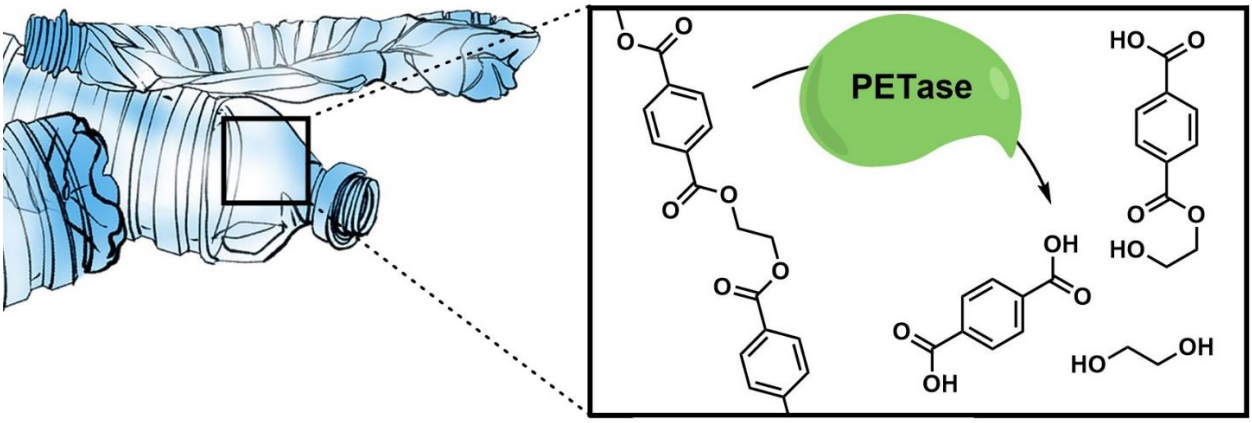
BCHM 421/422 Project – 2022-23

Project Title: Identification and optimization of novel plastic degrading enzymes from thermophilic bacteria

Project Outline: Plastics are a double-edged sword. The chemical inertness that makes these polymers so useful as containers is the same feature that makes plastic waste so pervasive and pernicious. Despite our best efforts, only 9% of plastics are recycled in Canada, with nearly 3 million tons of plastic waste going into landfills every year and 30,000 tons of this waste leaking into the environment. Similarly distressing rates of plastic pollution are found around the world, and it is now estimated that the oceans now contain 1 kg of plastic waste for every 5 kg of fish. Clearly, more efficient plastic recycling technologies are desperately needed. With the introduction of vast quantities of plastic wastes into most ecosystems, it is not entirely surprising that some organisms have apparently evolved the ability to metabolize plastics. This project is focused on the identification of the enzymes involved in the metabolism of plastic polymers and the subsequent optimization of these enzymes as industrially useful biocatalysts. Initial efforts will focus on the discovery of novel PETases – enzymes that breakdown polyethylene terephthalate (PET) into constituent monomers. PET is a major component of plastic soda and water bottles and makes up a significant component of the global plastic waste stream. While several known PETases have already been characterized, many of these enzymes are poorly active and/or insufficiently thermostable for industrial applications. We will use these known enzymes as seed sequences for genome mining efforts to identify PETase variants from thermophilic organisms in an attempt to find more thermostable PETases. Following characterization of these new enzymes, we will employ directed evolution to develop PETases with enhanced catalytic activities that will be amenable to large-scale industrial recycling efforts.



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Project Goals:

- Identify new PETase variants from thermostable organisms
- Clone, heterologously overexpress, and purify putative PETases
- Confirm catalytic activities of putative PETases using *in vitro* assays

- Engineer a variant PETase with high catalytic activity *and* thermostability

Experimental Approaches:

- Bioinformatics (BLAST, EFI-EST, InterPro, Clustal Omega, Phyre2.0, etc.)
- Molecular biology (cloning, PCR, gel electrophoresis, protein expression/purification, etc.)
- Enzymology (*in vitro* kinetic assays, thermal shift assays, site-directed mutagenesis, directed evolution)
- Organic/green chemistry (flash chromatography, distillation, switchable solvents, etc.)