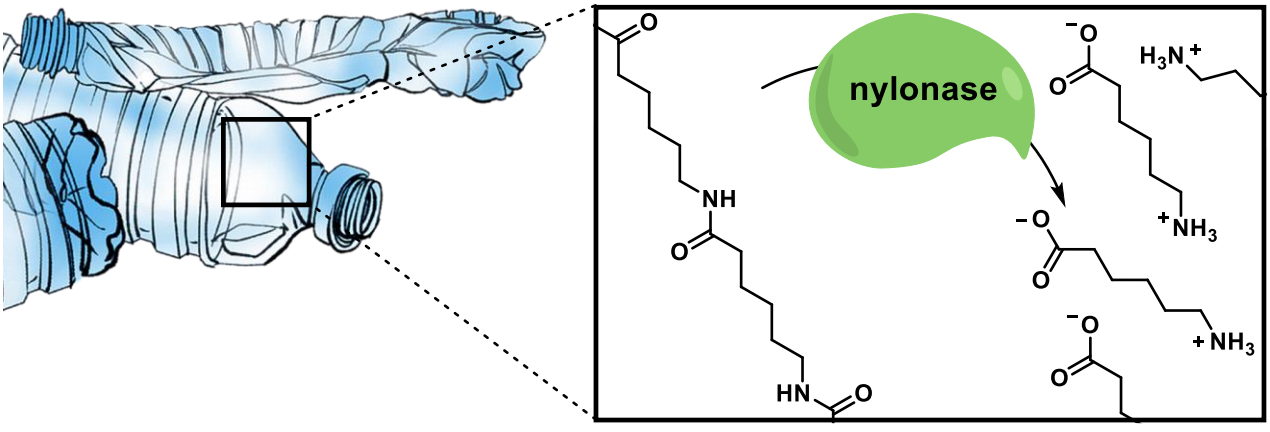
BCHM 421/422 Project – 2023-24

Project Title: Identification and optimization of novel nylon 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 nylon polymers and the subsequent optimization of these enzymes as industrially useful biocatalysts. Initial efforts will focus on the discovery of novel nylonases – enzymes that breakdown polyamides into constituent monomers. Nylon is a major component of fabrics and fibers, molded plastics, and in films used in food packaging. The utility of this thermoplastic has led to approximately 5 million tons being produced every year. Clearly, nylon makes up a significant component of the global plastic waste stream. While there are a few nylonases 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 nylonase variants from thermophilic organisms to find more thermostable enzymes. Following characterization of these new enzymes, we will employ directed evolution to develop nylonases with enhanced catalytic activities that will be amenable to large-scale industrial recycling efforts.



Supervisor: Dr. Graeme Howe

Project Goals:

- Identify new nylonase variants from thermostable organisms
- Clone, heterologously overexpress, and purify putative nylonases

- Confirm catalytic activities of putative nylonases using *in vitro* assays
- Engineer a variant nylonase 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.)