Identification and Characterization of Potential Plastic-Degrading Enzymes in Microbial Genomes

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BASS CONNECTIONS
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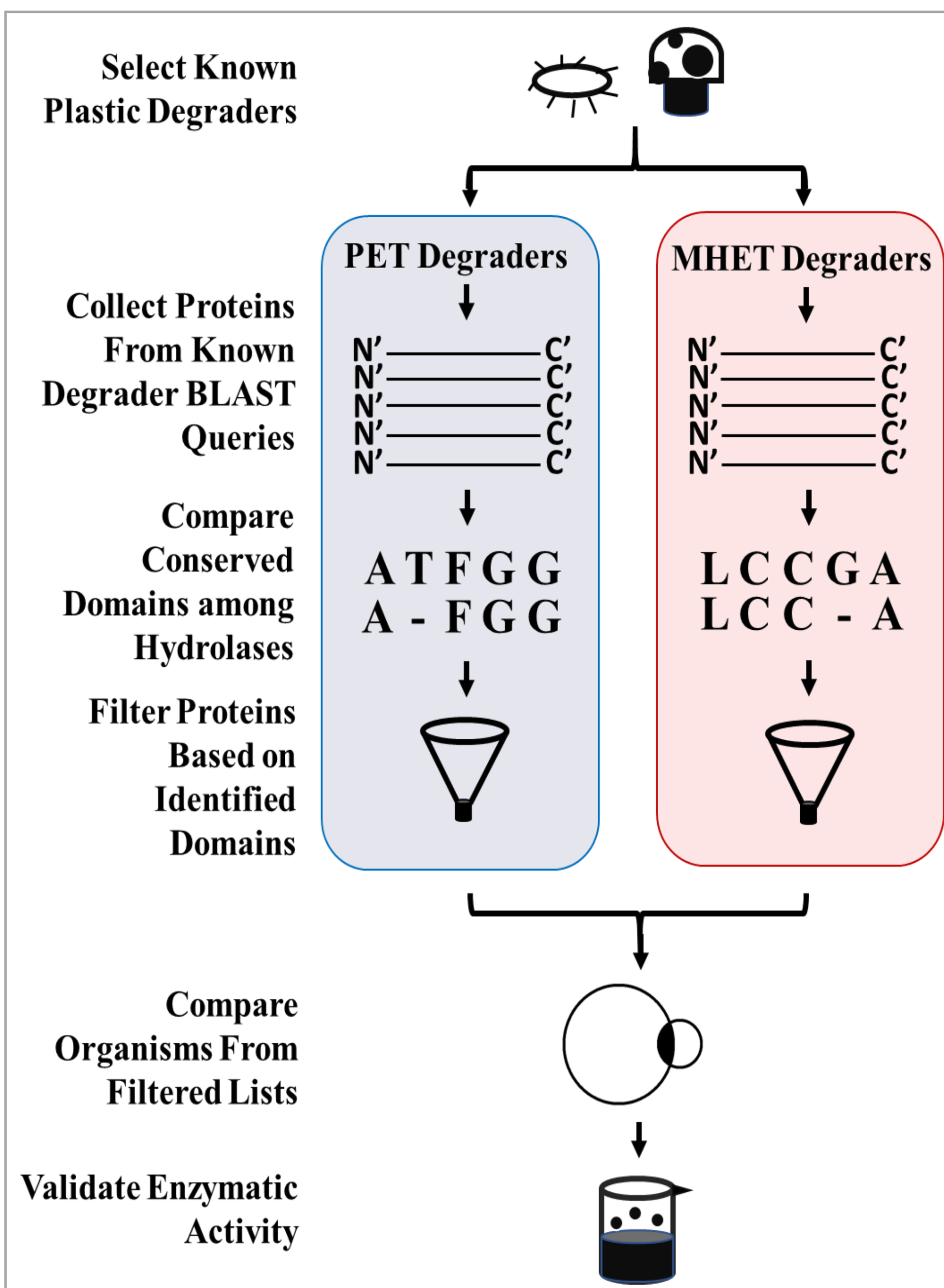
Introduction

