

## Decursin Production from Hairy Root Culture of *Angelica gigas*

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*Angelica gigas* Nakai, commonly known as Korean Angelica, is a perennial herb belonging to the Umbelliferae family and one of the most important medicinal plants in Korea. For thousands of years, the roots of *A. gigas* has been used as traditional Oriental herbal medicine to treat anemia, abdominal pain, injuries, migraine, arthritis, and female afflictions, and the material has also been prescribed for health-promoting effects [Chi and Kim, 1970; Choi *et al.*, 2003; Sarker and Nahar, 2004]. Decursin (Fig. 1) and decursinol angelate (a decursin isomer), the major secondary metabolites of *A. gigas*, have significant neuroprotective, anticancer, and anti-androgen-receptor signaling activities [Kang *et al.*, 2005; Yim *et al.*, 2005; Guo *et al.*, 2007].

Various species of bacteria can transfer genes to higher plants, among which *Agrobacterium rhizogenes*, a Gram-negative soil bacterium, is one of the most widely studied. *A. rhizogenes* infects the plant cell and leads to the formation of hairy roots [Signs and Flores, 1990]. In many plants, hairy root cultures have proven to be an efficient production system for secondary metabolites. Such cultures have genetic and biochemical stabilities, rapid growth rate, and the ability to synthesize natural compounds at levels comparable to those of intact plants [Giri and Narasu, 2000; Guillon *et al.*, 2006]. To the best

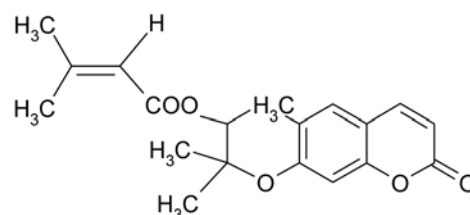


Fig. 1. Chemical structure of decursin.

of our knowledge, this is the first report on decursin production by hairy root culture of *A. gigas*. Here, we describe the production of decursin by hairy root cultures of *A. gigas* transformed with *A. rhizogenes* R1000.

*A. rhizogenes* strain R1000 cultures were grown to the log phase at 28°C on a gyratory shaker (180 rev/min) in liquid Luria-Bertani medium. The bacterial cells were collected by centrifugation for 10 min at 2000 rpm, and resuspended at a cell density of A600 = 0.5 in MS [Murashige and Skoog, 1962] liquid inoculation medium. Young leaf and stem of *A. gigas* were taken from plants grown *in vitro* and were cut at the ends into sections of 0.7 × 0.7 cm<sup>2</sup> and 0.7 cm. Excised leaves and stems were dipped into *A. rhizogenes* culture in the liquid inoculation medium for 10 min, blotted dry on a sterile filter paper, and incubated in the dark at 25°C on the agar-solidified MS medium. After 2 days of co-cultivation, the explant tissues were transferred to a hormone-free solid MS medium containing 200 mg/L Timentin. Within 4 weeks numerous hairy roots were observed emerging from the wound sites. The hairy roots were separated from the explant tissue and subcultured in the dark at 25°C on the agar-solidified MS medium. After repeated transfers to fresh media, rapidly growing hairy root cultures were obtained. Wild type root cultures were established by inoculating MS liquid medium with excised roots from *A. gigas* seedlings grown *in vitro*.

Isolated wild type and hairy roots (200 mg) were transferred to 30 mL MS liquid medium, containing 30 g/L sucrose, in 100 mL flasks. Wild type and hairy root cultures were maintained at 25°C on a gyratory shaker (100 rev/min) in a growth chamber. After 24 days of culture, hairy roots were harvested. Dry weight and decursin contents were determined. Three flasks were used for each culture condition, and the experiments were performed in duplicates.

Hairy roots were dried at -80°C in brown paper bags for at least 48 h using a freeze-dryer. Dried samples were ground into a fine powder using a mortar and pestle. Samples (0.5 g) were extracted with 30 mL of 70:30 (% v/v) ethanol-water at 50°C in a water bath for 1 h. After

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**Fig. 2.** Development of hairy roots from a stem of *A. gigas* after inoculation with *A. rhizogenes* strain R1000 ( $\times 10$ ).

centrifugation, the supernatant (25 mL) was concentrated under vacuum, and the residue (5 mL) was mixed with 5 mL of chloroform in a vortex mixer. The chloroform fraction was dried under vacuum and dissolved in 1 mL methanol. HPLC quantification of decursin was performed using a Futecs model NS-4000 HPLC apparatus (Daejeon, Korea) and an Optimapak ODS column (250  $\times$  4.6 mm; RStech, Daejeon, Korea) at room temperature. The solvent gradient consisted of 50% acetonitrile and 50% water (both v/v). The flow rate of the solvent was kept constant at 0.8 mL/min. Twenty  $\mu$ L samples were filtered through 0.45- $\mu$ m poly filters and injected, and the absorbance was monitored at 330 nm. All samples were run in triplicates.

