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Effecting of medium composition on biomass and ginsenoside production in cell suspension culture of *Panax vietnamensis* Ha et Grushv.

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Abstract. We established cell suspension culture on *Panax vietnamensis* and some attempts have been made to increase ginsenoside yield of ginseng cell culture through manipulation various culture factors and process variable. Half and full strength MS medium were found to be equally suitable for both biomass as well as ginsenoside production. The biomass production and ginsenoside yield were obtained 9.8 g/L DW and 6.81 mg/g DW, respectively. The effect of initial sucrose concentrations were also investigated in suspension cultures of *P. vietnamensis* for biomass and production of ginseng saponin (secondary metabolite). The final dry cell weight was increased from 5.4 to 10.3 g/L with an increase of initial sucrose concentration from 20 to 50 g/L, but an even higher sucrose concentration of 60 g/L seemed to repress the cell growth, further increase of sucrose concentration upto 70 g/L led to a decrease in ginsenoside accumulation and biomass production. The maximum growth and ginsenoside production was obtained for nitrogen concentration of 30 mM.

Keywords: MS strength, sucrose, nitrogen, auxin, and cytokinin.

1. Introduction

Vietnamese ginseng was found at highland of Central Vietnam in 1973, and was regarded as a new species as *Panax vietnamensis* Ha et Grushv. (1985). This is the most southern distribution of *Panax* genus (Araliaceae). It is a secret medicine of the Sedang ethnic group as a miraculous, life-saving plant drug used for the treatment of many serious diseases and for enhancing body strength in long journeys in high mountains.

The demand for ginseng has increased dramatically worldwide and ginseng becomes very expensive because of its long-term conventional (5-7 years) and troublesome production cycles. The annual turnover of ginseng in the United States was $98 million with a growth rate of 26% [1]. Therefore, plant cell and tissue culture methods have been

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explored as potentially more efficient alternatives for the mass production of ginseng and its active components. Research into ginseng cell and tissue culture started in the early 1960s and commercial application has underway since the late 1980s. The powder and extracts from ginseng cell culture were used to make health foods, drinks and cosmetics. The ginseng culture has continued to attract considerable research and development effort in recent years and scientists seek to understand and optimize the culture conditions [2].

As earlier reported [3] that *P. ginseng* callus produces almost the same pharmacologically active saponins, ginsenosides as that of cultivated ginseng root. In a 30-liter jar fermentor culture, the increase of the growth ratio and dry weight were not accompanied by an increase of the saponin content. Using MS medium minus NH_4NO_3 and plus 0.5% glucose and 2% sucrose and 2% sucrose added after 2 weeks of culture resulted in a higher growth ratio and higher dry weight than using regular MS medium containing 3% sucrose.

Effects of application sole nitrate (NO_3^-) and in combination with ammonium (NH_4^+) on production of ginseng saponin and polysaccharides by suspension cultures of *Panax ginseng* were observed by [4]. The results indicated that the specific production (content) of ginseng polysaccharide was not significantly affected by alteration of the N source and the saponin production was relatively higher within the initial N concentration of 5 mM with nitrate alone or a $(NO_3^-)/(NH_4^+)$ ratio of 2:1.

In this paper, we established cell suspension culture of ginseng cell and some attempts have been made to increase biomass and ginsenoside yield of Ngoc Linh ginseng cell culture.

2. Materials and methods

Induction of callus

Fresh mountain ginseng roots were collected from Ngoc Linh mountain, Quang Nam province. Selected root were washed with a detergent solution for 5-10 min and then rinsed with running tap water for 5-10 min. They were rinsed with sterilized water after being soaked in 70% aqueous EtOH for 0.5-3 min under reduced pressure, further sterilized with 1% sodium hypochloride for 10-30 min, and then rinsed repeatedly with sterile distilled water. The sterilized roots were cut into sections of 2-10 mm and then were inoculated into MS solid medium (Murashige and Skoog, 1962) containing 30 g/L sucrose, 1 mg/L 2,4-D, and 0.1 mg/L kinetin. After 1 month callus were induced. The callus were subcultured into above medium after every 20 days for proliferation of callus. After 5 times of subculture into the solid medium the callus were inoculated into liquid medium (same with above).

Stock cell culture and culture condition

Suspended cells of *P. vietnamensis* were initiated through callus induction from the cultivated plant root [5]. The cell line was maintained in MS liquid medium supplemented with 3 mg/L indole-3-butyric acid (IBA), 0.1 mg/L of kinetin and 30 g/L sucrose. The pH was adjusted to 5.8 before autoclaving.

Cells were cultivated in 300 ml conical flasks with a working volume 100 ml on a rotary shaker in darkness at a rotation speed of 105 rpm and a culture temperature of 25°C. Cells cultivated for 15 days were used in the experiment and the inoculum size 6 g/flask (fresh weight). The other cultural conditions were done as described by [6].

