ABSTRACTS (MASTER THESIS)

A new *O*-methyltransferase gene involved in antitumor lignan biosynthesis in *Anthriscus sylvestris*

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Lignans are phenylpropanoid dimers that are linked at C-8 position of their propyl side chains [1] and isolated from stem, heartwood, leaf, root, flower and fruit of a wide variety of plants [2]. Some lignans are known for having various physiological activities including antitumor, antiviral and antioxidative. Especially, podophyllotoxin is known as an antitumor lignan and used as a precursor for the chemical synthesis of the anticancer drugs etoposide, teniposide and etopophos [3]. However, the availability of this lignan is limited because of overharvesting podophyllotoxin-forming plants. Hence, stable and large scale production of podophyllotoxin has been paid attention [3].

Biosynthetic pathways of antitumor lignans have been studied using several plants, and some pathways were proposed. In a biosynthetic pathway of *A. sylvestris*, Sakakibara et al. proposed the pathway from matairesinol to yatein based on feeding experiment using stable isotope-labeled precursors [4]. Recently, cDNA encoding thujapilcatin *O*-methyltransferase (TJOMT), which catalyzes a conversion from thujaplicatin to 5-*O*-methylthujaplicatin, was identified from *A. sylvestris* [5]. But, the 5-*O*-methylthujaplicatin *O*-methyltransferase (5MTJOMT), catalyzes a conversion from 5-*O*-methythujaplicatin to 4,5-*O*,*O*-dimethylthujaplicatin, has not been identified. A set of genes involved in the podophyllotoxin biosynthetic pathway was not completely isolated in any plants including *A. sylvestris*. Isolation of all genes enables biological productivity of podophyllotoxin in future.

In this study, based on a correlation analysis between patterns of 5MTJOMT activity and of gene expression among different organs of *A. sylvestris*, several putative OMT sequences which had more than 0.2 correlation coefficient were selected as 5MTJOMT candidate genes. Then, recombinant proteins were prepared from each gene, and checked whether the recombinant protein had any activity with potential substrate. A lysate of the recombinant protein having OMT activity for 5-*O*-methylthujaplicatin was purified by affinity chromatography and desalted by gel filtration chromatography, and kinetic analysis for 5-*O*-methylthujaplicatin was conducted.

Eight genes were selected as 5MTJOMT candidate genes by correlation analysis. Among 5MTJOMT candidate genes, AsOMT95 having a moderate correlation coefficient (R = 0.43) showed OMT activity for 5-*O*-methylthujaplicatin and 4-demethylyatein. In addition, the catalytic efficiency (k_{cat}/K_m) of AsOMT95 for 5-*O*-methylthujaplicatin and 4-demethylyatein was 1.78 and 4.07 min^{-1} μM^{-1}, respectively. In *A. sylvestris*, 4-demethylyatein has not been detected and lignan biosynthetic pathway via 5-*O*-methylthujaplicatin was proposed based on feeding experiments. Therefore, it was suggested that *AsOMT95* is a gene involved in methylation of 5-*O*-methylthujaplicatin, i.e., *As5MTJOMT*.

References

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