ABSTRACTS (MASTER THESIS)

Stepwise enzymatic fractionation and structural analysis of lignin-carbohydrate complex

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Lignocelluloses are mainly composed of cellulose, hemicelluloses, and lignin. Lignin is a heterogeneous aromatic polymer with high potentials to utilize as resources for energy, chemicals, and materials supply. Lignin associates with hemicelluloses in plant cell walls. The amphipathic complex is called Lignin-Carbohydrate Complex (LCC). LCC contributes to the physical strength of plant cell walls, but at the same time, it hinders the separation of the plant cell wall components. Chemical structures of LCC, especially covalent linkages between lignin and carbohydrates have not been fully understood because of the low frequency of lignin-carbohydrate (LC) linkages, compared with its polymer backbones, lignin and hemicellulose moieties. Therefore, the concentration of LC linkages is essential for the entire structural analysis. Recently, ester-type LC linkages have been elucidated from pine wood [1]. In this study, we aimed at purifying the LC fragments with increased LC linkages by combining two steps enzymatic treatments degrading lignin and hemicelluloses from wood.

In the lignin degradation, we used five kinds of lignin-degrading enzymes derived from a marine microorganism, *Novosphingobium* sp. These five enzymes catalyze three-step reactions cleaving β-*O*-4 linkages, a major linkage in lignin. It is shown that the dimeric lignin model compounds can be degraded into aromatic phenylpropanone monomer. We proved these enzymes act on natural lignin substances and produce monomer in one pot [2]. The advantage in using these enzymes is selective cleavage of the interunit linkages between phenylpropane units in lignin without the scission of LC bonds, which facilitates purification of the LCC fragments with decreased molecular mass. We evaluated the enzyme reactivity towards LCC fractions by LC-ESI-MS and 2D-NMR.

References

[1] Nishimura, H., Kamiya, A., Nagata, T., Katahira, M., Watanabe, T. (2018). Direct evidence for α ether linkage between lignin and carbohydrates in wood cell walls. *Scientific Reports*, 8, 6538.

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