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BRIEF COMMUNICATION

Effect of carbon dioxide on cell growth and saponin production in suspension cultures of *Panax ginseng*

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Abstract

The effects of carbon dioxide supply within the range of 1 - 5 % (along with purified air), on cell culture of *Panax ginseng* were investigated in a balloon type bubble bioreactor containing 4 dm^3 of Murashige and Skoog (MS) medium supplemented with 7.0 mg dm^{-3} indolebutyric acid, 0.5 mg dm^{-3} kinetin and 30 g dm^{-3} sucrose. A 1 % CO_2 supply was found beneficial for the production of cell mass; however, increasing CO_2 concentration to 2.5 and 5 % decreased the biomass accumulation. CO_2 enrichment was not beneficial for saponin production and 1, 2.5, and 5 % CO_2 supply resulted in decrease in saponin accumulation up to 11.6, 19.5, and 50.6 %, respectively.

Additional key words: bioreactors, ginseng, plant cell culture, secondary metabolites.

Ginseng (*Panax ginseng*), a member of *Araliaceae* family, is traditionally considered one of the most important medicinal plants. Saponins (ginsenosides) have been regarded as the most important active components in ginseng roots (Tang and Eisenbrand 1992).

In recent years, plant cells, tissues, organs or plantlets are cultured in large-scale bioreactors for the production of secondary metabolites (Rao and Ravishankar 2002). Growth and accumulation of secondary metabolites in large-scale bioreactor is influenced by various factors including gas composition. In bioreactors, forced aeration is needed to supply oxygen and to improve fluid mixing. However, it may also lead to the removal of some known (such as CO_2 and ethylene) or unknown gaseous compounds. Such gaseous compounds were proven or suggested to be important for cell growth and/or synthesis of secondary metabolites in plant cell cultures (Gao and Lee 1992, Schlatmann *et al.* 1997). Carbon dioxide is often considered an essential requirement for the culturing plant cells (Ducos *et al.* 1988, Maurel and Parilleux 1985). In this study, we have established *Panax ginseng* cell cultures in balloon type bubble bioreactors and the interaction between carbon dioxide supply, cell growth, and ginsenoside production was investigated.

Panax ginseng C.A. Meyer cell suspensions were maintained in Murashige and Skoog (1962, MS), medium supplemented with 2.0 mg dm^{-3} α-naphthalene acetic acid (NAA), 0.1 mg dm^{-3} kinetin, and 30 g dm^{-3} sucrose. The medium pH was adjusted to 5.8 before autoclaving. Cells were grown in 300 cm^3 flasks with a working volume of 100 cm^3 and were maintained on rotary shaker at 105 rpm, in dark at temperature of 25 °C. Cells were maintained by subculturing to fresh medium once in fifteen days.

Bioreactor cultures were established containing 4 dm^3 of MS medium (working volume) supplemented with 7.0 mg dm^{-3} indolebutyric acid (IBA), 0.5 mg dm^{-3} kinetin and 30 g dm^{-3} sucrose. Sixty grams cell fresh mass per dm^3 was added as inoculum. In the bubble bioreactor, a sinter glass was used for aeration. The airflow rate was controlled at 0.1 by flow meter (*Dwyer Inc.*, Mich City, USA), and adjusted during cultivation to homogenous mixing state. To investigate the effects of different CO_2 concentrations on cell biomass and saponin production the inlet air was mixed with different

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Abbreviations: IBA - indolebutyric acid; MS medium - Murashige and Skoog medium; NAA - α-naphthalene acetic acid.
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concentrations of carbon dioxide *i.e.*, 0.03 (control), 1.0, 2.5, and 5.0 %. The cultivation temperature in bioreactors was controlled at 25 °C and continuous darkness was maintained. Three identical cultivation vessels were operated under each condition and the cultivation data shown represent average values with standard error.

The cell suspensions were filtered and washed several times with distilled water before weighing. After that the cells were weighed as fresh mass. The fresh cells were dried at 50 °C to a constant mass, and then the dry mass was determined.

Extraction and analysis of saponins were done by the method of Yu *et al*. (2000). The saponin fraction was analyzed using HPLC system (*Waters 2690* separation module; *Waters 996* photodiode array detector; *Waters millennium 2010* chromatography manager, Milford, MA, USA) on an *Altec* platinum C18 column (particle size 1.5 μm, 33 mm × 7 mm), eluting with water and acetonitrile at 3:1 (v/v) for 10 min then 63:37 (v/v) for 25 min with a flow rate low rate of 1.2 cm^3 min^{-1}. Saponin was detected at 203 nm. Authentic samples of saponins were from *Chromadex*, Santa Ana, CA, USA. Total saponin content was calculated as the sum of saponin fractions.

