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PRODUCTION OF GINSENOSIDE SAPONINS BY CULTURING GINSENG (*PANAX GINSENG*) EMBRYOGENIC TISSUES IN BIOREACTORS[1)]

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SUMMARY

Ginseng (*Panax ginseng*) embryogenic tissues were cultured in three types of reactors and the ginsenoside productivities in these tissues were compared. As a result, the saponin productivity was the best when an airlift reactor was used, and more than twice of that when a paddle or internal turbine reactor was used. The tissues grew 9 fold during 42 days, and the ginsenoside pattern resembled that of ginseng leaves.

INTRODUCTION

Panax ginseng has been widely used for the treatment of several diseases as one of the most important medicinal plants. The saponins named ginsenosides were isolated and characterized as major active constituents (Iida et al., 1968, Nagai et al., 1971, Sanada et al., 1974). However, the cultivation of ginseng needs a long time and is troublesome. Thus, studies of saponin production by plant tissue culture have been carried out. Furuya et al. (1973) reported that saponins were produced by a ginseng callus culture. Several conditions were investigated for mass production of the calli (Furuya et al., 1983a, Furuya et al., 1983b, Furuya et al., 1983c, Furuya et al., 1984). Recently, these ginseng calli have been

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[1)]Part 98 in the series 'Studies on Plant Tissue Cultures' For Part 97 see Orihara, Y., and Furuya, T., (1993) submitted for publication.

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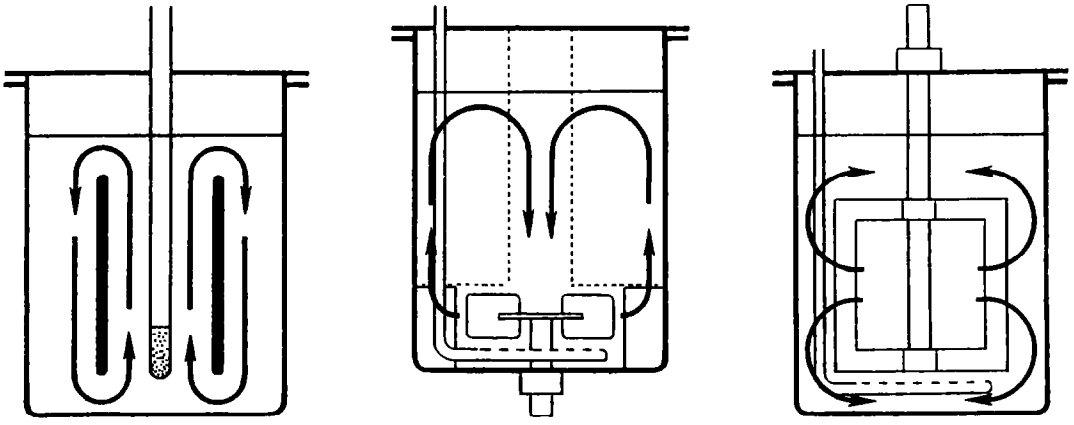
used as an industrial source of ginseng saponins and a ginseng extract (Furuya, T., 1993).

On the other hand, we have studied propagation methods of ginseng by plant tissue culture and reported an induction method for ginseng embryoids by a moderate high temperature treatment (Asaka et al., 1993a) and a proliferation method for the embryoids by culturing on media containing high concentrations of sugar (Asaka et al., 1993b). Furthermore, we have determined the ginsenoside contents in the ginseng embryoids and in the regenerated plantlets from the embryoids, and found that the regenerated plantlets were consistent with the seedlings in the quality and quantity of saponin components, and the ginsenoside components of both tissues resembled those of ginseng leaves (Asaka et al., 1993c). Ginseng leaves have a hypoglycemic activity and an effect of quenching human thirst, and are used to prepare ginseng tea in China. However, ginseng leaves are expensive due to low harvest, similar to ginseng roots. In the present work, we have applied three types of reactors to culture of the ginseng (*Panax ginseng*) embryogenic tissues, and determined ginsenoside productivities in the cultures by HPLC, in order to investigate the availability of the embryogenic tissues as an industrial source of ginseng leaf saponins.

MATERIALS AND METHODS

Induction of embryogenic tissues. Ginseng multiple shoots were induced by the methods described in a previous paper (Furuya et al., 1986). Embryogenic tissues were obtained from the ginseng multiple shoots by a treatment at 35°C for 24 h and the culture at 20 °C under 14 h light (5,000 lux) / 10 h dark condition for 6 weeks on Murashige and Skoog's basal media supplemented with sucrose 30 g/l and gellan-gum 2 g/l (MS). To proliferate the embryoids, the embryogenic tissues were transferred on MS media containing sucrose (80 g/l) and cultured under the above conditions of temperature and light. The embryogenic tissues, which included a lot of embryoids, were subcultured under the same conditions for 28 days, or in reactors for 42 days.

Mass culture of embryogenic tissues in three types of reactors. Airlift, internal turbine and paddle reactors were examined to culture the ginseng embryogenic tissues (Fig. 1). The airlift reactor was the Model CP reactor (3 l, ABLE Corp. Tokyo). The internal turbine pump and paddle turbine reactor were Bioreactor TBR-2-1 (Sakura Finetechnical Co., Ltd. Tokyo) and Mini-jar fermenter KMJ (3 l, Mituwa Rikagaku Kogyo Co., Ltd. Osaka) respectively. The embryogenic tissues (35–40 g) were transferred into these reactors containing 2 l of MS liquid media supplemented with 80 g/l sucrose, and cultured for 42 days at 20°C under room light. Each aeration rate was 1.0 VVM. Rotation speeds of turbines in



Airlift reactor Internal turbine reactor Paddle reactor

Fig. 1 Three types of reactors used in culture of embryogenic tissues. Arrows showed the streams of media in the reactors.

paddle and internal turbine reactors were set at 50 rpm and 400rpm respectively, at each optimal speed.

