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Influence of inoculum density and aeration volume on biomass and bioactive compound production in bulb-type bubble bioreactor cultures of *Eleutherococcus koreanum* Nakai

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ABSTRACT

This study deals with the effects of initial inoculum density and aeration volume on biomass and bioactive compound production in adventitious roots of *Eleutherococcus koreanum* Nakai in bulb-type bubble bioreactors (3-L capacity). While the fresh and dry weights of the roots increased with increasing inoculum density, the highest percentage dry weight and accumulation of total target compounds (eleutheroside B and E, chlorogenic acid, total phenolics, and flavonoids) were noted at an inoculum density of 5.0 g L^{-1}. Poor aeration volume (0.05 vvm) stunted root growth, and high aeration volume (0.4 vvm) caused physiological disorders. Moreover, an inoculum density of 5.0 g L^{-1} and an aeration volume of 0.1 vvm resulted in the highest concentration of total target compounds and least root death. Such optimization of culture conditions will be beneficial for the large-scale production of *E. koreanum* biomass and bioactive compounds.

1. Introduction

Eleutherococcus koreanum Nakai, which belongs to the Araliaceae family, is an endemic medicinal plant grown on Jeju Island, located off the southern coast of South Korea. Extracts from this plant have traditionally been used in Korea as a tonic and for the treatment of rheumatism, diabetes, and hepatitis (Kang et al., 2005). The root and stem of this plant contain bioactive compounds such as eleutherosides, chlorogenic acid, and other phenolic compounds. Eleutheroside B and E are considered the most important bioactive compounds from *E. koreanum*, and they have been shown to produce strong stimulant and anti-stress effects (Slacanin et al., 1991). In addition, chlorogenic acid from this plant has been shown to have a protective effect against gastric ulcers and to stimulate eleutheroside E activity (Fujikawa et al., 1996).

Because of excessive random harvesting from natural stands for medical purposes, *E. koreanum* has been listed as an endangered species (Park et al., 2005). Propagation of *E. koreanum* through seeds is difficult because over 18 months of stratification are required for germination of the zygotic embryos to be induced (Ko et al., 2003), and the quantity of bioactive compounds obtained from field-grown plants is affected by harvest time and environmental conditions (Jwa et al., 2000). Thus, the production of *E. koreanum* via seed germination is not a suitable method for use in the pharmaceutical industry, which requires large-scale production of uniform products.

Plant tissue culture techniques have been explored as a potential alternative for the production and conservation of *E. koreanum*. In particular, research has shown that culturing of adventitious roots in bioreactors is an effective means of biomass and bioactive compound production because of the fast growth rate, regular annual production, and stable metabolite productivity achieved (Murthy et al., 2008; Wu et al., 2006). However, many physical and chemical factors need to be optimized for successful bioreactor cultures. Inoculum density and aeration volume are two such factors associated with the agitation of explants and the amount of dissolved oxygen in the media, which affect the performance of bioreactor cultures (Ahmed et al., 2008; Jeong et al., 2009a,b).

Few studies have reported the factors affecting root growth and bioactive compound accumulation in *E. koreanum*. These researches have shown that the effects of plant growth regulators (Ahn et al., 2005; Lee et al., 2010) and jasmonic acid (Ahn et al., 2006) on root biomass and bioactive compound production and the use of somatic embryogenesis for plant regeneration (Park et al., 2005). Studies using pilot-scale bioreactor systems (over 500-L capacity) for commercial production found that the production of biomass and bioactive compounds from *in vitro* explants of *E. koreanum* was lower than that from explants of other medicinal plants (Choi et al., 2000; Murthy et al., 2008; Wu et al., 2007).

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Further, there have been no reports on the effect of various physical conditions within bioreactors on the physiological responses of *E. koreanum* adventitious roots.

Therefore, the objective of the present study was to optimize the inoculum density and aeration volume for the production of root biomass and bioactive compounds in *bulb-type bubble bioreactor* cultures of *E. koreanum* Nakai. In addition, the physiological responses of adventitious roots in relation to the changes in gas components (CO_2 and O_2) within the bioreactors were determined.