- Plastic is ubiquitous in our daily lives. From its uses in medicine to its applications in packaging and manufacturing of consumer products, the material has become an essential part in almost every industry.
- A prominent plastic polymer found in beverage containers, films, fibers, and other items is polyethylene terephthalate (PET).
- Only 26.8% of PET bottles and jars were predicted to have been recycled in 2018 (EPA, 2020).
- With the large presence of plastics, there is a need to remove the current plastics from the environment and halt the production of new plastics.
- The bacterial species *Ideonella sakaiensis*, discovered in landfills, has become a particular species of interest after it was found capable of growing on PET and using the polymer as both an energy and carbon source at room temperature (Yoshida et al., 2016).
- Two enzymes were identified to be important in PET degradation for *I. sakaiensis*: polyethylene terephthalate hydrolase (PETase) and monohydroxyethyl terephthalate hydrolase (MHETase).
- Other bacterial species such as *Thermobifida fusca* and *Thermobifida halotolerans* have been identified to also have alpha/beta hydrolase proteins able to degrade PET (Kawai et al., 2019).
- These known protein PET degraders share functional domains vital to their activity.
- *We hypothesized that publicly-available databases contain protein sequences from microbial species with potential plastic-degrading capability.*

Methods



Results

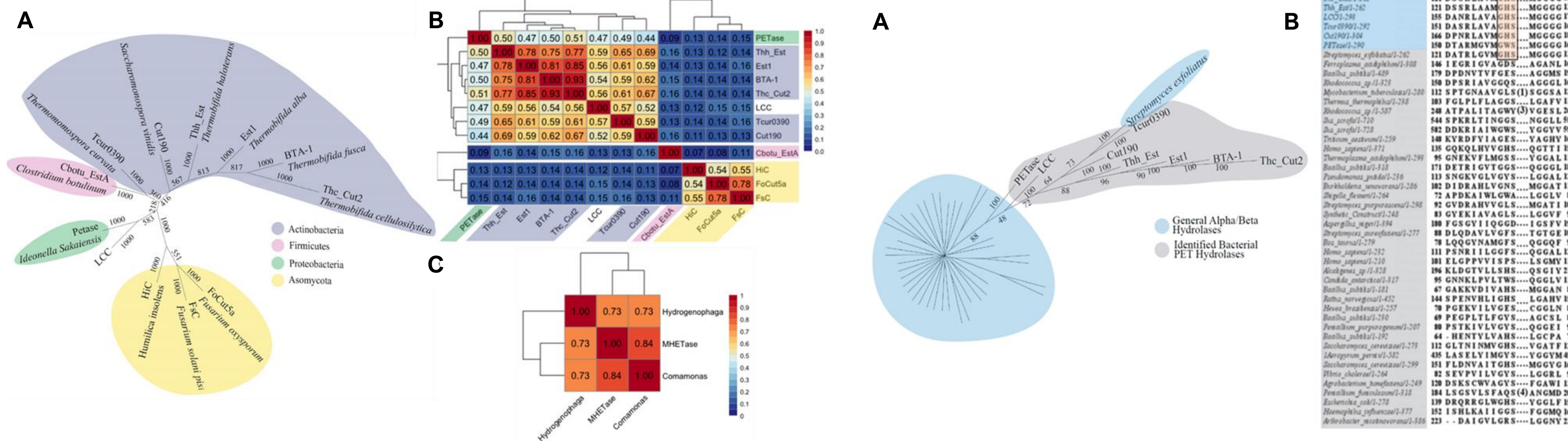


Figure 1. Initial selection of known plastic-degrading query enzymes or associated proteins. (A) Unrooted maximum likelihood phylogenetic tree of the nine bacterial and three fungal enzymes selected as initial plastic degrader queries for this study. (B) Percent identity heat map of the nine bacterial species and the three fungal species serving as queries. (C) Percent identity heat map of the three MHET degraders chosen in the study

Figure 3. Known PET degraders compared to general alpha/beta hydrolases. (A) Phylogenetic tree of known bacterial PET hydrolases and general alpha/beta hydrolases. Using previous research studies, the general hydrolases were chosen as representatives for the alpha/beta hydrolase families. (B) Multiple sequence alignment of PET hydrolase and general hydrolase list. I evaluated the conserved nature of the GXSMGGGG motif.

