RECENT RESEARCH ACTIVITIES

Characterization of lignocellulose in *Erianthus arundinaceus* and identification of the genes that encode antitumor lignan biosynthetic enzymes (Laboratory of Metabolic Science of Forest Plants and Microorganisms, RISH, Kyoto University)

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Wood biomass is the most abundant renewable resource on the earth, and therefore, the better utilization and efficient production of wood biomass are the key factors to establish a sustainable society. In this context, our laboratory is involved in analyzing metabolic functions of forest plants from a wide variety of aspects, including organic chemistry, biochemistry, molecular biology, and metabolomics, aiming at the elucidation of mechanisms of lignocellulose formation of biomass plants and their biotechnological application. Here we describe some of the recent research topics of our laboratory.

1. Characterization of lignocellulose in *Erianthus arundinaceus*

Lignin is a major component of the secondary cell walls of vascular plants, and an obstacle in the enzymatic saccharification of cell wall polysaccharides. *Erianthus* spp. are large gramineous plants of interest as potential feedstock. However, lignocelluloses of the species have not been chemically characterized. In this study, we analyzed lignins, related compounds, enzymatic saccharification efficiencies (ESEs), and minerals in the ash of the inner and outer parts of the internode, leaf blade, and leaf sheath of *Erianthus arundinaceus*. Lignins in the four organs consisted of guaiacyl, syringyl, and *p*-hydroxyphenyl units. The ratios of syringyl to guaiacyl lignins and lignin contents ranged from 0.43 to 0.79 and 20 to 28%, respectively, with values highest in the outer part of the internode. The amounts of ferulic acid unit were similar (7.3–11.8 mg g^{-1} dry weight of cell-wall material) in all four organs, while there was more *p*-coumaric acid units in the inner part of the internode (44.7 mg g^{-1} dry weight of cell-wall material) than in other organs (25.7–28.8 mg g^{-1} dry weight of cell-wall material). The ESE (24 h reaction time) of the leaf blade was 21.6%, while those of the other organs ranged from 10.0 to 15.2%. The inner part of the internode did not show a negative correlation between lignin contents and ESEs, suggesting that enzymatic saccharification of inner part was reduced by not only lignin but also other factors. The leaf blade had the highest ash content (17.1%); the main inorganic element was silicon. This study provides the first fundamental knowledge of *E. arundinaceus* lignins [1].

2. Identification of cDNAs that encode antitumor lignan biosynthetic enzymes

There have been a number of reports on the isolation and characterization of cDNAs encoding enzymes involved in lignan biosynthesis. In our research, we isolated and characterized three cDNAs that encode *O*-methyltransferases (OMTs), which are involved in lignan biosynthesis of *Anthriscus sylvestris* and *Forsythia koreana*. The first OMT, AsTJOMT, is responsible for the first *O*-methylation step in yatein biosynthesis from matairesinol in *A. sylvestris* [2]. Yatein is a precursor to podophyllotoxin, an aryltetralin lignan used for semi-synthesis of cancer-treating drugs. The other OMTs, each from *A. sylvestris* and *F. koreana*, catalyzed the *O*-methylation of matairesinol into arctigenin and isoarctigenin, respectively. Arctigenin is known to have high pharmacological significance such as: being a lead structure for inhibitors of human immunodeficiency virus type-1 integrase, having immunomodulatory effect on tumor necrosis factor-α and nitric oxide production, and lymphocyte production, and as an activator of AMP-activated protein kinase, which controls whole-body glucose homeostasis.

References

[1] M. Yamamura, S. Noda, T. Hattori, A. Shino, J. Kikuchi, K. Takabe, S. Tagane, M. Gau, N. Uwatoko, M. Mii, S. Suzuki, D. Shibata, T. Umezawa, "Characterization of lignocellulose of *Erianthus arundinaceus* in relation to enzymatic saccharification efficiency." *Plant Biotechnology* 30, 25-35, 2013.

[2] S. K. Ragamustari, T. Nakatsubo, T. Hattori1, E. Ono, Y. Kitamura, S. Suzuki, M. Yamamura, T. Umezawa, "A novel *O*-methyltransferase involved in the first methylation step of yatein biosynthesis in *Anthriscus sylvestris*," *Plant Biotechnology*, in press.