Acta Physiol Plant
DOI 10.1007/s11738-013-1476-6

ORIGINAL PAPER

Several factors affecting hypericin production of *Hypericum perforatum* during adventitious root culture in airlift bioreactors

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Received: 12 September 2013 / Revised: 12 November 2013 / Accepted: 27 December 2013

Abstract *Hypericum perforatum* L. is a traditional medicinal plant for the treatment of depression and wound healing, and hypericin is one of the main effective active substances. To optimize the culture system for producing hypericin in adventitious root, this study used balloon-type airlift bioreactors to investigate the effect of air volume, inoculation density, indole-3-butyric acid (IBA) concentration and methyl jasmonate (MeJA) concentration on hypericin content and productivity during adventitious root culture. Hypericin content and productivity were improved with increasing air volume, and 0.1 vvm (air volume/culture volume/min) was optimal for hypericin production. Inoculation density also had a great effect on hypericin accumulation. Hypericin content and productivity were favorable in an inoculation density of 5.0 g l^{-1} and decreased when inoculation densities were lower or higher than 5.0 g l^{-1}. Furthermore, 1.25 mg l^{-1} IBA enhanced hypericin content and productivity, but too low ($\leq$0.50 mg l^{-1}) or too high ($\geq$1.50 mg l^{-1}) IBA concentrations decreased hypericin accumulation. MeJA concentration significantly affected biomass accumulation and hypericin production. The biomass decreased and hypericin production increased with increasing MeJA concentration. Optimum hypericin content (1.61 mg g^{-1} DW) and productivity (15.57 mg l^{-1}) were obtained at 350 μM MeJA. The hypericin content in bioreactor-grown adventitious roots was lower than in 3-year field-grown plants, but significantly higher than that in in vitro-grown plantlets and 1-year field-grown plants. Thus, the bioreactor culture of adventitious roots can realize rapid and mass production of hypericin in *H. perforatum*.

Keywords Adventitious root · Air volume · Bioreactor · Inoculation density · Hypericin · MeJA

Abbreviations

vvm	Air volume/culture volume/min
DW	Dry weight
FW	Fresh weight
IBA	Indole-3-butyric acid
HPLC	High-performance liquid chromatography
HIV-1	Human immunodeficiency virus type 1
MeJA	Methyl jasmonate
MS	Murashige and Skoog
rpm	Revolutions per minute
SA	Salicylic acid
SE	Standard error

Introduction

Hypericum perforatum L. (St. John's wort) is a perennial herb native to Europe and also a traditional medicinal plant which is gaining popularity mainly for the treatment of depression and wound healing (Lawvere and Mahoney 2005). The most characteristic constituents of *H. perforatum* are naphtodianthrones (hypericin and pseudohypericin)

Communicated by K.-Y. Paek.

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Published online: 09 January 2014

and phloroglucinols (hyperforin and adhyperforin) (Butterweck 2003). *H. perforatum* has potential in the treatment of mild to moderate form of depression due to the active constituents 'hypericin and hyperforin' and has become a popular medicinal plant due to its antiviral, anticancer, bactericidal, antiinflammatory and sedative properties (Linde 2009). Moreover, photodynamic hypericin activities displayed under the influence of light are used for therapy in various diseases. These properties allow hypericin to act as an antiviral agent. Attention has been focused on its use against human immunodeficiency virus type 1 (HIV-1) (Meruelo et al. 1988) and to enhance radiolytic sensitivity of tumor cells (Hadjur et al. 2008).

However, the level of hypericin in *H. perforatum* plants often varies with growth under different field environments (Southwell and Campbell 1991; Büter et al. 1998). The limited area of occurrence of this plant, seasonal harvesting, loss of biodiversity, variability in quality and contamination issues trigger the search for alternative methods of hypericin production (Kirakosyan et al. 2000; Sirvent and Gibson 2002; Walker et al. 2002; Kornfeld et al. 2007). In recent years, plant cell culture technology has been successfully applied to the production of many useful secondary metabolites, including pharmaceuticals, pigments and other fine chemicals (Verpoorte et al. 2002). The culturing of adventitious root tissues is an efficient means of biomass production because of fast growth rates and stable metabolite productivity (Murthy et al. 2008). Bioreactors are often used for large-scale production of plant metabolites as these automated or semi-automated processes save labor and production costs (Chakrabarty et al. 2003). For *H. perforatum*, active compounds such as phenolics, flavonoids, chlorogenic acid, quercetin and hyperoside also have been produced by culturing adventitious roots with bioreactors (Cui et al. 2010; Cui et al. 2011). During bioreactor culture, there have been many factors affecting adventitious root growth and accumulation of active compounds in *H. perforatum*. Auxin and auxin/cytokinin combinations, inoculation sizes and Murashige and Skoog (MS) medium dilutions affect adventitious root biomass and accumulation of phenols and flavonoids (Cui et al. 2011). Chlorogenic acid, polysaccharides, as well as phenolics and flavonoids are also affected by inoculation density, aeration volume and culture period (Cui et al. 2010). However, the information on the factors affecting hypericin production of *H. perforatum* by bioreactor culture of adventitious root has been limited. Cui et al. (2010) indicated that hypericin could be produced by adventitious root culture in a bioreactor, but a detailed analysis and research of the factors affecting hypericin production and increase of hypericin content have not been made.