- I identified a total of nine bacterial species and three fungal organisms that contained PET hydrolases. I also selected three known MHET degraders: *Ideonella sakaiensis*, *Comamonas* sp., and *Hydrogenphaga* sp. (**Figure 1**; Knott et al., 2020).
- From the BLAST searches, a total of 33,601 proteins were identified to be similar to the known chosen bacterial PET hydrolases. Of the fungal protein searches, I collected 11,678 proteins.
- The three MHET searches resulted in a total of 18,855 proteins.
- All selected bacterial proteins aligned at their oxyanion holes and eight of the nine proteins aligned to the catalytic triad and the GXSMGGGG motif (**Figure 2A**).
- A general alpha/beta hydrolase, *Streptomyces exfoliatus*, was grouped among the PET degraders, however the PET degraders were distinct from the majority of general alpha/beta hydrolases (**Figure 3**).
- A total of 2,327 proteins aligned to the three (i.e., the catalytic triad, oxyanion hole, and the GXSMGGGG motif) domains. Of these specific domain-containing proteins, I identified 1,141proteins that were greater than or equal to 50% identical to the original query (**Figure 4**).
- The sequences collected from the MHET degrader proteins did not align to the functional domains used in their filter.
- I identified one organism, *Pseudomonas stutzeri*, to have a protein in the PET Degrader BLAST list that aligned to three domains and were greater than or equal to 50% identical (72.408%) to the original query and also contained a protein in the MHET Degrader BLAST list greater than 50% identical to original query (**Figure 5**).

