ABSTRACTS (MASTER THESIS)

Characterization of an *O*-methyltransferase involved in antitumor lignan biosynthesis in *Thujopsis dolabrata*

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Lignans are phenylpropanoid dimers that are linked at C8 positions of their propyl side chains and known for having various physiological activities. For examples, podophyllotoxin, aryltetralin lignan, is known as an antitumor lignan and used as a precursor for the chemical synthesis of the anticancer drugs etoposide, teniposide and etopophos [1]. *Podophyllum hexandrum* and *Anthriscus sylvestris* are known as plants that produce antitumor lignans including podophyllotoxin. Biosynthetic pathway of antitumor lignans has been studied using several plants, and some pathways were proposed. Sakakibara et al. [2] carried out feeding experiments using several stable isotope-labeled precursors to *A. sylvestris*, and proposed the pathway from matairesinol to yatein via 4,5-*O*,*O*-dimethylthujaplicatin. Recently, Lau and Sattely [3] were successful in isolation many genes involved in lignan biosynthesis of *P. hexandrum* based on RNA-seq gene expression analysis, and demonstrated that each of isolated genes can catalyze each reaction step from matairesinol to deoxypodophyllotoxin via bursehernin by agroinfiltration into tabacco leaves and biochemical characterization using recombinant protein.

Recently, author's laboratory cloned putative lignan OMT genes (TdOMT1, 3, 4, 5, and 6) from *Thujopsis dolabrata*, a closely related to *T. occidentalis*, as a collaboration with Shizuoka University [4]. Recombinant protein of TdOMT6 showed OMT activities for 4-*O*-demethylyatein and 5-*O*-methylthujaplicatin, while the functions of other genes (TdOMT1, 3, 4, and 5) were also unknown. On the other hand, although *T. dolabrata* produces deoxypodophyllotoxin [5], the biosynthetic pathway leading to the compound has not been elucidated. In this study, the author performed biochemical characterization of TdOMTs and estimated the biosynthetic pathway.

In this study, several lignans including matairesinol and deoxypodophyllotoxin were found in leaves of *T. dolabrata*. Furthermore, recombinant TdOMT6 catalyzed *O*-methylation of hydroxy group at C4 position of 4-*O*-demethylyatein, 5-*O*-methylthujaplicatin, and pluviatolide. The OMT activity for 4-*O*-demethylyatein was much greater than those for 5-*O*-methylthujaplicatin and pluviatolide, when the mixture of equal amounts of these three lignans was used as a substrate for enzyme assay. These results strongly suggest that TdOMT6 functions as 4-*O*-demethylyatein OMT, and biosynthetic pathway from matairesinol to deoxypodophyllotoxin via 4-*O*-demethylyatein in *T. dolabrata* exists.

References

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