CHARACTERISTICS OF *PANAX GINSENG* STRAINS AND GROWTH OF CELL SUSPENSIONS IN BIOREACTORS

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Presently, a consequently high attention is drown to natural plant products, which are important for medicine, food, and cosmetic industry. The method of plant cell culture offers new prospects to study biogenesis of these products and also to create new sources of various commercially important substances.

As the producents of commercially profitable products, the cultured plant cells have some advantages in comparison with a whole plant : (1) independence of substance production on climate, season, weather ; (2) preservation of genotypes of rare or disappearing plants ; (3) obtaining overproducing cells by genetic manipulations ; (4) reduction of the product cost due to optimization and automatization of the processes of fermentation and post - fermentation.

The commercial success of the plant cell industrial technology depends on the high price of products and their high biological activity at rather low concentration. Our biotechnological group of Cell Biology and Biotechnology Department maintains in the collection some medicinal plant cell strains belonging to Araliaceae family (Table 1), which contain complex of biologically active substances.

Table 1. Collection of Araliaceae species cultured *in vitro*

Panax ginseng C.A. Meyer
Panax quinquefolius L
Panax japonicus C.A. Meyer
Polysceas filicifolia Moor a. Fouenier
Eleutherococcus senticosus (*Rup. et Maxim.*) *Maxim*
Aristolochia manshuriensis Kom. (*Aristolochiaceae*)

Among them there are cell cultures of *Panax ginseng* C.A. Meyer. The pharmacological effect revealed for the active princiles of *P. ginseng* root and cultivated cells represented in Table 2.

It is well known that, as a rule, cultured plant cells have lower content of secondary substances in comparison with a whole plant. To obtain high productive cell lines the successive screening is usually used. It includes the choice of species with high content of valuable secondary substances (*P. ginseng*) using the productive individual plant (root of forest wild plant in this case), the correct choice of proper organ and tissue as explants (tissue from middle zone of the root). Then genetic manipulations with cultured cells could be performed. This last step, from my point of view, is very important because it gives the possibility to create the productive cell lines by the methods, which are unacceptable for a whole plant. Screening of variants and mutants and somatic hybridization are among them. Some characteristics of various *P. ginseng* strains obtained by our group from initial wild strain on the basis of cell somaclonal variation and mutagenesis are represented in the Table 3. All these strains are polyploid or aneuploid and differ in the modal and maximum number of chromosomes, the range of cell aggregation in suspension culture, and biomass productivity.

Biomass productivity of all strains in suspension culture was considerably higher than in callus culture on agar medium.

The chemical composition of cultured cell biomass and fresh root of *P. ginseng* are represented in Table 4. The group of substances numbered from 7 to 11 (Table 4) forms the biologically active complex. The sum of ginsenosides is approximately equal in both cases and all additional components with the biological activity are the same.

Table 2. The pharmacological value of *Panax ginseng* tinctures

Species, strains	Active principles	Effect revealed
P. ginseng C.A. Mey St. G - 1 St. G - 2	ginsenosides (dammarane - type saponins)	stimulative, antifatigues antistress, immunoactive antihypotonic, antineurotic, against sex impotence

Aglycones of ginsenosides protopanaxadiol and protopanaxatriol from *P. ginseng* root and cell suspension culture were identified by our group by GC - MS (Table 5, Fig. 1). Cell biomass of *P. ginseng* (Sts. G - 1 and G - 2) cultivated in suspension and callus cultures was used as a raw material to obtain tinctures for medicine. We studied tinctures from different sources. As the result of research, we revealed that all tinctures contained

Table 3. Some characteristics of various *P. ginseng* strains (2n=48)

Strain	Mode of obtaining	Nutrient medium	Number of chromosomes	Number of cells in suspension aggregates	Dry mass $g\ \ell^{-1}\ day^{-1}$	
					callus	suspension
IPhR G1	Root of wild plant	MS, Kinetin, NAA, m-insoitol, CH	50-100	6-10	0.42	1.5 (frem.)
IPhR G2	Var. selected from IPhR G1	MS, NAA	41-136	6-20	0.50	2.0 (frem.)
IPhR G3	〃	MS	28-400	11-30	0.21	0.7 (flask)
IPhR G11	mutant from IPhR G1 (N-NMM)	MS, kinetin, NAA	54->200	-	-	-
IPhR G12	〃	〃	30-90	-	0.60	-
IPhR G13	〃	〃	30-107	-	-	-
IPhR G14	〃	〃	28-112	-	-	-
IPhR G15	〃	〃	48-154	-	-	-
IPhR G16	〃	〃	43->200	-	-	-

