Treatment of the fermentation inhibitors from wood by *Trametes versicolor* RC3 Laccase

Keisuke Nishi
Laboratory of Biomass Conversion, RISH, Kyoto University

INTORODUCTION

For an efficient ethanol production from wood biomass resources, a pretreatment step is essential to remove lignin which covers cellulose in the plant cell wall. Ethanolysis is one of the proposed pretreatment steps and separates wood into a pulp fraction and soluble fraction (SF). We have demonstrated that the SF contains furfural, vanillin, syringaldehyde, 5-hydroxymethylfurfural (5-HMF), 3-methyl-2,5-furandion, levulinic acid which are produced in the pretreatment step and that they inhibit the following fermentation to produce ethanol [1]. To obtain high yield of the ethanol production from wood biomass, it is necessary to minimize the influences of these wood-originated fermentation inhibitors produced in the pretreatment process. We founded that a white rot fungus *Trametes versicolor* RC3, which was isolated from Thailand [2], secreted laccase isozymes and they abolished the inhibitory effect of the compounds and promoted the fermentation efficiency of the SF by *Pichia stipitis*[3]. In the present study, to elucidate the removal mechanism of fermentation inhibitors by *T. versicolor* RC3 laccase, we conducted gene cloning and heterologous expression of a RC3 laccase isozyme and analyses of the reaction products of the fermentation inhibitors treated by *T. versicolor* RC3 laccase fractions using GC-MS.

MATERIALS and METHODS

We purified a laccase isozyme with pI value 8.35, using the gel filtration and ion-exchange chromatography and the N-terminal amino acid sequence was determined. Using degenerated primers for the gene encoding this isozyme, a DNA fragement was amplified by PCR and cloned. After cloning of the flunking sequences, we constructed an expression plasmid which has *P. ostreatus sdi1* promoter followed by the coding sequence of the cloned laccase gene from *T. versicolor* RC3. The expression plasmid was introduced in *P. ostreatus* and extracellular laccases were characterized. On the other hand, 5-HMF, vanillin, syringaldehyde and the SF was treated with the acidic or alkaline fractions of laccase from culture filtrate of *T. versicolor* RC3, at 45°C for 24 hour, and the resulting compounds were analyzed by GC-MS.

RESULTS and DISCUSSION

The determined nucleotide sequence demonstrated that the cloned gene encodes a new laccase isozyme, but not isozyme of pI 8.35, and the recombinant gene was expressed to produce an isozyme of pI 2.94 in *P. ostreatus*. We also found that acidic fraction of *T. versiclor* RC3 laccases degraded 5-HMF, vanillin, syringaldehyde as well as, or better than, the other laccases from the same strain. Moreover, it was demonstrated that the acidic fraction was significantly stable against incubation with SF compared to any other laccases tried, suggesting possible contribution of this fraction in the high removing activity of fermentation inhibitors by RC3 laccases.

REFERENCES

[1] Nakamoto H. (2005) Div. Appl. Life Sci., Graduate School of Agric Sci., Kyoto Univ., Master thesis.
[2] Khanongnuch, C. et al. (2004) Fungal Diversity, 15, 187-196
[3] Wada M. (2004) Div. Appl. Life Sci., Graduate School of Agric Sci., Kyoto Univ., Master thesis.