Determination and analyses

Extraction and determination of ginsenoside production were determined as reported previously [5,6].

Experimental design and data analysis

All experiment were repeated three times with 3 replicates. Data were subjected to Duncan's multiple range test using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

3. Results and discussion

1. Effects different strength of MS medium on biomass and ginsenoside production

Table 1 shows the effects of different strength of MS medium on biomass and ginsenoside production. Half and full strength MS medium were found to be equally suitable for both biomass as well as ginsenoside production. The highest biomass production and ginsenoside yield were obtained 9.8 g/L DW and 6.81 mg/g DW, respectively. High salt strength (2.0) inhibited a cell growth and ginsenoside production accumulation. Such a phenomenon was also described in provious cultures of *Panax ginseng* adventitious roots [7].

Table 1. Effect of different strength of MS medium on biomass and ginsenoside production

MS medium concentration (g/L)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
			Rg	Rb	Total
0.5	153 a[z]	9.5 a	2.39	4.42	6.81
1.0	162 a	9.8 a	2.27	4.39	6.66
1.5	120 b	7.3 b	1.95	3.88	5.83
2.0	89 c	5.4 c	1.52	2.92	4.42

[z]Mean separation by Duncan's multiple range test at $p \leq 0.05$

2. Effect of different sucrose concentrations on cell growth and ginsenoside production

The effect of initial sucrose concentration (0, 20, 30, 50, 60 and 70 g/L) was also investigated in suspension cultures of *P. vietnamensis* for biomass and production of ginseng saponin (secondary metabolite). The final dry cell weight was increased from 5.4 to 10.3 g/L with an increase of initial sucrose concentration from 20 to 50 g/L, but an even higher sucrose concentration of 60 g/L seemed to repress the cell growth. Further increase of sucrose concentration upto 70 g/L led to a decrease in ginsenoside accumulation and biomass production (Table 2). On the contrary of our results, several authors suggested that a relatively high sucrose level was benificial to secondary metabolite synthesis [8]. For example, [9] reported that the triacylglycerol content of the cells of oil seed rape could be increase about 8-fold on a fresh weight basis when sucrose concentration in the growth medium was raise from 2 to 22% (w/v). [10, 11] found that the optimal concentration of sucrose for cell growth was between 30 and 50 g/L and upto 70 g/L sucrose inhibited cell growth, while the ginsenoside content showed a steady increase with sucrose concentration of upto 60 g/L. Based on our results it can be concluded that high sucrose level and secondary metablite production is not a general phenomenon and depends on plant species.

Table 2. Effect of different sucrose concentrations on cell growth and ginsenoside production

Sucrose concentr. (g/L)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
			Rg	Rb	Total
0	89c[z]	5.4c	1.52	2.92	4.42
20	158a	9.6a	2.32	4.31	6.63
30	165a	9.9a	2.95	4.01	6.96
50	171a	10.3a	2.13	4.69	6.82
60	134b	8.1b	1.49	3.42	4.91
70	93c	5.6c	1.25	2.81	4.06

[z]Mean separation by Duncan's multiple range test at $p \leq 0.05$

3. Effect of different nitrogen concentration on cell growth and ginsenoside production

The effect of the initial nitrogen concentration in the medium for cell growth and metabolite production was studied in *P. vietnamensis* cell cultures. The initial nitrogen level was adjusted to 0, 10, 30, 60, 90 and 120 mM. The kinetics of growth (based on dry weight) in various cultures is shown in (Table 3). It is apparent that growth was inhibited at a high initial N concentration. The highest dry weight reached 10.2 g/L at an initial nitrogen concentration of 30 mM. The highest ginsenoside production was (7.35 mg/g DW) at initial medium nitrogen concentration of 30 mM after 25 days of culture.

Table 3. Effect of different nitrogen concentration on cell growth and ginsenoside production

Nitrogen concent. (mM)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
			Rg	Rb	Total
0	79c[z]	5.2c	1.47	2.81	4.28
10	122b	8.1b	2.25	4.33	6.58
30	176a	10.2a	2.81	4.54	7.35
60	156a	10.1a	2.52	4.59	7.11
90	119b	7.9b	1.95	4.02	5.97
120	86c	5.4c	1.21	3.34	4.55

[z]Mean separation by Duncan's multiple range test at $p \leq 0.05$

In cell cultures of *P. quinquefolium*, [12] reported that the final dry cell weight was relatively low with the low nitrogen concentration. Maximum cell dry weight obtained (15 g/L) at a total initial nitrogen concentration of 40 mM and the cell growth was inhibited at a high initial nitrogen concentration of 80 mM. Similarly, the accumulation of total saponin and polysaccharide were also influenced by initial nitrogen concentration in the medium. The maximum production of ginseng saponin and polysaccharide obtained (1.5 g/L and 2.19 g/L) at the initial nitrogen concentration of 40 mM [12]. In the simultaneous production of ginseng saponin and polysaccharide by suspension cultures of *P. ginseng*, [4] reported that production of ginseng saponin was related with the total nitrogen concentration. The result suggested that a low nitrogen concentration was beneficial for the stimulation of total saponin production.

