Biosensor-based directed evolution of methanol dehydrogenase from *Lysinibacillus xylanilyticus*

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Methanol dehydrogenase(Mdh), is a crucial enzyme for utilizing methane and methanol as carbon and energy sources in methylotrophy and synthetic methylotrophy. Engineering of NAD-dependent Mdh, has thus been actively investigated to enhance methanol conversion. However, its poor catalytic activity and low methanol affinity limit its wide application. In this study, we applied a transcriptional factor-based biosensor for direct evolution of Mdh from *Lysinibacillus xylanilyticus*(Lxmdh). A random mutant library of Lxmdh was constructed in Escherichia coli and was screened using formaldehyde-detectable biosensors by incubation with low methanol concentrations. Positive clones were selected by fluorescence-activated cell sorting(FACS) system, and their catalytic activities toward methanol were evaluated. The selected mutants showed high activity, particularly at very low methanol concentrations. In kinetic analysis, mutant E396V,K318N, and K46E had superior methanol conversion efficiency with 79,23, and 3-fold improvement compared to the wild type, respectively. These mutant enzymes could thus be useful for synthetic methylotrophy and for enhancing methanol conversion to useful products.