Studies on extracellular metabolites of white rot basidiomycete involved in selective lignin degradation

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Photosynthetic plants are the principal solar energy converter sustaining life on Earth. In particular, woody plant is the most abundant carbon fixed in terrestrial and an important renewable organic resource. In recent years, the global warming and environmental pollution caused by excessive use of fossil fuels became serious problem for our environment. Hence, it is an urgent task to utilize wood biomass as energy and chemical resources, in harmony with environmental safeguard.

In biological conversion of woody biomass, selective lignin degradation is a key process because cell wall polysaccharides in wood are surrounded by lignin. In nature, the degradation of lignin in wood occurs primarily through the action of lignin-degrading basidiomycetes called white rot fungi; consequently, this ecological group has received a considerable amount of research attention. Most of white rot fungi simultaneously decompose lignin and cellulose, accompanied by erosion of wood cell walls, while some fungi called selective white rot fungi, such as *Ceriporiopsis subvermispora* are able to degrade lignin without intensive damage of cellulose [1, 2].

Previous studies revealed that selective lignin degradation by *C. subvermispora* is catalyzed by low molecular weight metabolites at a site far from extracellular enzymes and fungal hyphae. For the possible wood decay system, an *in situ* radical generating system by lipid peroxidation initiated with manganese peroxidase (MnP) or its oxidation products, diffusible manganic chelates has been proposed. In selective white rot, control of cellulolytic active oxygen species, hydroxyl radicals by ceriporic acids has also been proposed as a central system to suppress cellulose depolymerization. However, mechanisms behind the selective ligninolysis after incipient stage of wood decay are not well understood. The present doctoral thesis aimed at elucidating the unique wood decay mechanism by analyzing structure and functions of key extracellular metabolites in sheath, a glucan matrix interfacing fungal hyphae and wood cell wall regions.

Regarding the extracellular metabolites of *C. subvermispora*, three novel alkylitaconic acids—tetradecylitaconic (ceriporic acid A), hexadecylitaconic (ceriporic acid B) and (*Z*)-7-hexadecenylitaconic acid (ceriporic acid C) have been isolated and identified [3–5]. Production of a trace amount of the other alkenylitaconic acids with different chain lengths has been suggested by GC–MS of crude extracts from eucalyptus wood cultures of *C. subvermisopra* [6] but only the three metabolites, ceriporic acids A, B and C were so far isolated. The molecular functions of ceriporic acids have been analyzed in relation to the control of production of active oxygen species. An important function of ceriporic acid is inhibition of cellulose degradation by hydroxyl radicals ($HO^{\bullet}$) produced in the Fenton reaction system [7–9]. In brown rot and non-selective white rot fungi, a cellulolytic active oxygen species, $HO^{\bullet}$ is a principal low-molecular mass oxidant that erodes wood cell walls to enhance the accessibility of the extracellular enzymes. This is because phenols are good reductants of Fe^{3+}, and reductive radicals such as formate anion and semiquinone radicals from oxalate and benzoquinones reduce molecular oxygen to yield superoxide, which in turn disproportionates into H_2O_2 or reduces Fe^{3+} to Fe^{2+}. Reduction of Fe^{3+} and formation of H_2O_2 leads to the production of $HO^{\bullet}$. In selective ligninolysis in an environment where free radicals and lignin-derived phenols are produced in the presence of O_2 and Fe ions, suppression of the Fenton reaction system by ceriporic acid is essential.

In this thesis, the author profiled metabolites pooled in the extracellular sheath, and found that *C. subvermispora* secretes a series of ceriporic acids and other metabolites. It was found that *C. subvermispora* produce a new ceriporic acid derivative bearing (*E*)-7-hexadecenyl chain from the culture of *C. subvermispora*. To identify the natural metabolite, (*E*)- and (*Z*)-7-hexadecenylitaconic acids were chemically synthesized. The new metabolite isolated was identical to the synthetic (*E*)-hexadecenylitaconic acid, and designated as ceriporic acid D. *De novo* synthesis of the *cis*- (ceriporic acid C) and *trans*-7-hexadecenylitaconic acids (ceriporic acid D) was demonstrated by feeding experiments

with [^{13}C-U]-glucose. The biosynthesis of ceriporic acid D was discussed with a focus on the formation of *trans*-desaturated bond, in comparison with structurally-related lichen acids [10]. Ceriporic acids have an asymmetric centre at carbon-3, but absolute configuration has not been determined. The author determined absolute configuration of ceriporic acid as *R* by measuring NMR spectra of the natural and synthetic compound with a chiral shift reagent. Considering an asymmetry of the chiral center and a possible biosynthetic pathway of ceriporic acids involving in the condensation of acyl-CoA and oxaloacetate, it was proposed that ceriporic acids are biosynthesized through attack of acyl-CoA to the *re* face of a keto carbonyl group of oxaloacetate. The stereospecificity is opposite to most of citrate synthase. Absolute configuration of ceriporic acids and structurally related lichen acids was reviewed [11].

In conclusion, the author analyzed metabolites pooled in the extracellular sheath, and found that *C. subvermispora* secretes a series of ceriporic acids and other metabolites. Structural analyses and function analyses of these metabolites involved in selective lignolysis were demonstrated and described the unique wood decay mechanism.

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