Production of Useful Compounds by Tissue Culture of Ginseng (*Panax ginseng*)

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Introduction

Ginseng (*Panax ginseng* C. A. Meyer) is a herbaceous plant belonging to the Araliaceae. Its root, called ginseng or Korean ginseng, is a favourite remedy in Chinese, Korean and Japanese medicines and it has been widely used as a tonic and health food in the orient since ancient times. However, ginseng root is very expensive because of its long-term, about 5-7 years, and troublesome cultivation. Therefore, we have studied the production of the crude and/or the active ingredients, that is a saponin, ginsenosides, by tissue culture since 1967. As a result, we have already shown that ginseng callus produces the same kind of saponins in a chemical and pharmacological sense as those of the original plant[1,2]. Moreover, we have investigated various culture methods for effective saponin production. A newly isolated strain, coded Pg-3 induced in 1978, was examined for growth and saponin production in a large scale culture, such as jar fermenter or tank cultures[3,4]. In the present time, the strain has been cultured in a 20 ton tank by Nitto Denko Co., and the cultured ginseng have been used as the commercial products.[5]

On the other hand, we have recently succeeded in the induction of the ginseng hairy root by the transformation with *Agrobacterium rhizogenes* using a co-culture method with callus[6]. The hairy roots grew vigorously in a liquid medium with numerous lateral branching and produced in large quantities the saponins resembled those of native roots. In this report, those studies of the production of ginseng saponins by various tissue culture methods are described.

Abbreviations: MS; Murashige and Skoog's basal medium, D; 2,4- dichlorophenoxy-acetic acid, IBA; indole-3-butyric acid, IAA; indole-3-acetic acid, NAA; 1-naphthaleneacetic acid, K; kinetin, P; N-phenyl-N^1-(4-pyridyl)urea.

Callus Induction and Sellection

Pg-1 Callus[1-3]

Pg-1 callus was induced on MS agar medium containing 0.1 ㎎/l of D from the petiole of 2-year-old ginseng in 1967. The callus has been subcultured on the same medium at 25℃ in the dark at 4-week intervals. Pg-1 callus has stably maintained the abilities to produce saponins and to differentiate tissues or organs up to now. Pg-1 callus was transferred into MS medium containing K 1 ㎎/l without D under illumination, 2,500-4,000 lux, 16hr/day, with warm fluorescent light in a phytotron cabinet (Pg-1 K 1 callus). This Pg-1 K1 callus gradually generated shoots and small roots. The roots were selected and subcultured on MS medium containing IBA 1 ㎎/l instead of K 1 in the dark, and continued to form roots only in the dark (Pg-1 IBA 1 callus).

Although the IBA 1 callus, which was mainly redifferentiated roots, was the most excellent in saponin production, it was thought that large lumps formed during suspension culture might be a problem for mass cultivation, in particular for the transfer from suspension culture to suspension or jar fermenter in a large-scale culture. So, Pg-3 callus was newly induced and established as an excellent strain for a large-scale culture according to the optimal conditions obtained by the investigation in Pg-1 callus.

Pg-3 Callus[4]

Slices of 5-year-old Korean ginseng root were placed on MS agar medium supplemented with 1 ㎎/l of D and 0.1 ㎎/l of K (named DK medium) in 1978. The developing callus, Pg-3, was transferred onto the same medium, maintained at 25℃ in the dark, and subcultured at intervals of 4 weeks (Pg-3 DK callus). After a third subculture, the callus was transferred onto MS medium containing IBA 2 ㎎/l and K 0.1 ㎎/l (B2K medium) to exclude 2,4-D, which may be thought to be toxic for human-life because of the use as an agricultural chemicals. The callus was kept at 25℃ in the dark and subcultured at intervals of 4 weeks for about a year, and eventually vigorous growth was achieved with small aggregates, 3-5 mm (Pg-3 B2K callus)[7].

Newly isolated Pg-3 callus with a lower degree of differentiation was examined with respect to culture conditions for growth and saponin production when cultured as a large-scale suspension.