Table 4. The chemical compositions of cell cultured biomass (St. G1) and root *P. ginseng*

No.	Substances	Content, % per dry mass	
		cell biomass	root
1	Protein N	1.18	1.68
2	Non-protein N	4.82	2.73
3	Sucrose	1.03	3.07
4	Glucose+Fructose	2.28	-
5	Starch	5.40	19.5
6	Cellulose	9.84	10.24
7	Pectins	15.40	15.80
8	Lipids	1.61	2.34
9	Volatile oils(Panacenes)	traces	0.05
10	Phytosterols	0.9	0.8
11	Ginsenosides	3.21	3.12

the ginsenosides Rb_1, Rd, and Re. Moreover, some tinctures contained also ginsenosides Rc, Rb_2, Rf, and Rg_1.

Table 5. Mass-spectra of damarane-type *P. ginseng* triterpenoids

Panaxadiol	M/Z : 109, 127, 175, 189, 341, 460
Panaxatriol	M/Z : 109, 127, 173, 187, 339, 476

The biological effect of *P. ginseng* biomass extracts from both native roots and culture was reaveled in experiments on proliferation of *Chinese hamster* fibroblasts cultivated under stress conditions (Table 6).

However, in control test, effect of *P. ginseng* biomass extracts on fibroblast proliferation was absent for both sources (Table 7).

The productivity of *P. ginseng* cell cultures could depend both on the strain variaties and conditions of cultivation (callus tissue on the surface of agar medium, suspension culture in flasks or bioreactors of various types). Regimes of cultivation, bath culture, continuous or semicontinuous culture, and composition of the medium are also very important both for product content and biomass productivity. *P. ginseng* culture St. G-2 was grown in MS medium containing 2 mg ℓ^{-1} NAA, 0.4 mg ℓ^{-1} thiamine, and 30 to 50 g ℓ^{-1} sucrose.

