

Toward a Circular Life Cycle for PLA: Engineering Microbial Production and Biological Degradation

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Polylactic acid (PLA) is a biodegradable bioplastic used in packaging, textiles, 3D printing, medical devices, and agricultural applications. Although derived from renewable resources, it degrades slowly under natural conditions, limiting its impact on plastic waste reduction. Enzymatic depolymerization offers a promising strategy to accelerate degradation and enable closed-loop recycling. Building on prior reports of a cutinase-like enzyme from *Cryptococcus* species that degrades PLA, we used public protein databases and BLAST to identify 14 homologous fungal enzymes with potential polyester-degrading activity. These genes were cloned into a yeast host for secretory expression and screened using a rapid solid plate assay. Three homologs showed higher hydrolysis efficiency than the *Cryptococcus* control, each with distinct optimal temperatures. These findings demonstrate the value of bioinformatics-guided enzyme discovery and establish a rapid screening platform for identifying efficient plastic-degrading hydrolases. Ongoing work extends degradation assays to structure-guided enzyme engineering, focusing on active sites residues and their interactions with the substrate. In parallel, we engineered *Escherichia coli* to produce lactic acid, the monomer of PLA, by overexpressing lactate dehydrogenase (LDH). The LDH gene was cloned into low-, medium-, and high-copy plasmids to evaluate gene dosage effects. Lower induction temperatures improved titers across constructs. At 20 °C, low- and high-copy plasmids achieved the highest production (approximately 1.0-1.2 g/L in 24 hours), while the medium-copy plasmid produced less. Together, these efforts integrate enzyme discovery and metabolic engineering to advance a sustainable, circular bioplastics economy.

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