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EFFECT OF OSMOTIC PRESSURE ON CELL GROWTH AND PRODUCTION OF GINSENG SAPONIN AND POLYSACCHARIDE IN SUSPENSION CULTURES OF *PANAX NOTOGINSENG*

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SUMMARY

The effects of initial osmotic pressure (IOP) on the production of ginseng polysaccharide and ginseng saponin were studied in suspension cultures of *Panax notoginseng* cells. At higher IOP, the specific saponin production and intracellular carbohydrate storage were increased, while the plant cell volume, the consumption rates of major medium components and the specific cell growth rate were decreased. The specific production of polysaccharide was reduced with an increase of IOP from 4.45 to 5.18 atm, and levelled off at an even higher IOP.

INTRODUCTION

Panax notoginseng is one of the most famous traditional Chinese medicinal plants. The pharmacological activities of ginseng saponin (a secondary metabolite) have been recognized for many years (Zhong and Zhu, 1995). Some researchers also claim that ginseng polysaccharide (a primary metabolite of the plant), whose main effective component is pectin, had immunological and antitumor activities (Zhu et al., 1990; Yang et al., 1992). Production of these useful metabolites by plant tissue and cell cultures may be a cost-effective approach to meeting the popular market demand.

The effect of osmotic pressure on the growth and differentiation of plant tissue and callus has been reported by a number of researchers (Thorpe and Murashige, 1968; Kimball et al., 1975). However, there are few papers regarding the effect of osmotic stress on the formation of secondary metabolites in plant cell cultures, such as alkaloids (Rudge and Morris, 1986) and anthocyanin pigments (Do and Cormier, 1990; Zhong et al., 1994). In this article, we report the effect of initial osmotic pressure on the production of both primary (ginseng polysaccharide) and secondary (ginseng saponin) metabolites in suspension cultures of *P. notoginseng* cells.

MATERIALS AND METHODS

Plant materials and culture medium: The cell cultures of *P. notoginseng*, initiated from the cultivated root, have been maintained in liquid suspension for more than two years (Zhong and Zhu, 1995). The cells were subcultured every 14 days by inoculating 1.8 g fresh cells into a 250 m*l* Erlenmeyer flask containing 50 m*l* Murashige and Skoog (MS) medium supplemented with 2 mg/*l* of 2,4-D, 0.7 mg/*l* of kinetin and 30 g/*l* of sucrose. The shake flask was incubated on a gyratory shaker (110 rpm) at 25°C in the dark. A modified MS medium containing 60 mM potassium nitrate as sole nitrogen source instead of KNO_3 and NH_4NO_3 in the original medium was used, and the inoculation size was controlled at 1.5 g fresh cells per flask.

Analyses: For measurement of cell wet weight, cells were filtered and washed with a large amount of distilled water, then collected and weighed. Dry cells were measured after dried at 50°C for a sufficient time. Ginseng saponin was assayed by TLC-colorimetric method (Zhong and Zhu, 1995). Ginseng polysaccharide was determined according to the carbazole-sulfuric acid reaction (Li et al., 1987). Residual sugar was analyzed as described elsewhere (Zhong et al., 1991). Phosphate and nitrate concentrations in the medium were determined according to Chen et al. (1956) and Hecht & Mohr (1990), respectively. The intracellular carbohydrate was measured as previously reported (Gulik et al., 1992).

Estimation of initial osmotic pressure: The initial osmotic pressure in medium was estimated according to the following formula (Salisbury and Ross, 1969):

$$P = [C_1/M_1 + C_2/M_2 + \cdots\cdots + C_n/M_n]\cdot RT \qquad (1)$$

where P is the osmotic pressure (atm), C_1~C_n are concentrations of solutes (g/*l*), M_1~M_n are the molecular weights of solutes, R is gas constant (0.082 *l*·atm/mol/K), T is the culture temperature (K).

RESULTS AND DISCUSSION

The initial osmotic pressure in medium (IOP) was altered by adding 5.32 g/*l* or 15.96 g/*l* of mannitol to the modified MS medium (IOP ca. 5.18 atm and 6.62 atm, respectively), while taking the medium without addition of mannitol as the control (IOP ca. 4.45 atm). Figure 1A shows the cell growth profile of *P. notoginseng* cell cultures at different IOP. The result indicates that the cell growth was reduced at a higher IOP. In addition, it was also observed that under the higher IOP, the cell aggregates became smaller and the culture broth turned turbid at the end of cultivation. Similar phenomena were also observed in suspension cultures of *Catharanthus roseus* (Rudge and Morris, 1986) and *Vitis vinifera* L. (Do and Cormier, 1990). However, in the case of soybean tissue cultures Kimball et al. (1975) claimed that the callus growth was increased by an addition of metabolically inert osmotica (mannitol, sorbitol).

Figure 1B shows the changes of the ratio of dry cell weight to fresh cell weight (in percentage) during the cultivation under different IOP. A higher IOP led to a higher percentage of dry cell weight/fresh cell weight. In addition, our microscopic observation confirmed that the individual cell volume was decreased with an increase of IOP. A similar phenomenon was also reported by several research groups (Kimball et al., 1975; Rudge and Morris, 1986; Zhong et al., 1994). The above fact suggests that regulation of

medium osmotic pressure may be useful for controlling morphological changes during cell cultivation. This point is also very interesting from the viewpoint of bioprocess engineering, as discussed previously by Zhong et al. (1994).