Production of Saponins

Isolation and analysis of saponins

According to the usual methods, the n-BuOH-soluble layer, crude saponin fractions, separated from MeOH extract of the Pg-1 callus, 10 kg fresh weight, was obtained. The saponins were separated on a column of Sephadex LH-20 using $CHCl_3$-MeOH(1:2), and then purified by silica gel column chromatography using $CHCl_3$-MeOH. Ginsenosides Ro, Rb_1, Re and Rg_1 were isolated from each fraction in the yleld of 260 mg, 295 mg, 220 mg and 1,750 mg, respectively. Four ginsenosides were confirmed by mp, IR, NMR and mass spectra in comparison with authentic ginsenosides. The isolation of ginsenosides Re and Ro from ginseng callus were first demonstrated. Futhermore, the presence of all ginsenosides Ro, Ra, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1 Rg_2 and Rh_1 were detected by TLC and HPLC, and determined by densitometry.

Determination of saponins

The crude saponins obtained from each calli or tissues were spotted together with the standard samples of ginsenosides Rb_1 and Rg_1 on Merck silica gel TLC plate 60 F254 and were developed with upper layer of n-BuOH-AcOEt-H_2O(5:1:4). The spots of saponins were detected by spraying with 10% H_2SO_4 followed by heating at 105℃ for 10 min and determined by densitomety using Shimadzu CS-910 dual-wavelength TLC scanner. The amount of the Rb group was calculated as the total of ginsenosides Ra, Rb_1, Rb_2, Rc and Rd, having protopanaxadiol as the sapogenin, and the Rg group was calculated as the total of ginsenosides Re,· Rf, Rg_1, Rg_2 and Rh_1, having protopanaxatriol. Total saponin in each culture shows the content of pure ginsenosides, determined by TLC densitometry. All data are the average

value of duplicate estimations in each of two or three different cultures, and especially in five cultures in the IBA series.

On the other hand, also by HPLC, ginsenosides were determined under the following conditions. Column; Senshu Pak NP (10×300 mm), mobile phase; 22% CH_3CN for ginsenosides with protopanaxatriol and 33% CH_3CN for ginsenosides with protopanaxadiol, flow rate; 5 ml/min for the former and 4 ml/ min for the latter, detector; photodiode array (UV 202 nm). Recently developed HPLC method made to be able to determine more correctly the amount of saponins. However, it takes too long time to determine the saponin contents, because ginsenosides having protopanaxadiol and protopanaxatriol as the sapogenin, respectively, must be separately treated and also the assay time for a sample is too long, about 1 hr. So, we were virtually unable to repeatedly measure a large amount of sample.

Effects of Various Culture Conditions

Each value shown in Table 1 indicates the average of all data obtained by culturing Pg-1 K 1 callus on various media. The effects of illumination, 2,500 to 4,000 lux, on the saponin content and growth were not observed, except the ratio of Rb group to Rg group, which is thought to show a qualitative evaluation. The difference of the ratio was due to the decrease in amounts of Rb group saponins under illumination.

In the liquid cultures, IBA 1 callus were used and cultured on a gyratory and reciprocal shaker. In suspension cultures on a gyratory shaker, the growth was excellent compared to cultures on a reciprocal shaker, but saponin production was lower in gyratory than in reciprocal cultures. The gyratory suspension cultures produced soft and brownish cell aggregates generating many roots and showed 1.8 times in the growth rate and increased production index compared with reciprocal suspension cultures. The production index, that is the product of growth ratio and total saponin, is thought to show the effectiveness of the production per culture. Also, cell suspension cultures produced 1.6 times higher amounts of saponins than the static culture. The gyratory suspension cultures of IBA 1 callus were the best condition for callus cultures, and produced almost the same saponin in qualitative as the roots of the original plant. Its total saponin contents % per dry mass were 5 times higher than that of the plant roots.

