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PETase Protein, Expression and Purification

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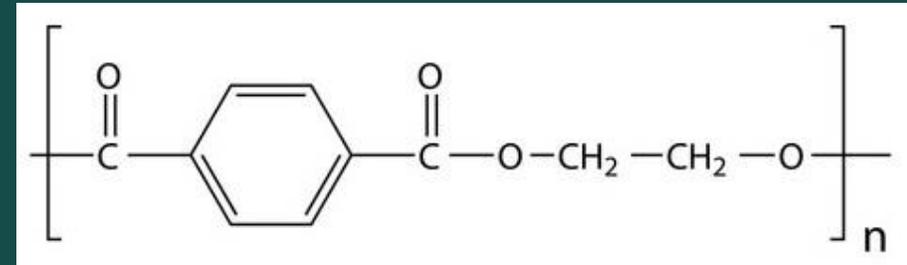
Recommended Citation

PETase Protein

Expression and Purification

Madelyn Degenhardt and Dr. Ruff

What is PETase?

- PETase is an enzyme that can break down PET plastic

- PET plastic is found in soft drink bottles, water bottles, other food packaging, and polymer fabrics

PET Plastic Structure
Image credit: https://omnexus.specialchem.com/selection-guide/polyethylene-terephthalate-pet-plastic

- **Our Goal:** To determine if PETase is a reliable protein for expression and if it can be used to complete further tests, specifically for use in Biochemistry labs

Expression and Purification

- Steps we took to achieve PETase purification
  - Growth of cells with the PETase plasmid
  - Purify cells with antibiotics
  - Cell lysis
  - Centrifuge lysate
  - Run a column
    - Nickel beads inside column collect PETase protein by histidine tag bonding
    - PETase is then removed from beads using imidazole

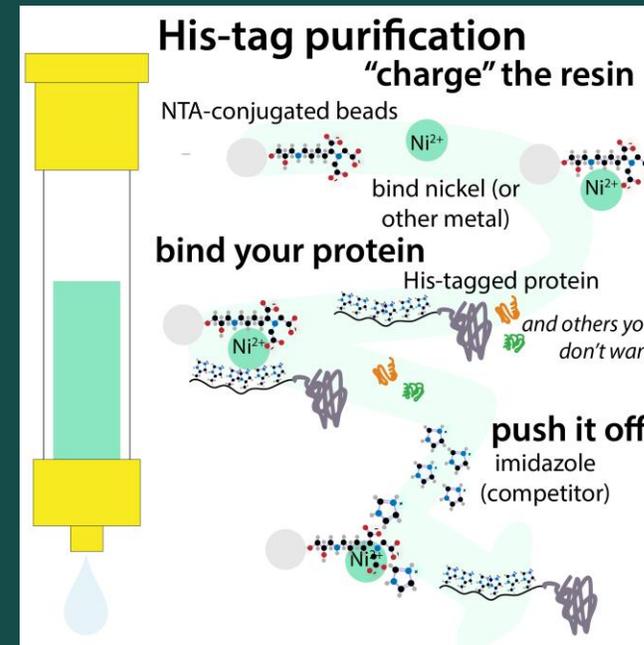Quick Bradford assay done on the column product. Blue indicates protein!

His-tag purification
"charge" the resin
NTA-conjugated beads
Ni²⁺
bind nickel (or other metal)
Ni²⁺