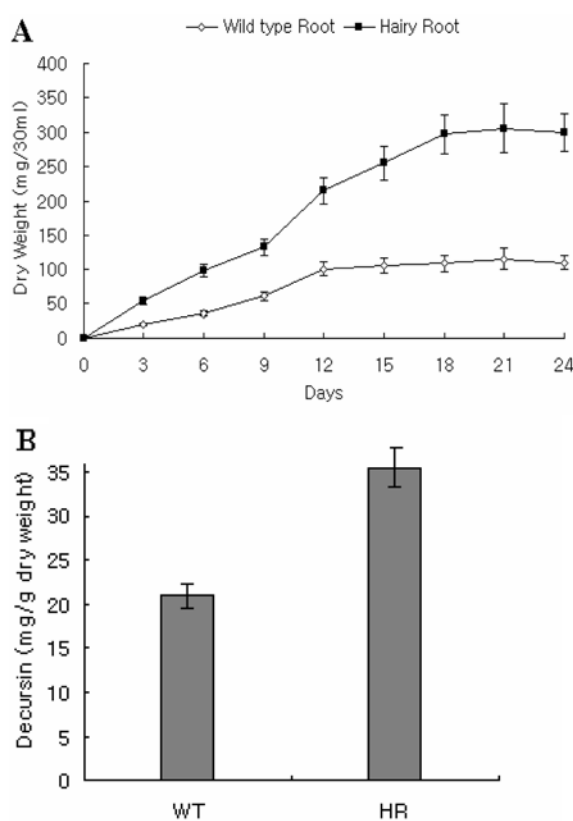
Hairy roots were induced from leaf and stem explants of *A. gigas*. After 2 days of co-cultivation with *A. rhizogenes* R1000, explant tissues were transferred to the agar-solidified MS medium containing 200 mg/L Timentin to remove *A. rhizogenes*. Two different explants, leaf and stem, were tested for hairy root production. Wounded explants were susceptible to infection by *A. rhizogenes* R1000, as shown by the percentages of explants from which hairy roots emerged. *A. rhizogenes* R1000 infected more than 60% of the leaf and stem explants after 30 days of inoculation (Fig. 2). Stems were chosen, as optimal explant tissues for co-cultivation with *A. rhizogenes*, because the frequency of bacterial infection was higher than that of the leaf explants and the resulting hairy roots grew more rapidly than those derived from the leaf (Table 1).

Young hairy roots emerged from the wound sites on the leaf and the stem within 15 days after bacterial inoculation. After 30 days of exposure to the bacteria, the hairy roots began to grow more rapidly. Rapidly growing hairy roots were excised from the explant tissues and

**Table 1.** Comparison of the frequency of infection and the induction of hairy root of the explant tissues of *A. gigas*

Explant tissue	Infection frequency (%)	Number of hairy roots	Root length (cm)
Leaf	60.8 $\pm$ 4.2	2.9 $\pm$ 0.2	1.1 $\pm$ 0.1
Stem	68.8 $\pm$ 5.7	4.2 $\pm$ 0.3	1.5 $\pm$ 0.1

Values represent means  $\pm$  SD values of three independent measurements made 30 days after bacterial inoculation.



**Fig. 3.** Time course of growth (A) and decursin production (B) of hairy root (HR) and wild type (WT) root cultures of *A. gigas* grown in MS medium for 24 days. Values represent the means  $\pm$  SD of six independent measurements.

subcultured at intervals of 3 weeks on a fresh agar-solidified MS medium containing 200 mg/L Timentin. After repeated subcultures for over 3 months, the hairy root clones were transferred to the MS liquid culture medium.

Hairy roots and wild type roots from *in vitro* grown plants were cultured in the MS liquid medium for 24 days. Growths of both hairy and wild type roots were investigated by harvesting at intervals of 3 days (Fig. 3-A). As the culturing time increased, the growths of both types of the root reached plateau. During the 24 days

culture period, the dry weight of the hairy root culture increased from the original inoculum level of 20 mg/30 mL to attain 306 mg/30 mL, where the maximum growth was attained after 20 days of culture and thereafter the root growth showed a decreasing trend. Though the increasing trend of the wild root growth was similar to that of the hairy root growth, the rate of increase was much lower compared with that of the hairy root. The maximum dry weight of the root was found at 21 days of culture irrespective of the root types. The amount of hairy root (306 mg/30 mL) was around three times higher than that of the wild type root (115 mg/30 mL). After 24 days of culture, the contents of decursin in hairy root and wild type root were investigated (Fig. 3-B). For the production of decursin, the hairy root was much better than that of wild type root. The amount of decursin (35.4 mg/g dry weight basis) found in the hairy root was 41% higher than that of the wild type root (20.9 mg/g dry weight basis).

In conclusion, the findings of this study suggest that the hairy root culture of *A. gigas* could be a good alternative for the production of decursin. Our current laboratory efforts are aimed at further improving the decursin production in the hairy root cultures of *A. gigas*.

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