Table 1. Effects of various culture conditions on growth and saponin content

Culture condition	Growth ratio*	Dry wt(g) per 100 g fr wt	Saponin content(mg)** per 100 g fr wt			Rb group / Rg group	Total Saponin % Dry wt	Production index***
			Rb	Rg group	Total			
Static dard	3.1	2.59	31.1	12.1	43.2	2.57	1.67	132.6
K 1 callus light	3.6	2.70	25.8	14.8	40.6	1.74	1.50	139.8
Liquid reciprocal	1.9	3.76	40.5	29.7	70.2	1.36	1.87	134.1
IBA callus gyratory	3.4	3.28	40.4	26.5	66.9	1.52	2.04	226.0
Plant aerial		9.53	21.7	62.1	83.8	0.35	0.88	
(stem, leaf) root		23.91	59.0	37.3	96.3	1.58	0.40	

* Growth ratio was determined by the increasement of fresh weight after 4 weeks culture. The value was expressed as number of times.

** The amount of Rb group was calculated as the total of ginsenosides, having protopanaxadiol as the sapogenin, and Rg group was culculated as the total of ginsenosides, having protopanaxatriol. The value shows the average of each callus cultured on the media supplemented with various plant growth regulators.

*** Production index is expressed the product of the growth ratio and total saponins.

Effects of plant growth regulators[3)]

The effects of auxins and cytokinins, alone or in combination, on saponin content and growth in static cultures using IBA 1 callus were examined. D, NAA, IAA and IBA were used as auxin, and K and P were used as cytokinin. The better growth ratio and increased amounts of saponins were observed in the combination of IBA and K. Although D showed the best growth in the D requiring Pg-1 callus, it inhibited the growth of IBA 1 callus and in 5 mg/l completely inhibited the growth.

Next, the comparison of saponin content and growth of IBA 1 callus, which generated rootlets in the dark, were made under various conditions in suspension culture. The results are summarized in Table 2. In gyratory shaking cultures, the growth was excellent in comparison with the

reciprocal ones, especially in the use of IBA, but saponin production was higher in reciprocal than in gyratory cultures, except in the presence of D. It was observed that the combination of IBA and K is lower in growth ratio and much higher in saponin production than that of IBA with P, namely the combination of IBA 2 and K 0.1 gives the best production index, which is expressed as the function of growth and saponin production, and IBA 5 with P 0.1 gives the best growth ratio 8.19. The production indices were 359 in IBA 2 K 0.1 and 346 in IBA 5 P 0.1.

Table 2 Effects of plant growth regulators on growth and saponin content of IBA 1 callus in a gyratory suspension culture

