Engineering thermostable LCC for enhanced PET hydrolysis at moderate temperatures

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The breakdown of polyethylene terephthalate (PET) is essential for prompting the goals of a circular bioeconomy aimed at reducing the environmental footprint of plastic materials. In this context, the enzymatic hydrolysis of PET becomes a pivotal procedure, allowing its bioconversion into monomers which could then serve as starting materials to yield valuable chemicals.
The most promising PET hydrolase, in terms of depolymerization efficiency, is the thermostable leaf-branch compost cutinase (LCC). Evolved variants of the enzyme possessing a higher activity on PET have been produced by a semi-rational design approach, such as the S101N/F243T ΔLCC variant which is able to fully depolymerize 1.3 g of untreated postconsumer PET waste in less than 3 days at 55 °C (*as previously published in Pirillo et al. (2023) FEBS J, 290: 3185-3202*).
Although this optimal temperature is lower than that for the wild-type one, and suitable for in vitro polymer biodegradation, the integration of LCC into one-pot cellular systems designed for the upcycling of PET (engineered whole cell biocatalysts) is hampered. To address this issue, we focused on evolving LCC variants with significant activity at moderate temperatures while retaining a significant thermal stability. To reach this goal a high-throughput screening protocol has been set up and protein engineering studies, supported by bioinformatic analysis, are carried out by various approaches, including site-directed mutagenesis, error-prone PCR, and DNA shuffling. Interesting clones are further evaluated for their activity on PET nanoparticles under different bioconversion conditions.
Within the framework of a circular bioeconomy, this research marks the beginning for the set-up of bioconversion systems with the objective of extracting value from post-consumer environmental pollutants.
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