Enzymatic Degradation of Polyethylene Terephthalate (PET) Plastic: A Sustainable Approach

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INTRODUCTION

Plastics and Pollutions

- 9.3 billion tonnes of plastic waste accumulated since 1950 (1,2)
- 6.9 billion (74.2%) not collected 14 million tonnes ended up in oceans yearly
- 33 billion tons will accumulate by 2050

PET Plastic

PET is an oil-based thermoplastic, one of the most widely used polymers (3)

- 41.56 million metric tonnes were produced in 2014 and 73.39 in 2020 (4)
- Over half a trillion plastic bottles were sold in 2020 (5)
- For the one million plastic bottles sold every minute, 91% are not recycled, only 6 out of the 9% recycled are reused

Enzymes degrading PET

PET Plastic can be degraded by biological activities of thermophilic microbial polyester hydrolases like:

1. TfH from *Thermobifida fusca* DSM43793
2. TfCut1 and TfCut2 from *Thermobifida fusca* KW3
3. LC cutinase a metagenome from plant sources
4. TCur from *Thermonospora curvata,* and the
5. Mesophilic IsPETase from *Ideonella sakaiensis, etc*

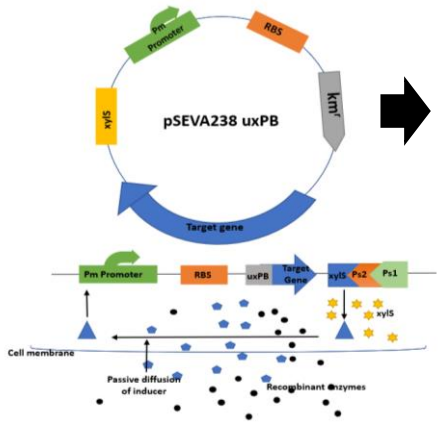
Open Research

Access to open research information and resources contributed immensely toward the success of this research, the outcomes were made public through workshops and conferences in support of research reproducibility and replicability.

METHODS

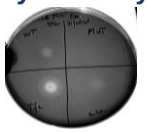
- We engineer and express PET esterases from a high-level expressing vector designed with uxPB, an N-terminal secretion system previously identified in *P. putida* KT2440, to transport the expressed enzymes out of the host cells, avoid avoiding the tedious and expensive enzyme purification steps.
- Recombinant enzymes secreted were assayed with optimized in-house PCL assay and on the amorphous PET
- Experimental degradation of 6.3% crystalline PET were performed at 70°C with 5mg of enzymes (protein) in 1mL of the diluted supernatants

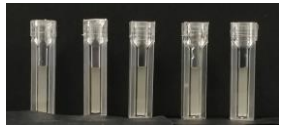
Cloning and expression of PET hydrolases

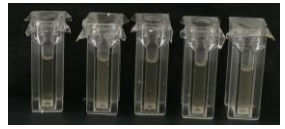


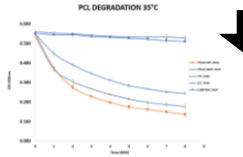
Engineering and induction for the expression of PET degrading enzymes

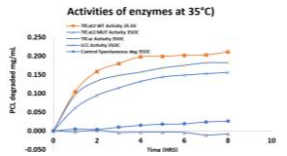
Enzyme assay







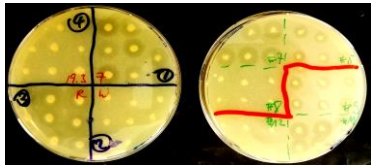




PERCENTAGE PCL LOSS AT 35°C

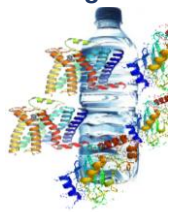
Time (HRS)	TfCut2 WT (%)	TfCut2 MUT (%)	TFC (%)	LCC (%)	Control (%)
0	0	0	0	0	0
1	35	0	33	20	2
2	53	0	46	32	1
3	60	0	51	38	3
4	66	0	54	44	5
5	66	0	58	48	6
6	67	0	60	50	6
7	67	0	63	51	8
8	70	0	63	52	9

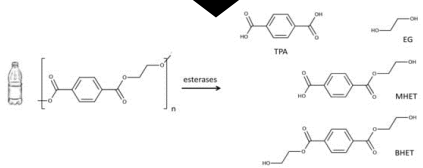
The assay of PET esterases on polycaprolactone (PCL)

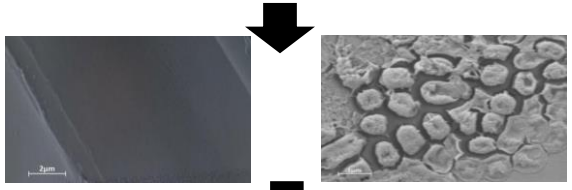


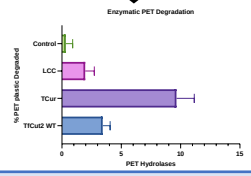
The assay of PET esterases on amorphous PET plastic

PET Degradation









CONCLUSION

The recombinantly expressed enzymes degrade PCL polymer, this is an indication of their strong hydrolytic activities. The degradation of partially crystalline PET plastic confirms that they are well expressed in their active confirmations and have the potential of hydrolysing PET plastic. Optimising the PET degradation protocol may improve their hydrolytic outcomes, and help in curtailing plastic pollution for a sustainable future through knowledge sharing.

1. Carniel *et al.,* 2017, 2. Statista, 2018, 3. Nace, 2017, 4. Schlossberg, 2017, 5. Parker, 2018