Medium	Growth ratio	Dry wt(g) per 100 g fr wt	Saponin content(mg) per 100 g fr wt			Rb group / Rg group	Total Saponin % Dry wt	Production index
			Rb	Rg group	Total			
IAA 1	3.74	3.41	20.4	34.7	55.1	0.59	1.62	206
IAA 2	4.41	3.93	14.9	22.0	36.9	0.68	0.94	163
IAA 5	4.12	3.90	7.6	9.5	17.1	0.80	0.44	70
IAA 1 K 0.1	4.35	3.73	23.8	17.6	41.4	1.35	1.11	180
IAA 2 K 0.1	5.73	3.52	24.3	21.8	46.1	1.11	1.31	264
IAA 5 K 0.1	4.12	4.05	24.4	17.9	42.3	1.36	1.04	174
NAA 1	4.68	3.70	19.3	19.5	38.8	0.99	1.05	182
NAA 2	4.36	3.75	22.0	20.8	42.8	1.06	1.14	187
NAA 5	5.83	3.57	17.5	19.9	37.4	0.88	1.05	218
NAA 1 K 0.1	4.31	3.55	19.2	18.6	37.8	1.03	1.06	163
NAA 2 K 0.1	4.89	3.72	23.3	13.9	37.2	1.68	1.00	182
NAA 5 K 0.1	5.26	3.60	20.9	13.3	34.2	1.57	0.95	180
D 1	3.41	3.44	21.4	11.7	33.1	1.83	0.96	113
D 2	2.06	3.72	22.2	13.0	35.2	1.71	0.95	73
D 5	1.75	3.82	21.0	14.3	35.3	1.47	0.92	62
D 1 K 0.1	3.47	2.92	21.0	23.1	44.1	0.91	1.51	153
D 2 K 0.1	2.23	3.56	23.5	20.5	44.0	1.15	1.24	98
D 5 K 0.1	1.57	3.74	20.9	11.7	32.6	1.79	0.87	51
IBA 1	4.11	4.15	18.1	38.7	56.8	0.47	1.37	233
IBA 2	4.80	4.42	16.5	42.0	58.5	0.39	1.32	281
IBA 5	3.42	4.32	26.2	43.1	69.3	0.61	1.60	237
IBA 1 K 0.1	3.17	4.96	33.7	47.3	81.0	0.71	1.63	257
IBA 2 K 0.1	4.66	4.95	32.8	44.2	77.0	0.74	1.56	359
IBA 5 K 0.1	4.14	4.11	16.3	41.8	58.1	0.39	1.41	241
IBA 1 P 0.1	5.48	3.38	19.1	16.8	35.9	1.14	1.06	197
IBA 2 P 0.1	6.34	3.59	22.3	16.5	38.8	1.35	1.08	246
IBA 5 P 0.1	8.19	3.44	25.3	17.0	42.3	1.49	1.23	346
Plant								
Main root		16.15	45.8	40.1	85.9	1.14	0.53	
Lateral root		15.45	46.7	32.6	79.3	1.43	0.51	

Each value show the average of 5 different cultures as shown in Table 1.

The saponin content of the 5-year-old native ginseng root cultivated in Korea was shown in the last column in Table 2 to compare with that of callus cultures. The best condition in callus cultures, the combination of IBA 2 with K 0.1, produced almost the same saponin content, especially in protopanaxatriol group saponins, as that of the crude drug. In protopanaxadiol group saponins, however, the content of Rb_1 was higher than that of crude drug, but Rc and Rd were lower.

Mass Culture[4)]

Thus, the newly established Pg-3 B2K callus showed a higher growth ratio and saponin content, especially in the culture on B2K medium. Pg-3 callus cultured on B2K agar medium was directly transferred to a 5 liter jar fermenter containing 4 liters of test medium. The aeration ratio was 1 volume(aeration) per volume (medium) per minute (VVM) at 100 rpm. The effect of carbon and nitrogen sources was examined with callus suspended in liquid B2K medium. In suspension culture containing 3% sucrose, the growth of Pg-3 B2K callus was slow for the first 2 weeks. This result was probably caused by low availability of sucrose at the initial stage of the culture. The callus was transferred to medium containing 1% sucrose and various amounts of glucose and cultured for 2 weeks. After an initial 2 weeks of culture, the growth ratio reached a maximum at 0.5-0.6% glucose concentration. Therefore, 0.5% glucose and various concentrations of sucrose were tested. Sucrose 2% was shown to produce maximum growth during the initial 2 weeks. These results indicated that a medium with 0.5% glucose and 2% sucrose is the best for growth during the initial 2 weeks.

On the investigation of mass culture, Pg-3 callus grown on agar also was transferred to 500 ml of medium in a 1-liter Erlenmeyer flask and cultured on a reciprocal shaker for 4 weeks. Cells cultured in this manner were then used as inocula for a 30-liter jar fermenter containing 25 liters of test medium. The inoculum size was 24 or 48 g/liter. The aeration ratio was 0.25 VVM. Three turbine types (disk, angled-disk, and anchor-type) and to speeds (100 and 150 rpm) were tested. The results are summarized in Table 3. MS medium without NH_4NO_3 was similar to regular MS medium for growth and saponin content. Use of MS medium without

NH_4NO_3, but with 0.5% glucose and 2% sucrose, and another 2% sucrose added after 2 weeks of culture, result in a higher growth ratio and higher dry weight (g/liter) than regular MS medium containing 3% sucrose, the relative amount of saponins (mg/liter) decreased only slightly.

