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A sustainable end-of-life solution for thermoset composites: Directed enzyme evolution for the degradation of vinyl-ester resins.

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PURPOSE OF THE ABSTRACT
The handling and manage of the end-of life of complex artificial materials, such as thermoset composites, is a rather significant challenge for nowadays sustainability. These materials are known for their durability due their exceptional mechanical, thermal and chemical resistance. As landfill storage and incineration are the most typical destinations – thus harming the surrounding ecosystems – environmentally-friendlier approaches need to be developed and implemented (1). We are currently working in engineering improved biocatalysts for degradation of these materials.

Cutinases (EC 3.1.1.74) exhibit many of the necessary qualities for a promising degrading biocatalyst, due to their natural activity to hydrolase ester bonds – a motif with a widespread of magnitude in several thermoset composites (1). In our study, the cutinase from Fusarium solani (FsC) was chosen as departure point for directed evolution towards the degradation of vinyl-ester and poly-ester resins. As these polymeric matrix show extreme complexity and high hydrophobicity, their direct use as a substrate in a high-throughput manner is precluded. Instead, a colorimetric HTS assay was developed based on p-nitrophenyl trimethylacetate (3-MA), which represents a structural soluble scaffold molecule present both in vinyl- and poly-ester resins.

Using this assay, we performed focused directed evolution by MORPHING (2) targeting the active site and surroundings to random mutagenesis and recombination, which rendered 5 different first-generation mutant winners. In a wise-step approach, beneficial mutations were recombined by Site-Directed Recombination (SDR) (3) while saturating hot spot-residues identified during the evolution campaign. As a result, we obtained a final mutant variant, referred to as RancoR, which displayed a 30-fold total activity improvement with respect to parental type FsC. Both FsC and RancoR were cloned into the overexpressing host Pichia pastoris for upscale production and ulterior purification and characterization, which revealed the improved biochemical characteristics of the evolved biocatalyst.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Thermoset composites | Directed Evolution | Cutinases | polyester resins

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