2. Methods

2.1. Plant material and adventitious root culture

Adventitious roots were induced from *in vitro*-grown plantlets derived from mature seeds that were maintained under low temperature for 2 years to break dormancy. The seeds were sterilized with 2% (w/v) sodium hypochlorite solution for 15 min and then washed 3 times with distilled water. Zygotic embryos were isolated from the sterilized seeds and placed on 1/2 Murashige and Skoog (MS) medium for germination (Murashige and Skoog, 1962). After 2 months, roots were excised from the seedlings and transferred to 1/2 MS medium supplemented with 3 mg L^{-1} indole-3-butyric acid (IBA), 0.01 mg L^{-1} thidiazuron (TDZ; *N*-phenyl-*N*′-1,2,3,-thidiazol-5-ylurea), 30 g L^{-1} sucrose, and 2.3 g L^{-1} gelrite (Ahn et al., 2005). Induced adventitious roots were propagated in 1/2 MS medium supplemented with 5 mg L^{-1} IBA, 0.01 mg L^{-1} TDZ, and 30 g L^{-1} sucrose in 250-mL shake flasks (containing 70 mL of liquid medium) using a gyratory shaker at 100 rpm in the dark at 22 ± 1 °C. In these experiments, adventitious roots were grown in 3-L bulb-type bubble bioreactors containing 2 L 1/2 MS medium supplemented with 5 mg L^{-1} IBA, 0.01 mg L^{-1} TDZ, and 30 g L^{-1} sucrose and maintained under the same culture conditions as provided above (Lee et al., 2010). Bioreactor cultures were initiated by inoculation with fresh adventitious roots at a density of 5.0 g L^{-1}, and the aeration volume in bioreactors was automatically adjusted to 0.1 vvm (air volume/culture volume per min) using air flow meters (RMA series; Dwyer Instruments, Inc., Michigan City, USA). The adventitious roots were maintained by subculturing to fresh medium every 5 weeks.

2.2. Determination of optimal inoculum density and aeration volume

To determine the optimal inoculum density required to promote both root biomass and bioactive compound production, we varied the initial inoculum density of the fresh roots (2.5, 5.0, 7.5, 10.0, and 15.0 g L^{-1}). Various aeration volumes of 0.05, 0.1, 0.2 or 0.4 vvm, and 0.05/0.1/0.2/0.3/0.4 vvm (gradually increased at a 1-week interval) were also tested. After 5 weeks, growth parameters (fresh weight, dry weight, percentage dry weight, and growth rate), the content of bioactive compounds (eleutheroside B and E, chlorogenic acid, total phenolics, and flavonoids), root death, and CO_2 and O_2 concentrations inside the bioreactors were assessed.

2.3. Determination of the root weight, percentage dry weight, and growth rate

After 5 weeks of culture, the roots were separated from the medium by passing them through a stainless steel sieve. The fresh weight was measured after blotting away the surface water. The dry weight was recorded after drying the fresh roots at 60 °C for 24 h. The growth rate was calculated as follows: [harvested dry weight (g) – inoculated dry weight (g)]/inoculated dry weight (g)/culture period (d) (Ahmed et al., 2008).

2.4. Preparation of root extract

Extraction was carried out in 100-mL round-bottom flasks fitted with a cod oil bath digestion system (LS-2050-S10; LS-TECH, Gwangju, Korea). Dried roots (2.0 g) were refluxed with 30 mL 40% (w/v) methanol at 80 °C for 1 h. After extraction, the cooled extract was filtered and adjusted to 30 mL with 40% (w/v) methanol. This extract was used to determine the content of bioactive compounds.

