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Application of an Airlift Bioreactor System for the Production of Adventitious Root Biomass and Caffeic Acid Derivatives of *Echinacea purpurea*

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Abstract In this study, we evaluated the feasibility of using mass cultivation of the adventitious roots of *Echinacea purpurea* in balloon type bubble (air-lift) bioreactors to produce caffeic acid derivatives, which have pharmaceutical and therapeutic values. An approximately 10 fold increase in biomass and secondary compounds was observed after 4 weeks of culture in balloon type bubble bioreactors (5 L capacity containing 4 L of half strength MS medium). In addition, a linear relationship was observed between the concentration of biomass and the sucrose and ion consumption rate. Furthermore, the concentration of biomass in the bioreactor culture was found to increase as the conductivity decreased. An inoculum density of 7 g/L FW and an aeration rate of 0.1 vvm were found to be suitable for inducing the accumulation of biomass and secondary metabolites. Of the three caffeic acid derivatives evaluated (caftaric acid, chlorogenic acid, and cichoric acid), the concentration of cichoric acid was the highest (26.64 mg/g DW). © KSBB

Keywords: balloon type bubble bioreactor, Echinacea*, caffeic acid, cichoric acid, adventitious roots*

INTRODUCTION

Echinacea purpurea (L.) Moench, which is also known as the purple coneflower, is an important medicinal plant native to North America that is grown worldwide for commercial purposes. The purple coneflower has gained international popularity in recent years as a result of claims that it beneficially stimulates the body immune system [1,2]. Extracts from the plant have been shown to have antioxidative, antibacterial, antiviral and antifungal properties and are often used to treat the common cold as well as respiratory and urinary diseases [1]. The most important potential active compounds in the purple coneflower are caffeic acid derivatives (especially cichoric acid), polysaccharides, alkamides, and glycoproteins [1,3]. Of the caffeic acid derivatives, cichoric acid has been found to have immunostimulatory properties such as the promotion of phagocyte activity *in vitro* and *in vivo*. In addition, cichoric acid has been shown to have antihyaluronidase activity, as well as to exert a protective effect on the free radical-induced degradation of collagen. Chichoric acid has also been shown to exert antiviral activity, [1] and has recently been found to inhibit HIV-1 integrase and replication [4].

Cell or organ cultures have emerged as a valuable route for biosynthesizing phytochemicals, and bioreactor-based systems have been developed for the production of ginsenosides [5-7], eleutherosides [8,9], alkaloids [10], and ethanol [11]. Many parameters such as inoculum size, medium components, efficient oxygen transfer and mixing and other physico-chemical factors exert a strong effect on production in cell/organ bioreactors [12,13]. In a previous study, we reported that scale-up cultures for the cultivation of adventitious roots of Echinacea were feasible [14]. However, the individual parameters affecting biomass and metabolite accumulation have not yet been studied. Therefore, in this study, we have established the optimal conditions for the production of phenolics and flavonoids, especially caffeic acid derivatives, in bioreactor cultures.

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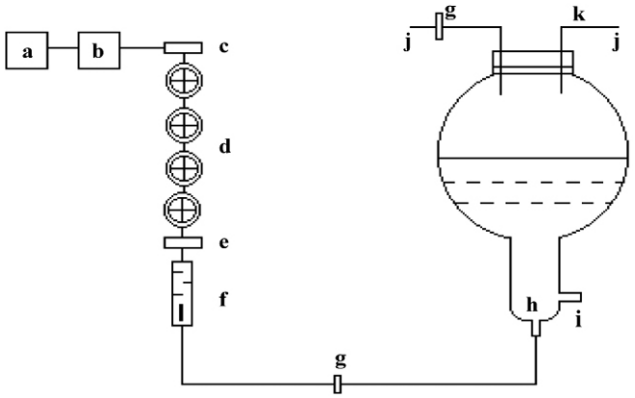

Fig. 1. Schematic diagram of an air lift bioreactor. a: air compressor, b: air reservoir, c: air cooling device, d: air filter system, e: air dryer, f: air flow meter, g: membrane filter, h: glass sparger, i: medium sampling port, j: vent, k: pre filter.

Specifically, we optimized the initial inoculum density, culture period, aeration and other chemical parameters required for the growth of adventitious roots.

MATERIALS AND METHODS

Maintenance of Adventitious Roots in Flask Cultures

Adventitious roots of *E. purpurea* (L.) Monech were induced according to the procedures described by Wu *et al.* [15] and maintained in half strength Murashige and Skoog (MS, 5:25 mM ammonium and nitrate ratio) medium [16] supplemented with 2 mg/L indole butyric acid (IBA) and 50 g/L sucrose. Adventitious roots were grown in 300 mL flasks containing 100 mL of medium that were maintained at 25°C in the dark on a rotary shaker at 100 rpm. The adventitious roots were maintained by sub-culturing to fresh medium every 4-weeks.

