

Development of high-throughput lignin determination and characterization methods and discovery of oxalate transporter from *Fomitopsis palustris*

**(Laboratory of Metabolic Science of Forest Plants and Microorganisms,
RISH, Kyoto University)**

Toshiaki Umezawa, Takefumi Hattori, and Shiro Suzuki

It is becoming more important to establish a sustainable society, which depends on renewable resources. 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 and microorganisms from a wide variety of aspects, including organic chemistry, biochemistry, molecular biology, and metabolomics, aiming at the elucidation of mechanisms of wood formation of biomass plants, degradation of wood by wood-rotting fungi, and symbiosis of ectomycorrhizal fungi with forest trees, and their biotechnological application. Here we describe the recent research topics of our laboratory.

1. Development of high-throughput lignin determination and characterization methods

In molecular breeding of wood biomass plants, straight-forward, high-throughput, and microscale characterization of wood biomass components is important to select the best recombinant lines at the stage of juvenile plantlets. Because lignin is one of the major components in wood biomass, its biosynthesis is one of the major targets in molecular breeding of wood biomass plants. However, the conventional methods for lignin analysis are low-throughput and employ complicate experimental steps. Recently we modified the thioglycolic acid lignin determination and nitrobenzene oxidation method suitable for large number and small quantity of samples [1-3]. Using the analytical methods, we are selecting the rice plants to enable the efficient conversion of wood biomass to energy.

2. Discovery of oxalate transporter from *Fomitopsis palustris*

An oxalate-fermenting brown-rot fungus, *Fomitopsis palustris*, secretes large amounts of oxalic acid during wood decay. The acid is indispensable for the degradation of wood cell walls. We isolated a cDNA, *FpOAR* (*Fomitopsis palustris* Oxalic Acid Resistance), from *F. palustris* by functional screening of yeast transformants with cDNAs grown on oxalic acid-containing plates. The yeast transformant possessing *FpOAR* (*FpOAR*-transformant) acquired resistance to oxalic acid and contained less oxalate than the control transformant with an empty vector. Biochemical analyses using membrane vesicles of the *FpOAR*-transformant showed the oxalate transport property of FpOAR. FpOAR is strongly suggested to play an important role in wood decay by acting as a secondary transporter responsible for secretion of oxalate by *F. palustris* [4].

References

- [1] S. Suzuki, Y. Suzuki, N. Yamamoto, T. Hattori, M. Sakamoto, T. Umezawa, "High-throughput determination of thioglycolic acid lignin from rice," *Plant Biotechnology*, vol. 26, pp. 337-340, 2009.
- [2] M. Yamamura, T. Hattori, S. Suzuki, D. Shibata, T. Umezawa, "Microscale alkaline nitrobenzene oxidation method for high-throughput determination of aromatic components," *Plant Biotechnology*, vol. 27, pp. 305-310, 2010.
- [3] M. Yamamura, S. Wada, N. Sakakibara, T. Nakatsubo, S. Suzuki, T. Hattori, M. Takeda, N. Sakurai, H. Suzuki, D. Shibata, T. Umezawa, "Occurrence of guaiacyl/p-hydroxyphenyl lignin in *Arabidopsis thaliana* T87 cells," *Plant Biotechnology*, vol. 28, pp. 1-8, 2011.
- [4] T. Watanabe, N. Shitan, S. Suzuki, T. Umezawa, M. Shimada, K. Yazaki, T. Hattori, "Oxalate efflux transporter from the brown-rot fungus *Fomitopsis palustris*," *Applied and Environmental Microbiology*, vol. 76, pp. 7683-7690, 2010.