Screening of extracellular oxidized metabolites produced by selective white rot fungus

**(Graduate School of Agriculture,
Laboratory of Biomass Conversion , RISH, Kyoto University)**

Yuichi Setogawa

Ceriporiopsis subvermispora is known as one of the best selective lignin-degrading fungi. Due to the unique wood decay system degrading lignin without intensive damage of cellulose, extensive research on the applications of the fungus to biopulping, preparation of feed for ruminant animals and pretreatments for enzymatic saccharification and fermentation have been carried out. In selective white rot by *C. subvermispora*, lignin degradation proceeds without penetration of extracellular enzymes into the wood cell wall regions. This unique phenomenon indicates that low molecular mass metabolites are principally responsible for the lignin degradation because these metabolites are able to diffuse into the wood cell wall regions where extracellular enzymes cannot penetrate.

Thus far, manganese peroxidase (MnP)-mediated lipid peroxidation has been proposed as a ligninolytic system at an incipient stage of wood decay by *C. subvermispora.* Production profiles of MnP, saturated and unsaturated fatty acids and their oxidation products, hydroperoxides and TBARS supported that the fatty acids are produced by the fungus and oxidized during the early stage of wood decay. In addition to the production and oxidation of fatty acids, new secondary metabolites suppressing ion redox cycle have been found. Thus far, four novel alk(en)ylitaconic acids; tetradecylitaconic (ceriporic acid A), hexadecylitaconic (ceriporic acid B), (*Z*)-7-hexadecenylitaconic (ceriporic acid C) and (*E*)-7-hexadecenylitaconic acid (ceriporic acid D) have been isolated and identified from the cultures of *C. subvermispora.* It was demonstrated that the alkylitaconic acids suppressed reduction of ferric ions, thereby attenuating production of celluloytic hydroxyl radicals by the Fenton reaction system.

In the present study, the author focused on oxidized metabolites produced by *C. subvermispora.* The selective white rot fungus, *C. subvermispora* ATCC90467 was grown on a potato dextrose agar medium at 25°C for 5 days. The preculture was inoculated into 200 ml of SDW medium in 500-ml Erlenmeyer flask and incubated statically at 28 °C for 3 weeks. After incubation, the culture was gently filtered with nylon mesh. The residues of fungal hyphae on the nylon filter were filtered off using vacuum pump and the metabolite solution was collected. Then, ten times amount of ethanol was added to the solution to give white precipitates. After removing precipitates by centrifugation, the supernatants were purified by solid phase extraction, evaporated, and analyzed by LC-IT-TOF MS.

Screening of the metabolites by LC-IT-TOF MS revealed that this fungus produced a wide range of oxidized derivatives from long-chain alkyl and alkenyl carboxylic acids. Some of the oxidized derivatives were also found from wood meal cultures of the fungus. The oxidized metabolites are trait of the extracellular oxidative reactions in selective white rot, and important to understand the roles of secondary metabolites in the wood decay at a site far from enzymes.