2.5. Determination of bioactive compounds: eleutheroside B and E and chlorogenic acid

The contents of eleutheroside B and E and chlorogenic acid in the methanolic root extract were determined using the method described by Ahn et al. (2005) with modifications. The fractions of these 3 compounds were analyzed using an HPLC system (Waters 2690 separation module; Waters, Mildfold, USA) equipped with a UV detector (Waters 996 photodiode array detector; Waters, Mildfold, USA) on a Nova pak C18 column (4.0 μm, 3.9 × 150 mm; Waters Chromatography, Mildfold, USA). Phosphoric acid (A) and acetonitrile (B) were used as the mobile phase, and the ratio of A and B for the initial 10, 25, 40, 50, and 60 min was 90:10, 80:20, 60:40, 50:50, and 90:10, respectively. The flow rate of the mobile phase was 0.5 mL^{-1}, and the injection volume for both samples and standards was 20 μL. The contents of the eleutherosides and chlorogenic acid were detected at 220 and 330 nm, respectively. Standards were obtained from CromaDex (USA). Measurements were integrated by comparison with each external standard calibration curve.

2.6. Determination of total phenolic content

The total phenolic content in the methanolic root extract was determined as described by Folin and Ciocalteu (1927) with modifications. To 0.05 mL of the extract with 2.55 mL distilled water, 0.1 mL (2 N) Folin–Ciocalteu reagent was added. The solution was thoroughly mixed and allowed to stand for 6 min before 0.5 mL of 20% (w/v) sodium carbonate solution was added. The color developed after 30 min at room temperature, and the absorbance was measured at 760 nm with a UV visible spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). Measurements were compared to a standard curve for prepared gallic acid solution (Sigma Chemical Co., St. Louis, MO, USA).

2.7. Determination of total flavonoid content

The total flavonoid content in the methanolic root extract was determined as described by Cui et al. (2010). To 0.25 mL of the methanolic root extract or a (+)-catechin standard solution (Sigma Chemical Co., St. Louis, MO, USA), 1.25 mL distilled water and 0.075 mL 5% (w/v) of sodium nitrate solution were added. After 6 min, 0.15 mL of 10% (w/v) aluminum chloride solution was added, and the mixture was allowed to stand for 5 min before 0.5 mL of 1 M sodium hydroxide solution was added. The absorbance was measured at 510 nm with a UV visible spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). The absorbance measurements were integrated by comparison with an external standard calibration curve.

2.8. Determination of root death

Root death was estimated according to the method described by Qin and Lan (2004) with modifications. After 5 weeks of culture, 50 mg of fresh roots was removed from the bioreactors. The roots were incubated for 10 min with 0.05% (w/v) Evans blue solution

(Sigma Chemical Co., St. Louis, MO, USA) and washed with distilled water to remove excess and unbounded dye. The dead cells, to which the dye was bound, were treated with 1 mL of 50% (w/v) methanol and 1% (w/v) sodium dodecyl sulfate (SDS; Sigma Chemical Co., St. Louis, MO, USA), and the suspension was allowed to stand for 10 min at 60 °C. The absorbance was measured at 600 nm with a UV visible spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan).

2.9. Determination of CO_2 and O_2 concentrations inside the bioreactors

To measure the CO_2 and O_2 concentrations inside the bioreactors, gas samples of 1 mL were taken from the vent line, which was connected to a filter. The CO_2 and O_2 concentrations were determined by gas chromatography (GC) using an HP 6890 system (Agilent Co., Wilmington, USA) equipped with a thermal conductivity detector (TCD) on a 60/80 Caboxen-1000 column (15″ × 1/8″, 4.5-m packed column; Supelco, Bellefonte, USA). Helium was used as the carrier gas at a constant flow rate of 40 mL^{-1}. The oven temperature was first maintained at 37 °C for 7 min and then gradually elevated by 20 °C min^{-1} to reach 200 °C; this temperature was maintained for 10 min. The injector and TCD temperatures were 200 °C and 230 °C, respectively. Standards of CO_2 and O_2 were obtained from DaeDeok Gas Co. (Republic of Korea), and the measurements were integrated by comparison with each external standard calibration curve.

2.10. Statistical analysis

Results are expressed as the mean values for each experiment. Error bars indicate the standard error (SE) of the mean for each replicate. Statistical significance of the difference between treatments was assessed by Duncan's multiple range test ($p = 0.05$). Data were analyzed using the SAS program (Version 6.21) (SAS Institute, 1989).

