A Synthetic Biology Solution To Polystyrene Degradation

University of Leicester iGEM Team

Introduction

Expanded polystyrene (EPS) is a low density, bulky material, and recycling currently economically unviable. Naturally occurring organisms using polystyrene (PS) as a carbon source have been identified by analysing the accumulation of polystyrene breakdown products (Atiq *et al.,* 2010).

The biochemical pathway used by these organisms is unknown, but the toluene degradation pathway is a possible contender, since toluene has the same structure as the phenyl side chain in PS. One toluene degradation pathway we concentrated on was encoded by the Tod operon. We intended to isolate genes in this operon to make biobricks using known strains of *Pseudomonas putida* and *Pseudomonas aeruginosa*.

We had two approaches to explore: isolating naturally occurring bacteria growing on PS, as well as identifying modifiable biochemical pathways and studying chemical degradation..

Chemistry

We explored chemical routes to PS degradation to inform on potential biochemical pathways that could be adapted. A number of potential mechanisms for potential chemical degradation pathways were considered.

Some mechanisms we researched focused on ways to make PS into polylactic acid, a feedstock for recycling PS into biofoam, an industrially biodegradable alternative. Another feasible mechanism (No. 4 see Figure 1.) used thermal energy to form free radicals on the hydrocarbon chain, then added a reactive oxygen species to ultimately form an organic peroxyl.

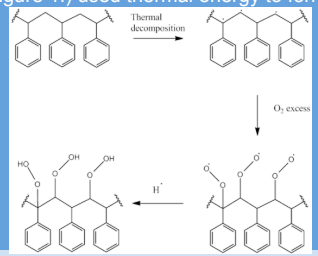


Figure 1. Diagram of mechanism 4

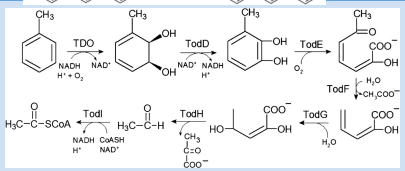
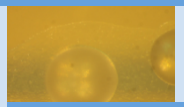


Figure 2: Diagram of the Tod operon of *P.putida*



As shown in Figure 2 the Tod operon encodes all the enzymes needed to degrade toluene to acetylCoA, which can then be degraded further by aerobic respiration. TDO is encoded by TodC1 and TodC2 to form the active enzyme, and TodA and TodB shuttle electrons from NADH to the active site. TodX is a toluene transporter (George *et al*., 2011) . Figure 3 shows small amounts of bacterial growth around PS "sugar.".

Citizen Science Experiment (CSE)

A large portion of the project involved enrolling members of the public to bury polystyrene chips in the ground for several months, before sending them back to us for analysis. The chips were swabbed and plated onto nutrient agar.The colonies that grew were tested for the ability to metabolise PS by plating them onto minimal media agar with 5% polystyrene.

Two colonies (dubbed "Orange" and "Yellow" for their distinctive colouring) were discovered growing on polystyrene beads from sample 01# 502. These were isolated, grown in broth, and boiled to extract DNA for PCR analysis. Additionally, they were tested in liquid culture to determine if they were indeed growing on polystyrene.

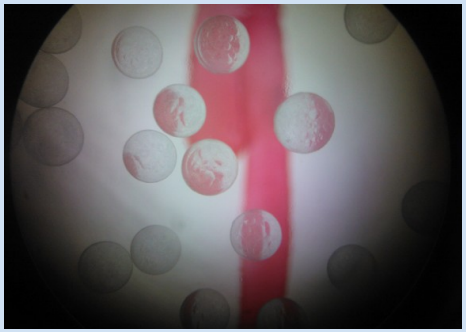


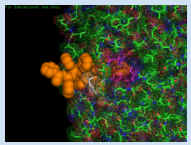
Figure 4. A photomicrograph of PS sugar in a PS minimal media plate,. The red line is drawn on the underside of the Petri dish.

The PS minimal media contains 9.5ml minimal salts media spread in a Petri dish with 0.5g PS sugar sprinkled on top to prevent the sugar from sinking while setting.

Toluene 2, 3-Dioxygenase (TDO) Modification

Pymol (Schrödinger, LLC) was used to predict the structure of TDO after the substitution of 7 bases of TodC1 (causing 4 amino acid changes), as illustrated in Figure 5.

Figure 5. Orange atoms are in the PS chain, pink residues are residues in the active site and modified residues are white. The mutations have increased the width of a gap bridging the active site and external conditions, allowing PS to reach the active site (the terminal PS phenyl group is in active site).



Modifying the gene by site directed mutagenesis would not be easy and the modified enzyme could adopt a different conformation when synthesised. However if it did function, it could also potentially act on of other bulky addition polymers.

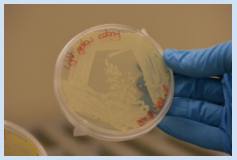


Figure 6 (left): the yellow colony. Figure 7 (right): the orange colony, Both extracted from CSE kit 01#502

16S and Tod PCR

The orange and yellow colonies isolated from sample 01#502 had their DNA extracted using CTAB and Maxwell protocols. To try to determine the genus of these colonies, we used Universal 16S primers to amplify around 300-350bp of the conserved 16S rDNA:

16S PCR primers

28f AAGAGTTTGATCCTGGCTCAGA

519R GWATTACCGCGGCKGCTG

BLAST (Altschul *et al.*, 1990) searches, showed all Tod operon genes are conserved, so primers with little degeneracy were designed to amplify them from *P. putida* strains. *In silico* PCR (http://insilico.ehu.es/) showed that at least one strain of *P. putida* should show amplification of its Tod genes using our primers.

Results

We were unfortunately unable to create a synthetic bacterium, but we have established a good base for future teams.

- Growth experiments showed the "Orange" and "Yellow" bacteria found on strip 01# 502 bacteria grew better in the presence of PS.
- The 16S rDNA sequence of the bacteria found on strip 01# 502 showed that Orange was in the genus *Pseudomonas*, and Yellow was in the genus *Paenibacillus*. Both bacteria may be degrading polystyrene, or working together.
- Primers and and a biobrick construction strategy were worked out for 3 of the 5 Tod genes. Time constraints and issues with PCR contamination meant that we did not successfully amplify the Tod genes from a lab strain of *P. putida* or the isolated Orange and Yellow bacteria.
- Bacteria, DNA and PCR primers have been archived at -80C for work by future iGEM teams…

CSE sample 01# 502 sent in by Sarah Curtis – thanks Sarah!

In partnership with GENIE

References:
Atiq, N., Ahmed, S., Ali, M., Andleeb, S., Ahmad, B., Robson, G., 2010. Isolation and identification of polystyrene biodegrading bacteria from soil. *African Journal of Microbiology Research*. **4(14),** 1537-1541.
George, K.W., Kagle, J., Junker, L., Risen, A., Hay, A.G., 2011. Growth of Pseudomonas putida F1 on styrene requires increased catechol-2,3-dioxygenase activity, not a new hydrolase. *Microbiology*. **157,** 89-98.
The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC. , Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Biol*. 215:403-410.