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Effect of Inoculum Size on Biomass Accumulation and Ginsenoside Production by Large-Scale Cell Suspension Cultures of *Panax ginseng*

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Abstract

Cell growth and ginseng saponin production by large-scale suspension (bioreactor) cultures of *Panax ginseng* were investigated under various inoculum sizes. Cell growth was low at an inoculum size of 40 g FW/L, and the maximum cell growth was obtained with increasing inoculum size up to 100 g FW/L. The cell density of 333 g FW/L and 12.7 g DW/L was obtained at inoculum size of 100 g FW/L after 30 days of cultivation. Maximum saponin production of 4.40 mg/g DW was achieved at 60 g FW/L of inoculum size. Thus, inoculum size 60 g FW/L was suitable for optimum biomass accumulation as well as saponin production during bioreactor cultivation of ginseng suspension cells.

Key words: Bioreactor, ginseng, inoculum size, saponin, suspension culture

Introduction

Ginseng saponin (a secondary metabolite, termed ginsenoside) is one of the principal bioactive ingredients of ginseng (*Panax ginseng* C. A. Meyer), and has cardio-protective, immunomodulatory, anti-fatigue, antitumor, and hepato-protective effects. Recently cells and roots are proliferated *in vitro* for efficient saponin production.

Plant cell suspension cultures are the alternative source for the production of high-value secondary metabolites. Bioprocess optimization and scale-up of suspension cultures require the understanding of inoculum size to increase the production of cell biomass and secondary metabolites (Rokem and Goldberg 1985). There is a minimum size of explant or quantity of separated cells per unit culture volume for successful culture initiation. Large explants generally survive more frequently and grow more rapidly than relatively small ones at the initial stage of culture (Paek et al., 2001).

Cell suspension cultures are initiated using relatively high cell concentration, since there is a minimum inoculation density below which growth does not occur or is preceded by a long lag phase. If the inoculum density is sufficiently high, lag phases may be eliminated so that the initial specific growth rate is equal to the maximum (van Gulik et al. 1994). Inoculation density has also been shown to affect the activities of individual enzymes in suspended plant cell culture for the synthesis of secondary metabolites (van Gulik et al. 1994). There are reports on the effect of inoculum size on cell biomass and anthocyanin production in carrot and strawberry (Ozeki and Komamine 1985; Sakurai et al. 1996), and taxol production in *Taxus chinensis* (Wang et al. 1997). The same experiment was also carried out in flask cultures of *Panax ginseng* (Akalezi et al. 1999) but the effect of inoculum density varies with the type and the vessel of culture and culture period (Paek et al., 2001). In this study, ginseng cells were cultured in 5-liter scale bioreactors to determine optimal inoculum size for cell biomass increase and saponin production.

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Materials and Methods

Stock cell cultures

Stock suspension cells of *Panax ginseng* were maintained in Murashige and Skoog (MS) medium supplemented with 2.0 mg L^{-1} α-naphthalene acetic acid (NAA), 0.1 mg L^{-1} kinetin, and 30 g L^{-1} sucrose. The medium pH was adjusted to 5.8 before autoclaving. Cell cultures were grown in 300 ml flasks with a working volume of 100 ml and were maintained on rotary shaker at 105 rpm, in dark at 25℃. Cells were maintained by sub culturing to fresh medium once in fifteen days.

Establishment of large-scale suspension cultures in Bioreactors

Five-liter capacity airlift bioreactors containing 4 l of MS medium (working volume) supplemented with 7.0 mg L^{-1} indolebutyric acid (IBA), 0.5 mg L^{-1} kinetin and 30 g L^{-1} sucrose were used. Cell density of 40, 60, 80 and 100 g fresh weight per liter was used as inoculum. In the airlift, a sinter glass was used for aeration, and the airflow rate was adjusted during cultivation to homogenous mixing state. The cultivation temperature in bioreactors was controlled at 25℃ and a darkness condition was maintained. Three identical cultivation vessels were operated under each condition, and the cultivation data was represented by average values with standard error.

