

Structure modification of Quercetin using Multiple expression with several genes

Bong-Gyu Kim , Kwang-Hee Shin and Joong-Hoon Ahn

Bio/Molecular Informatics Center, Department of Molecular Biotechnology, KonKuk University, Seoul

TEL : +82-2-450-3764, FAX +82-2-446-9001

Flavonoids are typical plant secondary metabolites belonging to a group of polyphenols. Over 6,000 natural flavonoids have been reported, and many of them are common in higher plants¹⁾. Flavonoids constitute a relatively diverse family of phenolic molecules. These compounds are modified by genes such as *O*-methyltransferase(OMT) and glycosyltransferase(GT)^{2),3)}. We investigated the multiple expressions of ROMT-9, SOMT-2, and BcGT-1. ROMT-9 and SOMT-2 are 3' and 4'-*O*-methyltransferase of quercetin and was cloned from Rice and Soybean, respectively. Also BcGT-1 is 7-glycosyltransferase and cloned from *Bacillus cereus*. Three genes were cloned to pET-21 vector system, respectively. In addition, ROMT-9 and SOMT-2 was independently cloned into one pET-21 vector and the resulting plasmid is named as RSOMT. ROMT-9, SOMT-2 and BcGT-1 conjugated vector is named as RSBK. Five constructs were expressed in *E. coli* BL21 to study multiple expression of RSOMT and RSBK for quercetin as substrate. HPLC analysis of reaction product from three construct having each gene showed one apparent product peak: 3'-*O*-methylated quercetin for ROMT-9, 4'-*O*-methylated quercetin for SOMT-2, and 7-*O*-glycosylated quercetin for BcGT-1. When RSOMT was expressed in BL21, three reaction products were detected, containing 3', 4'-*O*-dimethylated quercetin. In case of RSBK reaction products showed six product peaks, which will be further identified through mass spectrometric and NMR analysis.

References

1. Harbone, J. R. B. and Williams, C. A. (2000) Advances in flavonoid research since 1992. *Phytochemistry* 55 : 481-504.
2. Daniel J. Kliebenstein, Virginia M. Lambrix, Michael Reichelt, Jonathan Gershenzon, and Thomas Mitchell-Olds. (2001) Gene duplication in the diversification of secondary metabolism: tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in *Arabidopsis*. *Plant Cell*. 13 : 681-693.
3. Ingrid Muzac, Jing Wang, Dominique Anzellotti, Hong Zhang and Ragai K. Ibrahim (2000) Functional expression of an *Arabidopsis* cDNA Clone Encoding a Flavonol 3'-*O*-Methyltransferase and Characterization of the Gene Product. *Archives of Biochem. and Biophys.* 375 : 385-388.