3. Results and discussion

3.1. Effect of inoculum density on biomass and bioactive compound production

Bioreactor cultures are used to enhance biomass and bioactive compound production in plant cell or organ culture (Jeong et al., 2009a,b). To determine the exact stage at which the maximum biomass is produced and to evaluate the accumulation of bioactive compounds, fresh roots were inoculated at densities varying from 2.5 to 15.0 g L^{-1}. The fresh and dry weights increased with the increasing inoculum density, and the highest percentage dry weight was achieved at an inoculum density of 5.0 g L^{-1} (Table 1). In contrast, the growth rate was negatively affected by an increase in inoculum density: the lowest inoculum density (2.5 g L^{-1}) resulted in the highest growth rate (0.35 d^{-1}), while densities exceeding 7.5 g L^{-1} yielded more than 50% lower growth rates (Fig. 1). The results indicated that inoculating *E. koreanum* adventitious roots with initial densities exceeding 5.0 g L^{-1} resulted in low efficiency of biomass accumulation. Additionally, the highest *in vitro*-growth rate of the plantlets over 5 weeks of culture occurred at the initial culture stage; this is probably because of the high cell division that occurred early in the culturing. In general, high initial inoculum densities result in shorter lag phases in roots and earlier onset of the declining phase and senescence of explants. Fig. 2 shows the effect of inoculum density on root death after 5 weeks of culture. Root death was positively associated with increasing inoculum density. This implies that roots inoculated at high initial densities reach senescence earlier than those inoculated at lower densities. Wu et al. (2006) and Min et al. (2007) also reported that high initial inoculum density had a positive effect on root biomass and a negative effect on the growth ratio in adventitious root cultures of *Echinacea augustifolia* and *Scoplia parviflora*, respectively. Cultured plant cells are fast growing and therefore require higher initial inoculum densities than organs, to initiate cell division. Previous studies have indicated that while the optimal inoculum densities for cell cultures exceed 60 g L^{-1}, those for adventitious root cultures are less 10 g L^{-1} because of the slow growth and metabolism of roots (Jeong et al., 2009a,b; Lee et al., 2006; Thanh et al., 2006). Similarly, an inoculum density of 5.0 g L^{-1} is suitable for generating the optimum adventitious root biomass in the case of *E. koreanum*.

The effect of inoculum density on the accumulation of bioactive compounds in the adventitious roots of *E. koreanum* is shown in Table 2. The contents of all bioactive compounds tended to decrease with increasing inoculum densities. The highest concentration of the total target compounds was obtained at an inoculum density of 5.0 g L^{-1}. This is probably because the amounts of eleutheroside B and E and chlorogenic acid in the adventitious roots were relatively low, and therefore higher inoculum densities significantly inhibited the accumulation of these bioactive compounds. Even if roots are cultured in the same medium, the synthesis of different bioactive compounds requires different metabolic pathways, and the accumulation of bioactive compounds in

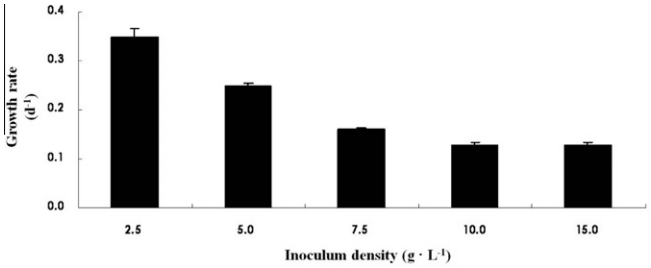

Fig. 1. Effect of inoculum density on growth rate in the adventitious roots of *E. koreanum* after 5 weeks of culture. Bars represent means ± SE ($n = 3$).

Table 1
Effect of inoculum density on biomass accumulation of *E. koreanum* adventitious roots after 5 weeks of culture.