Determination of cell weight

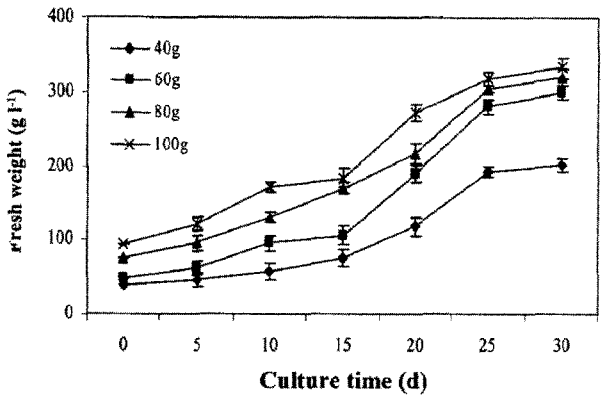
The cell suspensions were filtered and washed several times with distilled water before weighing. After that the cells were weighed as fresh weight. The fresh cells were 50℃ to a constant weight, and then the dry weight determined. The electrical conductivity was measured the exhausted medium using conductivity meter Wissenwerkstalten model LF-54 (WTW GmbH, Wielhalm, Germ

Determination of saponin content

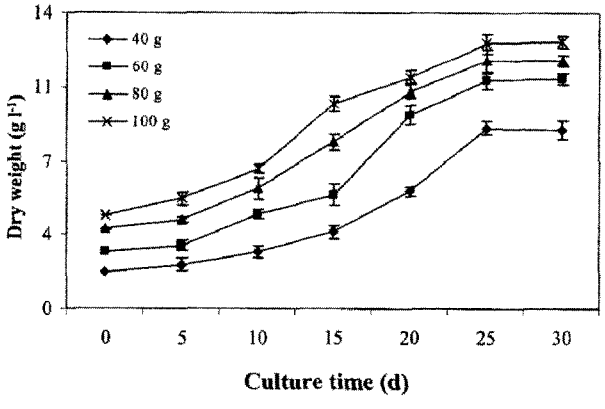
Extraction and analysis of saponin were done by the method of Yu et al. (2002). The saponin fraction was analyzed using HPLC system (Waters 2690 separation module; Waters 996 photodiode array detector; Waters millennium 2010 chromatography manager) on an Altec Platinum C_{18} column (Φ1.5 μm, 33 mm × 7 mm), with water and acetonirile as the mobile phase. The ratio of water and acetonitrile for the first 10 min and last 25 min were 75:25 and 63:37, respectively. Flow rate of the mobile phase was 1.2 ml min^{-1} and the saponin was detected at 203 nm. Authentic saponins were purchased from Karl Roth, Germany to compare with accumulation pattern of cultured cells. Total saponin content was calculated as sum of saponin fractions. The saponin content of cultured cells was calculated as: Saponin (mg g^{-1}) = sample saponin concentration (from HPLC) (mg L^{-1}) x sample volume (L)/cells dry weight (g).

Results and Discussion

The effects of inoculum size within the range of 40 to 100 g FW L^{-1} on the cell cultures of *Panax ginseng* were investigated on a bioreactor scale. Differences in biomass accumulation by the use of different inoculum sizes are shown in Figure 1A for fresh weight, and in Figure 1B for dry weight. The results reveal that all the cultures exhibited exponential growth kinetics for more than 25 days of the



A



B

Effect of inoculum size on biomass accumulation of *Panax ginseng* cells cultivated in large-scale suspension cultures. A) fresh dry weight. Results represent the average of three determinations; error bars are the difference of the three determinations

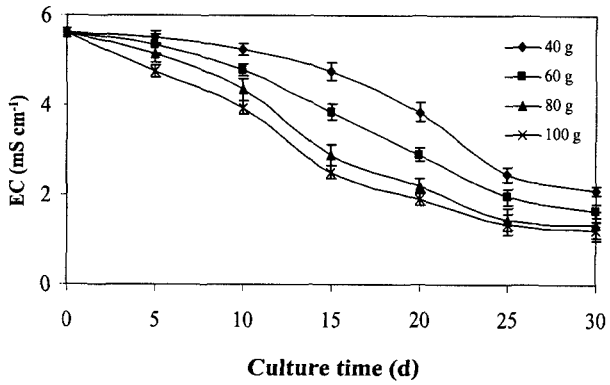


Figure 2. Time profiles of medium conductivity of large-scale suspension cell cultures of *Panax ginseng*. Results represent the average of three determinations; error bars are the difference of the three determinations.