Extraction of saponin fraction. Each embryogenic tissue was lyophilized. A part of dried material (0.5 g) was extracted with 60 % MeOH (20 ml) at 100 °C for 3 h and filtered in vacua. The MeOH extract was evaporated to dryness and dissolved in H_2O (10 ml). The solution was adsorbed on a Sep-Pak C 18 cartridge and washed with H_2O (20 ml). The crude saponin was eluted with MeOH (10 ml). The eluate was passed through a Sep-Pak NH2 cartridge and evaporated to dryness.

HPLC analyses. The saponin fraction was analyzed by HPLC. Each sample was dissolved in MeOH (5 ml). Column; Senshu Pak NP (10 x 300 mm), mobile phase; 22 % CH_3CN for ginsenosides Rg_1 and Re, and 33 % CH_3CN for ginsenosides Rb_1, Rb_2, Rc and Rd, flow rates of 5 ml/min for the former and 4 ml/min for the latter, detector; photodiode array (UV 202 nm). Each ginsenoside was identified by comparison with authentic ginsenosides purchased from Funakoshi Chemical Co. (Tokyo). Quantitative analyses were carried out by the one-point curve method by external standards of authentic ginsenosides. Ginsenoside contents were expressed in mg per g dry wt.

RESULTS AND DISCUSSION

For industrial mass production, the static culture has the disadvantage that it needs a troublesome transplantation and harvest. Then, we examined the growth ratio and ginsenoside productivities of three types of bioreactors which allow us to culture the tissues continuously. The results are summarized in Table 1, together with those from the static culture. The growth ratio of the airlift reactor reached 9.1, much higher than those of the paddle turbine and internal turbine cultures. The results are similar to those of Wanger and Vogelman (1977), reporting on production of anthraquinones with *Morinda citrifolia*. It is generally accepted that embryoids can not resist high shear stress (Kodama, T. and Yamakawa, T., 1993) and the high growth ratio of culture in the airlift reactor may be due to the lower shear stress. The total ginsenoside content and productivity of the harvest in the airlift reactor were more than twice of those of paddle turbine or internal turbine culture, though the ginsenoside content was lower on a dry weight basis (also see Table 2). This is more obvious, when compared to the static culture. The productivity of saponins in the airlift reactor was no higher than 20 % of that in the static culture, for the total ginsenoside

Table 1. Harvest, growth ratio and saponins productivity in various culture systems.

culture system	inoculum (g fresh wt.)	harvest (g fresh wt.)	harvest (g dry wt.)	growth[a] ratio	total[b] ginsenoside (mg)	ginsenoside[c] productivity (mg / g·day)
airlift	37.2	338.0	48.4	9.1	149.0	0.095
paddle	40.9	149.9	23.2	3.7	77.7	0.045
internal turbine	44.2	80.0	14.5	1.8	65.7	0.036
static culture	42.2	63.4	11.5	1.5	92.8	0.079

† Culture periods were 42days, except 28 days for static culture.
[a]Growth ratio is expressed as g fresh wt. of harvest per inoculum. [b]Total ginsenoside is a sum of the quantities of detected ginsenosides per harvest, calculated by (g dry wt. of harvest) x (total ginsenoside, mg per g dry wt.) in Table 2. [c]Ginsenoside productivity is expressed as mg per g inoculum wt. per a day of culture.

Table 2. Saponin components of the ginseng embryogenic tissue in various culture systems.

culture system	saponin fraction	ginsenoside						total * ginsenoside
		Rb_1	Rb_2	Rc	Rd	Re	Rg_1	
airlift	11.8	0.17	0.08	0.16	0.04	1.44	1.19	3.08
paddle	12.1	0.29	0.09	0.19	0.03	1.67	1.08	3.35
internal turbine	2.3	0.74	0.28	0.34	0.12	1.78	1.27	4.53
static culture	15.5	0.59	0.55	0.45	0.33	2.93	3.22	8.07

† All values are expressed as mg per g dry wt. tissue.
*Total ginsenoside content was sum of the quantities of detected ginsenosides.

per dry wt. of airlift culture was much lower (Table 2). Therefore, to maximize the saponin productivity in airlift culture, the change to a lower growth condition may be useful.

Table 2 shows the results of ginsenoside analysis per each g of dry wt. tissue. The relative contents of ginsenosides in each culture were not so different among the cultures. In all cultures, the contents of ginsenoside Rg group which has a protopanaxatriol, were higher than the contents of ginsenoside Rb group which has a protopanaxadiol as a sapogenin. This is characteristic of ginsenoside components in ginseng leaves (Yahara et al., 1979).

Profumo et al. (1991) reported on aescin production by the embryoids of *Aesculus hippocastanum*, which is the only example of saponin production by embryoids. We have clarified ginsenoside production by ginseng embryoids in this report, and our results suggest a new possibility for ginseng embryoids and embryogenic tissues, as an industrial source of saponins, especially of ginseng leaf saponins.

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