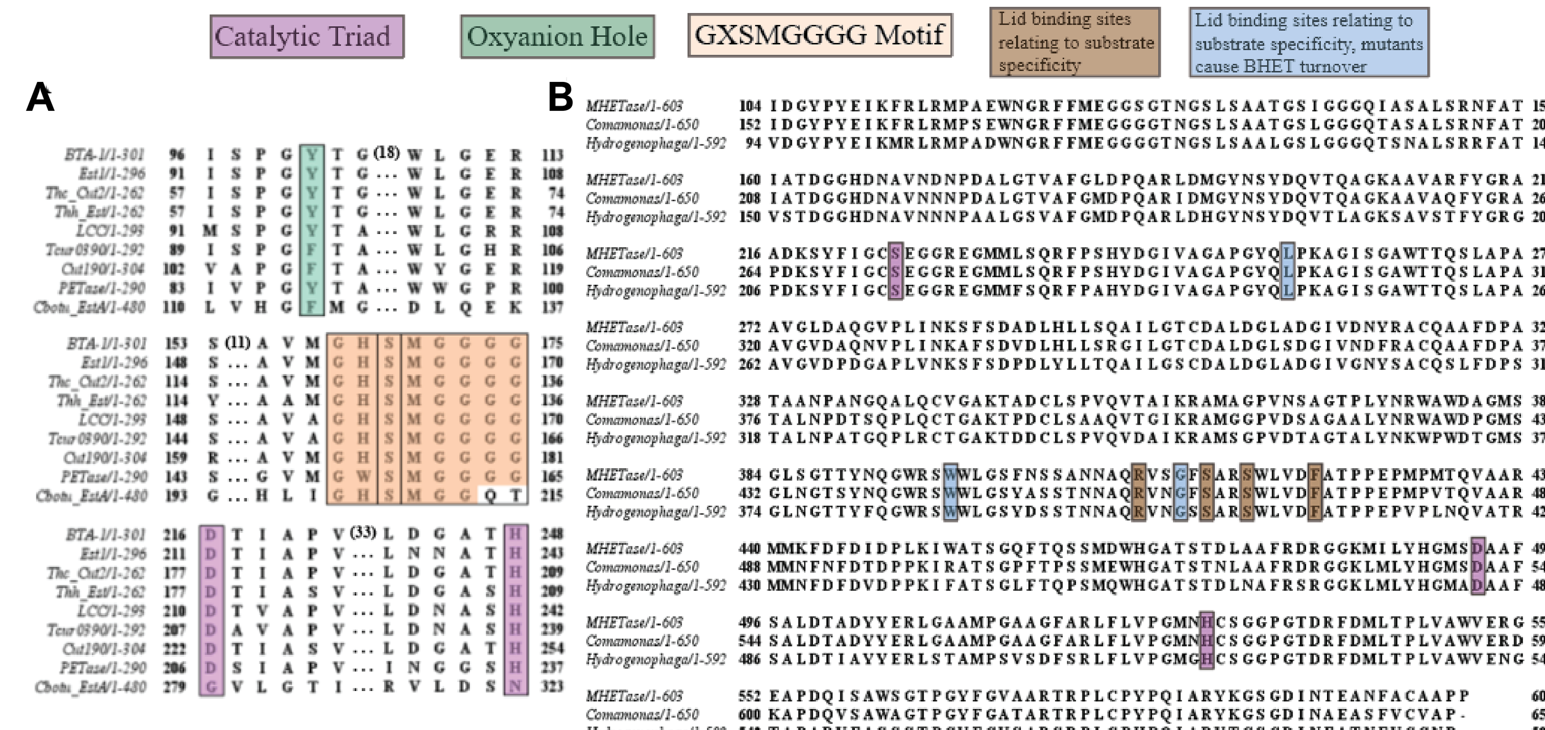


Figure 2. Functional sites and motif selection of PET and MHET hydrolases. (A) Multiple sequence alignment of known plastic degraders from bacterial species. Domains shown include the catalytic triad, oxyanion hole, and the GXSMGGGG motif. Functional sites were identified based on locations identified in previous research. (B) Multiple sequence alignment of the 3 known MHET degrading enzymes

