Production of Red Ginseng Specific Ginsenosides (Rg2, Rg3, Rh1 and Rh2) from Agrobacterium - transformed Hairy Roots of Panax ginseng by Heat Treatment

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It was reported that Red ginseng contains specific ginsenoside-Rg2, -Rg3, -Rh1 and -Rh2, which show various pharmacological effects. However, production of these specific ginsenosides from Red ginseng is not commercially applicable because of high cost of the raw material, roots. This work was carried out to examine the production of Red ginseng specific ginsenosides from Agrobacterium-transformed hairy roots. Hairy roots were induced from 3 year-old root segments of Korean ginseng (Panax ginseng C.A. Meyer) after infection with Agrobacterium rhizogenes A4. Among many lines of hairy roots, KGHR-8A was selected. Steam heat treatment of hairy roots was resulted in the changes of ginsenoside composition. Eleven ginsenosides were detected in heat-treated hairy roots but eight in freeze dried hairy roots. In heat treated hairy root, content of ginsenoside-Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 were decreased compared to those of freeze dried hairy roots. However, heat treatment strongly enhanced the amount of Red ginseng specific ginsenosides (ginsenoside-Rg2, -Rg3, -Rh1 and -Rh2). Amounts of ginsenoside-Rg3, -Rh1 and -Rh2 in heat-treated hairy roots were 2.58, 3.62 and 1.08 mg/g dry wt, respectively, but these were detected as trace amount in hairy roots without heat treatment. Optimum condition of heat treatment for the production of Red ginseng specific ginsenoside was 2 h at 105°C. This result represents that Red ginseng specific ginsenoside can be produced from hairy roots by steam heat treatment.

Key words: Red ginseng, heat treatment, hairy root, ginsenoside

INTRODUCTION

Korean ginseng (Panax ginseng C.A. Meyer) is a perennial herbaceous plant and has been recognized as a miraculous medicinal plant. Various pharmacological effects of Korean ginseng were reported, such as tonic, adaptogenic, antistress, hypothermic, minor hyperglycemic, and anticancer activity [1]. The pharmacologically active main component of ginseng was largely accepted as saponin such as panaxadiol, panaxatriol, and oleanolic acid [2]. More than 20 of ginsenosides was reported in Korean ginseng and each ginsenoside has specific pharmacological effect [1].

Red ginseng is made from steam heat treatment of fresh ginseng roots. The red ginseng has some superior pharmacological effects compared to fresh or dried ginseng roots [3]. The red ginseng contains specific ginsenoside-Rh1, ginsenoside-Rh2, 20(S) - ginsenoside Rg2, 20(S) - ginsenoside Rg3, and these are not detected or even as trace amount in fresh and dried ginseng roots [4]. Ginsenoside-Rg3 showed anti-tumor [5], neuroprotective activity [6], and relaxing activity of vascular smooth muscle [7,8]. Ginsenoside-Rh1 and -Rh2 showed apoptosis of human Hepatoma [9], and anti-tumor [10-13]. These specific ginsenosides of Red ginseng can be used for important medicine. However, commercial use of Red ginseng specific ginsenosides is very difficult because of high price of the root.

Ginseng hairy roots induced by Agrobacterium rhizogenes-transformed show vigorous growth and produce equal amounts or more saponins than non-transformed ginseng roots [14], which shows that ginsenoside can be produced from ginseng hairy roots. It has not been reported, so far on the production of Red ginseng specific ginsenosides from ginseng hairy roots.

This paper was carried out to examine the production of Red ginseng specific ginsenosides (Rg and Rh groups) from transformed hairy roots of Panax ginseng by heat treatment.

MATERIALS AND METHODS

Culture of hairy roots Root segments excised from three year-old roots of Korean ginseng (Panax ginseng C.A. Meyer) were infected with Agrobacterium rhizogenes A4 as the same protocol reported by Yang et al. [14]. Among several hairy roots lines, KGHR-8A was used as material. Maintenance of hairy roots was performed by transferring the 1.0 g hairy roots into MS [15] liquid medium containing B5 vitamins [16], 3% sucrose in 100 ml Erlenmeyer flask by 4 weeks of subculture intervals (23°C under complete dark).

Steam heat treatment of hairy roots Hairy roots were washed
twice with sterilized distilled water and then kept on the iron mesh tray for removing water. Thereafter hairy roots were transferred to the steam heater. Temperatures and times of heat treatment was performed at 105°C for 30, 60, 120, and 180 min, 121°C for 30 and 120 min. The heat-treated roots were dried in oven at 60°C.

