephthalate

Right: DuraPETase Structure Including Mutations from Original PETase
Cyan: Mutations - L117F, Q119Y, T140D, W159H, G165A, I168R, A180I, S188Q, S214H, R280A



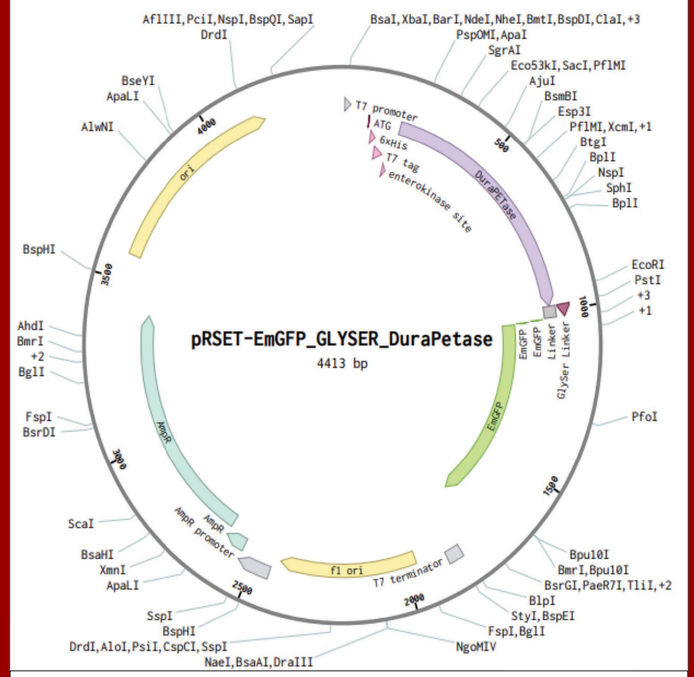
pET30 Plasmid with DuraPETase Recombinant

PROTEIN STRUCTURE MODELING

Protein sequences with no characterized structures were viewed in Modeller[2], a protein structure prediction program that created models based on homologous templates. ChimeraX/Chimera[3] served as protein visualization tools vital in annotating differences between the original enzyme (PDB: 6EQE) and the DuraPETase variant (PDB:6ky5). Active sites (His237, Ser160, Asp206), disulfide bridges(Cys 203, Cys 239), and other important binding sites (Ser 214, Ser 238, Trp 159) found in almost all PETase variants were visualized. Structural Analysis tools were then applied to overlay both structures for identification of sequence differences and their effect on the structure. The research performed inspired the concept of introducing DuraPETase into fungi degraders to heighten their degradation potential and observe their interactions with other plastics.

FUTURE RESEARCH

After both expression systems have been ligated with the DuraPETase gene and expressed in cells, the DuraPETase enzyme will be tested for its degradation ability. The method chosen will have to have to be very precise as the degradation rate of these biodegradable enzymes are slow. One proposed method is to test for the metabolites of PET degradation using chromatography. Once a reliable a method for detecting degradation has been proven, more organisms and enzymes can be tested such as white-rot fungus. The workflow process being developed is critical to the study of plastic degrading enzymes. When these plastic degrading enzymes can be efficiently expressed and tested, research can continue into various enzymes and species.



pRSET Plasmid with DuraPETase Recombinant

EXPERIMENTAL

The objective of the experimental analysis was to successfully express DuraPETase and test its degradation. To express the enzyme, two vector systems will be used: pET30 and pRSET. A plasmid with the DuraPETase gene was designed and ordered containing sites for the restriction enzymes: BamHI, HindIII, and NcoI. Three restriction sites were designed so both expression vectors could be used. pET30 has complimentary sites for BamHI and HindIII while pRSET has complimentary sites for BamHI and NcoI. The plasmid also had a glycine-serine linker before the NcoI site because of a green fluorescent protein (GFP) gene in pRSET. This allows the transformation to be qualitatively analyzed. Both expression systems are induced under IPTG.

The plasmid was transformed into DH5α cells. These cells were cloned and their DNA was extracted. Gel electrophoresis confirmed the presence of the plasmid in each sample and a restriction enzyme digest (pictured below) confirmed the restriction sites worked. The slight difference between the NcoI and HindIII digests represents the small number of bases difference in their location on the plasmid.

Gel Electrophoresis Restriction Enzyme Digest

Lanes 1, 4 & 6: HyperLadder
Lane 2: IDT plasmid digested with BamHI & NcoI
Lane 4: IDT plasmid digested with BamHI & HindIII
Lane 5: Uncut IDT plasmid

CONSIDERATIONS

Consideration should also be explored into what the effect of a large-scale plastic breakdown would be. With microplastics already found in organisms, including humans, care must be taken when furthering this breakdown process. For example, there are some findings that show these by-products can activate certain hormone receptors in humans. Care must also be taken to limit plastic degradation to only intended targets that match the enzymes specificity pocket.

REFERENCES

1. Yinglu et al. **Computational Redesign of a PETase for Plastic Biodegradation under Ambient Condition by the GRAPE Strategy**. ACS Catalysis 11 (3), 1340-1350, 2021, https://doi.org/10.1021/acscatal.0c05126
2. **UCSF Chimera--a visualization system for exploratory research and analysis**. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.
3. A. Šali and T. L. Blundell. **Comparative protein modelling by satisfaction of spatial restraints**. J. Mol. Biol. 234, 779-815, 1993.
4. J. Kaushal, M. Khatri, and S. Arya. **Recent insight into enzymatic degradation of plastics prevalent in the environment: A mini - review**,Cleaner Engineering and Technology, Volume 2, 2021. https://doi.org/10.1016/j.clet.2021.100083