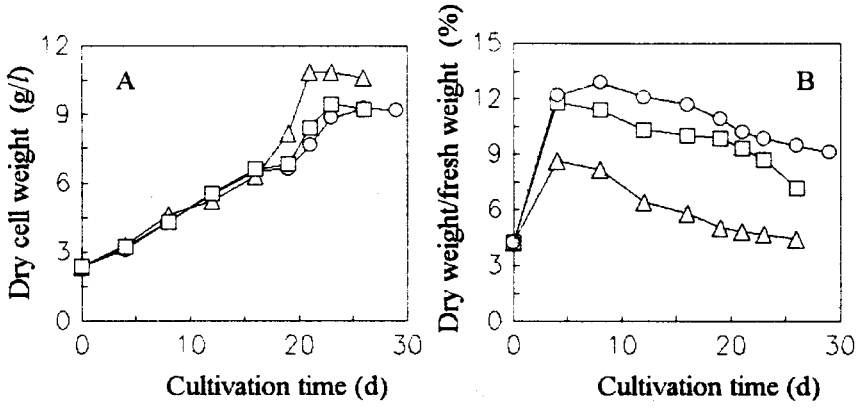

Figure 1. Time profiles of dry cell weight (A) and the ratio of dry cell weight to fresh cell weight (in percentage) (B) at a different initial osmotic pressure in suspension cultures of *P. notoginseng*. Initial osmotic pressure (atm): △, 4.45; □, 5.18; ○, 6.62.

Figure 2 indicates the consumption of major medium components during cultivation. It was found that the consumption rates of both sugar and phosphate were decreased a little at a higher IOP (Figs. 2A, 2C). The nitrate utilization percentage was also slightly reduced at a relatively higher IOP. The above result was in good agreement with the response of the cell growth to the alteration of IOP.

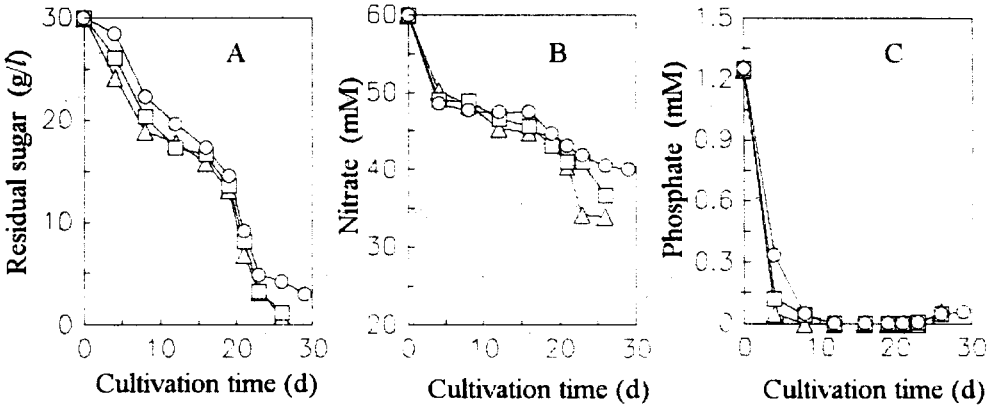

Figure 2. Time courses of the consumption of major medium components under a different IOP in suspension cultures of *P. notoginseng*. A, sugar; B, nitrate; C, phosphate. The symbols are the same as those in Fig. 1.

The data presented in Table 1 indicate the effects of IOP on the content of intracellular carbohydrate and the highest production of ginseng saponin and polysaccharide in suspension cultures of *P. notoginseng*. It indicates that the storage of intracellular carbohydrate (including starch, sucrose, glucose, and fructose) was increased with an increase of IOP. A relatively higher IOP was favorable to the specific production

of saponin (i.e. saponin content). However, the total production of saponin was not enhanced due to the decrease of cell growth under the condition (as shown in Fig. 1). For the ginseng polysaccharide, both its specific production and total production were reduced with an increase of IOP from 4.45 to 5.18 atm, and they were leveled off at an even higher IOP (i.e. 6.62 atm). Here, a further study is necessary to understand the mechanism how the osmotic pressure affects the biosyntheses of ginseng saponin and polysaccharide in the cell cultures. In the case of *C. roseus* cell cultures, it was reported that the cells under high osmotic pressure were packed with starch granules, and a relatively higher IOP was good for serpentine content, but it did not have a great effect on that of ajmalicine (Rudge and Morris, 1986). In addition, an increase of osmotic pressure could result in a significant increase of anthocyanin accumulation in grape cell cultures (Do and Cormier, 1990), and it even caused excretion of the intracellular red pigments in cell cultures of *Perilla frutescens* (Zhong et al., 1994).

Table 1. Effect of initial osmotic pressure on the formation of intracellular carbohydrate, ginseng saponin and polysaccharide in *P. notoginseng* cell cultures

Initial osmotic pressure (atm)	Content of intracellular carbohydrate (%)	Saponin		Polysaccharide	
		content (%)	production (g/l)	content (%)	production (g/l)
4.45	9.8	7.8	0.85	16.2	1.76
5.18	20	8.2	0.69	14.2 (23 d)	1.34 (23 d)
6.62	24	10.3	0.79	14.7 (26 d)	1.36 (26 d)

*Except those indicated in the table, all the data shown were on the 21st day of cultivation when the highest production of saponin and polysaccharide was obtained.

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