Adventitious Root Culture in Bioreactors

Bioreactor cultures (balloon type bubble bioreactors with a 5 L capacity) were filled with 4 L of half strength modified MS medium (ammonium and nitrate ratio is 5:25 mM) supplemented with 2 mg/L IBA and 50 g/L sucrose. A schematic diagram of the entire experimental system is shown in Fig. 1. The bioreactors were maintained in the dark at 25°C for eight weeks. The bioreactor cultures were then initiated by inoculation with adventitious roots at a density of 10 g/L, after which they were aerated at 0.2 vvm (air volume/culture volume per min). Twenty four bioreactors were maintained to establish the growth kinetics of the adventitious roots. The medium (spent medium) was sampled at weekly intervals from three bioreactors in each treatment group, with the experiment in that were sampled being terminated at the time of sampling. The medium was then used to determine the specific growth rate, as well as the phenolic and flavonoid content, including the concentration of caffeic acid derivatives.

Optimization of the Inoculum Density

To determine the optimal inoculum density, different levels of the inoculants [2.5, 5.0, 7.0, 10.0, and 15.0 g/L (fresh weight)] were added to separate reactors, which were then aerated at 0.2 vvm and maintained in the dark at 25°C for five weeks.

Optimization of the Aeration Rate

In another experiment, the medium was aerated at 0.05, 0.1, 0.2, and 0.3 vvm for five weeks or at 0.05 vvm during the first three weeks of culture and then at 0.1, 0.2, or 0.3 vvm for the following two weeks to determine the optimum air flow. In the aeration experiments, reactors were inoculated with adventitious roots at a concentration of 10 g/L and the cultures were maintained in the dark at 25°C for five weeks. Each treatment contained three replicates and each experiment was repeated twice.

Determination of the Root Weight and Growth Ratio

The root fresh weight (FW) and dry weight (DW) were determined as follows. Roots were separated from the medium by passing it through a 1 mm stainless steel sieve. The root FW was then measured after rinsing the roots once with sterile water and then blotting away the surface water. The roots were then dried at 70°C for several days, after which the root DW was recorded. The growth ratio was then calculated as follows: harvested dry weight (g)/inoculated dry weight (g).

Determination of the Specific Growth Rate of Adventitious Roots

The specific growth rate (μ) of the adventitious roots is defined as:

$$\mu = 1/X \cdot dX/dt$$

where X is the adventitious root weight (g/L), t is the time (day), and μ is the specific growth rate (per day). The doubling time (T_d) of the adventitious roots is defined as $T_d = \ln 2/\mu = 0.693/\mu$.

Measurement of Conductivity and Hydrogen Ion Concentration of the Medium

The electrical conductivity (EC) of the medium was measured using a conductivity meter (Model LF-54, WTW GmbH, Germany) and the hydrogen ion concentration (pH) of the culture medium was measured using a pH meter (Model Inolab, WTW GmbH, Germany) at weekly intervals.

Determination of Residual Sugars and Ions in the Media

The amount of residual sugar and ions in the media was determined using a previously described method [17,18].

Determination of Total Phenolic and Flavonoid Contents

The concentration of the total phenolics in the roots was determined spectrophotometrically [15]. The total flavonoid content was determined using a colorimetric method [19].

Determination of the Content of Caffeic Acid Derivatives

The extraction and analysis of caffeic acid derivatives was conducted using the method described by Pellati *et al*. [20]. The caffeic acid fractions were analyzed using an HPLC system with an XTerra RP 18 column (particle size 3.0 μm, 150 mm × 3 mm). The mobile phase was (A) water and (B) acetonitrile. The gradient elution was modified as follows: initial 10% B for 40 min; 25% B for the next 11 min; 50% B for 1 min; after which to the mobile phase was returned to its initial conditions for 8 min with a flow rate 0.3 mL/min. The caffeic acid derivatives were detected at 330 nm and then compared to standard caftatic acid, chichoric acid and chlorogenic acid, which were obtained from ChromaDex (Laguna Hills, CA, USA).

Statistical Analysis

The results shown are the mean values of three independent experiments. One-way analysis of variance (ANOVA) was used to determine if the groups differed significantly. Statistical assessments of the difference between mean values were then assessed by the least significant difference (LSD) test. A p of < 0.05 was considered to indicate statistical significance and all data were analyzed using the SAS program (SAS Institute, Inc., USA).

RESULTS AND DISCUSSION

Time Course of Adventitious Root Growth

Bioreactor cultures are often used to enhance biomass as well as metabolic productivity in plant cell/organ cultures. To determine the exact stage at which maximum biomass production occurs and to evaluate the accumulation of bioactive compounds, bioreactors were inoculated with adventitious root biomass at a concentration of 7 g/L FW (0.8 g/L DW). The cultures were incubated in the dark at 25°C and the amount of biomass that accumulated was then monitored. As shown in Fig. 2, the adventitious roots of *E. purpurea* grew well in liquid medium. The growth patterns of the adventitious roots typically showed a lag phase from 0~1 weeks, an exponential phase from 2~7 weeks and then remained stationary from 7 weeks onwards. After 7 weeks of cultivation, the biomass reached its peak concentration (10.5 g/L DW), which was approximately 13 times higher than that of the dry weight of the initial inoculum. The specific growth rate (μ) of the *E. purpurea* adventitious roots was rapid during the initial days, with the peak rate (0.128) occurring at 14 days, which gave a doubling time (T_d) of 5.42 days (Table 1). In addition, the productivity of the dry adventitious root mass was found to be much higher in the bioreactor cultures (10.5 g/L DW) than in the shake flask cultures (6.6 g/L DW).