Inoculum density (g L^{-1})	Fresh weight (g L^{-1})	Dry weight (g L^{-1})	Percentage dry weight
2.5	34.09 e[a]	4.64 d	13.61
5.0	46.65 d	6.73 c	14.43
7.5	50.90 c	7.02 bc	13.79
10.0	57.27 b	7.63 b	13.33
15.0	74.20 a	9.61 a	12.94

[a] Mean separation within columns by Duncan's multiple range test, 5% level.

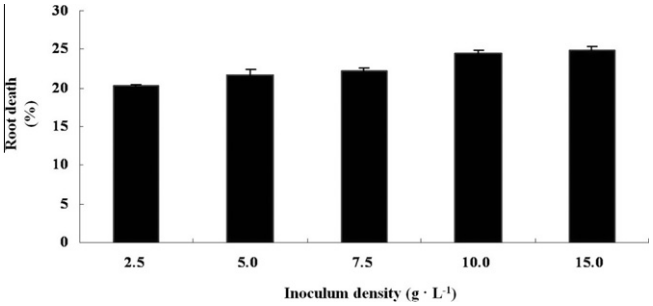

Fig. 2. Effect of inoculum density on root death in the adventitious roots of *E. koreanum* after 5 weeks of culture. Bars represent means ± SE ($n = 3$).

Table 2
Effect of inoculum density on accumulation of bioactive compounds in the adventitious roots of *E. koreanum* after 5 weeks of culture.

Inoculum density ($g\ L^{-1}$)	Amount of eleutheroside B ($\mu g\ g^{-1}$ DW)	Amount of eleutheroside E ($\mu g\ g^{-1}$ DW)	Amount of chlorogenic acid ($mg\ g^{-1}$ DW)	Amount of total phenolics ($mg\ g^{-1}$ DW)	Amount of total flavonoids ($mg\ g^{-1}$ DW)	Amount of total target compounds ($mg\ g^{-1}$ DW)
2.5	46.21 a[a]	108.53 a	2.69 a	9.95 a	4.60 a	17.40 a
5.0	27.59 b	106.61 a	2.42 a	10.06 a	4.91 a	17.53 a
7.5	26.42 b	108.89 a	2.71 a	9.12 b	4.03 b	15.99 b
10.0	15.95 c	87.47 b	2.64 a	9.38 b	4.16 b	16.28 b
15.0	6.71 d	89.65 b	0.99 b	8.26 c	3.41 c	12.76 c

[a] Mean separation within columns by Duncan's multiple range test, 5% level.

explants is affected by the culture stage (Ahmed et al., 2008; Cui et al., 2010). This may explain why eleutheroside B and E, which belong to the lignan group (Ahn et al., 2005), showed less bioaccumulation with increasing inoculum density compared with other bioactive compounds. Wu et al. (2006) reported that high initial inoculum density resulted in low phenol and flavonoid contents in the adventitious roots of *E. augustifolia*, and Min et al. (2007) found that high inoculum densities resulted in decreased production of scopolamine, a tropane alkaloid belonging to the phenol group.

During the culture period, *in vitro* plantlets consume O_2 inside the culture vessel for anabolism and release CO_2 as an outcome of catabolism. Fig. 3 shows the CO_2 and O_2 concentrations inside the bioreactor after 5 weeks of culture. The CO_2 concentration significantly increased with the increasing inoculum density; with all the densities, there was at least a 3-fold increase in the CO_2 concentration compared with the initial CO_2 concentration of the air in the bioreactor. Moreover, the highest inoculum density ($15.0\ g\ L^{-1}$) produced the highest CO_2 concentration inside the bioreactor (approximately 5-fold increase over the initial concentration). These high CO_2 concentrations probably contributed to root death and inhibited the accumulation of bioactive compounds in the adventitious roots. However, high CO_2 concentration inside a bioreactor does not always negatively affect bioactive compound accumulation. For instance, Ali et al. (2006) reported that the accumulation of phenolic compounds in the adventitious roots of *E. angustifolia* was enhanced with CO_2 supplements up to 1%. In another study, the authors reported that subjecting ginseng adventitious roots in a bioreactor to 1% CO_2 promoted the formation of root biomass but inhibited the accumulation of bioactive compounds (Ali et al., 2008). Since ginseng and *E. koreanum* belong to the same family (Araliaceae), it is not surprising that the adventitious roots of these plants showed the same pattern of response to CO_2 concentration. No significant differences were observed in the O_2 concentrations at the various inoculation densities, since fresh air was supplied into the bioreactor throughout the culture period (Fig. 3). Thus, CO_2 accumulation inside bioreactors is an important factor affecting the root physiology of *E. koreanum*.

