

Expression of Isoprenoid Pathway Genes during Saikosaponin Biosynthesis in the Hairy Roots of *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, μ -Bondapak C18 ; Solvent, Acetonitrile:H₂O 3:7→7:3 gradient

Results and Discussion

To establish an efficient system to produce saikosaponins in *Bupleuri Radix*, different root culture medium were previously tested and 3RCM and MS medium were used in this experiment. Saikosaponin was hardly detected in the hairy roots grown in MS medium but about 5 times higher amount in 3RCM medium. So, the hairy roots growing in MS medium were subcultured into 3RCM medium. Then, the accumulation of saikosaponin was observed at 0, 2, 4, 8, 10, 15, 25, and 30 days of incubation. 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 in 3RCM medium.

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 remained during the remaining period. The correlation between the SS and OSC transcript levels and the amount of saikosaponin in the hairy roots suggests that transcriptional regulation plays an important role in controlling saikosaponin synthesis.