

Thermostable metagenomic esterases: discovery and development for polyester depolymerization

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Plastic pollution has become one of the most urgent environmental and health challenges of our time, necessitating the development of innovative solutions to mitigate its impact. Among the promising approaches is enzyme-driven biodegradation, which targets synthetic polymers such as polylactic acid (PLA), a widely used biodegradable plastic. In this study, we identified a novel PLA-degrading enzyme, MGY, from a metagenomic database. While this discovery held significant potential, the enzyme's practical application was hindered by several limitations, including low solubility, poor expression, and insufficient thermostability. To overcome these challenges, we employed ancestral sequence reconstruction, a cutting-edge protein engineering technique, to generate three ancestral MGY variants. These variants not only retained high PLA-degrading activity but also exhibited enhanced functional properties. Notably, one variant demonstrated the ability to degrade polycaprolactone (PCL), a structurally distinct polyester. PLA and PCL, though both polyesters, differ significantly in their chemical structures: PLA is derived from lactic acid and possesses a relatively rigid backbone, while PCL, synthesized from caprolactone, is more flexible. This structural divergence makes PLA and PCL an excellent pair of substrates for investigating enzyme adaptability and substrate promiscuity, providing valuable insights into how enzyme structure and flexibility influence substrate recognition and hydrolysis. The ancestral variants exhibited remarkable improvements in key properties, including solubility, expression levels, and thermostability. Differential scanning calorimetry (DSC) analysis revealed that one variant achieved a melting temperature (T_m) of 84 °C—the highest reported for any PLA-degrading enzyme to date. These enhancements underscore the potential of ancestral sequence reconstruction as a powerful tool for engineering enzymes with superior functional properties.