S2-2

Sesquiterpenoids Bioconversion Analysis by Wood Rot Fungi

Su-Yeon Lee[1], Sun-Hwa Ryu[1], In-Gyu Choi[2] and Myungkil Kim[*]

*[1]Division of Wood Chemistry & Microbiology, Department of Forest Resources Utilization, Korea Forest Research Institute, Seoul 130-712, Republic of Korea; [2]Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, South Korea; *Email: mkkim0201@korea.kr*

Sesquiterpenoids are defined as C_{15} compounds derived from farnesyl pyrophosphate (FPP), and their complex structures are found in the tissue of many diverse plants (Degenhardt et al. 2009). FPP's long chain length and additional double bond enables its conversion to a huge range of mono-, di-, and tri-cyclic structures. A number of cyclic sesquiterpenes with alcohol, aldehyde, and ketone derivatives have key biological and medicinal properties (Fraga 1999).

Fungi, such as the wood-rotting *Polyporus brumalis*, are excellent sources of pharmaceutically interesting natural products such as sesquiterpenoids. In this study, we investigated the biosynthesis of *P. brumalis* sesquiterpenoids on modified medium.

Fungal suspensions of 11 white rot species were inoculated in modified medium containing $C_6H_{12}O_6$, $C_4H_{12}N_2O_6$, KH_2PO_4, $MgSO_4$, and $CaCl_2$ for 20 days. Cultivation was stopped by solvent extraction via separation of the mycelium. The metabolites were identified as follows: propionic acid (1), mevalonic acid lactone (2), β-eudesmane (3), and β-eudesmol (4), respectively (Figure 1). The main peaks of β-eudesmane and β-eudesmol, which were indicative of sesquiterpene structures, were consistently detected for 5, 7, 12, and 15 days These results demonstrated the existence of terpene metabolism in the mycelium of *P. brumalis*. *Polyporus spp.* are known to generate flavor components such as methyl 2,4-dihydroxy-3,6-dimethyl benzoate; 2-hydroxy-4-methoxy-6-methyl benzoic acid; 3-hydroxy-5-methyl phenol; and 3-methoxy-2,5-dimethyl phenol in submerged cultures (Hoffmann and Esser 1978). Drimanes of sesquiterpenes were reported as metabolites from *P. arcularius* and shown to exhibit antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus* (Fleck et al. 1996).

The main metabolites of *P. brumalis, β-Eudesmol and β-eudesmane, were categorized as eudesmane-type sesquiterpene* structures. The eudesmane skeleton could be biosynthesized from FPP-derived IPP, and approximately 1,000 structures have been identified in plants as essential oils. The biosynthesis of eudesmol from *P. brumalis* may thus be an important tool for the production of useful natural compounds as presumed from its identified potent bioactivity in plants. Essential oils comprising eudesmane-type sesquiterpenoids have been previously and extensively researched (Wu et al. 2006). β-Eudesmol is a well-known and important eudesmane alcohol with an anticholinergic effect in the vascular endothelium (Tsuneki et al. 2005). Additionally, recent studies demonstrated that β-eudesmol acts as a channel blocker for nicotinic acetylcholine receptors at the neuromuscular junction, and it can inhibit angiogenesis *in vitro* and *in vivo* by blocking the mitogen-activated protein kinase (MAPK) signaling pathway (Seo et al. 2011).

Variation of nutrients was conducted to determine an optimum condition for the biosynthesis of sesquiterpenes by *P. brumalis*. Genes encoding terpene synthases, which are crucial to the terpene synthesis pathway, generally respond to environmental factors such as pH, temperature, and available nutrients (Hoffmeister and Keller 2007, Yu and Keller 2005). Calvo *et al*. described the effect of major nutrients, carbon and nitrogen, on the synthesis of secondary metabolites (Calvo *et al*. 2002). *P. brumalis* did not prefer to synthesize sesquiterpenes under all growth conditions. Results of differences in metabolites observed in *P. brumalis* grown in PDB and modified medium highlighted the potential effect inorganic sources such as $C_4H_{12}N_2O_6$, KH_2PO_4, $MgSO_4$, and $CaCl_2$ on sesquiterpene synthesis. β-eudesmol was apparent during cultivation except for when *P. brumalis* was grown on $MgSO_4$-free medium. These results demonstrated that $MgSO_4$ can specifically control the biosynthesis of β-eudesmol. Magnesium has been reported as a cofactor that binds to sesquiterpene synthase (Agger *et al*. 2008). Specifically, the Mg^{2+} ions bind to two conserved metal-binding motifs. These metal ions complex to the substrate pyrophosphate, thereby promoting the ionization of the leaving groups of FPP and resulting in the generation of a highly reactive allylic cation. Effect of magnesium source on the

sesquiterpene biosynthesis was also identified via analysis of the concentration of total carbohydrates. Our current study offered further insight that fungal sesquiterpene biosynthesis can be controlled by nutrients.

To profile the metabolites of *P. brumalis,* the cultures were extracted based on the growth curve. Despite metabolites produced during mycelia growth, there was difficulty in detecting significant changes in metabolite production, especially those at low concentrations. These compounds may be of interest in understanding their synthetic mechanisms in *P. brumalis*. The synthesis of terpene compounds began during the growth phase at day 9. Sesquiterpene synthesis occurred after growth was complete. At day 9, drimenol, farnesol, and mevalonic lactone (or mevalonic acid lactone) were identified. Mevalonic acid lactone is the precursor of the mevalonic pathway, and particularly, it is a precursor for a number of biologically important lipids, including cholesterol hormones (Buckley et al. 2002). Farnesol is the precursor of sesquiterpenoids. Drimenol compounds, bi-cyclic-sesquiterpene alcohols, can be synthesized from trans-trans farnesol via cyclization and rearrangement (Polovinka et al. 1994). They have also been identified in the basidiomycota *Lentinus lepideus* as secondary metabolites. After 12 days in the growth phase, β-elemene caryophyllene, δ-cadiene, and eudesmane were detected with β-eudesmol. The data showed the synthesis of sesquiterpene hydrocarbons with bi-cyclic structures. These compounds can be synthesized from FPP by cyclization. Cyclic terpenoids are synthesized through the formation of a carbon skeleton from linear precursors by terpene cyclase, which is followed by chemical modification by oxidation, reduction, methylation, etc. Sesquiterpene cyclase is a key branch-point enzyme that catalyzes the complex intermolecular cyclization of the linear prenyl diphosphate into cyclic hydrocarbons (Toyomasu et al. 2007). After 20 days in stationary phase, the oxygenated structures eudesmol, elemol, and caryophyllene oxide were detected. Thus, after growth, sesquiterpenes were identified. Per these results, we showed that terpene metabolism in wood-rotting fungi occurs in the stationary phase. We also showed that such metabolism can be controlled by magnesium supplementation in the growth medium. In conclusion, we identified *P. brumalis* as a wood-rotting fungus that can produce sesquiterpenes.

To mechanistically understand eudesmane-type sesquiterpene biosynthesis in *P. brumali*s, further research into the genes regulating the dynamics of such biosynthesis is warranted.