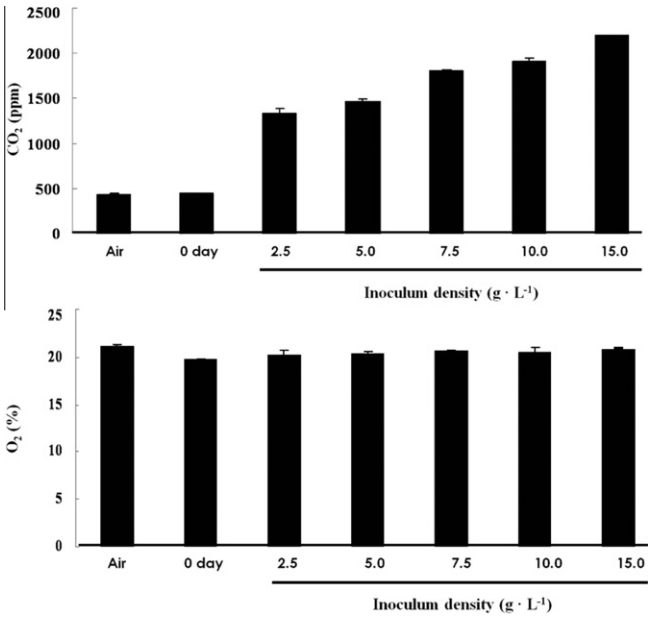

Fig. 3. Effect of inoculum density on CO_2 and O_2 concentrations inside the bioreactors after 5 weeks of culture. Bars represent means ± SE ($n = 3$).

The results of the present study indicate that an inoculum density of $5.0\ g\ L^{-1}$ is optimal for both root biomass and bioactive compound production in the case of *E. koreanum*, as evidenced by the growth parameters (high root biomass and low root death), accumulation of high amounts of total target bioactive compounds, and the absence of physiological disorders caused by high CO_2 concentrations.

3.2. Effect of aeration volume on biomass and bioactive compound production

Optimizing aeration volume is one conventional way of controlling the gaseous composition, which can affect root growth. Although the percentage dry weight increased slightly with the aeration volume, there were no significant differences in root biomass production between the different aeration volumes (0.05–0.4 vvm), except between the lowest (0.05 vvm) and highest (0.4 vvm) (Table 3, Fig. 4). Supply of aeration at a high volume into a bioreactor may cause excessive shear stress to roots and thicker cell walls (Fischer and Alfermann, 1995). Fig. 5 shows the effect of different aeration volumes on root death after 5 weeks of culture. Root death showed a similar pattern as root biomass accumulation: it was higher at the lowest and highest aeration volumes than at the other aeration volumes. Increasing the aeration volume improves oxygen transfer and the mixing efficiency of explants with the culture medium. In the present study, a poor aeration volume (0.05 vvm) resulted in stunted root growth and stimulated root senescence because of low oxygen transfer and mixing ratio. Similarly, the highest aeration volume (0.4 vvm) inhibited accumulation of biomass, probably because of physiological damage due to

Table 3
Effect of aeration volume on biomass accumulation in *E. koreanum* adventitious roots after 5 weeks of culture.

Aeration volume (vvm)	Fresh weight ($g\ L^{-1}$)	Dry weight ($g\ L^{-1}$)	Percentage dry weight
0.05	44.21 b[a]	5.51 c	12.46
0.1	47.13 a	5.99 ab	12.70
0.2	47.52 a	6.22 a	13.09
0.4	43.74 b	5.81 bc	13.27
0.05–0.4	48.89 a	6.37 a	13.02

[a] Mean separation within columns by Duncan's multiple range test, 5% level.

