Synthesis of Ermanin, 5,7-Dihydroxy-3,4'-dimethoxyflavone from Kaempferol, 
3,5,7,4'-Tetrahydroxyflavone with Two O-Methyltransferases Expressed in E. coli

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Enzymatic modifications of natural compounds have been drawn attention because they provide regioselectivity and chiral selectivity, which are hardly achievable with chemical synthesis. Using a mobilized enzyme or a whole cell containing a particular gene delivers useful tools for modification of natural compounds. In addition, using a whole cell, known as biotransformation, has advantage of saving expensive cofactors.

Flavonoids and alkaloids are secondary metabolites produced mainly in plants. Their biological activities have an impact on human health so that they serve as target molecules to develop new drugs. One of common modification reactions found in these two groups of compounds is O-methylation due to free hydroxyl groups. Various O-methylation reactions have been reported and a number of genes (O-methyltransferases: OMTs) that mediated O-methylation have been cloned and characterized. Some of OMTs that use flavonoids as substrates are highly specific and thus they could be used for the regioselective modification of flavonoids. O-methylation of flavonoids resulted in reduction of chemical reactivity and increase of antimicrobial activity. So far, O-methylated flavonoids have been mainly isolated from plants, which could be a limitation for biological assays. Kaempferol (3,5,7,4'-tetrahydroxyflavone) is one of commonly found flavonoids in nature. Even though several biological activities of kaempferol have been established, its dimethoxy form, ermanin (5,7-dihydroxy-3,4'-dimethoxyflavone) was known to have several activities including antiviral, anti-inflammatory, cytotoxic, and antibacterial activity. Lists of its biological activity might be extended if large amount of ermanin is supplied.

Previously, we cloned and characterized two OMT genes, SOMT-2 (soybean O-methyltransferase-2) and ROMT-9 (rice O-methyltransferase-9). The transgenic E. coli expressing SOMT-2 transferred a methyl group to 4'-hydroxyl group of flavonoids. ROMT-9 expressed in E. coli showed different regioselectivity depending on the availability of 3'-hydroxyl groups. It transferred a methyl group to 3'-hydroxyl group if flavonoids have 3'-hydroxyl group. But, when 3'-hydroxyl group is not present, it methylated a 3'-hydroxyl group. Here we report the biological synthesis of ermanin from kaempferol with two OMTs expressed in E. coli.

ROMT-9 and SOMT-2 were cloned in one expression vector and both proteins were induced from E. coli containing both ROMT-9 and SOMT-2 by adding IPTG at 100 μM. In addition, ROMT-9 and SOMT-2 were induced separately from E. coli containing either ROMT-9 or SOMT-2. After 4 hr induction, the cells from each culture were harvested and resuspended in LB containing ampicillin (50 μg/mL). 100 μM of kaempferol (3,5,7,4'-tetrahydroxyflavone) was added. The mixture was further incubated for 15 hrs at 28 °C. Analysis of culture filtrates from three reactions using high performance liquid chromatography (HPLC) revealed that kaempferol was converted into a new product which had different HPLC retention time from kaempferol itself. ROMT-9 produced a new peak at 15.2 min (Fig. 1B) and SOMT-2 produced a new peak at 19.9 min.

Figure 1. HPLC analysis of kaempferol reaction products with SOMT-2 and ROMT-9. A, authentic kaempferol; B, reaction product of kaempferol with ROMT-9; C, reaction product of kaempferol with SOMT-2; D, reaction product of kaempferol with SOMT-2 and ROMT-9.
The complete assignment of the NMR data of the reaction product was 3,4'-OCH₃ 3.84 (s) 55.3 C-4' H-3'.

Production of ermanin was monitored periodically for 128 hrs. SOMT-2 and ROMT-9 was induced as above. The culture was collected periodically and the amounts of reactant and product quantified with HPLC. As shown in Figure 2, the amount of kaempferol continued to decrease over time and was completely metabolized after 24 hrs. In contrast, 3-methoxy kaempferol increased in quality until 8 hrs and 4'-methoxy kaempferol increased until 16 hrs. The ermanin appeared after 8 hrs and increased to increase until 128 hrs. After 48 hrs incubation, more than 90% of kaempferol was converted into ermanin. Thus, 13.1 mg of ermanin was obtained from 14.3 mg of kaempferol with a yield of 91%. This approach does not require an enzyme purification step and S-adenosyl-L-methionine as a cofactor. Thus, it may be suitable for the production of large amounts of ermanin.

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References.