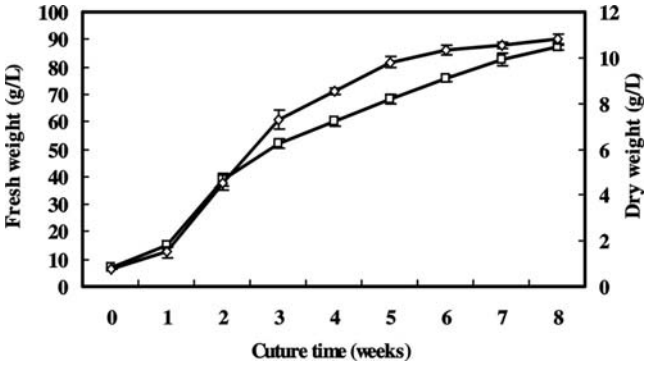

Fig. 2. Time profiles for adventitious root growth (-◦- fresh weight; -▫- dry weight) of *Echinacea purpurea* cultured in 5 L balloon-type bubble bioreactors.

Table 1. Specific growth rate (μ) and doubling time (Td) of *Echinacea purpurea* adventitious roots grown in 5 L capacity air lift bioreactors containing 4 L of half strength MS medium

Days	7	14	21	28	35	42	49	56
μ	0.103	0.128	0.107	0.086	0.073	0.061	0.053	0.048
$T_d = \ln 2/\mu$max (day)			5.41					

Conductivity and Hydrogen Ion Concentrations in Bioreactors

Figure 3 shows the electrical conductivity (EC) and hydrogen ion concentration (pH) of the medium during the course of the experiment. The conductivity of the culture medium decreased over time, which reflects the increase in biomass accumulation that occurred over time. This finding is similar to the results of studies conducted by Ryu *et al.* [21] and Taya *et al*. [22], who found that conductivity parameters were correlated with the cell biomass.

The pH profiles observed in this study are characteristic of plant cell/organ cultures [23,24], although plant cell/organ cultures are effectively maintained when the medium pH is between 5.0 and 6.0. The results of a previous study indicated that the hydrogen ion concentration increased during nitrate uptake and decreased during ammonia assimilation [25]. In addition, the results of studies conducted by Tauto-

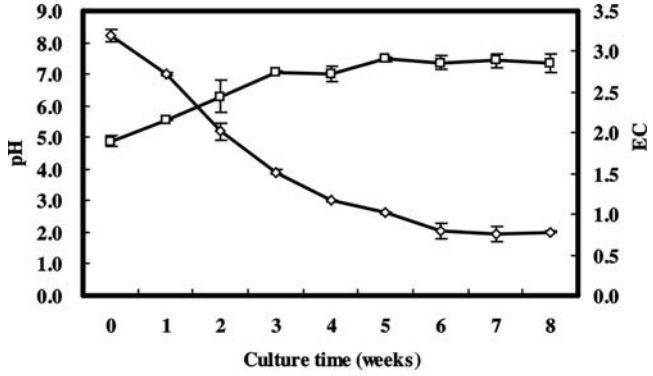

Fig. 3. Changes in electrical conductivity (EC) and hydrogen ion concentration (pH) in adventitious root cultures of *E. purpurea* cultured in 5 L balloon type bubble bioreactor (-▫- pH; -◦- EC).

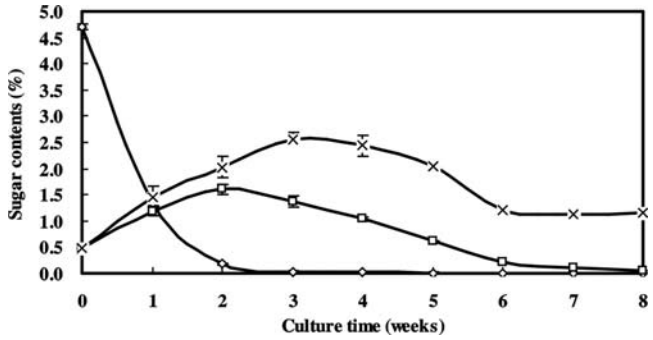

Fig. 4. Time profiles for residual sugars in *E. purpurea* adventitious root suspensions cultured in 5 L balloon type bubble bioreactor (-◊- sucrose; -▫- glucose; -x- fructose).

rus *et al.* [26] suggested that pH may be useful for determining specific growth stages, with a pH of 6.5~7.0 corresponding to a stationary phase in their system. In the present study, the pH of the medium increased gradually to 7.0 after 5 weeks, after which it remained steady. This may have occurred as a result of nutrient depletion in the culture medium and the accumulation of metabolites in the cultured roots.

Sugar and Mineral Status of the Bioreactor Cultures

The sugar concentration in the media was measured at weekly intervals during the culture period to determine the sugar uptake patterns of the adventitious roots (Fig. 4). The sucrose concentration steadily decreased as adventitious root growth proceeded, with a sharp drop from 5 to 1.35% occurring after 1 week of culture. In addition, even though only sucrose was added to the medium, glucose and fructose were detected in the medium, with the glucose concentrations increasing significantly during the first two weeks and then decreasing. These findings indicate that extracellular hydrolysis of the sucrose occurred, which lead to the formation of glucose and fructose. This hydrolysis may have been induced by acid invertase that was secreted from the adventitious root tissue into the medium, as previously reported [27,28].

