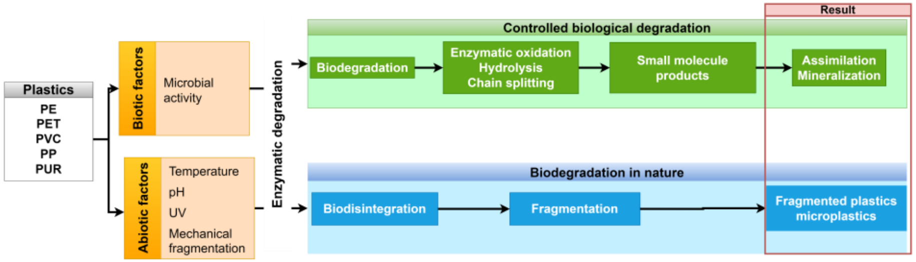
Microbial degradation of polyester-model urethane

JORDÁN Anikó[1,2]* VISKOLCZ Béla[1,2] SZŐRI-DOROGHÁZI Emma[1,2]*

[1] Institute of Chemistry, University of Miskolc, Egyetemváros, Miskolc-, Hungary-3515
[2] Advanced Materials and Intelligent Technologies Higher Education and Industrial Cooperation Centre (HEICC), University of Miskolc, 3515 Miskolc, Hungary
Email: *aniko.jordan@uni-miskolc.hu, *emma.szori-doroghazi@uni-miskolc.hu

Introduction

Plastics improve the quality of human life and provide technological advances, but the waste they generate contributes to many environmental problems. It is necessary to neutralize this waste. One approach to addressing it is through degradation, which is also influenced by oxo-biotic factors such as mechanical fragmentation, light, hydrolysis and biodegradation [1]. The latter process can be achieved through the activity of microorganisms in basically 3 main steps: attachment, fragmentation and mineralization. Among the petrochemical plastics, polyurethane (PUR) is a synthetic polymer used in foams, insulating materials, textile coatings and anti-corrosion paints [2, 3]. Several microorganism and enzyme involved in the degradation of polyurethane have been investigated in literature, but the specific mechanism of biodegradation is not fully understood. Most of the identified enzymes belong to esterase family, which can degrade ester bonds in the polymer molecules [4, 5].



Experiments and Results

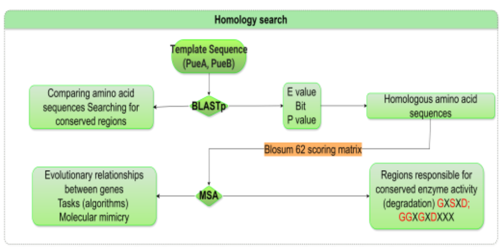


Figure 1 Method for identifying potential degrative enzymes, and sequence homology search using BLASTp and MSA programs. (PueA and PueB are Polyurethane esterase type enzymes of *Pseudomonas chlororaphis*)

The microorganisms capable for the degradation processes can be selected using bioinformatic methods.
For this purpose, we utilized template proteins previously reported in the literature (e.g., PueA and PueB) and performed BLAST search on the UniProt database to find proteins with amino acid sequences highly similar to the templates [5].
Based on literature, we know that proteins with similar amino acid sequences often exhibit functional similarities. As a result, we identified several microorganisms possessing PueA or PueB homologues, although no literature data were available regarding the polyurethane-degrading abilities of their proteins [4].

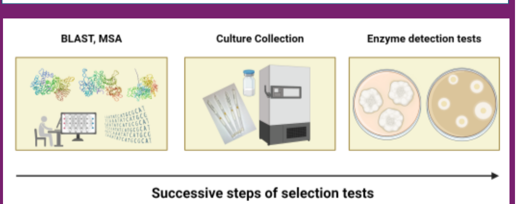


Figure 2 Preliminary tests for the selection of strains capable for PUR degradation

Model substrates, which are chemically similar to real plastics but with simpler structures, are often used to study plastic degradation capacity of bacteria. Imprantil DLN substrate (as nutrient source in the medium) and p-nitrophenyl acetate (a compound used to detect extracellular esterase activity, *p*-NPA) are commonly used for preliminary studies on the biodegradation of PET and PUR [6].
If the bacteria produce an extracellular esterase during Imprantil DLN SD degradation, it can break down *p*-NPA's acetate chain, producing p-nitrophenol (*p*-NP). *p*-NP is yellow and has maximal absorption at 405-410 nm. The product of this reaction can be followed spectrophotometrically.
We applied this Imprantil DLN SD degradation test to the bacteria in our strain collection that were predicted, based on bioinformatics tests, to possess PUR-degrading enzymes.

