Utilizing ancestral variants of polylactic acid-degrading enzymes to enhance solubility and expression yields

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L. Brtan[I], M. Aubel[II], E. Bornberg-Bauer[II], I. Kekez[I], A. Maršavelski[I]

[I]University of Zagreb, Faculty of Science, Department of Chemistry, Horvatovac 102A, Zagreb, Croatia, [II]Molecular Evolution and Bioinformatics, Institute for Evolution and Biodiversity, University of Münster, Münster, Germany

Over the years, plastic pollution has become an increasing problem for both the environment and human health. The urgent need for sustainable solutions to plastic pollution has led to a surge in research on enzyme-driven biodegradation of synthetic polymers, particularly polylactic acid (PLA). In this study, we identified a novel enzyme, MGY, from a metagenomic database for its PLA-degrading ability. However, limited solubility and low yield of the MGY enzyme hindered further structural and functional analysis. To improve its solubility and yield, we applied ancestral sequence reconstruction, generating three ancestral MGY variants. These ancestral variants retained high PLA-degrading activity, as verified through activity assays on emulsified PLA plates, making them more suitable for practical applications and detailed structural studies. Transformation of electrocompetent bacteria with the vector containing synthetic genes of the specific enzymes, followed by cultivation and protein expression, demonstrated that sequence modification through ancestral reconstruction increases protein solubility and contributes to higher expression yields. The MGY enzyme and its ancestral variants were assayed using differential scanning calorimetry (DSC) and a thermal shift assay with fluorescent dye SYPRO® Orange. The enhanced solubility and expression of these ancestral MGY variants highlight ancestral sequence reconstruction as a powerful tool, providing a pathway for developing effective biocatalysts for PLA recycling and environmental sustainability and facilitating both applied and structural studies.