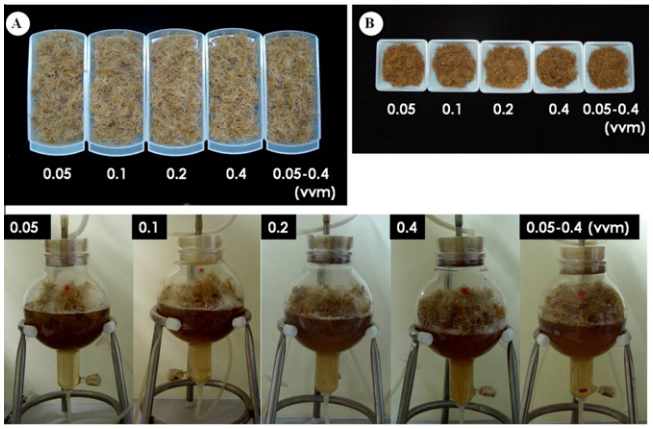

Fig. 4. Effect of aeration volume on growth of *E. koreanum* adventitious roots after 5 weeks of culture. (A) Fresh roots; (B) dry roots.

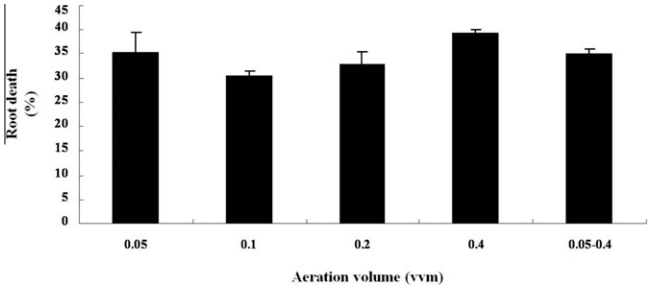

Fig. 5. Effect of aeration on root death in the adventitious roots of *E. koreanum* after 5 weeks of culture. Bars represent means ± SE (n = 3).

the excessive agitation and shear stress (Meijer et al., 1993). In general, cultured cells require less oxygen than microorganisms because of their larger vacuoles and metabolism (Min et al., 2007). However, each plant species requires a different aeration volume and responds differently to shear stress. In the present study, the least root death and highest accumulation of root biomass were achieved at an aeration volume of 0.1 vvm. This suggests that 0.1 vvm is optimal for *E. koreanum* adventitious roots, because it supplies sufficient O_2 for anabolism, prevents accumulation of large amounts of CO_2 inside the bioreactor, and does not cause shear stress or root physiological disorders such as changing to a dark color or inhibiting root elongation.

The accumulation of bioactive compounds in the adventitious roots of *E. koreanum* is shown in Table 4. Similar to the findings for root growth, no significant differences were found in the amount of the total target compounds at different aeration volumes except the lowest (0.05 vvm) and highest (0.4 vvm). In particular, high aeration volumes significantly inhibited the

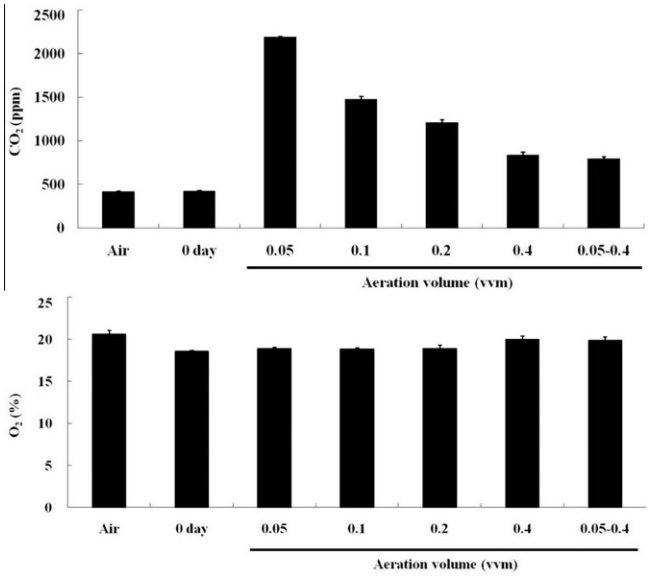

Fig. 6. Effect of aeration volume on CO_2 and O_2 concentrations inside the bioreactors after 5 weeks of culture. Bars represent means ± SE (n = 3).

