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Optimization of ginseng cell culture in airlift bioreactors and developing the large-scale production system

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ABSTRACT

Ginseng cell suspension culture was established in 5 l capacity balloon type airlift bioreactors using Murashige and Skoog medium supplemented with 7 mg l^{-1} indole butyric acid, 0.5 mg l^{-1} kinetin and 30 g l^{-1} sucrose. Various parameters such as types of bioreactors (cylinder, balloon, bulb and cone type bioreactors), aeration volume (0.05, 0.1, 0.2, 0.3 vvm constant air supply or the amount air supply was increased from 0.05 to 0.3 vvm at 6-day intervals), and inoculum density (40, 60, 80 and 100 g l^{-1}) were optimized. Balloon type airlift bioreactors, aeration volume of 0.1 vvm, and inoculum density of 60 g l^{-1} were found suitable for accumulation of ginseng cell biomass and ginsenosides. Based on optimized results, 500 l capacity large-scale drum type and balloon type bioreactors were established for the production of ginsenosides. Optimal yield of 187 kg and 400 kg fresh biomass and 6.2 kg and 13.3 kg dry biomass, 7.86 mg g^{-1} DW and 7.75 mg g^{-1} DW total ginsenosides could be achieved in 500 l capacity drum and balloon type bioreactors respectively. These results are useful for commercial production of ginseng cell biomass and secondary metabolites.

1. Introduction

Ginseng (*Panax ginseng* C. A. Meyer) is one of the most valuable oriental herbs and it has been used as health tonic and food ingredient in China, Korea and Japan. Major active components of ginseng are ginsenosides, a group of saponins with triterpenoid dammarane structures. Ginsenosides are divided into three groups by their structure i.e. Rb group (panaxadiols including Rb1, Rb2, Rc, Rd, etc.), the Rg group (panaxatriols including Rg1, Re, Rf, Rg2, etc.) and the Ro group (olenolic acid) (Park et al., 2005). Ginsenosides are reported to possess various pharmacological activities such as anti-cancerous, anti-diabetic, cardioprotective, immunomodulatory, antistress and antioxidant activities (Park et al., 2005).

Wild ginseng is rare and scarce commodity, cultivation of ginseng is possible, however, it takes 5–7 years from seedling to the final harvest and further the productivity is affected by many environmental factors including soil, shade, climate, pathogens and pests. The use of the plant tissue culture process has been viewed as a potential alternative for more efficient production of ginseng and its active ingredients, such as ginseng saponins. Cell culture may also offer better selectivity and yield for the desired bioactive products, since the cell strains may be selected from tissue or organs can be more productive than other parts of the plant (Mathur et al., 1994, 1999; Wu and Zhong, 1999; Zhong et al., 1996). Recent investigations revealed that *Panax ginseng* (Korean ginseng) possesses about 3–4% saponins and more than 38 kinds of ginsenosides, which is highest when compared to *P. notoginseng* (Chinese ginseng), *P. quinquifolium* (American ginseng), *P. vietmensis* (Vietnamese ginseng) and *P. japonicas* var. repens (Japanese ginseng) (Choi, 2008; Park, 1996; Thanh et al., 2007). Korean ginseng is also reported to be superior in its pharmacological activities than that of American ginseng and Chinese ginseng (Choi, 2008).

We initiated cell cultures of Korean ginseng (*Panax ginseng* C. A. Meyer) in bioreactors with an objective of production of ginsenosides (Thanh, 2005). In this report, our successful results of parameters affecting the ginseng cell culture such as bioreactor configuration, aeration volume, inoculum density are presented. We have also established large scale/pilot scale bioreactor cultures for the production of ginseng cell biomass and bioactive compounds.

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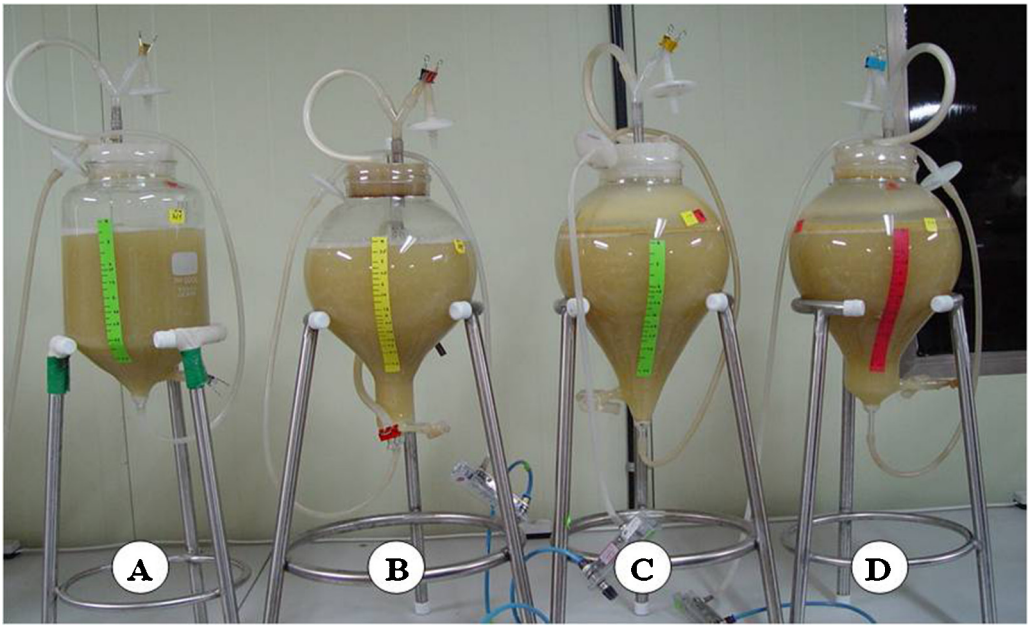