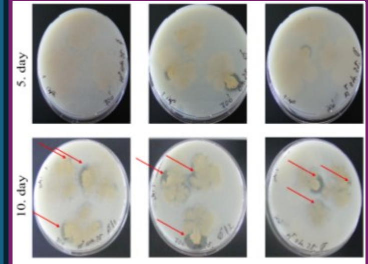


Figure 3 Potential PUR degrading bacteria showing clearing zones after 5 and 10 days of incubation on Imprantil complemented solid medium
Red arrows point at clearing zones.

Imprantil DLN SD was used as a substrate at a concentration of 1% (V/V) both in Luria-Bertani solid and in liquid medium [7, 8].
On solid medium, the appearance of clear zones around bacterial colonies indicated degradation (**Figure 3**). The size of these zones indicates the efficiency of the breakdown.

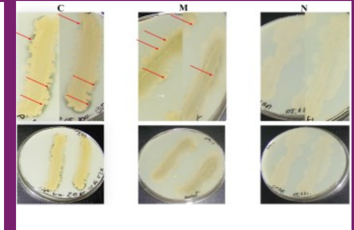


Figure 5 Mixed culture (M) on LB agar plate, bacteria causing clearing zone (C) and the other only growing (N) on LB agar plate complemented with Impranl substrate.

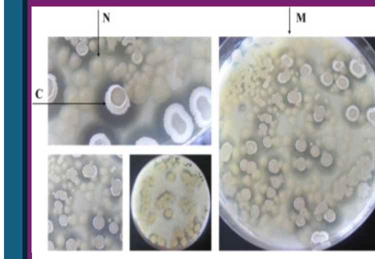


Figure 4 Consortium of bacteria (M, mixed culture) on LB agar plates supplemented with Imprantil. (C) bacteria that produce a clearing zone, (N) bacteria do not produce a clearing zone. Black arrows indicate the clearing zones.

On Imprantil-supplemented plates, two types of colonies were observed. One type, labeled C, showed a well-defined, relatively large clearing zone along with the appearance of a crystalline white ring, while the other type, labeled N, did not form a clearing zone and produced more uniform, yellowish colonies (**Figure 4**). On LB agar without Imprantil, no difference in the morphology of the two colony types was observed (M labeled plate **Figure 5**).
We could conclude that we have a bacterial consortium, and at least one member of the consortium is responsible for the degradation of Imprantil.

Figure 6 Separated bacterial culture on 1% Imprantil DLN SD agar. Causing the clearing zone (C, left) and the other only growing (N, right).

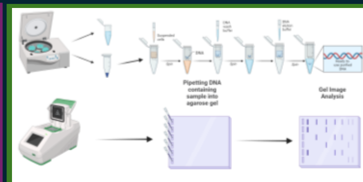


Figure 7. Preliminary tests for the selection of strains capable of degradation

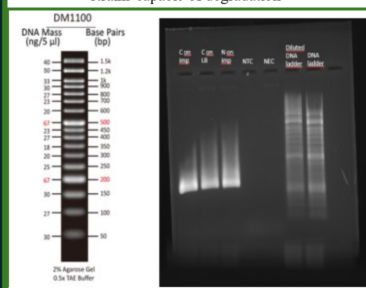


Figure 8. PCR results with *Bacillus* spp. BacF/R primer pair

C on imp: gDNA isolated from a bacterium colony causing the clearing zone on LB+Imprantil plate
C on LB: gDNA isolated from C type bacterium grown on LB agar plate
N on imp: gDNA isolated from bacterial colony without clearing zone
NTC: Non Template Control
NEC: Non Enzymatic Control

To identify the potential members of the bacterial consortium, genomic DNA (gDNA) was isolated (**Figure 6**) from colonies capable of degrading Imprantil (colonies C). The isolated gDNA was used as template for PCR reactions (**Figure 8**).
Several primer pair was tested in PCR reactions, but PCR product was obtained only with the primer pair specific to the *Bacillus* group (**Figure 8**).

