ABSTRACT

Waste created by fossil-fuel-based single-use plastics grows tremendously each year. Global statistics show production of single-use plastics 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 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 fungus, 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.

EXPERIMENTAL

The objective of the experimental analysis was to successfully express DuraPETase and test its degradation. To express the enzyme, two vector systems were 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 glycin-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.

REFERENCES


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

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