ENZYME ENGINEERING FOR VALORIZATION OF AGROWASTE-DERIVED LEVULINIC ACID TO VERSATILE 4-HYDROXYVALERIC ACID

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Levulinic acid, C5 γ-ketoacid, is considered as a useful building block obtained from biomass and can be converted to value-added fuels and chemicals. Nonetheless, enzymatic valorization of levulinic acid has been limited thus far. Herein, we aimed to valorize levulinic acid to 4-hydroxyvaleric acid, which is a precursor of bio-jet fuel, biodegradable polymer, and biopharmaceuticals, by enzymatic hydroxylation. However, there is no enzyme for catalyzing hydroxylation of γ-ketoacid in nature. Accordingly, we aimed to broaden the substrate specificity of 4-hydroxybutyrate dehydrogenase that is an enzyme for catalyzing hydroxylation of acetoacetate, (i.e., C4 β-ketoacid), a substrate analog of levulinic acid. Through genome-mining and structure-based molecular simulation, 2 mutation sites were determined and site-directed mutagenesis was performed. As a result, the mutants successfully catalyzed not only their primary substrate (i.e., acetoacetate) but also levulinic acid to varying extent; kinetic parameters were determined. In addition, levulinic acid was produced from various agrowastes via acidic hydrothermal reaction and the reaction parameters (i.e., catalyst concentration, reaction temperature, and reaction time) were optimized by response surface methodology. Finally, the mutants catalyzed agrowaste-derived levulinic acid to versatile 4-hydroxyvaleric acid using co-produced formic acid as a hydrogen resource for NADH regeneration. This results might provide insights on enzyme engineering to broaden the substrate specificity and furthermore can contribute to cope with recent climate crisis by construction of circular carbon economy.