**Extraction and analysis of ginsenoside**

Extraction of ginsenoside was followed by the method of Ando et al. [17]. Milled powder (1 g) of hairy roots were soaked in 80% MeOH at 60°C. After evaporation, the residue were dissolved in H₂O and washed twice with ether followed by extraction with n-BuOH saturated with water. The BuOH layer was evaporated to give crude saponins. Each sample was dissolved in EtOH and this liquid were filtrated and subjected to HPLC. For TLC, total ginsenosides (5 µl) was spotted together with the standard samples on TLC plate (silica gel 60 F₂₅₄, Merck) containing CHCl₃-MeOH-H₂O (65:35:10, v/v), thereafter stained by spraying with 30% H₂SO₄, followed by heating at 105°C. Ginsenoside were analyzed using HPLC (Model) on LiChrospher-NH₂ column (Merk Co., 10 µm, 0.46 cm I.D, 25 cm) in CH₃CN-H₂O-BuOH (80:20:10, v/v) and CH₃CN-H₂O (90:10, v/v) with monitoring using refractive index (RI) (Waters R401) at 202 nm. Flow rate was 0.3 ml/min for the former and 2.0 ml/min for the latter. Each ginsenoside was calculated by comparison with the authentic ginsenoside provided from Korean Ginseng and Tobacco Research Institute. Quantitative analysis was performed by the one-point curve method by external standards of authentic ginsenosides and the data were expressed in mg/g dry wt.

**RESULTS AND DISCUSSION**

When one gram of hairy roots of KGHR-8A line was cultured on MS medium for 4 weeks, 430 mg of dry weight of roots was produced. About 15 mm long hairy root grew to 87 mm after 4 weeks of culture and the number of lateral roots was 19 (Figure 1).

In the line of hairy roots (KGHR-8A), the content of Rg₁ (19.67 mg/g dry wt) was exceptionally high compared to normal hairy roots (3.43 mg/g dry wt), indicating highly useful for the utilization of Rg₁ for chemical and medicinal purpose. The other ginsenosides showed similar content to the normal hairy roots.

Heat treatment of hairy roots was resulted in the changes in the content and composition of ginsenoside compared to freeze-dried roots (Figure 2, Table 1). Contents of ginsenosides-Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁ were decreased in heat treated-roots (Table 1). Ginsenoside-Rg₃, -Rh₁ and -Rh₂ were detected as trace amount in ginseng roots without heat treatment, but heat treatment strongly stimulated the production of those ginsenoside (Table 1). The production of each ginsenoside was depended on the treatment of temperature and its duration (Table 1). Optimum heat treatment to produce the ginsenoside-Rg₃, -Rh₁

![Figure 1. Hairy roots (KGHR-8A) selected from Agrobacterium-transformed ginseng hairy roots, KGHR-8. A. Hairy roots cultured in 1/2 MS solid(A) and liquid(B) media with 3% sucrose in Petridish and 100 ml Erlenmyer flask.](image1.png)

![Figure 2. TLC profiles of crude ginseng saponins by BuOH extration from hairy roots (KGHR-8A) with and without heat treatment. Mobile phase: CHCl₃-MeOH:H₂O (65:35:10, v/v, lower phase). Lanes 1: freeze dried hairy roots, 2: heating for 60 min at 90°C, 3: heating for 30 min at 105°C, 4: heating for 1 hour at 105°C, 5: heating for 2 hours at 105°C, 6: heating for 3 hours at 105°C, 7: heating for 30 min at 121°C, 8: heating for 60 min at 121°C, 9: ginsenoside-Rh₁, Rh₂ standard, 10: ginsenoside-Rg₂, Rg₁ standard, 11: ginsenoside-Rb₁-Rg₁ standard.](image2.png)
Production of Red Ginseng Specific Ginsenosides from Hairy Roots

Table 1. Change of ginsenoside composition according to steam heat treatment in ginseng hairy roots.

<table>
<thead>
<tr>
<th>Temp (°C)-time (min)</th>
<th>Ginkenoside composition %/total ginkenoside</th>
<th>Total mg/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rb₁</td>
<td>Rb₂</td>
</tr>
<tr>
<td>Natural fine root No treatment</td>
<td>22.93</td>
<td>15.28</td>
</tr>
<tr>
<td>No treatment</td>
<td>20.92</td>
<td>14.64</td>
</tr>
<tr>
<td>Transformed hairy root 105-60</td>
<td>17.26</td>
<td>11.71</td>
</tr>
<tr>
<td>105-180</td>
<td>13.15</td>
<td>8.50</td>
</tr>
<tr>
<td>121-60</td>
<td>10.83</td>
<td>5.92</td>
</tr>
</tbody>
</table>

All roots were freeze-dried except for steam heat treatment.
Data were the mean of three independent experiments.
T: Trace amount, PD: Ginsenoside-Rb₁+Rb₂+Re+Rd+Rh₂+Rg₃, PT: Ginsenoside-Re+Rf+Rg₁+Rg₂+Rh₁.

Figure 3. HPLC profile of crude ginseng saponins by BuOH extraction from hairy roots with heat treatment for 2 hours at 105°C. HPLC conditions: RI detector, LiChrospher-NH₂ column (4.6x250 mm), CH₃CN/H₂O/BuOH (80:20:10, v/v).

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REFERENCES


