Evolutionary Analysis of Plastic-Degrading Enzyme Polyethylene Terephthalate Hydrolase (PETase) in the Endophytic Microbiome of Viridiplantae for Phytoremediation

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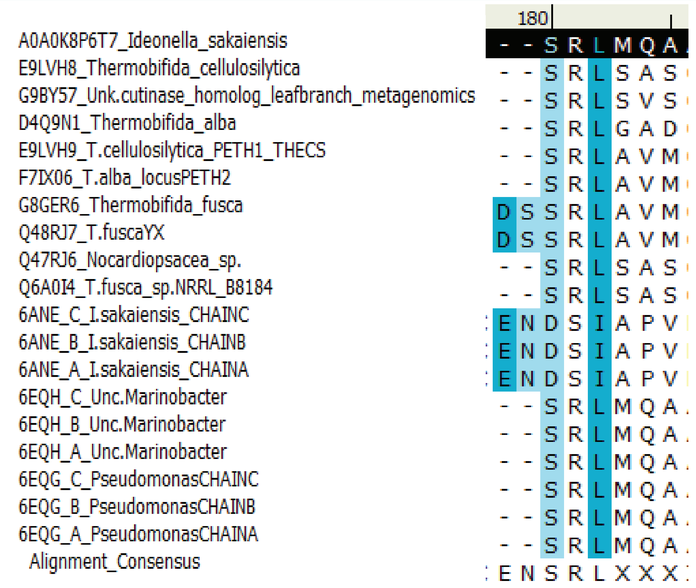
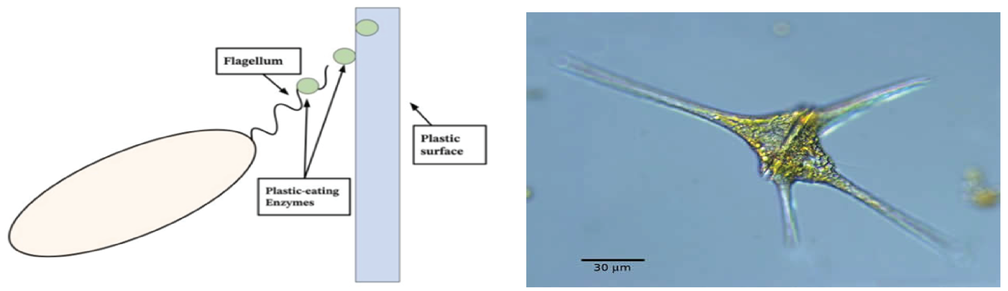
INTRODUCTION

Polyethylene terephthalate (PET) is a clear plastic designed for single-use consumer packaging. PET plastics are 100% recyclable but only 31% of PETs are recycled[1]. These durable plastics do not decompose for up to 450 years in disposal facilities, resulting in the clogging of natural landscapes with phthalate leaching litter. *Ideonella sakaiensis*, a bacteria discovered in 2016 in the sludge a plastic-recycling facility in Japan, exhibited successful decomposition and metabolization of PETs by binding to the surface of plastic consumer products and delivering enzymes to catabolize the plastic into its original structural components[2]. The discovery of PETase in fungi, *Pestalotiopsis microspora,* by Yale in 2008 opened the door for new models of plastic degradation[3]. By exploiting the lineage between fungi, endophytes, and marine plants with endophytic microbiomes, we could determine if natural PETase activity could be the solution to the hydrolysis of microplastics in our natural local wetland environment.

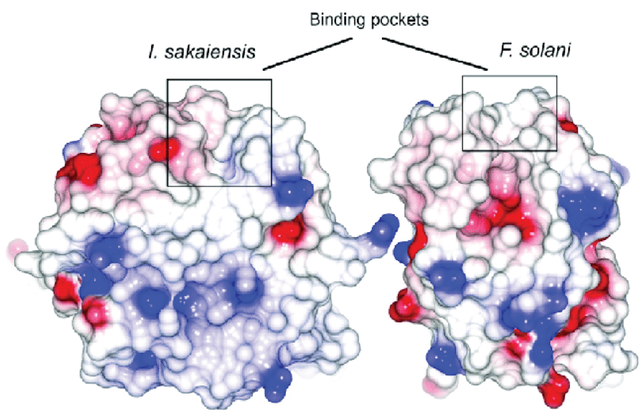




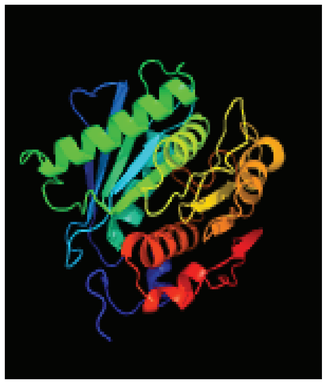
Carty, S. (2014). Freshwater Dinoflagellates of North America. Cornell University Press: 1 ed.

