# Functionality Improvement of Fungal Lignin Peroxidase by DNA Shuffling

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The lignin peroxidase was subjected to multiple rounds of directed evolution in an effort to produce a mutant suitable for use as a xenobiotics degradator in watertreatment. The wild-type peroxidase is rapidly inactivated under the high concentrate 2,4 DCP and high peroxide concentration(5-10 mM). Rapid screening method using color reaction was developed to quantitatively evaluate the lignin peroxidase (LiP) activity. For 2,4-dichlorophenol (DCP) degradation activity, this method correlated well with the HPLC method. The kinetics of wild type Lignin peroxidase was determined at 2,4-DCP 1 mM, H2O2 50 mM. (Vmax : 59.880, Km : 0.359, Vmax/Km : 1.67 x 105 and Initial degradation rate : 59.822 (μmol /min/mg of protein)). The lignin peroxidase H2 gene was isolated from *Phanerochaete chrysosporium*, a white-rot fungus. The molecular weight of the recombinant LiP was ca. 38 kDa, which corresponded to the predicted mature LiP H2 gene. We performed cloning into pYD vector for yeast cell surface display of the gene. The surface expression was checked by the rapid, colorimetric screening method. The supernatant did not change the color, but the washed yeast cells changed the color from white to red, which confirmed the enzymes were located on the cell surface. Five colonies expressing the LiP H2 gene at high levels were selected. The average amount of 2,4-DCP degradation was about 23%. It was approximately 63% degradation level of the LiP from the wild type fungus. And the average amount of total surface displayed LiP H2 protein concentration was 34.57 (mg/ml).

We performed improvement of 2,4-DCP degradation activity of LiP H2 by DNA shuffling. The catalytic efficiency toward 2,4-DCP(vs. wild type) of the variant pYD using surface display system was improved by DNA shuffling. The Vmax value increased 8.35 fold but Km value decreased ca. twice. And Vmax/Km value increased 3.96 fold at high concentrate 2,4-DCP 1 mM and H2O2 50 mM. The Km value for H2O2 was increased vs. wild type. (4.08 x 10-3, 88 folds)