Changes in the concentration (mg/L of ions) of anions and cations in the medium were monitored continuously during the culture cycle (Fig. 5). The kinetic changes in ion concentrations in the media revealed a rapid depletion of NH_4^+, Ca^{2+}, Na^+, K^+, NO_3^-, Mg^{2+}, SO_4^{2-}, and HPO_4^- and a steady absorption of Cl^-. HPO_4^- was rapidly depleted from the medium and its concentration was near zero after one week, whereas NH_4^+ and Mg^{2+} were depleted within two weeks. The preferential uptake of NH_4^+, HPO_4^-, and Mg^{2+} at the beginning of the culture has been observed for many species [18,28,29]. In addition, the uptake of these compounds has been shown to depend on the level of differentiation of the organ cultured *in vitro* [30]. The results of the present study indicate that the bioreactor cultures adequately met the nutritional needs of the *E. purpurea* adventitious root cultures.

Accumulation of Phenolics, Flavonoids, and Caffeic Acid Derivatives

The profiles of the total phenolic and flavonoid production are shown in Fig. 6. The accumulation of total phenolics and flavonoids increased linearly with time for the first 5 weeks of the experiment, with the highest values being approximately 60.0 mg/g DW and 32.8 mg/g DW, respectively. In addition, there was a gradual depletion in the accumulation of phenolics and flavonoids in the adventitious root cultures of *E. purpurea* after 5 weeks of culture. These findings indicate that the optimum biomass production and phenolic and flavonoid accumulation occurred in the five week old cultures.

The time courses of caftaric acid, chlorogenic acid, and cichoric acid accumulation in the *E. purpurea* adventitious root cultures are shown in Fig. 7. The amount of caftaric acid was initially low after the first week of culture (0.8 mg/g DW); however, it was 5.2 mg/g DW at the end of the third and fourth week, after which point it gradually decreased. The concentration of chlorogenic acid was low at the end of the first week (3.9 mg/g DW), but then rose to 5.5 mg/g DW at the end of third week, after which it was maintained at this concentration for the duration of the experiment. The cichoric acid and total caffeic acid content was low at the end of the first week (5.4 mg/g DW and 10.1 mg/g DW), but these values then increased gradually in subsequent weeks, with the optimum concentration (27.5 mg/g DW and 38.1 mg/g DW) being observed at the end of the fifth week. These findings demonstrate that culture for five weeks produced the optimum caffeic acid content. Furthermore, these findings indicate that, cichoric acid was produced in the greatest quantity among the various caffeic acid derivatives (Fig. 7). The production of caffeic acid derivatives by adventitious roots grown in bioreactors was found to be much higher (5.28 mg/g DW caftraic acid, 5.53 mg/g DW chlorogenic acid, and 27.51 mg/g DW cichoric acid) than that of hairy roots cultivated in flask cultures (3.56 mg/g DW of caftaric acid, 0.93 mg/g DW chlorogenic acid, and 19.21