Structurally similar projections between *I. sakaiensis* PETase delivery (top) and microalgae *Ceratium candelabrum* (bottom).

Encoded by Serine-Argenine-Leucine sites as an evolutionary artefact.

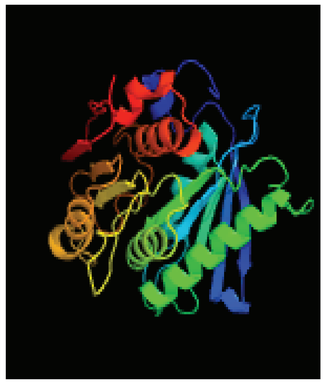


H., Lihui, L., Changcheng, H., Liu, L., Yunzi, B., Rui. (2018). Protein Crystallography and Site-Direct Mutagenesis Analysis of the Poly(ethylene terephthalate) Hydrolase PETase from Ideonella sakaienss. ChemBioChem; 19: 10-1002.



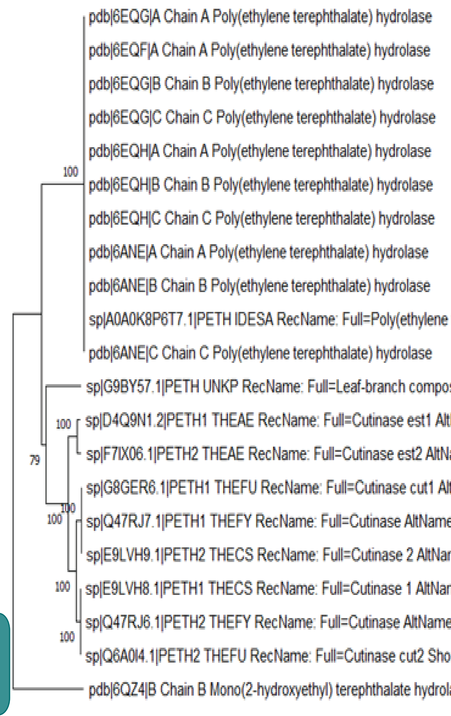
Ideonella sakaiensis (PETase)

Rhizobacter gummiphilis (PETase)



Rhizobacter gummiphilis (cutinase)

Maize root ferredoxin oxidoreductase



METHODS

Literature Search

Collect sequencing data from GenBank, a NCBI Database[5].

Alignment & Development

Test for regions of similarity using BLAST. Align sequences in Discovery Studio.

Transform MSA into a phylogenetic tree using MegaX[4]. Determine major linkages.

3D Homology Models

Use PHYRE2 to develop 3D homology models for folding comparison.

RESULTS

SRL Alignment as an Artefact of Microbial Evolution

SRL, an amino acid sequence associated with "dinoflagellates", exploits the linkage between microalgae and bacterial enzyme delivery.

Bacterial to Fungi Linkage

Major active bacteria and fungi identified are: *Aspergillus sp., Bacillus amyloliquefaciens, Nocardia sp., & Thermobifida sp.*

DISCUSSION

Limitations of Novel Enzyme

Lack of information about sequencing made production of alignment difficult.

Artificial Manipulation

Majority of sequences in GenBank are lab-created strains of "unknown origin".

Better Together

Literature suggests endophytes cease degradation when supplemented with growth-promoting nutrients.

Looking Forward…

Identifying organisms in local environments for sampling and DNA extraction.

SOURCES

[1] Yoshida, S., Kazumi, H., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K. Kimura, Y., Oda, K.. 2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351: 1196–1199.

[2] Hemani, G., Zheng, J., Wade, K.H., Laurin, C., Elsworth, B., Burgess, S., Bowden, J., Langdon, R., Tan, V., Yarmolinsky, J., F, Hashem. (2018). The MegaX platform supports systematic causal inference across the human phenome. eLife.

[3] Kelley, L., Mezulis, S., Yates, C. et al. (2015). The PHYRE2 web portal for protein modeling, prediction and analysis. Nature Protocols. 10: 845 858.

[4] Wilm A.F., Dineen D.G., Gibson T.J., Karplus K., Li W., Lopez R., McWilliam H., Remmert M., Söding J., Thompson J.D., Higgins (2022). Molecular Systems: Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Sievers Biology. 7: 539.

[5] Sayers E.W., Bolton E.E., Brister J.R., Canese K., Chan J., Comeau D.C., Connor R., Funk K., Kelly C., Kim S., Madej T., Marchler Bauer A., Lanczycki C., Lathrop S., Lu Z., Thibaud-Nissen F., Murphy T., Phan L., Skripchenko Y., Tse T., Wang J., Williams R., Trawick B.W., Pruitt K.D., Sherry S.T.. (2022). Database resources of the national center for biotechnology information. Nucleic Acids Res. 50: D20-D26.