Table 3. Effect of medium conditions and turbine types in 30-liter jar fermenter culture of Pg-3 B2K callus*

Medium**	Turbine (rpm)	Inoculum		Dry weight		Total saponin content		
		size (g/liter)	Growth ratio	g/100g (fr wt)	g/liter	mg/100g (fr wt)	mg/100g (dry wt)	mg/liter
MS sucrose 3%	Disk (100)	24	4.45	5.71	6.1	41.1	720	43.9
	Disk*** (50)	48	4.33	5.64	11.7	23.1	410	48.0
MS Sucrose 3%	Disk (100)	24	5.00	8.86	10.6	41.3	466	49.4
		48	4.10	5.56	10.9	18.6	335	36.5
MS sucrose 2% + glucose 0.5%	Disk (100)	24	4.33	7.54	7.8	55.3	733	57.2
		48	6.12	3.17	9.3	18.4	580	53.9
MS sucrose 2% + glucose 0.5% add sucrose 2% after 2 weeks	Disk (100)	48	5.04	5.95	14.4	20.7	348	50.1
	Anchor (100)	48	4.66	6.86	15.3	19.5	284	43.5
	Angled (100)	48	6.45	5.48	17.0	9.7	177	30.1
	Disk (150)	48	3.86	5.82	10.8	26.7	459	49.6
	Angled (150)	48	3.81	7.74	14.2	24.2	313	44.4

* Aeration ratio, 0.25 VVM; culture period, 28 days; disk, disk-turbine, anchor, anchor-type turbine; angled, angled-disk turbine

** MS medium used in this experiment all were excluded NH_4NO_3

*** 0.5 VVM, two-disk turbines.

Among three turbine types, the angled-disk turbine provided the best growth ratio and dry weight increase (g/liter) but the lowest saponin content. When the agitation was accelerated to 150 rpm, the growth ratio and dry weight decreased, but the saponin content increased, giving a saponin production (mg/liter) similar to that at 100 rpm.

In a 30-liter jar fermenter culture, the increase of the growth ratio and dry weight were not accompanied by an increase of the saponin content. This observation indicated that the saponin production per culture was about equal. A jar fermenter culture is not comparable to a shake-flask suspension culture. Therefore, it is necessary to further examine the culture conditions for cells in jar fermenters. Thereafter, repeated selection of cell lines from Pg-3 callus and the engineering of a new device for mass culture in a ton-scale tank were carried out in detail. In the present time, Pg-3 callus has been cultured in a 20-ton tank by Nitto Denko Co., and the cultured ginseng has been used as the commercial products[5]

Table 4. Comparison of the saponin contents between various ginseng cultured tissues differentiated from Pg-3 callus and the source plant

Tissue	Growth ratio	Dry wt(g) per 100g fr wt	Saponin content(mg) per 100g fr wt			Rb group / Rg group	Total Saponin % Dry wt	Production index
			Rb	Rg group	Total			
Static								
DK callus	4.30	2.48	2.4	8.4	10.8	0.29	0.44	46
K 1 Shoot	5.81	2.96	15.1	22.3	37.4	0.67	1.26	217
IBA 1 root	3.40	3.09	27.6	25.1	52.7	1.10	1.71	179
Liquid								
B2K root	6.22	6.31	41.6	38.8	80.4	1.07	1.27	500
Plant								
Aerial part		9.53	21.7	62.1	83.8	0.35	0.88	
Root		23.91	59.0	37.3	96.3	1.58	0.40	

Each value show the average of 5 different cultures as shown in Table 1.

Differentiation and Saponin Production

From Pg-3 callus cultures, shoots and roots were formed at a high rate, in all flasks, under the optimal conditions according to the methods descr-ibed in Pg-1 callus. The systematic differentiation of shoots and roots from Pg-3 callus are shown in Fig. 1.

