Identification of esterase in Aspergillus flavus during degradation of polyurethane
Quentin Wong¹, Alina Ma¹, Dr. Mohini Sain², Dr. Robert Jeng²
¹University of Toronto Schools, ²University of Toronto Faculty of Forestry

Introduction

The abundance of synthetic polymers poses many environmental concerns; with the invention of polyurethane (PUR) in 1937 by Otto Bayer, over 2 million tonnes of PUR are produced in the European Union annually. Since polyester PUR tends to withstand heat, wear and tear better than polyether PUR and is more easily biodegraded by fungi (Cullen, Gaustad, and Yanful, 2007), the focus of past investigations have been with the degradation of polyester PUR using fungi. Although they can be recycled or incinerated cleanly, such practices are too expensive; thus, they are disposed of unsustainably.

Russell et al. (2011) hypothesized that one of the serine hydrolases secreted by Pestalotiopsis microspora was the enzyme behind the degradation of polyester PUR. Able to grow in an anaerobic environment, this Amazon fungus relied on polyester PUR as a sole carbon source by cleaving ester bonds.

Hameed, et al. (2009) tested other fungi from Aspergillus, a more accessible fungus than P. microspora. They proved, through the means of the weight loss of PUR, that Aspergillus flavus was more successful in PUR degradation than Aspergillus fumigatus or Aspergillus terreus.

Mathur and Prasad (2012) used A. flavus (ITCC 6051) and suggested that the esterase detected in its extracellular fluid may be the reason behind its activity; a 60.6% ± 0.3% reduction in the weight of the PUR was noted.

The ultimate objective of this project is to create a genetically engineered variant of Pichia pastoris which will be able to digest polyester PUR using the esterase from A. flavus. Once the active esterase is identified and purified, it can be reversed engineered to produce a gene that can be inserted into P. pastoris.

Materials & Methodology

Water based polyester PUR was created using an air dried film made from a mixture of low and high gloss water based PUR (Michelman Water Based Coatings). Thermoplastic polyester polyurethane (TPU) is a granular form of PUR. The fungus, Aspergillus flavus UAMH 8757, was ordered from the University of Alberta, Microfungus Collection and Herbarium.

TPU was placed into 2 flasks, water-based PUR in another two. NaNO₃, K₂HPO₄, KH₂PO₄, KCl, MgSO₄·7H₂O and distilled water were mixed to make the culture medium. After steam autoclaving the 4 flasks, A. flavus was added into two of them. After incubation with shaking at 120 rpm, the plastic was filtered out with a water vacuum pump. The post-incubation weight was compared with the pre-incubation mass. After freeze drying, Native PAGE and SDS-PAGE gels were conducted with a NuPAGE Novex Bis-Tris 4-12% gel in a Novex XCELL mini-electrophoresis apparatus using supplier instruction. FTIR spectroscopy was conducted for control and experimental water-based PUR films.

Discussion

No weight loss was observed in the first 2 trials; neither fungal growth nor esterase activity was observed in the experimental TPU flasks. However, both fungal growth and esterase activity were observed in the experimental water-based PUR flasks. Thus, esterase is at least an active enzyme in PUR degradation. A. flavus was unable to produce esterase in the presence of TPU, suggesting that this strain of A. flavus is unable to use TPU as a substrate under the conditions listed or that a different type of esterase is needed.

There is a difference in the FTIR spectra between samples 1 and 4 at the 1000–1200 frequency range which corresponds to the ester bonds. Since the absorption peak in this area has been reduced, this suggests that ester bonds have been broken, likely by esterase.

Lastly, a large amount of orange fungus, yet to be identified, was found growing on the water-based PUR of two control groups that were not steam autoclaved. This is likely an airborne fungus contamination, but its growth is comparable, if not greater than that of A. flavus.

Results & Observations

Figure 1: Chemical structure of polyurethane. It is formed by reacting diisocyanates with glycols.

Figure 2: Structure of esterase as determined by X-ray crystallography. http://www.bbmg.ox.ac.uk/Science-Articles/Archives/FRD/FHM-grants.html

Figure 3: A. flavus culture

Figure 4: Water-based polyester polyurethane

Figure 5: TPU, a granular form of thermoplastic polyester polyurethane

Figure 6: SDS-PAGE gel of yeast strain Ulster of first trial. The arrow indicates esterase activity observed in the water-based PUR flask. When compared to the control, the esterase has a molecular weight of around 20 kDa.

Figure 7: Native PAGE gel shows esterase activity (the two dark bands) present in the water-based PUR. The last four columns of the gel are a repeat of the first four columns.

Figure 8: Fungal activity was only witnessed on the surface of the water based PUR, but not on the TPU.

Figure 9: FTIR Spectra of water-based PUR films. Row 1 is a film from the control sample. Rows 2 and 4 are films samples that had A. flavus growing on its surface; samples were cleaned before spectrosopy though. Row 3 is a film without fungal growth removed from its surface.

Next Steps & Applications

The DNA can be reverse engineered from the protein capable of degrading the PUR. After inserting the DNA into a plasmid, P. pastoris can incorporate the plasmid into its own DNA. After growing the mutated P. pastoris to observe whether it will secrete the same protein observed in A. flavus, the same PUR degradation experiment will be conducted to determine the success of this P. pastoris. We will subject the digested sample to matrix assisted laser desorption ionization (MALDI), conduct SEM on the PUR, use RT-PCR to have the completed DNA sequence of the esterase, and do N-terminal amino acid sequencing. The DNA sequence can be compared to the esterase, also from A. flavus mentioned in patent EP0157894 B1 to see whether they are identical. If not, this enzyme can be tested for PUR degradation ability.

If the DNA sequence of the esterase is sequenced then patented, we would be able to make an artificial enzyme, capable of degrading the PUR. However, with current technology, it is likely that the genetically modified P. pastoris would be more successful with degradation, since artificial enzymes have lower catalysis rates than natural enzymes. The production of esterase by P. pastoris provides substantial benefits – if it can secrete a large amount of esterase, it would be able to degrade PUR faster. If deemed safe for the environment, it may be grown in landfills; if not, it can be used in a controlled environment like a waste treatment plant. If the enzyme responsible for degradation is commercialized, P. pastoris offers a cheap and abundant source for generating such enzymes.

Conclusion

Our hypothesis was that esterase secreted in the extracellular fluid of the A. flavus would be able to break down the ester bonds in both the water-based PUR and TPU. Through the FTIR spectra and the presence of an extra esterase band shown in the gel electrophoresis, it can be concluded, that the esterase plays a role in the degradation of water-based PUR.

Biodegradation of PUR, a pollutant, is an important step in bioremediation and overall reduction of plastic pollution. The ability of A. flavus to degrade certain types of PUR was demonstrated in this study.

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Works Cited


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Figure 10: A. flavus on the surface of water-based PUR

Figure 11: FTIR Spectra of water-based PUR films.