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Optimization of Nutritive Factors in Culture Media for Growth and Tropane Alkaloid Production from *Anisodus acutangulus* Hairy Roots

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ABSTRACT

Anisodus acutangulus hairy roots were grown in N6 medium which was optimal for growth and alkaloid production. The cell biomass and alkaloid yield reached 2.7 g l^{-1} and 3.9 mg l^{-1} (dry weight) respectively in sucrose medium, higher than those obtained in other carbon source media. 90 mM nitrogen (NH_4^+/NO_3^- = 4:1) gave the highest cell yield (4.5 g l^{-1}) and the maximum alkaloid production (9.9 mg l^{-1}). The cell yield (4.1 g l^{-1}) of hairy roots grown at pH 6.5 was 2 times higher than that at pH 4.5. However, the maximum alkaloid production (7.2 mg l^{-1}) was yielded at pH 4.5.

Abbreviations: pH: Potential of Hydrogen; HPLC: High-Performance Liquid Chromatography; MS: Murashige and Skoog.

INTRODUCTION

Tropane alkaloids, such as hyoscyamine, anisodamine, scopolamine and anisodine, are widely used as anticholinergic agents (Kai et al, 2009; Kai et al, 2011). The rapidly increasing pharmaceutical market and economic value of these alkaloids have prompted efforts towards the production of these alkaloids (Kang et al. 2005; Kai et al 2011b).

Anisodus acutangulus is an attractive resource plant species for the production of tropane alkaloid, because the content of total alkaloid in *A. acutangulus* is higher than other common *Solanaceae* species (Kai et al, 2009; Li et al, 2008), along with detection of most of tropane alkaloid in the root. With the help of recent technological advancements in the field of plant biotechnology, better yield of these useful secondary metabolites from plant cells, tissue and organ cultures is possible (Bensaddek et al, 2001).

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Hairy root cultures obtained by genetic transformation using *Agrobacterium rhizogenes* have been recognized as an important strategy for enhancement of secondary metabolites (Sevon et al, 2008). Nutritive factors, like the carbon source, the nitrogen source and the pH of the culture medium are important parameters, influencing the production of alkaloid. The effect of carbon source on the production of various secondary metabolites in plant cell suspension cultures has been known for many years (Mantell and Smith, 1983).

The nitrogen concentration and NH_4^+/NO_3^- molar ratio of the culture medium often influence the synthesis of alkaloid (Sugimoto et al, 1988). It is reported that the culture medium pH can affect ion uptake (Pasqua et al, 2002). As far as we know in the previous studies, only simple optimization of culture conditions was examined and there are no published reports regarding the effect of the basal medium, the carbon source, the nitrogen concentration and the culture medium pH on growth and alkaloid production in *A.acutangulus*. Therefore, the aim of the study reported here was to optimize the above mentioned factors towards enhance alkaloid yield from *A. acutangulus*.

MATERIALS AND METHODS

Hairy root cultures

A hairy root line which was induced from leaves of *A.acutangulus* was used in the study and grown in the basal medium at pH 5.8. All experiments were performed in 250 ml shake flasks, containing 100 ml of medium on a rotary shaker with 100 rpm at 25°C under darkness. The cultures were subcultured every 30 days and used for further studies.

Optimization of nutritive factors

Four kinds of different media including MS medium (Murashige and Skoog, 1962), N6 medium (Chu et al, 1975), B5 medium (Gamborg et al, 1968) and White medium (White et al, 1963) were used to optimize the basal medium. Effect of different carbon sources on liquid cultivation of *A.acutangulus* hairy root was studied by adding 30 g l^{-1} of one of the following carbon sources, i.e. sucrose, glucose, fructose and galactose to the basal medium. The nitrogen supply in the medium was modified by altering the total nitrogen concentration. The different medium pH values of 4.5, 5.6, 6.5 and 7.3 were made by using hydrochloric acid or sodium hydroxide, respectively.

RESULTS AND DISCUSSION

Effect of different kinds of basal media

N6 medium was found optimal for growth and alkaloid production of hairy root cultures of *A.acutangulus*, as compared to B5, MS and White medium (Table 1 and Fig. 1). This is coincident well with the notion that the N6 medium belongs to the high potassium medium which is beneficial to the growth of plant tissues (Hayashi et al, 1988).

Effect of different carbon sources

Carbohydrates are important carbon and energy sources for cultured cells (Yu et al, 1996). Earlier report indicated that several carbohydrates were less effective than sucrose for the growth and alkaloid production in *Colchicum autumnale* (Hayashi et al, 1988).

To test the effect of carbon sources on the growth and alkaloid yield, medium containing different carbon sources including sucrose, glucose, fructose and galactose were compared. As shown in Table 1 and Fig. 2, 3% sucrose gave the best yield among diffrent carbon sources used.