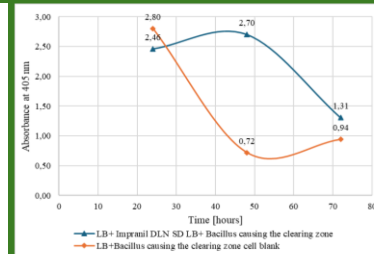


Figure 9 Spectrophotometric analysis of Imprantil fermentation at 405 nm after 24, 48, 72 h of fermentation (using 0.1 mmol/mL p-NPA as the substrate).

For the fermentation experiments, a sample of the fermentation supernatant was taken, and the change in absorbance of the Imprantil-containing medium was monitored spectrophotometrically at 405 nm using p-nitrophenyl acetate (**Figure 9**).

In the reaction with Imprantil inoculated with bacteria showing a clearing zone (C), the absorbance peaked at 48 hours. In the fermentation without Imprantil, absorbance was lowest after 48 hours. These results suggest that the bacterium exhibiting the clearing zone produces an extracellular enzyme capable of degrading Imprantil, with maximum protein production occurring on day 2.

[1] S. Lambert and M. Wagner, "Characterisation of nanoplastics during the degradation of polystyrene," *Chemosphere*, vol. 145, pp. 265–268, Feb. 2016, doi: 10.1016/J.CHEMOSPHERE.2015.11.078.
[2] S. Lambert and M. Wagner, "Formation of microscopic particles during the degradation of different polymers," *Chemosphere*, vol. 161, pp. 510–517, Oct. 2016, doi: 10.1016/J.CHEMOSPHERE.2016.07.042.
[3] F. Xie, T. Zhang, P. Bryant, V. Kurusingal, J. M. Colwell, and B. Laycock, "Degradation and stabilization of polyurethane elastomers," *Prog Polym Sci*, vol. 90, pp. 211–268, Mar. 2019, doi: 10.1016/J.PROGPOLYMSCI.2018.12.003.
[4] K. Skleničková, S. Abbrent, M. Halecký, V. Kočí, and H. Beneš, "Biodegradability and ecotoxicity of polyurethane foams: A review," *https://doi.org/10.1080/10643389.2020.1818496*, vol. 52, no. 2, pp. 157–202, 2020, doi: 10.1080/10643389.2020.1818496.
[5] Y. Tokiwa, B. P. Calabia, C. U. Ugwu, and S. Aiba, "Biodegradability of Plastics," *International Journal of Molecular Sciences 2009, Vol. 10, Pages 3722-3742*, vol. 10, no. 9, pp. 3722–3742, Aug. 2009, doi: 10.3390/IJMS10093722.
[6] Recombinant porcine intestinal carboxylesterase: cloning from the pig liver esterase gene by site-directed mutagenesis, functional expression and characterization. Protein Engineering Design and Selection, 16(12), 1139–1145 | 10.1093/protein/gzg120." Accessed: Oct. 14, 2024. [Online]. Available: https://sci-hub.se/10.1093/protein/gzg120
[7] Esterase Activities ofBrevihacteriumsp. R312 andBrevihacterium linens62. Bioscience, Biotechnology, and Biochemistry, 59(8), 1464–1471 | 10.1271/bbb.59.1464." Accessed: Oct. 14, 2024. [Online]. Available: https://sci-hub.se/https://doi.org/10.1271/bbb.59.1464
[8] Q. Ying and J. Kameoka, "ISOLATION AND CHARACTERIZATION OF POLYURETHANE-DEGRADING MICROORGANISMS," 2022.