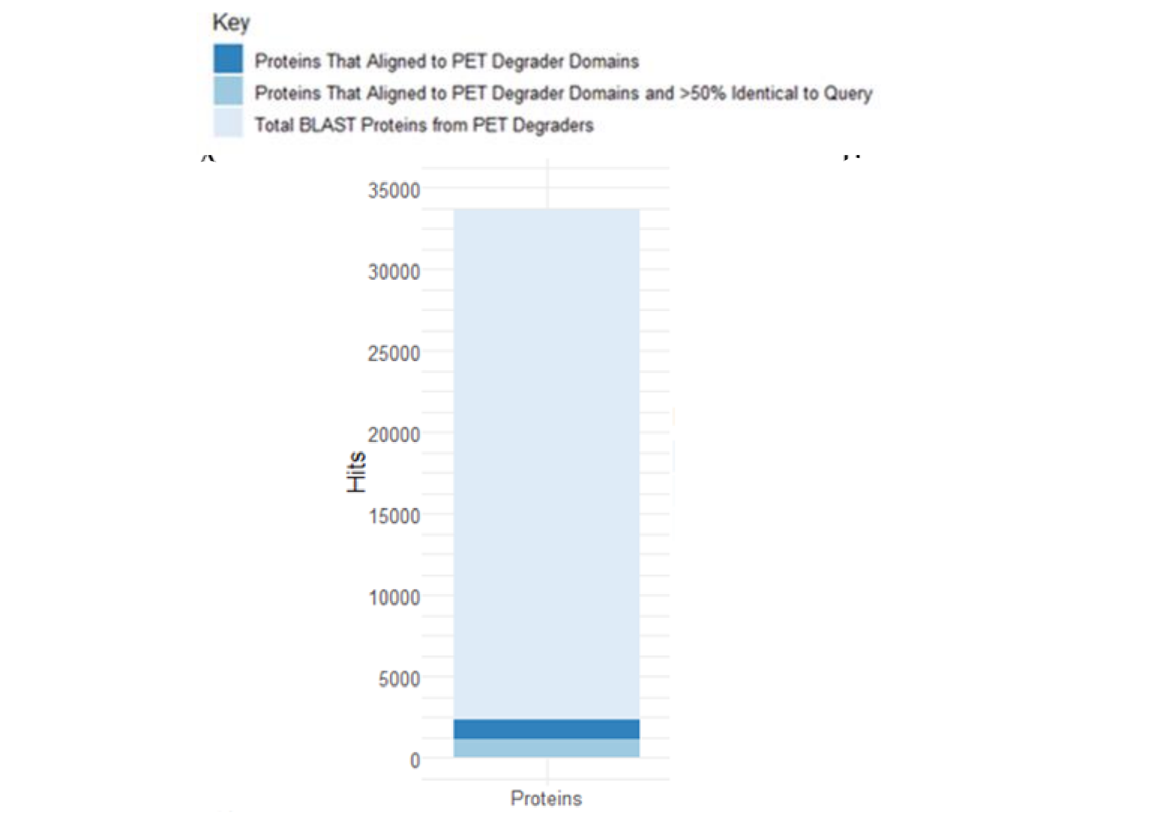


Figure 4. Identification of proteins containing PET degrading functional domains that were deemed important to plastic degradation. Resulting proteins originated from all three initial hydrolase query lists.

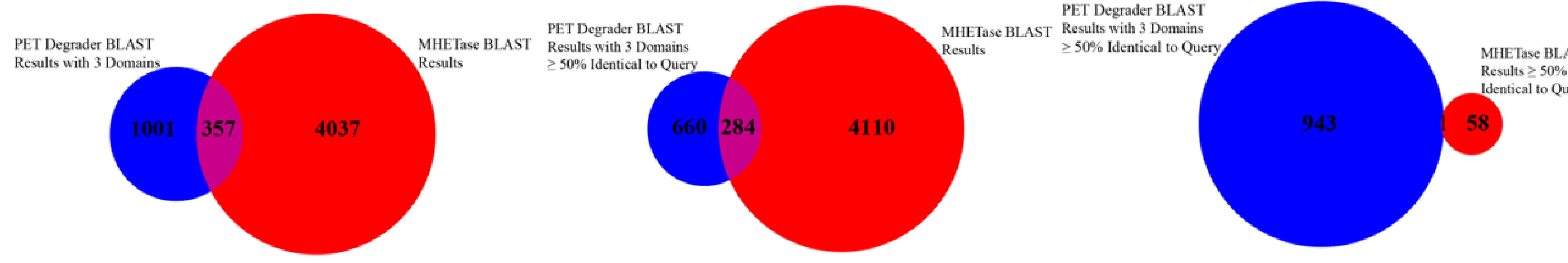


Figure 5. Organismal overlap between proteins from the PET degrader and MHET degrader BLAST searches. With no results from the MHET degrader filter, we were limited to the initial list.

Discussion

- I observed consensus across all three domains for the PET degraders from bacterial species excluding Cbotu_EstA due to the protein being 176 amino acids larger than the second largest sequence.
- The alignment among all lid-domains of the MHET degraders indicates an importance for such amino acids and their placements.
- I was able to reveal a more significant relationship between the GXSMGGGG motif and PET degradation by highlighting the uniqueness of the motif via the created multiple sequence alignment.
- Although the hydrolase from *Streptomyces exfoliatus* aligned with the GXSMGGGG motif, it has previously been identified to function similar to a cutinase as well as degrade another type of polyester (Kawai et al., 2019).
- The findings suggest a possible uniqueness of the ability to degrade MHET compared to the hydrolysis of PET. There is also a possibility that the PET degrader filter was not as strong as that of the MHET degrader filter.
- *Pseudomonas stutzeri* has previously been identified to degrade polyethylene glycol (Obradors & Aguilar, 1991).
- Future studies will need to explore the relationship among these fungal species.

Literature Cited

Kawai, Fusako, Takeshi Kawabata, and Masayuki Oda. "Current knowledge on enzymatic PET degradation and its possible application to waste stream management and other fields." *Applied Microbiology and Biotechnology* 103.11 (2019): 4253-4268.

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