It could be possible that different carbon sources could affect the plant cell growth and metabolism resulting in alteration in alkaloid productivity.

Effect of different nitrogen concentrations

Cell growth and alkaloid yield was inhibited at low or high total nitrogen concentrations and the most favorable concentration of nitrogen for the maximum biomass (4.5 mg l^{-1}) and the highest alkaloid yield (10.4 mg l^{-1}) was found to be 90 mM ($NH4^{+}$/$NO3^{-}$ = 4:1)(Fig. 3).

Results in Table 2 indicated that total nitrogen concentration in the medium had distinct effects on growth and alkaloid production in *A.acutangulus*.

Effect of pH of the culture medium

pH of the culture medium is very important for the cell growth. Many studies have shown that low pH levels can inhibit cation uptake (Mantell S. H and Smith H, 1983). In particular, much attention has been focused on the effect of pH on uptake of nitrogen and the way nitrogen in the nutritive solution influences the uptake of the other ions (Lang and Kaiser, 1994).

The optimum pH for *A.acutangulus* hairy root growth was found to be 6.5. However, the maximum alkaloid production of hairy root was yielded at pH 4.5 (Table 1 and Fig. 4).

It could be possible that plants' own resistance could stimulate hairy roots to produce more secondary metabolites in the acidic environment.

Table. 1: Effect of different kinds of basal media and carbon sources on growth and alkaloid yield from *A.acutangulus* hairy roots.

Hairy root	Nutritive factors in culture media							
	Kind of medium				Carbon source			
	MS	B5	N6	White	Galactose	Glucose	Fructose	Sucrose
Dry weight (g l^{-1})	2.9±0.4	4.3±0.6	5.6±0.6	2.2±0.4	0.9±0.2	2.2±0.4	0.4±0.1	2.7±0.2
Alkaloid yield (mg l^{-1})	4.5±0.2	7.2±0.2	9.5±0.3	2.1±0.1	0.45±0.1	1.7±0.2	0.21±0.1	3.9±0.2

Hairy root cultures were maintained in different basal media or in N6 medium with different carbon sources. The biomass was measured on a dry weight basis after the cells had been dried to constant weight, and the determination of tropane alkaloid production in the cells was performed by HPLC as described (Li et al. 2008). Data are shown as mean ± standard deviation of three replicates (ANOVA).The validity criteria p for the difference between experiment and appropriate control measurements was <0.05.

Table 2 Effect of the nitrogen concentration and the culture medium pH on growth and alkaloid yield from *A.acutangulus* hairy roots.

Hairy root	Nutritive factors in culture media							
	Nitrogen concentration				Culture medium pH			
	30mM	60mM	90mM	120mM	7.3	6.5	5.6	4.5
Dry weight (g l^{-1})	2.2±0.1	3.5±0.2	4.5±0.1	3.6±0.1	3.2±0.2	4.1±0.1	2.8±0.1	2.4±0.1
Alkaloid yield (mg l^{-1})	2.9±0.2	4.3±0.3	9.9±0.4	7.3±0.3	3.9±0.21	6.9±0.5	4.3±0.3	7.2±0.5

Hairy root cultures were maintained in modified N6 medium by altering the total nitrogen concentration or the culture medium pH. The biomass was measured on a dry weight basis after the cells had been dried to constant weight and the determination of tropane alkaloid production in the cells was performed by HPLC as described (Li et al. 2008). Data are shown as mean ± standard deviation of three replicates (ANOVA).The validity criteria p for the difference between experiment and appropriate control measurements was <0.05.

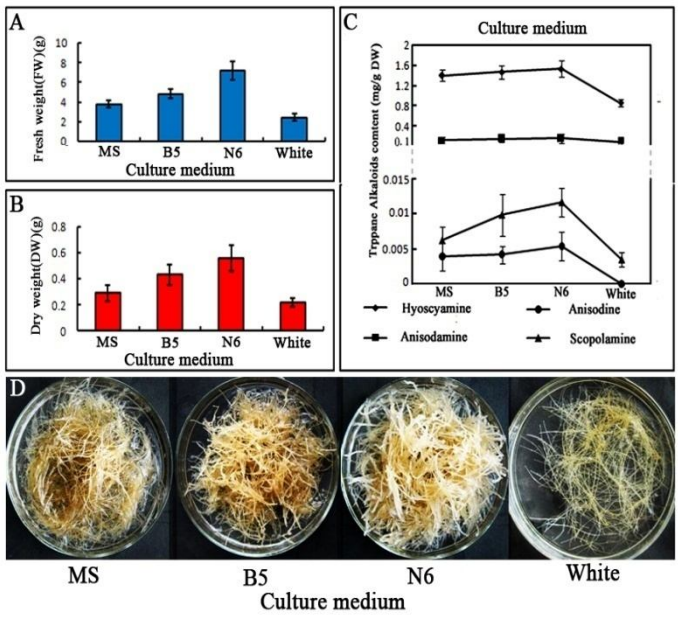

Fig. 1: Effect of different kinds of media on *A.acutangulus* hairy roots growth and alkaloid production after 30- day culture. a. fresh weight (FW) b. dry weight (DW) c. Tropane alkaloid content d. hairy roots.

