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Gene co-expresson network analysis and tropical tree biotechnology

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It is becoming more important to establish a sustainable society, which depends on renewable resources. Because wood biomass is the most abundant renewable resource, studies of wood formation is the key to improve forest biomass production. In this context, we are involved in analyzing metabolic functions of forest plants and microorganisms from a wide variety of aspects, including organic chemistry, biochemistry, molecular biology, and metabolomics, in order to conduct basic investigations contributing cultivation and protection of forest resources. These projects are conducted in collaboration with Assistant Professor Shiro Suzuki, Institute of Sustainability Science, Kyoto University.

1. Gene co-expression network analysis

During the last two decades, significant advances have been made in molecular biology of wood formation. For example, many genes involved in lignin biosynthesis have been cloned and their functions have been unequivocally identified. Having the genes in our hands, the major concern in tree biotechnology is the elucidation of comprehensive gene expression control mechanisms for cell-wall formation. Identification of transcription factors (TFs), as well as microRNAs, controlling expression of genes encoding enzymes involved in cell-wall formation is one of the most important steps. Following the completion of genome sequences of *Arabidopsis thaliana*, a large number of microarray data sets of *A. thaliana* gene expression are open to public, which can be exploited to analyze co-expression of genes. We are conducting gene co-expression network analysis starting with the genes encoding enzymes of the cinnamate/monolignol pathway providing monolignols. This allowed us to find out several genes encoding TFs which possibly control the gene expression of the pathway enzymes. Next, in order to confirm the roles of the TFs, we prepared transgenic *A. thaliana* cells where the individual TF genes were upregulated. Lignin characterization of the transgenic cells thus obtained and *A. thaliana* T-DNA tag line mutants with the target genes being downregulated are under way by the use of Forest Biomass Analytical System, so that we can identify TFs which controls the metabolic flow of the cinnamate/monolignol pathway in *A. thaliana*.

2. Mechanisms for organic acid metabolism of wood-rotting fungi and ectomycorrhizal fungi

Biodegradation of wood components by wood-rotting (WR) fungi including white- and brown-rot basidiomycetes is important as a first process leading to humus production, which in turn contributes greatly to sustainable forest ecosystems. Oxalate excreted from WR fungi plays a wide variety of roles in the degradation owing to its various chemical natures. We have proposed that oxalate metabolism is an important biochemical device to produce energy for fungal growth of WR fungi. Previously, we purified terminal enzyme for oxalate synthesis, cytochrome *c* dependent glyoxylate dehydrogenase (FpGLOXDH), from brown-rot fungus *Fomotpsis palustris*. Recently, cDNA encoding the FpGLOXDH was cloned. The deduced FpGLOXDH possessed the peroxisomal target signal S-K-L at the C-terminus, suggesting that FpGLOXDH is localized in the peroxisome. The peroxisomal localization of FpGLOXDH was shown by electron microscopic and immunocytochemical analysis with an anti-FpGLOXDH antibody. In addition to our previous results on cytosolic oxalate production by oxaloacetase, the present results strongly suggest that oxalate is also formed by FpGLOXDH in the peroxisome of *F. palustris*.

Cytochemical and molecular approaches for enzymes and transporters involved in organic acid metabolism are being investigated for WR fungi. Furthermore, comprehensive study for elucidation of regulatory mechanisms for organic acid metabolism in WR and ectomycorrhizal fungi has just begun.