Synthetic biology for a sustainable recycling and upcycling of PET

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Despite increasing recycling efforts, 65% of plastic waste collected in Europe (amounting to 30 million tons/year) is still incinerated or released into the environment, posing a significant threat to human health. In the context of a circular bioeconomy, newly discovered enzymes capable of depolymerizing polyethylene terephthalate (PET) represent invaluable tools for novel processes aimed at sustainable and environmentally friendly plastic life cycle management.

Within our research group, we set up a modular workflow for the evolution of the PET hydrolyzing enzyme (PHEs) which was utilized in the engineering of the two most promising enzymes of this family: the Ideonella sakaiensis PET hydrolase (IsPET) and the thermostable leaf-branch compost cutinase (LCC). An improved variant of IsPET, exhibiting enhanced stability and affinity for PET, was generated by a directed evolution approach (previously published in Pirillo et al. (2022), Int. J. Mol. Sci. 2022, 23, 264). Subsequently, we produced variants of LCC, the most promising PHE for industrial applications due to its thermostability and high hydrolyzing activity. These variants showed significantly superior performances for the biodegradation of PET even at moderate temperatures. Indeed, 1.25 mg of the S101N/F243T-ΔLCC variant were able to fully depolymerize 1.3 g of untreated postconsumer PET in less than 3 days at 55 °C.

These evolved PHE variants represent the ideal biocatalyst for the initial step of novel synthetic metabolic pathways exploited both in vitro and in engineered prokaryotic (E. coli) and eukaryotic (i.e., black soldier fly larvae) organisms. In detail, novel integrated and transversal biotechnological processes for the simultaneous removal of PET from the environment (e.g., bioremediation from solid organic urban wastes or freshwaters) and the upcycling of degradation products (i.e., terephthalic acid and ethylene glycol) into high added-value compounds (e.g., amino acids) were designed.