The saponin content in various calli and differentiated tissues were deter-mined according to the methods described above. The values are shown in

Table 4 and are compared to the saponin content of the source plant. As a result, it was demonstrated that the shoots (K 1, Fig 1d, e) and the roots (IBA 1, Fig. 1f) produced larger amounts of saponins than the original callus (DK, Fig. 1a): 3.5 times more in K1 and 4.9 times more in IBA 1 tissues.

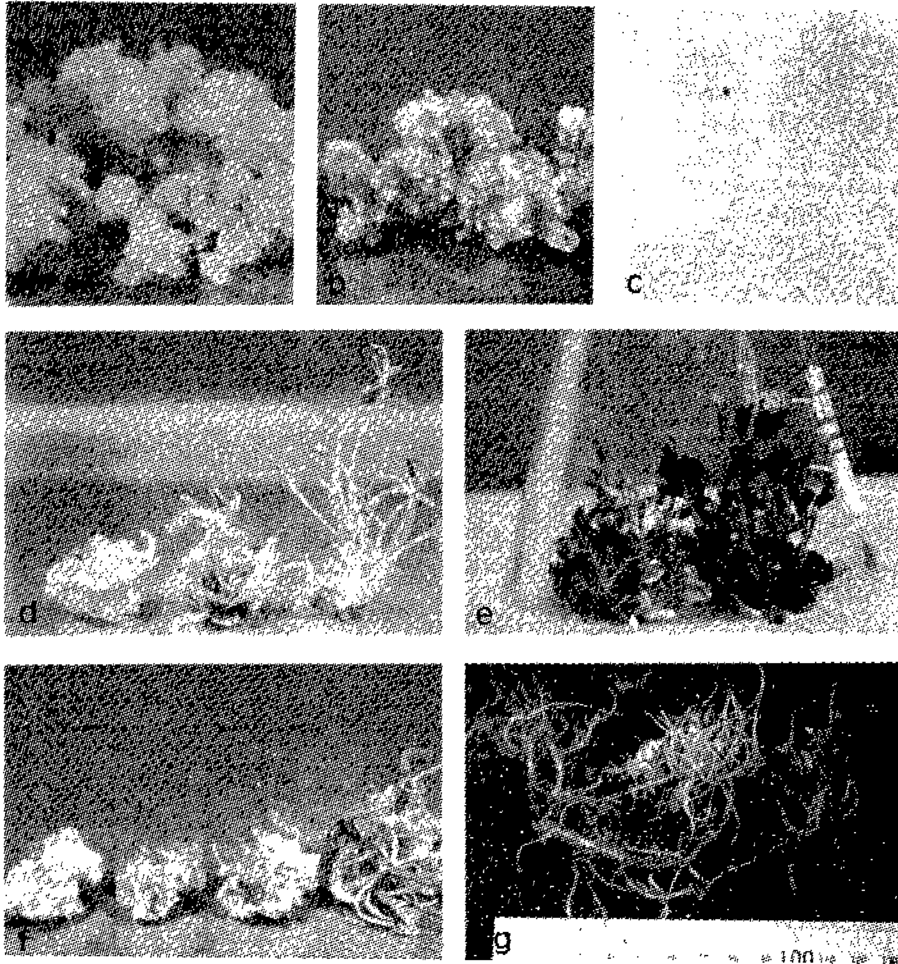

Fig. 1. Systematic formation of shoots, roots and plantlets from callus cultures of *Panax ginseng*. a; Ginseng callus derived from native ginseng root on MS medium containing D 1 mg/l and K 1 mg/l (DK medium). b; Meristemoids induced from the DK callus (a) on the medium without 2,4-D. c; A cross section of the meristemoids (b), X 20. d; Shoots formed from the meristemoids (b) on the medium containing K 1 mg/l, stepwise from left to right. e; Cloning plantlets developed form shoots (d) on the K 1 medium under illumination. f; Roots formed from meristemoids (b) on the medium containing IBA 1 mg/l, stepwise from left to right. g; Roots cultured in the liquid medium containing IBA 2 mg/l and K 0.1 mg/l in the dark.