Two types of stirred tank bioreactor were used : (1) MF-

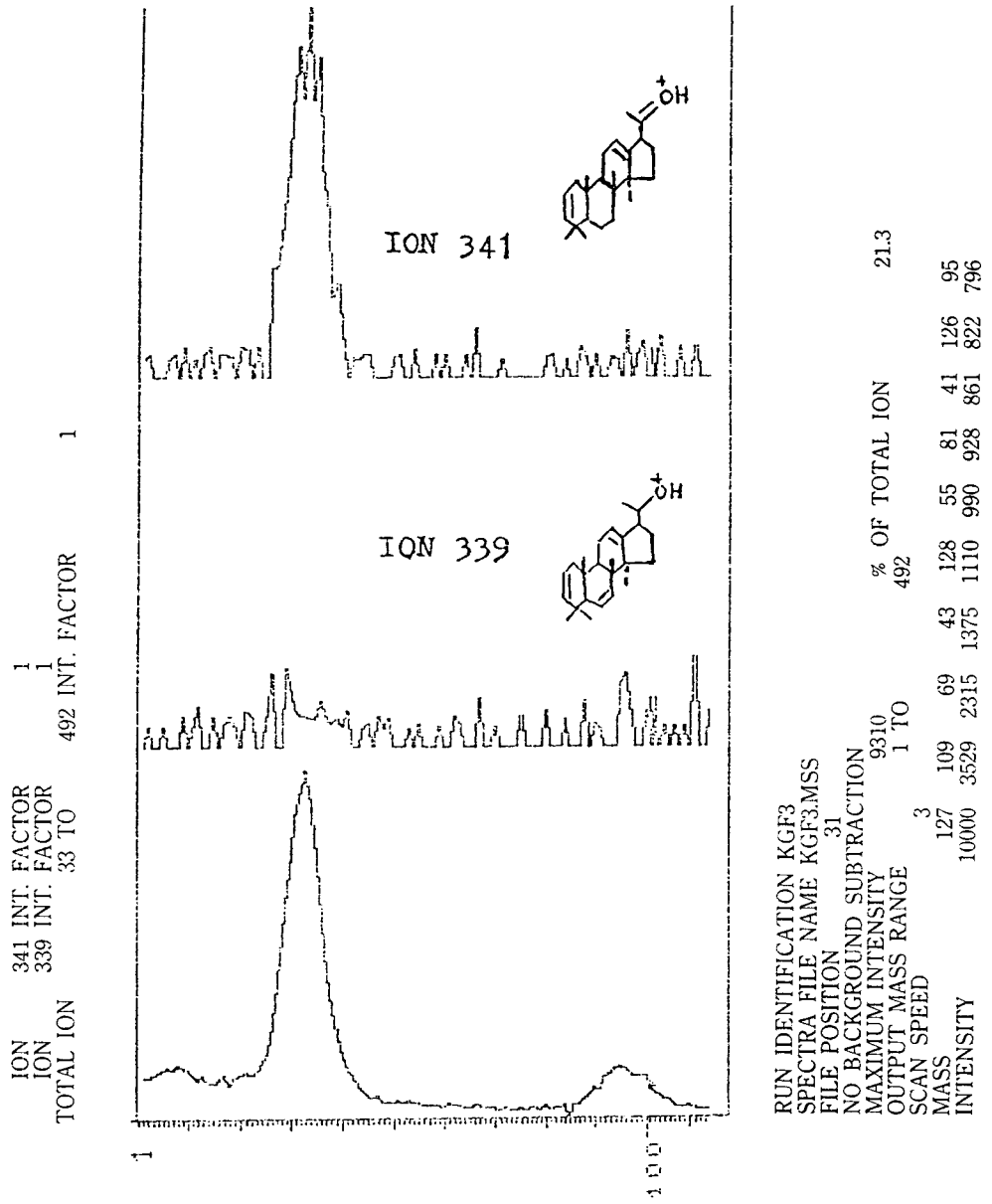

Fig. 1 Chromatogram of crude saponin from cell suspension of panax ginseng C.A. Mey(after acid hydrolysis)

Table 6. The effect of *P. ginseng* biomass extracts on proliferation of cultivated *Ch. hamster* fibroblasts (St. 7 - 84) under stress

Dose ml × 5% extr.	Control, number of cells $1 \cdot 10^4$/ml	P. ginseng root extract		P. ginseng cell extract	
		numner of cells $1 \cdot 10^4$/ml	stimulation effect, %	number of cells $1 \cdot 10^4$/ml	stimulation effect, %
0.0005	55	175	215	183	229
0.005	55	137	146	145	161
0.01	66	142	115	107	62
0.1	76	76	0	207	164
0.25	76	125	64	228	200
0.5	76	91	20	223	193

Table 7. The effect of *P. ginseng* biomass extracts on proliferation of normally growing *Ch. hamster* fibroblasts (St. 7 - 84)

Dose ml × 5% extr.	Control, number of cells $1 \cdot 10^4$/ml	P. ginseng root extract		P. ginseng cell extract	
		numner of cells $1 \cdot 10^4$/ml	stimulation effect, %	number of cells $1 \cdot 10^4$/ml	stimulation effect, %
0.005	245	280	14	690	18
0.01	252	219	-6	273	18
0.1	251	279	11	262	4
0.25	237	234	-1	269	13
0.5	206	203	-1	219	6

107 (New Brunswick) with operating volume of 5 ℓ and three six - flatbladed turbine impellers operating at 350 - 650 rpm, the rotation speed of 90 - 170 cm s^{-1}, and the aeration rate of 0.06 - 0.12 $Nm^3 h^{-1}$; and (2) EL - 75 (Novaferm AB, Sweden), operating volume of 50 ℓ with the marine type impellers working at 150 - 240 rpm, 120 - 330 cm s^{-1}, and 0.6 - 1.35 $Nm^3 h^{-1}$. Cultivation was conducted at 26℃.

The semicontinuous regime with the ratio of initial to final biomass concentration of 30% gave 1.5 - to 2 - fold increase in cell biomass productivity for *P. ginseng* as compared with the bath culture. Our data showed that cell suspension of *P. ginseng* was very sensitive to the shear stress. Nevertheless, in bioreactors with marine impeller and rotation speed up to 330 cm s^{-1} this suspension grew very well. The strain with a high content of the commercially important products and active growth in various bioreactors can be lost in the course of long - term cultivation.

On the one hand, a size of genome might be one of the positive features to increase the multiplying index and long - term cultivation can result in prevailing diploid and near diploid cells in the population. On the other hand, meanwhile, we could not found that in the course of long - term cultivated (since 1960) cell population of *P. ginseng*. In the partially synchronized with 5 - aminouracyl (5 - AU) callus culture after removing of the blockage of cell division one could clearly observe the separation of the population into two subpopulations. The subpopulations had a different duration of G_2 phase. In one subpopulation the transition to mitoses was observed at 16th hour after 5 - AU removing. In another one it occurred at 32nd hour (Fig. 2). Mitotic cycle of the first subpopulation was 32 hours.