The cell growth and biomass accumulation was gradually increased with increment of time and optimum biomass accumulation was reached after 30 d. Similar growth kinetics pattern was reported in *Panax notoginseng* cell cultures (Zhong *et al*. 1999). The fresh mass of cells with 0.03 % CO_2 (control) was 227 g dm^{-3} and corresponding dry mass was 11.6 g dm^{-3} (Fig. 1). It was found that optimum accumulation of fresh (258 g dm^{-3}) and dry mass (12.1 g dm^{-3}) was with the supply of 1 % CO_2 in the bioreactors. Thus 13.7 and 4.3 % increase in fresh and dry cell mass was evident with supply of 1 % CO_2.This finding is in agreement with earlier reported results that carbon dioxide is required for the growth of plant suspension cultures (Gathercole *et al*. 1976, Maurel and Parilleux 1985). The biomass accumulation declined with the further increase in CO_2 concentration. Fresh and dry cell mass was decreased by 30.1 and 38.3 %, respectively with the supply of 5 % CO_2. In contrast, reduced CO_2 concentration had no effect on cell growth of *Panax notoginseng* (Han and Zhong 2003). The present study demonstrates that 1 % CO_2 supply is beneficial for biomass accumulation of *Panax ginseng* cells, whereas higher concentrations of CO_2 (2.5 and 5 %) showed negative effect on biomass accumulation.

The ginsenoside production increased with the progress of the experiment and significantly higher saponin content was observed in control condition. After 30 d of culture increased CO_2 supply to 1, 2.5 and 5 % decreased saponin accumulation up to 11.6, 19.5 and 50.6 %, respectively. The maximum total saponin

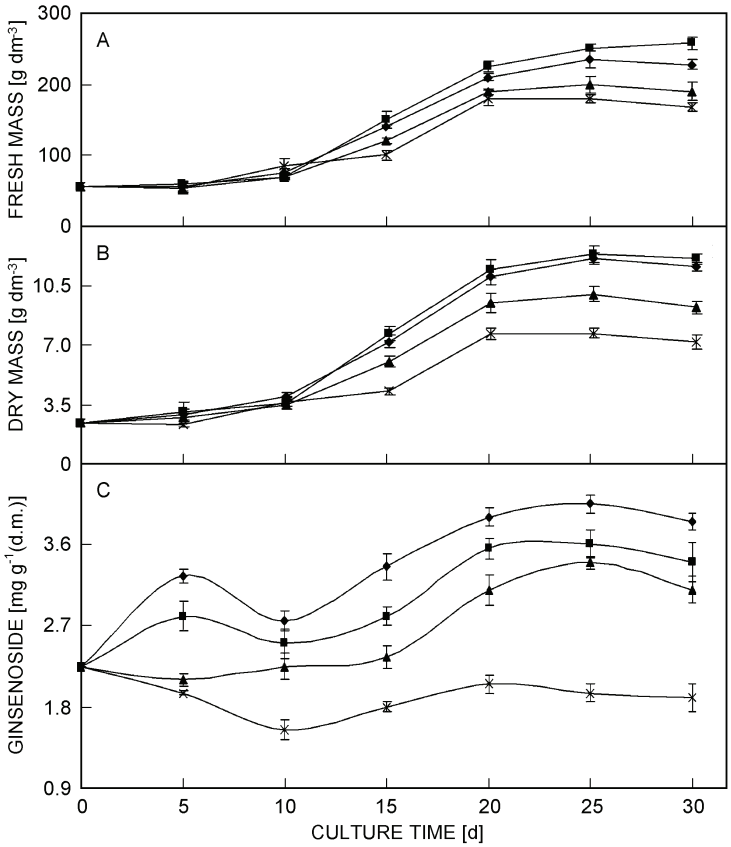

Fig. 1. Effect of CO_2 concentration on accumulation of cell fresh mass (*A*), dry mass (*B*), and production of saponins (*C*) of *Panax ginseng* cells cultivated in balloon type bubble bioreactors (*rhomb* - control, *square* - 1 % CO_2, *triangle* - 2.5 % CO_2, *cross* - 5 % CO_2).

concentration was 4.04 mg g^{-1} (d.m.) at 0.03 % CO_2 supply (Fig. 2). Contrary to the present report, beneficial role CO_2 on secondary metabolite production has been demonstrated in the cell cultures of *Thalictrum minum* (Kobayashi *et al*. 1991*), T. rugosum* (Kim *et al*. 1991), *Stizolobium hassjoo* (Huang and Chou 2000) and *Catharanthus roseus* (Scragg *et al*. 1987).

High cell density and fluid viscosity could significantly reduce transfer efficiencies of gaseous components in bioreactor and conventional way of improving transfer rate gaseous components is to increase agitation speed and/or aeration rate. However, these approaches have several limitations, such as high power consumption, cell damage due to mechanical shear stress, potential reduction of productivity because of the loss of CO_2 and other essential volatiles from the system. An alternative approach is improving the quality of incoming air by the concentration gaseous components. The results presented in this report emphasize the importance of the gaseous environment in large-scale plant suspension cultures. A distinct positive effect on biomass accumulation is evident with the supply of 1 % CO_2, however higher levels of CO_2 (2.5 and 5 %) was detrimental for biomass accumulation. Supplementation of CO_2 was found to be not beneficial for the accumulation of saponins in ginseng suspension cultures. The role of other important gaseous components like oxygen and ethylene on cell growth and saponin accumulation in ginseng suspension cultures is underway in our laboratory.

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