The saponin content in suspension culture (B2K medium, Fig 1g) was comparable to those of the aerial part and the root of the plant on a fresh-weight basis. On the other hand, the saponin content of the cultured roots on a dry-weight basis was 1.71% in static culture and 1.27% in suspension, and those were 3-4 times higher than that in the plant root, 0.40%. Moreover, the ratio of ginsenoside Rb group to Rg group was calculated for a quality evaluation of ginseng saponin. The ratios in DK callus and K 1 shoot resembled those in aerial part of native plant, whereas in the root cultures, i.e., IBA 1 root and B2K suspension, they resembled those in plant root.

Isolation of Hairy Root[6)]

Hairy root culture of ginseng was established after roots were induced on Pg-4 callus, which was induced on DK medium from 2-year-old root cultivated in Japan in 1980, following infection with *Agrobacterium rhizogenes*. After the Pg-4 calli (rigid small cell aggregates) were cultured on B2K medium for 3 weeks, the calli were collected by filtration with 20 mesh Nylon cloth. The calli were incubated in MS medium with 2% cellulase, 0.5% macerozyme, and 0.3 M mannitol at 30℃ for 2 hr, that is formation of the protoplasts only on the surface parts of callus aggregates. The calli were inoculated in the bacterial suspension cultured in YEB medium for 3 days. After infection for 15 hr, the calli were collected by filtration on Nylon cloth and throughly washed with a sterile water. Thereafter, to eliminate the bacteria the calli were incubated in MS medium supplemented with Vancomycin, Carbenicillin and Tetracycline, at 25℃ for 4 days. After washing with a sterile water, the calli were placed on MS agar medium without phytohormone and cultured at 25℃ in the dark. Approximately 6 weeks after infection, roots which appeared from several points of the calli, were isolated and transferred to liquid hormone-free MS medium. The hairy root cultures slowly grew without bacterial contamination and were maintained by transferring on the fresh medium at 3 weeks intervals. The hairy roots grew more actively with a high degree of lateral branch after transferring into MS medium supplemented with 0.5-2.0 ㎎/1 IBA.

Characteristics of Hairy Roots

The morphological property of the transformed hairy roots was compared with that of the ordinary cultured roots, which were induced by hormonal control from Pg-1 callus, IBA 1 callus (roots). The Pg-1 roots cultured on the MS medium supplemented with IBA2 mg/l and K 0.1 mg/l, called B2K medium, grew vigorously without ranching, and sometimes formed callus like aggregates and/or shoot and leaf. On the other hand, the hairy roots grew with extensive lateral branching and never differentiated tissues other than fine root. Subsequently, by long term culture over 4 weeks the hairy roots actively elongated colorless aerial roots in the liquid medium, whereas the ordinary cultured roots formed a ball-like aggreate of light yellow color without aerial root. The most important difference is that the hairy roots

Table 5. Growth and saponin contents of callus, ordinary cultured roots, hairy roots and native root of *Panax ginseng*

Tissue Medium	Growth ratio	Dry wt(g) per 100g fr wt	Saponin content (mg) per 100g fr wt			Rb group / Rg group	Total Saponin % Dry wt	Production index
			Rb	Rg group	Total			
Callus B2K	2.85	5.97	28.3	10.6	38.9	2.67	0.65	110.9
Ordinary roots								
hormone-free	1.26	5.31	15.3	4.9	20.2	3.15	0.38	25.5
B 2 K 0.1	3.96	5.57	25.7	25.0	50.7	1.03	0.91	200.3
Hairy roots								
hormone-free	3.07	10.09	24.8	11.1	35.9	2.24	0.36	110.2
K 0.1	2.25	10.29	21.7	14.6	36.3	1.48	0.35	81.7
B 2	5.11	9.62	39.6	26.6	66.2	1.49	0.69	338.3
B 0.5 K 0.1	4.30	10.45	56.3	40.0	96.3	1.41	0.92	414.1
B 2 K 0.1	6.20	10.58	55.7	44.5	100.2	1.25	0.95	621.2
Native root		23.91	59.0	37.3	96.3	1.58	0.40	