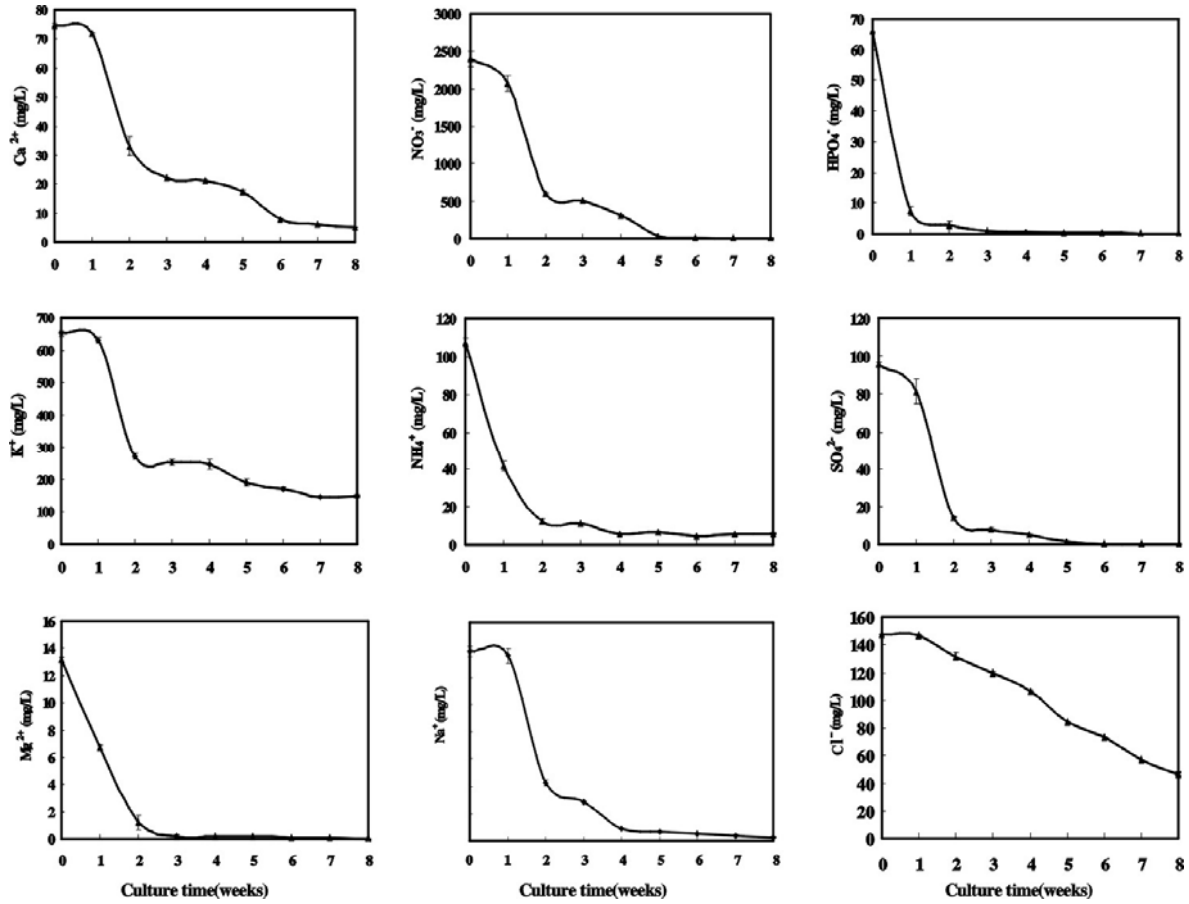

Fig. 5. Time profile of anion and cation contents during bioreactor culture of adventitious roots (throughout culture cycle).

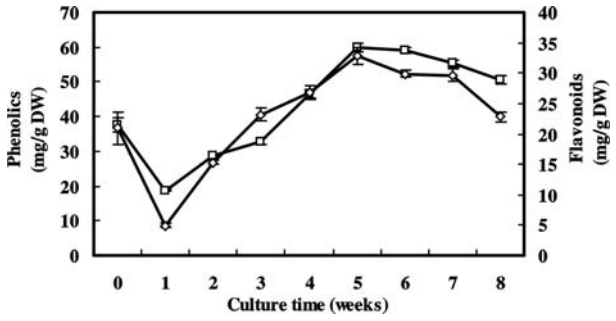

Fig. 6. Kinetics of phenolic and flavonoid production by bioreactor cultured *E. purpurea* adventitious roots (-▫- phenolics; -◊- flavonoids).

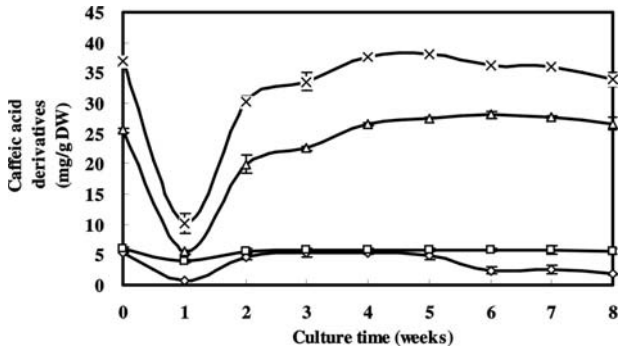

Fig. 7. Kinetics of production of caffeic acid derivatives by bioreactor cultured *E. purpurea* adventitious roots (-◊- caftaric acid; -▫- chlorogenic acid; -Δ-cichoric acid; -x- total caffeic acid derivatives).

mg/g DW cichoric acid) [31]. Therefore, bioreactor cultures are suitable for the large scale production of caffeic acid derivatives.

The Effects of Different Inoculum Densities on Biomass and Metabolite Accumulation

One of the factors that determine the accumulation of biomass and the productivity of bioactive compounds by *in vitro* cultures is the optimal inoculum density [24,32]. In this study, the inoculum density was found to have a profound effect on the growth of adventitious roots and the accumulation of phenolics and flavonoids (Table 2). The maximum biomass was obtained (79.0 g/L FW and 10.4 g/L DW)

Table 2. Effect of inoculum size on the adventitious root growth of *E. purpurea* and the productivity of phenolics and flavonoids after five weeks of culture using 5 L balloon type bubble bioreactors containing 4 L of half strength MS medium[a]

Inoculum density (g/L FW)	Fresh weight (g/L)	Dry weight (g/L)	Growth ratio	Total phenolics (mg/g DW)	Total flavonoids (mg/g DW)
2.5	55.6e	4.5e	15.4	34.2e	25.8d
5.0	59.2d	6.3d	10.5	55.2b	33.8b
7.0	67.2c	9.0c	10.8	58.5a	38.6a
10.0	74.8b	10.1b	8.2	45.5c	27.6c
15.0	79.0a	10.4a	5.4	40.6d	23.4e

[a]Mean separation within each column as determined by Duncan's multiple range test at a level of 5%.

