ABSTRACTS (MASTER THESIS)

Bioethanol production process incorporating expression of laccase bearing lignin-binding peptide

**(Graduate School of Agriculture
Laboratory of Biomass Conversion, RISH, Kyoto University)**

Kento Masuda

In recent year, production of bioethanol and chemicals from lignocellulosic biomass attracts a great deal of interest due to global warming caused by excess usage of fossil fuels. Process of bioethanol production from lignocellulosics consists of pretreatment, saccharification and fermentation. In the conversion process, lignin hiders access of (hemi)celluolytic enzymes to the cell wall polysaccharides, and the residual lignin adsorbs non-productively onto (hemi)cellulase to lose hydrolytic activity of the enzyme. In addition, pretreatments under harsh conditions produce enzyme and fermentation inhibitors. Therefore, removal of the lignin fragments and inhibitors is necessary to improve the bioethanol yield. For this purpose, we planned to develop the conversion process incorporating expression of laccase bearing lignin-binding peptide sequence C416 which had been found by phage display technique [1]

First, we expressed laccase from *Trametes versicolor* bearing the tandem dimer of lignin-binding peptide C416 [2] at N- or C-terminal end in *Saccharomyces cerevisiae* to assess the effects of mutant enzyme on bioethanol production. Next, we expressed the recombinant laccase in *Pichia pastoris*, and effects of the addition of mutant enzyme on bioethanol fermentation was evaluated. In pre-saccharification and simultaneous fermentation (PSSF) process using the laccase bearing the tandem dimer of lignin-binding peptide C416 at C-terminal end, ethanol production was increased compared with PSSF process using the laccase without the lignin biding peptide sequence.

References

[1] Yamaguchi, A., Isozaki, K., Takaya, H., Watanabe, T., “Discovery of 12-mer peptides that bind to wood lignin”, *Scientific Reports*, **6**, 21833, 2016.

[2] Oshiro, S., Yamaguchi, A., Watanabe, T., “Binding behaviour of a 12-mer peptide and its tandem dimer to gymnospermae and angiospermae lignins”, *RCS Advance*, **7**, 31338-31341, 2017.