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References

[1] B.K. Vogler, M.H. Pittler, E. Ernst, The efficacy of ginseng: a systematic review of randomised clinical trials, *European J. of Clinical Pharmacology* 55 (1999) 567.

[2] J. Wu, J.J. Zhong, Production of ginseng and its bioactive components in plant cell culture: current technological and applied aspects, *J. Biotechnology* 68 (1998) 89.

[3] T. Furuya, T. Yoshikawa, Y. Orihara, Oda Hirohiko, Studies of the culture conditions for *Panax ginseng* cells in jars fermentors, *J. Natural Products* 47 (1984) 70.

[4] S. Liu, J.J. Zhong, Simultaneous production of ginseng saponin and polysaccharide by suspension cultures of *Panax ginseng*: Nitrogen effects, *J. Enzyme and Microbial Technology* 21 (1997) 518.

[5] N.T. Thanh, L.T. Son, K.Y. Paek, Induction and proliferation of callus of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv): Effects of plant growth regulators, *VNU Journal of Science*, Natural Sciences and Technology 23, No.1S (2007) 167.

[6] N.T. Thanh, L.V. Can, K.Y. Paek, The adventitious root cultures of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv), *Proceeding of National Conference on Life Sciences, Vietnam*, 2007, pp 828-831.

[7] K.W. Yu, Production of the useful metabolites through bioreactor culture of Korean ginseng (*P. ginseng* C. A. Meyer). Ph.D. thesis, Chungbuk National University, South Korea, 2000.

[8] C.O. Akalezi, S. Liu, Q.S. Li, J.T. Yu, J.J. Zhong, Combined effects of initial sucrose concentration and inoculum size on cell growth and ginseng production by suspension cultures of *P. ginseng*. *J. Pro Biochem.* 34 (1999) 639.

[9] R.J. Weselake., S.D. Byers, J.M. Davoren, A. Laroche, D.M. Hodges, M.K. Pomeroy and T.L. Furukawa-Stoffer, Triacylglycerol biosynthesis and gene expression in microspore derived cell suspension cultures of oilseed rape, *J. Exp. Bot.*, 49 (1998) 33.

[10] K.T. Choi, C.H. Lee, I.O. Ahn, J.H. Lee, J.C. Park, Characteristics of the growth and ginsenosides in the suspension culture cells of Korean ginseng (*P. ginseng* C.A. Meyer). In W.G. Bailey, C. Whitehead, J.T.A. Proctor, J.T. Kyle. (eds), Proce Int Ginseng Con., Vancouver, 1994, pp. 259-268.

[11] K.T. Choi, I.O. Ahn, J.C. Park, Production of ginseng saponin in tissue culture of ginseng (*P. ginseng* C. A. Meyer), *Russ. J. Plant Physiol*. 40 (1994) 784.

[12] J.J. Zhong, S.J. Wang, Effects of nitrogen source on the production of ginseng saponin and polysaccharide by cell cultures of *P. quinquefolium*, *J. Pro. Biochem.* 33 (1998) 671.

Ảnh hưởng môi trường nuôi cấy đến sự tăng trưởng sinh khối và sự tích lũy sản phẩm ginsenoside trong nuôi cấy tế bào lỏng của Sâm Ngọc Linh (*Panax vietnamensis* Ha et Grushv.)

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Để sản xuất sinh khối và sản phẩm trao đổi chất thứ cấp ginsenoside, các thí nghiệm nuôi cấy tế bào lỏng của Sâm Ngọc Linh (*Panax vietnamensis* Ha et Grushv.) đã được tiến hành với các thành phần khác nhau của môi trường nuôi cấy. Đối với nồng độ môi trường MS cho thấy với tỷ lệ 50 hoặc 100% là thích hợp cho sự tích luỹ sinh khối tế bào và sự tổng hợp sản phẩm thứ cấp ginsenoside. Nồng độ đường trong môi trường nuôi cấy cũng được thay đổi, kết quả cho thấy 30 g/L là thích hợp cho sự tích luỹ sinh khối tế bào và sự tổng hợp sản phẩm ginsenoside. Sinh khối khô tăng từ 5.4 đến 10.3 g/L khi tăng nồng độ đường từ 0 đến 50 g/L. Tiếp tục tăng nồng độ đường sẽ kìm hãm sự sinh trưởng tế bào cũng như sự tổng hợp ginsenoside. Tương tự, ở nồng độ 30 mM nitrogen là tối ưu cho sự sinh trưởng tế bào và sự tích luỹ sản phẩm trao đổi chất thứ cấp ginsenoside.

Từ khóa: Nồng độ môi trường MS, đường, nitơ, auxin và cytokinin.