bind your protein
His-tagged protein
Ni²⁺
and others you don't want

push it off
imidazole
(competitor)
Ni²⁺

Image credit: https://thebumblingbiochemist.com/365-days-of-science/his-tag-protein-purification-with-imac-immobilized-metal-affinity-chromatography/
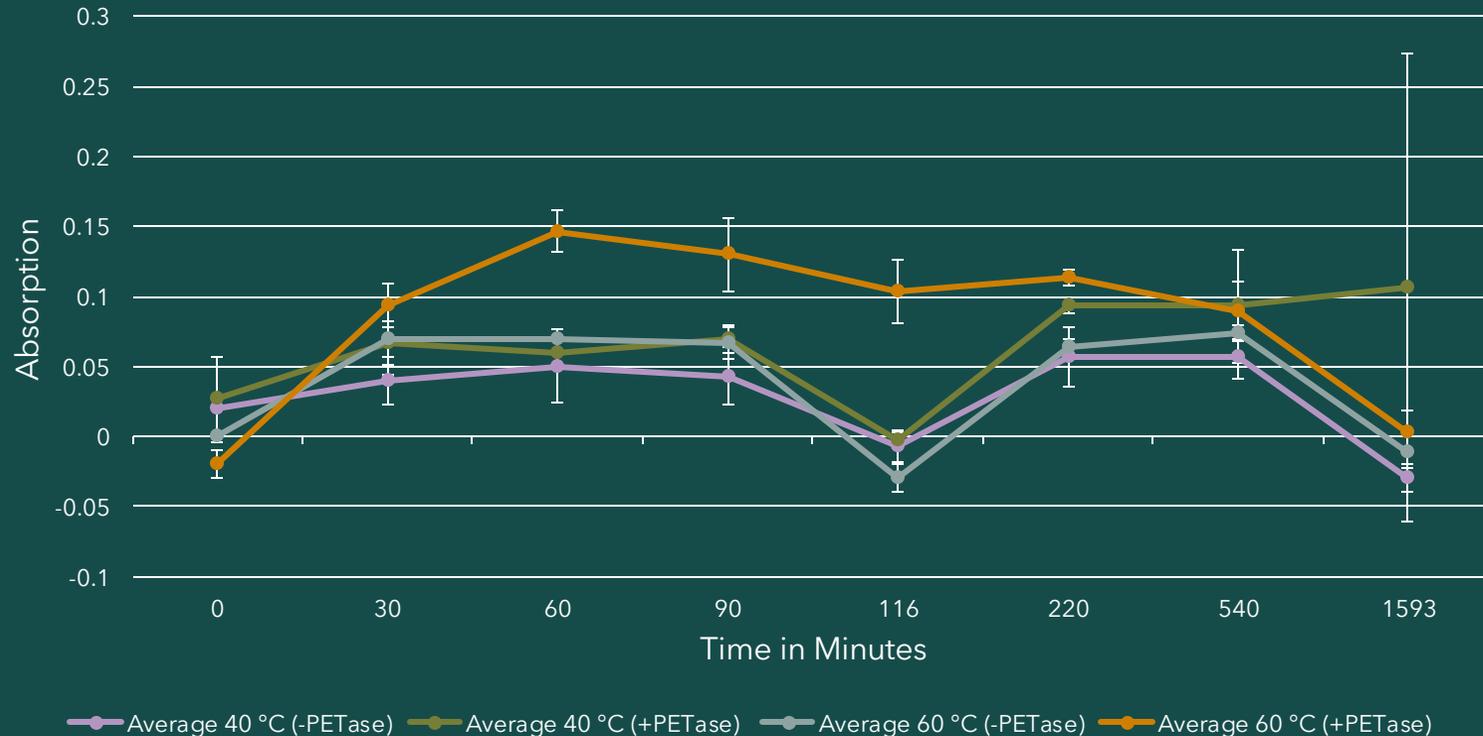
Results – SDS Page Gel

This gel showed one very clear band at the weight we would expect to find PETase indicating the purification's success!

Lane 1: ladder
Lane 2: lysate
Lane 3: column flowthrough
Lane 4: wash
Lane 5: E2
Lane 6: E3
Lane 7: E4
Lane 8: 50k in
Lane 9: 50k
Lane 10: PETase

Plastic Digestion

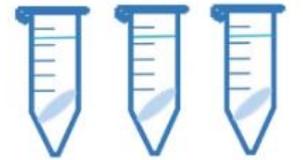Plastic Degradation - Absorbance vs. Time

- To each test tube, we added a small circle of PET plastic

-We periodically tested the absorbance using the nanodrop machine

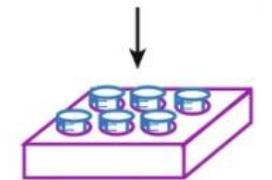-Between absorption tests, we incubated the tubes at either 40°C or 60°C
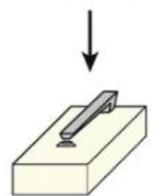
-Absorbance collected at 260 nm

Overall, we saw higher levels of absorption with the tubes containing PETase, which indicates some digestion but not to the level we would like to see

Conclusions and Next Steps

- We have a procedure that is able to express and purify PETase.

- However, the plastic degradation experiments associated with this procedure do not yield the expected results.

- Parallel experiments in Chem 408 suggest plastic degradation experiments can work, but further troubleshooting is needed to determine a reliable procedure.

- Variables to test: purification buffers, reaction buffers, temperatures,…