Fig. 1. Ginseng cell suspension cultures: effect of (A) cylinder, (B) balloon, (C) cone and (D) bulb type bioreactors on accumulation of biomass after 30 days of culture. 60 g l^{-1} cells were cultured in 4 l of MS medium supplemented with 7 mg l^{-1} of IBA, 0.5 mg l^{-1} kinetin and 30 g l^{-1} sucrose.

2. Methods

2.1. Plant material and establishment of cell suspension culture

Callus was induced from 6 years old field cultivated Korean ginseng roots (*Panax ginseng* C. A. Meyer) on Murashige and Skoog (MS 1962) semi-solid medium (0.8%, w/v agar) supplemented with 1.0 mg l^{-1} 2, 4-dichlorophenoxyacetic acid (2,4-D) and 30 g l^{-1} sucrose in the dark at 25 °C (Thanh, 2005). The callus proliferation was achieved on MS semi-solid medium (0.8%, w/v agar) supplemented with 2.0 mg l^{-1} naphthaleneacetic acid (NAA), 0.1 mg l^{-1} kinetin, and 30 g l^{-1} sucrose. Suspension cultures were established in 300 ml conical flasks containing 100 ml MS medium by adding 6 g callus and were incubated on rotary shaker at 105 rpm, in the dark at 25 °C. Cells were maintained by subculturing to fresh medium once in every 15 days.

2.2. Bioreactor cultures and optimization of culture conditions

Bioreactors of various configuration viz. cylinder (Fig. 1A), balloon (Fig. 1B), bulb (Fig. 1C) and cone type (Fig. 1D) airlift bioreactors of 5 l capacity were established with 4 l of MS medium supplemented with 7 mg l^{-1} indole butyric acid (IBA), 0.5 mg l^{-1} kinetin and 30 g l^{-1} sucrose. 60 g l^{-1} of cells were used as inoculum, the aeration volume in bioreactors was adjusted to 0.1 vvm (air volume per culture volume per minute) using air flow meters (RMA series; Dwyer Instruments Inc., Michigan, USA). In another set of experiment, cultures were established in 5 l capacity balloon type airlift bioreactor (Fig. 3A) containing 4 l MS medium as above and cultures were aerated with various aeration volumes of 0.05, 0.1, 0.2, 0.3 vvm constant air supply or the amount air supply was increased from 0.05 to 0.3 vvm at 6-day intervals to verify the suitable aeration volume on the accumulation of biomass and ginsenosides. In one more set of experiment, cultures were established in 5 l capacity balloon type airlift bioreactors containing MS medium as above with variable inoculum density viz. 40, 60, 80 and 100 g l^{-1} to test the effect of inoculum density on the accumulation of biomass and ginsenosides. Large scale cultures were also operated for the cultivation of ginseng cells by using 500 l capacity drum type and balloon type bioreactors (Fig. 3B and C). All the bioreactors were maintained at 25 ± 1 °C under dark for 30 days. After 30 days, growth parameters (fresh weight, dry weight and growth ratio) and the content of Rg and Rb group ginsenosides were assessed.

2.3. Determination of cell biomass

After 30 days of culture, the cells were separated from the medium through a stainless steel sieve. The fresh biomass was measured after blotting away the surface water. The dry weight was recorded after drying cells at 60 °C for 24 h. The growth ratio was calculated as follows: [harvested dry weight (g) – inoculated dry weight (g)/inoculated dry weight].

Table 1
Cell suspension cultures of *Panax ginseng*: effect of bioreactors on biomass accumulation and ginsenoside production after 30 days of culture.[a,b]

Bioreactor type	Initial $k_L a$ (h^{-1})	Fresh weight (g l^{-1})	Dry weight (g l^{-1})	Growth ratio	Ginsenosides (mg g^{-1} DW)		
					Rg	Rb	Total
Cylinder	5.25	240b	9.1b	4.14	1.45 ± 0.2	2.37 ± 0.3	3.82 ± 0.4
Balloon	6.98	255a	10.6a	4.82	1.35 ± 0.2	3.38 ± 0.6	4.73 ± 0.6
Bulb	6.95	252a	10.1a	4.59	1.09 ± 0.3	3.07 ± 0.5	4.16 ± 0.5
Cone	5.69	245ab	9.8ab	4.45	1.35 ± 0.3	2.59 ± 0.1	3.95 ± 0.2

[a] Cells (60 g l^{-1}) were cultured in 5 l bioreactors containing 4 l of MS medium supplemented with 7 mg l^{-1} indole butyric acid, 0.5 mg l^{-1} kinetin and 30 g l^{-1} sucrose.
[b] Data represents mean values of 3 replicates; each experiment was repeated twice. Mean separation within column by Duncan's multiple range test at $P \leq 0.05$, significance shown by different letters in columns.