Engineering of *Pediococcus acidilactici* D-Lactate Dehydrogenase for Enhancing Activity of 2-Hydroxy Acids with Bulky C_3 Functional Groups.

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Engineering D-lactic acid dehydrogenases for higher activity on diverse 2-oxo acids is necessary for the synthesis of 2-hydroxy acids. The application of 2-hydroxy acids includes biopolymers, pharmaceuticals and cosmetic compounds. Although there are many D-lactate dehydrogenases (DLDH) from various origins, they have low activities for 2-oxo acids with large functional group at C_3. In this study, the D-LDH from *Pediococcus acidilactici* was rationally designed by modulating the intermolecular interactions between the substrates and the residues in the active site. As a result, Y51L mutant with the catalytic ability on phenylpyruvate of 2200 s^{-1} mM^{-1} and Y51F mutant on 2-oxobutryate and 3-methyl-2-oxobutyrate of 37.2 and 23.2 s^{-1} mM^{-1}, were found, which were 138-, 8.5-, and 26-fold higher than the wild type, respectively. Structural analysis explained that the distance and the properties of the interactions between the side chain of the residue 51 and the substrate C_3 substituent group were related with the kinetic parameters.