Checking up this population beyond ten years after the first study showed the presence of the same two subpopulations (Fig. 3). However, both subpopulations had longer G_2 period (28th and 52nd hours, respectively). Mitotic cycle of the first subpopulation was 48 hours.

It is difficult to explain why the longer time was necessary for G_2 period of the second subpopulation of *P. ginseng* and why it was not a point of negative selection of these cells during an extensive period of subcultivation. We assume that prolonged G_2 phase could compensate this shortcoming by a higher stability to growth stresses. This assumption is also supported by an

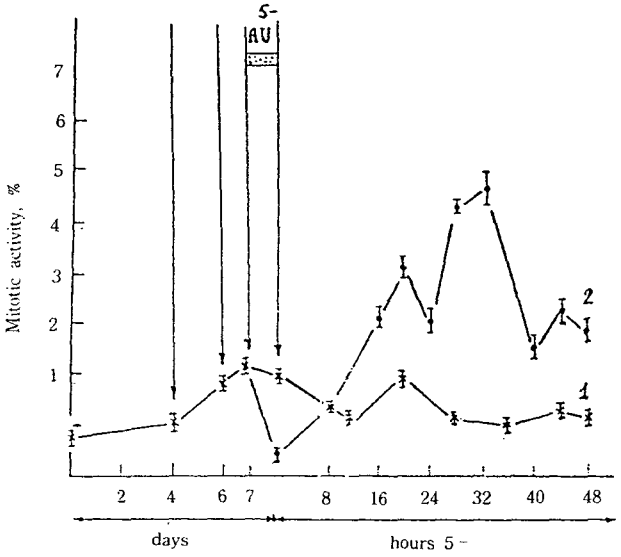

Fig. 2 Revealed two subpopulations with different periods of G_2 phase in the suspension culture P. ginseng. Mitotic cycle in the initial population have been partially syncronized by treatment with 6mM - 5AU.

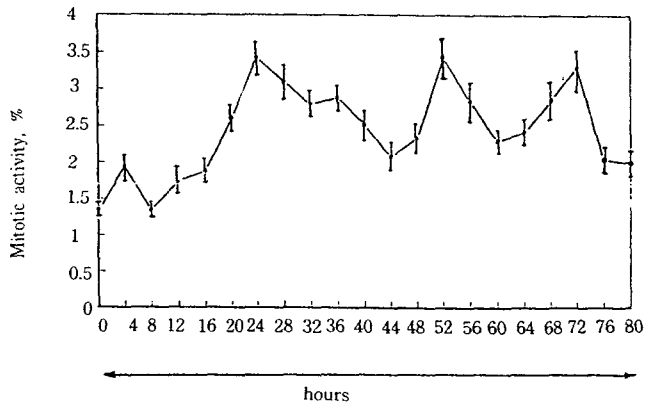

Fig. 3 The same two subpopulations of P. ginseng after 10th years of subcultivation. The period of the G_2 phase in mitotic cycle both subpopulations has increased.

Table 8. Cryo - bank of plant cells and meristems

No.	Species	Cell lines	Storage in liquid nitrogen, years
1	*Daucus carota* L.	M - 34	12.5
2	*Dioscorea deltoidea* Wall.	IPhR D1	8.5
3		IPhR DM - 0.5	7.5
4		IPhR DM - 1.0	6
5		IPhR DM - $8.0_{A^{\cdot}K^{\cdot}}$	7
6	*Panax ginseng* C.A. Mey	IPhR G - 1	8
7		IPhR G - $2_{A^{\cdot}K^{\cdot}}$	4
8		IPhR G - $3_{A^{\cdot}K^{\cdot}}$	5
9	*Panax quinquefolius L.*		4
10	*Nicotiana sylvestris L.* salt - resistant cell mutant	NrFs - 1	5
11	*Medicago sativa* L.	UIPhR L - 1	4
12	*Solanum tuberosum*L.	KT - 3	1
13		meristems var. 5	3
14		pollen of 70 specimens	7
15		seeds	3

increase of the G_2 period in both subpopulations after a long - term cultivation.

The valuable strains could be preserved in liquid nitrogen (cryo - bank preservation). Some valuable strains including the strains of *P. ginseng* are preserved in our collection (Table 8).

Dr. Popov and his collaborators in our Department showed that the original characteristics of strains could be preserved completely by cryopreservation for many years. This method allows us to believe that the best productive strains can be preserved for industrial cultivation.