Table 3. Effect of inoculum size on the production of caffeic acid derivatives after five weeks of culture of *E. purpurea* adventitious roots using 5 L balloon type bubble bioreactors containing 4 L of half strength MS medium[a]

Inoculum Density (g/L FW)	Amount of caffeic acid derivatives (mg/g DW)			
	Caftaric acid	Chlorogenic acid	Cichoric acid	Total[b]
2.5	2.3 ± 0.1	4.2 ± 0.2	16.5 ± 0.1	23.0 ± 0.3
5.0	2.4 ± 0.1	4.5 ± 0.1	27.2 ± 0.1	34.1 ± 0.1
7.0	4.1 ± 0.1	5.1 ± 0.1	28.1 ± 0.4	37.3 ± 0.3
10.0	2.7 ± 0.1	4.5 ± 0.1	27.2 ± 0.1	34.4 ± 0.1
15.0	2.3 ± 0.1	2.7 ± 0.1	25.2 ± 0.1	30.2 ± 0.1

[a]Values shown are the means of three replicates ± the standard error

[b]Total caffeic acid derivatives = caftatic acid + chlorogenic acid + cichoric acid

Table 4. The effects of air supply on the adventitious root growth of *E. purpurea* and the productivity of phenolics and flavonoids after five weeks of culture using 5 L balloon type bubble bioreactors containing 4 L of half strength MS medium[a]

Air supply (vvm)	Fresh weight (g)	Dry weight (g)	Growth ratio	Total phenolics (mg/g DW)	Total flavonoids (mg/g DW)
0.05	70.5ab	8.9ab	10.6	60.9a	37.8a
0.1	70.1ab	9.0ab	10.8	60.7a	38.8a
0.2	63.1de	8.2c	9.7	61.1a	38.3a
0.3	61.4e	8.1c	9.5	58.7b	35.6b
0.05-0.1	66.1cd	8.7b	10.4	60.2a	38.2a
0.05-0.2	67.5bc	8.8b	10.5	61.1a	38.9a
0.05-0.3	71.9a	9.1a	10.9	61.1a	38.7a

[a]Mean separation within each column as determined by Duncan's multiple range test at a level of 5%.

when 15 g/L FW of adventitious roots were fed into the bioreactors. However, the maximum phenolic and flavonoid content (58.5 mg/g DW and 38.6 mg/g DW, respectively) were obtained when the inoculum density was 7 g/L FW. As shown in Table 3, the concentration of the caffeic acid derivatives produced when the inoculums density was 7 g/L FW varies, with caftaric acid, chlorogenic acid, and cichoric acid being produced in concentrations of 4.1 mg/g DW, 5.1 mg/g DW, and 28.1 mg/g DW, respectively. These results clearly demonstrate that the inoculum size also affected the relative percentage of each derivative of caffeic acid. The present results also suggest that an inoculums density of 7 g/L FW is suitable to generate the optimum biomass production of adventitious roots, as well as the optimum accumulation of phenolics, flavonoids and caffeic acid derivatives.

The Effects of Different Aeration Rates on Biomass and Metabolite Accumulation

The factors that influence effective oxygen transfer in plant cell cultures must be carefully analyzed when designing bioreactors for the production of metabolites [33]. The adventitious root growth of *E. purpurea* under the four models of aeration (0.05, 0.1, 0.2, and 0.3 vvm) over five weeks is presented in Table 4. The optimal biomass of the root (70.1 g/L FW and 9.0 g/L DW, respectively) were obtained at an aeration rate of 0.1 vvm. The highest accumulation of phenolics and flavonoids (60.7 mg/g DW and 38.8 mg/g DW, respectively), caftaric acid (4.7 mg/g DW), chlorogenic acid (5.9 mg/g DW), and cichoric acid (26.6 mg/g DW) (Tables 4 and 5) were also obtained when the aeration rate was 0.1 vvm. The results of this study also indicated that higher aera tion rates of 0.2 and

Table 5. The effects of air supply on the production of caffeic acid derivatives after five weeks of culture of *E. purpurea* adventitious roots using 5 L balloon type bubble bioreactors containing 4 L of half strength MS medium[a]

Air supply (vvm)	Amount of caffeic acid derivatives (mg/g DW)			
	Caftaric acid	Chlorogenic acid	Cichoric acid	Total[b]
0.05	4.7 ± 0.1	5.6 ± 0.1	25.0 ± 0.1	35.3 ± 0.1
0.1	4.7 ± 0.1	5.6 ± 0.1	26.6 ± 0.1	37.2 ± 0.2
0.2	3.8 ± 0.1	5.5 ± 0.1	24.1 ± 0.1	33.4 ± 0.1
0.3	3.6 ± 0.1	5.2 ± 0.1	22.5 ± 0.6	31.3 ± 0.5
0.05-0.1	4.8 ± 0.1	5.8 ± 0.1	27.6 ± 0.1	38.2 ± 0.1
0.05-0.2	4.8 ± 0.1	6.0 ± 0.1	28.3 ± 0.2	39.1 ± 0.3
0.05-0.3	4.9 ± 0.1	6.0 ± 0.3	28.1 ± 0.3	39.0 ± 0.4

[a]Values shown are the mean of three replicates ± the standard error

[b]Total caffeic acid derivatives = caftatic acid + chlorogenic acid + cichoric acid

0.3 vvm were not suitable for both biomass and metabolite accumulation. This may have been due to the stripping of essential gaseous components such as carbon dioxide and ethylene, as reported by Gao and Lee [34], and Schlatmann *et al.* [35]. To verify the effect of differential aeration rates, cultures were aerated at 0.05 vvm for the first 3 weeks following inoculation, after which they were aerated at 0.1, 0.2, or 0.3 vvm (Tables 4 and 5). The differential aeration rate of 0.05 vvm for three weeks and 0.3 vvm during the subsequent two weeks was found suitable for both biomass production and metabolite accumulation. Indeed, the highest biomass yields (71.9 g/L fresh weight and 9.1 g/g dry weight) and phenolic and flavonoid production (61.1 mg/g DW and 38.7 mg/g DW, respectively) were recorded when these aeration rates were used.