Each tissue was cultured in MS liquid medium containing the hormone(s) shown in Table on a gyratory shaker at 25℃. Each value show the average of 5 different cultures as shown in Table 1.

could grow in hormone-free medium, higher growth ratio; 3.07, while the ordinary cultured roots did not grow in hormone-free medium, growth ratio; 1.26.

Saponin Production by Hairy Roots

The production of ginseng saponins by the hairy roots cultures was examined. The saponin contents in both the hairy roots and ordinary cultured roots are shown in Table 5. The ratio of Rb group to Rg gorup also was shown as an indication of the quality evaluation. As a result, it was demonstrated that the hairy roots cultured in hormone free medium produced almost the same amount of saponin on a fresh weight basis and the constituent of saponins as the original callus. On the contrary, in the medium supplemented with IBA, the hairy roots produced a larger amount of saponins, 66.2 ㎎/100g fr wt, than those of the original callus, 38.9 ㎎ and the ordinary cultured roots, 50.7 ㎎. Furthermore, the growth of the hairy roots was stimulately promoted up to 6.20 times, when cultured in the medium supplemented with IBA. Finally, the production index in B2K medium showed 621.2, the maximum in all culture conditions carried out in our laboratory. The saponin content, 0.95% on a dry wt, was 2.4 times higher than that of the native root, 0.40%, and the quality also was almost the same as the native root. Thereafter, its toxicological safety in the hairy root cultures was proved by two mutagenicity tests, that is the Ames and chromosome aberration tests.

Use for a Bioreactor

When the hairy roots were cultured in the medium supplemented with IBA and kinetin, they produced a larger amount of saponins than the original callus and the regenerated ordinary roots. Since both the ordinary roots and the callus had the ability to glycosylate various compounds, such as phenylcarboxylic acid, to form its glycosides as we previously reported, it was expected that the hairy roots also have the same high potentiality to

glycosylate as the callus and the ordinary roots[8,9]. Therefore, we investigated the glycosylation of various organic compounds, such as phenylcarboxylic acid[8,9], digitoxigenin[10] and 18β-glycyrrhetinic acid[11] by the ginseng hairy root cultures, which must have the highest glycosylation activity.

In fact, the hairy roots converted digitoxigenin to 5 glucosides and 18β-glycyrrhetinic acid to 4 glucosides and its 2 malonyl derivatives. The conversion abilities to (RS)-2-phenylpropionic acid (PPA) were compared between 3 different cultures, that is callus, regenerated ordinary roots and hairy roots. The callus showed lower glycosylation ability than those of the roots, and the product is only glucosyl-ester. Both the ordinary and hairy root cultures showed the greatest glycosylation potential, about 100% in the conversion ratio, and about a half of the glucosyl-ester was excreted into the medium.

Moreover, the hairy roots cultures were especially excellent in the application to a bioreactor, because they grew with extensive lateral branching and never formed callus-like tissues, which were frequently generated from the ordinary root and caused to choke the outlet-filter of the reactor. So, we tried to continuously form the glycosides from PPA using bioreactor with hairy roots. The bioreactor in this experiment was designed by revising a reversed flask type. As a result, we succeeded in the continuous reaction during about 2 months by the exchange of the reaction mixture every 3 days. The hairy root used in the bioreactor continued to convert for about 2 months, at maximum conversion ratio 45% and at 15% in average ratio[12].

Conclusion

Thus, we succeeded in the production of useful compounds, mainly ginsenosides, by various sellected ginseng cultured tissues and by means of the revised culture methods for the production. Now, we are further in progress the investigation about the production of the useful compounds with better quality and device for more effective production system.

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