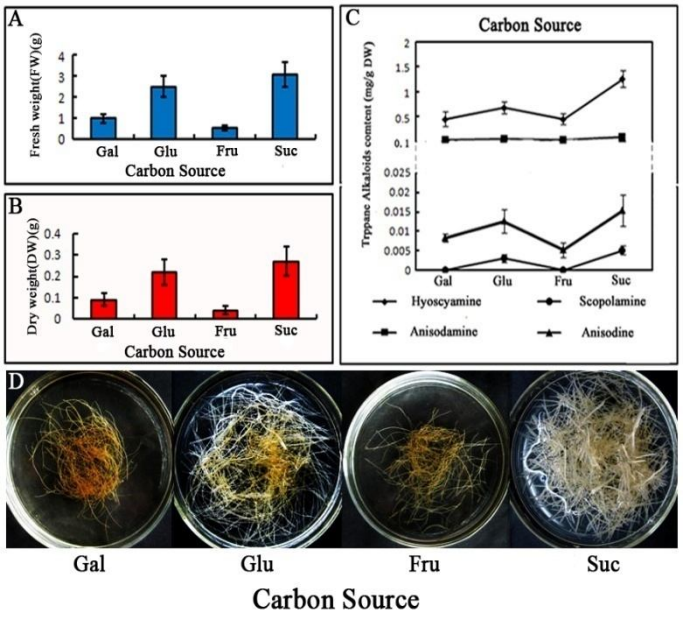

Fig. 2: Effect of different carbon sources on *A.acutangulus* hair roots growth and alkaloid production after 30-day culture. a. fresh weight (FW) b. dry weight (DW) c. Tropane alkaloid content d. hairy roots.

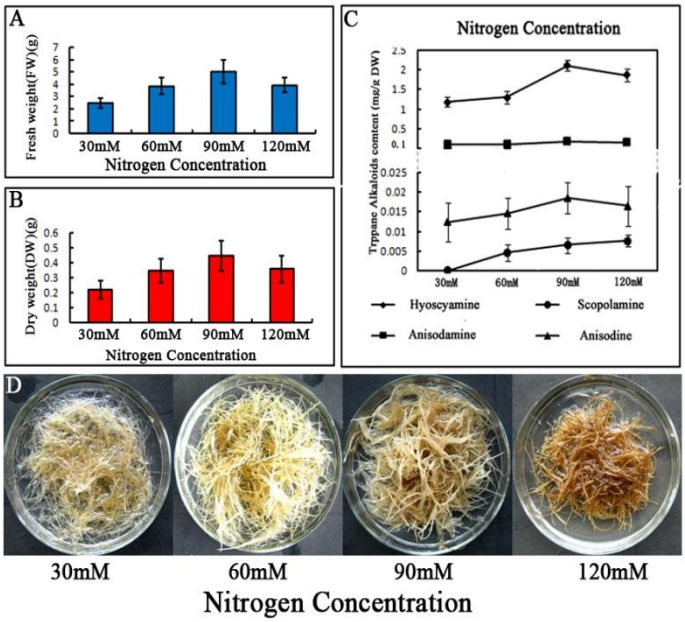

Fig. 3: Effect of the nitrogen concentration on hair roots growth and alkaloid production after 30- day culture. a. fresh weight (FW) b. dry weight (DW) c. Tropane alkaloid content d. hairy roots.