It has been reported that the amount of phenolic compounds in *E. purpurea* natural plants (roots and aerial parts) is 23.3 mg/g DW [18], whereas transformed roots contain 18.9 mg of phenolic compounds/g DW [36]. In this study, the concentration of phenolics and flavonoids in the adventitious roots was 60.7 mg/g DW and 38.8 mg/g DW, respectively, which is much higher than those found in naturally produced plants. Similarly, the concentration of cichoric acid was also found to be much higher (26.6 mg/g DW, Table 5) in adventitious roots grown in the bioreactors than in naturally grown plants (1.4~20.5 mg/g DW) [37].

CONCLUSION

In this study, a bioreactor technology that enabled the mass cultivation of adventitious roots of *E. purpurea* for the production of phenolics and flavonoids, especially caffeic acid derivatives, was established. An inoculum density of 7 g/L FW and an aeration rate of 0.1 vvm were found to be suitable for the accumulation of biomass and the production of caffeic acid derivatives. In addition, the amount of phenolics and flavonoids produced by the adventitious roots cultured in bioreactors were found to be much higher than the concentrations produced by natural stands. Therefore, the results of this study can be used for the scale-up of adventitious root culture for the production of derivatives of caffeic acid.

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REFERENCES

1. Barrett, B. (1989) Medicinal properties of Echinacea: A critical review. *Phytomedicine* 10: 66-86.
2. Baurer, R. and H. Wagner (1991) Echinacea species as potential immunostimulatory drugs. *Econ Medic Plant Res*. 5: 253-321.
3. Hobbs, C. (1989) *The Echinacea Handbook.* pp. 1-118. Elcectic Medical Publications. Portland, OR, USA.
4. Lin, Z., N. Neamati, H. Zhao, Y. Kiryu, J. A. Turpin, C. Aberham, K. Strebel, K. Kohn, M. Witvrouw, C. Pannecouque, Z. Debyser, E. D. Clercq, W. G. Rice, Y. Pommier, and T. R. Burke (1999) Cichoric acid analogues as HIV-1 integrase inhibitors. *J. Med. Chem*. 42: 1301-1414.
5. Kim, Y. S., E. J. Hahn, H. N. Murthy, and K. Y. Paek (2004) Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. *Biotech. Lett*. 26:1619-1622.
6. Thanh, N. T., H. N. Murthy, E. J. Hahn, and K. Y. Paek (2005) Methyl jasomonate elicitation enhanced synthesis of ginsenoside by cell suspension cultures of *Panax ginseng* in 5-l balloon type bubble reactors. *Appl. Microbiol. Biotechnol.* 67: 197-201.
7. Yu, K.W., E. J. Hahn, and K. Y. Paek (2000) Production of ginseng adventitious roots using bioreactors. *Korean J. Plant Tissue Cult*. 27: 309-315.
8. Park, S. Y., J. K. Ahn, W. Y. Lee, H. N. Murthy, and K. Y. Paek (2005) Mass production of *Eleutherococcus koreanum* plantlets via somatic embryogenesis from root cultures and accumulation of eleutherosides in regenerants. *Plant Sci.* 168: 1221-1225.
9. Shohael, A. M., D. Chakrabarty, K. W. Yu, E. J. Hahn, and K. Y. Paek (2005) Application of bioreactor system