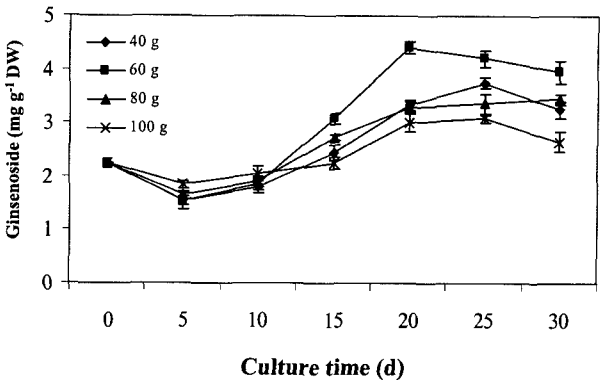


Figure 3. Effect of inoculum size on saponin accumulation in *Panax ginseng* cells cultivated in large-scale suspension cultures. Results represent the average of three determinations; error bars are the difference of the three determinations.

culture period; the growth continued even after this time. The final cell biomass was very low at a low inoculum size and cell growth was greatly enhanced with increase of inoculum size from 40 to 100 g FW L^{-1}. On the inoculum size of 40 g L^{-1} and 100 g L^{-1}, final fresh cell biomass was 201 g L^{-1} and 333 g L^{-1} and dry cell biomass was 8.5 and 12.7 g L^{-1}, respectively.

Electrical conductivity measurements (EC) have been used as an indirect method of biomass estimation in continuous on line monitoring of plant cell cultures in bioprocess engineering studies for its accuracy and efficiency (Ryu et al. 1990). In the present studies, medium conductivity gradually decreased with culture time (Figure 2), and this is due to increase in cell concentration. Similarly, Ryu et al. (1990) have shown that increase in cell concentration can be correlated directly with decrease in medium conductivity in the case of cell cultures in shake flasks. The electrical conductivity of the medium also reflects the uptake of medium salts (ions) by the cultured cells and linear decrease was observed with increase in cell density during cultivation, which indicated a high metabolic activity of cultured cells.

As reported by some researchers, inoculum size greatly affects not only cell growth kinetics but also secondary metabolite formation in plant cell cultures. For example, Su and Lei (1993) reported that rosmarinic acid productivity in *Anchusa officinalis* cell culture increased in proportion to the inoculum size up to 4 g dry cell L^{-1} whereas the maximum cell concentration was not affected by inoculum size between 1 and 11 g dry cells L^{-1}. Stimulatory effects of inoculum size on secondary metabolite production have been observed in anthocyanin accumulation in a carrot suspension culture (Ozeki and Komamine 1985) and shikonin production in *Lithospermum erythrorhizon* cell cultures (Fujita and Hara 1985). In addition, Matsubara et al. (1989) demonstrated that for high density fed-batch cultures of *Coptis japoniaca*, the cell concentration and berberine production increased with inoculum size up to 8 g dry cell L^{-1}, a high cell concentration of 55 g DW L^{-1} and a berberine production of 3.5 g L^{-1} was obtained at the inoculum size. Figure 3 shows the effects of inoculum size on saponin production. Saponin accumulation was enhanced in 10 days of culture by the inoculum size of 60 g L^{-1}. During the whole cultivation, a sharp increase of saponin content was detected from 10 to 20 days of culture (Figure 3). The maximum saponin production (4.4 mg g^{-1} DW) was achieved at an incoulum size of 60 g FW L^{-1}. However, a further increase of inoculum size did not yield a higher saponin accumulation. A similar phenomenon was also observed in *Anchusa officinalis* cell cultures for rosmarinic acid production (Su and Lei, 1993).

In conclusion, the present study revealed that biomass accumulation was affected by initial inoculum size in bioreactor culture of *Panax ginseng*. 100 mg DW L^{-1} inoculum size yielded optimum cell biomass yield. However, the production of saponin was greatest at the inoculum size of 60 g FW L^{-1}, which indicated to be suitable for both biomass and saponin accumulation. Further works are in progress regarding physical environments such as air volume, gaseous composition and medium supply method to maximize biomass and saponin accumulation in large-scale cell cultures of *Panax ginseng*.

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