accumulation of eleutheroside B and E and chlorogenic acid in the adventitious roots; the highest amount of the total target compounds was obtained at an aeration volume of 0.1 vvm. Studies in which the adventitious roots of *Scopolia parviflora* (Min et al., 2007) and *E. purpurea* (Jeong et al., 2009a,b) were cultured have reported that root growth and metabolism in these plants were affected by shear stress more than those in *E. koreanum* tested in the present experiment. It is possible that various chemical factors (such as salt strength, nitrogen sources, and sucrose concentration) had a greater influence on both root growth and accumulation of bioactive compounds than the physical factors (initial inoculum density and aeration volume) tested in this study did. The influence of chemical factors on adventitious root cultures of *E. koreanum* will be tested in future experiments.

The CO_2 and O_2 concentrations in the bioreactor were highly affected by aeration volume (Fig. 6). Only the CO_2 concentration was influenced by the aeration volume at the time of measurement, not root biomass accumulation and root death. The CO_2 concentrations in bioreactors with poor aeration volumes (0.05 vvm) were 5.2 times higher than those of the air; this probably caused inhibition of respiratory function and affected root development. A high aeration volume (0.4 vvm) was detrimental to the accumulation of bioactive compounds. This may have been due to the stripping of essential gaseous components, such as carbon dioxide and ethylene, which are needed to stimulate the production of bioactive compounds (Gao and Lee, 1992; Schlatmann et al., 1993). No significant changes were found in the O_2 concentrations inside the bioreactors with respect to aeration volume.

Table 4
Effect of aeration volume on accumulation of bioactive compounds in the adventitious roots of *E. koreanum* after 5 weeks of culture.

Aeration volume (vvm)	Amount of eleutheroside B (μg g^{-1} DW)	Amount of eleutheroside E (μg g^{-1} DW)	Amount of chlorogenic acid (mg g^{-1} DW)	Amount of total phenolics (mg g^{-1} DW)	Amount of total flavonoids (mg g^{-1} DW)	Amount of total target compounds (mg g^{-1} DW)
0.05	49.35 b[a]	106.88 a	3.02 a	9.09 a	5.34 a	17.60 ab
0.1	59.25 a	107.70 a	3.36 a	9.84 a	5.52 a	18.88 a
0.2	59.55 a	108.23 a	3.34 a	9.64 a	5.26 a	18.41 a
0.4	49.05 b	90.68 b	1.44 b	9.72 a	5.10 a	16.39 b
0.05–0.4	52.65 ab	98.78 a	2.96 a	9.58 a	5.20 a	17.89 a

[a] Mean separation within columns by Duncan's multiple range test, 5% level.

Thus, the results of the present study indicate that 0.1 vvm is the optimal aeration volume to prevent the stripping of essential gaseous components and to supply adequate air to maximize the accumulation of root growth and bioactive compounds in the adventitious roots of *E. koreanum*.

4. Conclusions

In vitro conditions strongly affect root growth and bioactive compound production in *E. koreanum* Nakai adventitious roots cultured in 3-L bulb-type bubble bioreactors. An inoculum density of 5.0 g L^{-1} and aeration volume of 0.1 vvm were found to be optimal for the accumulation of root biomass and bioactive compounds, as evidenced by growth parameters (high root biomass and low root death), high accumulation of bioactive compounds, and the absence of physiological root disorders. Culture protocols using these optimized culture conditions will prove beneficial for the large-scale production of *E. koreanum* Nakai-derived bioactive compounds in bubble-column-like bioreactors.

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