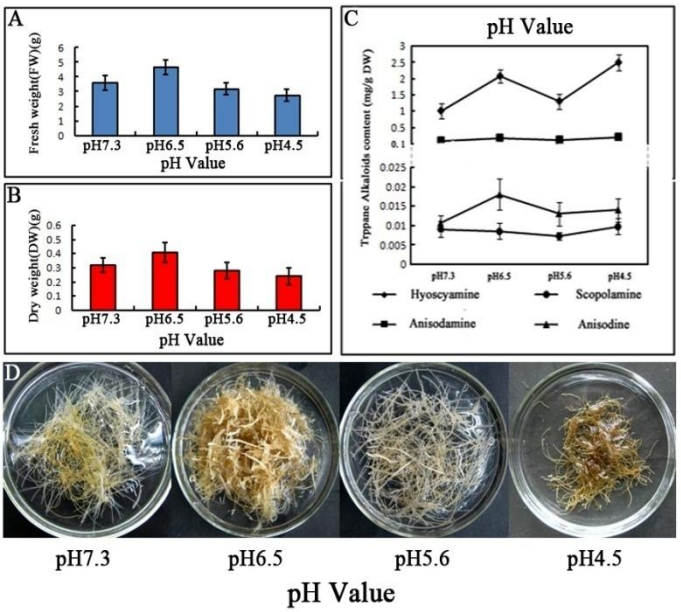

Fig. 4: Effect of the culture medium pH on hair roots growth and alkaloid production after 30-day culture. a. fresh weight (FW) b. dry weight (DW) c. Tropane alkaloid content d. hairy roots.

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REFERENCES

Bensaddek L., Gillet F., Saucedo J. E. N., Fliniaux M. The effect of nitrate and ammonium concentrations on growth and alkaloid accumulation of *Atropa belladonna* hairy roots. J Biotechnol. 2001; (85):35-40.

Chu C. C., Wang C. C., Sun C. S., Chen H., Yin K. C., Chu C. Y. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci Sin 1975 ; (18): 659-668.

Hayashi T., Yoshida K., Sano K.. Formation of alkaloids in suspension cultured *Colchicum autumnale*. Phytochem 1988 ; (27): 1371-1374.

Kai G. Y., Li L., Jiang Y. X., Yan X. M., Zhang Y., Lu X., Liao P., Chen J. B. Molecular cloning, characterization of two tropinone reductases in *Anisodus acutangulus* and enhancement of tropane alkaloids production in AaTRI-transformed hairy roots. Biotechnol Appl Bioc. 2009; 54（3）：177-186

Kai G. Y., Liu Y. Y., Wang X. Y., Ni X. N., Yang S., Fu X. Q., Luo X. Q., Liao P. Functional identification of hyoscyamine 6β-hydroxylase from *Anisodus acutangulus* and overproduction of scopolamine in engineered *Escherichia coli*. Biotechnol let. 2011; 33(7):1361-5.

Kai G. Y., Yang S., Luo X. Q., Zhou W. T., Fu X. Q., Zhang A., Zhang Y., Xiao J. B. Co-expression of AaPMT and AaTRI effectively enhances the yields of tropane alkaloids in *Anisodus acutangulus* hairy roots. BMC Biotechnol. 2011; 11:43.

Kang Y. M., Lee O. S., Jung H. Y., Kang S. M., Lee B. H., Karigar C., Prasad T., Bahk J. D., Choi M.S. Overexpression of hyoscyamine 6β-hydroxylase (h6h) gene and enhanced production of tropane alkaloids in *Scopolia parviflora* hairy root lines. J Microbiol Biotechnol. 2005; (15):91-98.

Lang B., Kaiser W. M. Solute content and energy status of roots of barley plants cultivated at different pH on nitrate or ammonium nitrogen. New Phytol. 1994; (128): 451-459.

Li L., Wang J., Wang W., Lu Y., Wang Y. L., Zhou G. Y., Kai G. Y. Optimization of induction and culture conditions and tropane alkaloid production in hairy roots of *Anisodus acutangulus*. Biotechnol Bioprocess Eng 2008 ; (13): 606-612.

Gamborg O.L., Miller R.A., Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res. 1968; (50):151-158.

Murashige T., Skoog F. A revised medium for rapid growth and biosaasys with tobacco culture. Physiol Plant. 1962; (15): 473-497.

Pasqua G., Manes F., Monacelli B., Natale L., Anselmi S. Effects of the culture medium pH and ion uptake in in vitro vegetative organogenesis in thin cell layers of tobacco. Plant Sci. 2002; (162): 947-955.

Sevon N., Oksman-Caldentey K. M. *Agrobacterium rhizogenes*-mediated transformation: root cultures as a source of alkaloids. Planta Med. 2002; (68): 859-868.

Mantell S. H., Smith H. Cultural factors that influence secondary metabolite accumulations in plant cell and tissue cultures. Cambridge Univ Press. (1983) 75-108.

Sugimoto Y., Sugimura Y., Yamada Y. Effects of culture conditions on bisbenzylisoquinoline alkaloid production in cultured roots of *Stephania cepharantha*. Agric Biol Chem. 1988; (52): 1495-1498.

White P. R. The cultivation of Animal and Plant Cells. The Ronald Press Co, New York. (1963): 10.

Yu S. X., Kwok K. H., Doran P. M. Effects of sucrose, exogenous production concentration and other culture conditions on growth and steroidal alkaloid production by *Solanum aviculare* hairy roots. Enzyme Microbiol Technol. 1996; (18): 238-240.

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