for large-scale production of *Eleutherococcus sessiliflorus* somatic embryos in an air-lift bioreactor and production of eleutheorsides. *J. Biotehnol.*120: 228-236.
10. Min, J. Y., H. Y. Jung, S. M. Kang, Y. D. Kim, Y. M. Kang, D. J. Park, D. T. Prasad, and M. S. Choi (2007) Production of tropane alkaloids by small-scale bubble column bioreactors of *Scopolia parviflora* adventitious roots. *Bioresource Technol*. 98: 1748-1753.
11. Jeon, B. Y., S. J. Kim, D. H. Kim, B. K. Na, D. H. Park, H. T., Tran, R. Zhang, and D. H. Ahn (2007) Development of a serial bioreactor system for direct ethanol production from starch using *Aspergillus niger* and *Saccharomyces cerevisiae*. *Biotechnol. Bioprocess Eng.* 12: 566-573.
12. Humphery, A. (1998) Shake flask to fermentor: What have we learned? *Biotechnol. Prog.* 14: 3-7.
13. Kim, Y., B. Wyslouzil, and P. Weathers (2002) Secondary metabolism of hairy root cultures in bioreactors. *In Vitro Cell. Dev. Biol. Plan*t 38: 1-10.
14. Wu, C. H., H. N. Murthy, E. J. Hahn, and K. Y. Paek (2007) Large-scale cultivation of adventitious roots of *Echinacea purpurea* in airlift bioreactors for the production of chichoric acid, chlorogenic acid and caftaric acid. *Biotechnol Lett.* 29: 1179-1182.
15. Wu, C. H., Y. H. Dewir, E. J. Hahn, and K. Y. Paek (2006) Optimization of culturing conditions for the production of biomass and phenolics from adventitious roots of *Echinacea anguistifolia. J. Plant Biol*. 49: 193-199.
16. Murashige, T. and F. Skoog (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*.15: 473-497.
17. Shin, K. S., D. Charkrabarty, J. Y. Ko, S. S. Hahn, and K. Y. Paek (2003) Sucrose utilization and mineral nutrient uptake during hairy root growth of red beet (*Beta vulgaris* L.) in liquid cultures. *Plant Growth Regl*. 39: 187-193.
18. Jeong, C. S., D. Chakrabarty, E. J. Hahn, H. L. Lee, and K. Y. Paek (2006) Effect of oxygen, carbon dioxide and ethylene on growth and bioactive compound production in bioreactor culture of ginseng adventitious roots. *Biochem. Eng. J*. 27: 252-263.
19. Zhishen, J., T. Mengcheng, and W. Jianming (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64: 555-559.
20. Pellati, F., S. Benvenuti, L. Magro, M. Melegari, and F. Soragni (2004) Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. *J. Pharm. Biomed. Anal.* 35: 289-301.
21. Ryu, D. D. Y., S. O. Lee, and R. J. Romani (1990) Determination of specific growth rate for plant cell cultures: comparative studies. *Biotechnol. Bioeng* 35: 305-311.
22. Taya, M., M. Hegglin, J. E. Prenosil, and J. R. Bourne (1989) Online monitoring of cell growth in plant tissue cultures by conductivity. *Enzyme Microb. Technol.* 20:469-274.
23. Hahn, J. J., A. C. Eschenlauer, M. H. Narrol, D. A. Somers, and F. Srienc (1997) Growth kinetics, nutrient uptake, and expression of the Alocaligenes europhus poly (beta-hydroxybutyrate) synthesis pathway in transgenic maize cell suspension cultures. *Biotechnol. Prog*. 13: 347-354.
24. Lee, C. W. T. and M. L. Shuler (2000) The effect of inoculum density and conditioned medium on the production of ajmalicine and catharanthine from immobilized *Cataranthus roseus* cell. *Biotechnol. Bioeng*. 67: 61-71.
25. McDonald, K. A. and A. P. Jackman (1989) Bioreactor studies of growth and nutrient utilization in alfalfa suspension cultures. *Plant Cell Rep.* 8: 455- 458
26. Tautorus, T. E., M. M. Lulsdorf, S. I. Kikcio, and D. I. Dunstan (1992) Bioreactor culture of *Picea mariana* Mill (black spruce) and the species complex *Picea glauca* Engelmannii (interior spruce) somatic embryo – growth parameters. *Appl. Microb. Biotechnol.* 38: 46-51.
27. Amino, S. I. and M. Tazawa (1988) Uptake and utilization of sugars in cultured rice cells. *Plant Physiol*. 29: 483-487.
28. Shin, K. S., H. N. Murthy, J. Y. Ko, and K. Y. Paek (2002) Growth and betacyanin production by hairy root cultures of *Beta vulgaris* L. in air lift bioreactors. *Biotechnol. Lett.* 24: 2067-2069.
29. Morad, P., C. Fulcheri, and M. Henry (1998) Kinetics of mineral nutrient uptake by *Saponaria officinalis* L. suspension cell cultures in different media. *Plant Cell Rep*. 18: 260-265.
30. Kintzios, S., E. Stavropoulou, and S. Skamneli (2004) Accumulation of selected macronutrients and carbohydrates in melon tissue cultures: association with pathways of *in vitro* dedifferentiation and differentiation (organogenesis, somatic embryogenesis). *Plant Sci.* 167: 655-664.
31. Liu, C. Z., B. H. Abbasi, M. Gao, S. J. Murch, and P. K. Saxena (2006) Caffeic acid derivatives production by hairy root cultures of *Echinacea purpurea. J. Agric. Food Chem.* 54: 8456-8460.
32. Huang, S. Y., C. H. Hung, and S. N. Chou (2004) Innovative strategies for operation of mist trickling reactors for enhanced hairy root proliferation and secondary metabolite productivity. *Enzyme Microb. Technol.* 35: 22-32.
33. Chattopadhyay, S., S. Farkya, A. K. Srivatava, and V. S. Bisaria (2002) Bioprocess considerations for production of secondary metabolites by plant cell suspension cultures. *Biotechnol. Bioprocess Eng*. 7: 138-149.
34. Gao, J. and J. M. Lee (1992) Effect of oxygen supply on the suspension cultures of genetically modified tobacco cells. *Biotechnol. Prog*. 8: 285-290.
35. Schlatmann, J. E., E. Fonck, H. J. G. ten Hoopen, and J. J. Heijnen (1994) The negligible role of carbon dioxide and ethylene in ajmalicine production by *Catharanthus roseus* cell suspensions. *Plant Cell Rep*. 14: 157-160.
36. Wang, B., G. Zhang, L. Zhu, L. Chen, and Y. Zhang (2006) Genetic transformation of *Echinacea purpurea* with *Agrobacterium rhizogenes* and bioactive ingredient analysis in transformed cultures. *Colloids Surf B.* 53: 101-104.
37. Wills, R., B. H. and D. L. Stuart (1999) Alkylaminde and cichoric acid levels in *Echinacea purpurea* grown in Australia. *Food Chem*. 67: 385-388.