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## Regulation of Isoprenoid Pathway Genes during Saikosaponin Biosynthesis in *Bupleurum falcatum*

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### Objectives

*Bupleuri Radix* (root of *Bupleurum* spp) is an important crude drug in oriental medicine. In the root, three major oleanane saponins, saikosaponin a, c and d, have been shown to exert various pharmacological effects such as anti-inflammatory and anti-tumor activity. In this experiment, hairy roots of *Bupleurum falcatum* were grown in MS and 3RCM liquid media to control saikosaponin production and then saikosaponin (a, c, and d) contents were measured in the roots. In this system, we examined expression pattern of 5 major genes in isoprenoid pathway (HMGR, IPP isomerase, FPP synthase, squalene synthase (SS), and oxidosqualene cyclase (OSC)) to get some insight into the regulatory mechanism controlling the triterpene branch (saikosaponin) of isoprenoid pathway

### Materials and Methods

1. Material : *Bupleurum falcatum* (cv. Siksiho)

2. Methods:

Roots culture: culture and maintenance of hairy roots BFHR2 accomplished as described previously (Ahn et al., 1999 Plant Biotechnology),

Cloning of 5 genes : partial cloning of 5 genes by RT-PCR by using degenerate primer

Saikosaponin analysis : Apparatus, HPLC of Waters Co.; Column,  $\mu$ -Bondapak C<sub>18</sub> ; Solvent, Acetonitrile:H<sub>2</sub>O 3:7→7:3 gradient

### Results and Discussion

Conserved regions of sequences from 5 isoprenoid pathway genes, HMGR, IPPI, FPP synthase, SS and OSC, were used to design degenerate primers for the cloning of the partial fragments from *Bupleuri Radix* by RT-PCR. Then, their expression patterns were examined in the hairy roots during the time course of saikosaponin biosynthesis. The expression of HMGR, FPP synthase and IPP isomerase genes was relatively constant during the time course but SS and OSC genes were dramatically induced at 8 days of incubation and maintained during the remaining period. On the other hand, quantitative HPLC analysis of saikosaponin content in the hairy roots revealed that the production of saikosaponin was getting started to increase from the 8 days of culture. The correlation between the SS and OSC transcript levels and the amount of saikosaponin in the hairy roots suggests that transcriptional regulation of SS and OSC genes plays an important role in controlling saikosaponin synthesis. We have further studied squalene synthase gene in the branch point of isoprenoid pathway.