To optimize the bioreactor culture for mass production of hypricin, the present study used airlift bioreactors to explore the effects of air volume, inoculation density, IBA concentration, culture period and MeJA on hypericin content and productivity during adventitious root culture. Finally, the hypericin contents from different source extracts were also compared.

Materials and methods

Plant material and adventitious root culture

Adventitious roots provided by Chungbuk National University (South Korea) were cultured in 250 ml conical flasks containing 70 ml MS (Murashige and Skoog 1962) medium supplemented with 30 g l^{-1} sucrose and IBA, and the pH was adjusted to 5.8. The cultures were maintained in the dark at 25 ± 2 °C on a gyratory shaker at 100 rpm (revolutions per minute), then subcultured to fresh medium once in 30 days.

Effects of air volume, inoculation density and IBA concentration on hypericin content and productivity

All the experiments were carried out in 5-l balloon-type airlift bioreactors, and the bioreactors were maintained in the dark at 25 ± 2 °C. Firstly, the 30-day adventitious roots cultured in conical flasks were cut to 1 cm long and transferred to bioreactors with 4-l working volume. To study the effect of air volume on hypericin production, the medium was aerated at 0.025, 0.05, 0.075 and 0.1 vvm (air volume/culture volume/min). 5 g l^{-1} adventitious roots (fresh weight, FW) were inoculated to each bioreactor. The culture medium was MS supplemented with 30 g l^{-1} sucrose and 1.0 mg l^{-1} IBA; the pH was adjusted to 5.8. Next, the inoculation density was tested, the initial density of adventitious roots was adjusted to 2.5, 5.0, 7.5, 10.0 and 12.5 g l^{-1} FW, air volume was adjusted at 0.1 vvm and other culture conditions were in keeping with the air volume experiment. Then, the IBA concentration in the culture medium was also studied and the MS medium supplemented with different concentrations of IBA (0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 mg l^{-1}) and 30 g l^{-1} sucrose (pH 5.8). 5 g l^{-1} of inoculation density and 0.1 vvm of air volume were applied. The hypericin content and productivity of adventitious root in the above three experiments were determined after 30 days of bioreactor culture.

To determine the changes of hypericin content during the culture period, adventitious roots were inoculated in the bioreactor with MS medium containing 1.25 mg l^{-1} IBA and 30 g l^{-1} sucrose (pH 5.8) at an inoculation density of 5.0 g l^{-1} and an air volume of 0.1 vvm for 50 days. The bioreactors were maintained in the dark at 25 ± 2 °C. The

adventitious roots were sampled at 5-day intervals from bioreactors and the fresh weight, dry weight (DW) and hypericin content were determined.

Effect of MeJA on hypericin production

MeJA (MUST Biotechnolog co. Ltd, Chengdu, China) was used as an elicitor for improving hypericin accumulation during bioreactor culture of adventitious root. Five-liter balloon-type airlift bioreactors were used to select a suitable MeJA concentration. Each bioreactor was inoculated with 5 g l^{-1} FW of adventitious roots, aerated at 0.1 vvm and filled with 4-l MS medium supplemented with 30 g l^{-1} sucrose and 1.25 mg l^{-1} IBA (pH 5.8). The different amounts of MeJA (0, 50, 100, 150, 200, 250, 300, 350 and 400 μM) were supplied to the bioreactors from the initial culture. The bioreactors were maintained in the dark at 25 ± 2 °C. After 40 days of culture, the adventitious roots were harvested and used for measuring the biomass and the hypericin content.

Comparison of hypericin content from the different sources

To define the hypericin production level in adventitious roots, the hypericin content of adventitious roots was compared with plantlets and 1-year or 3-year plants. The bioreactor-grown adventitious roots (treated with 350 μm MeJA) and in vitro-grown plantlets were obtained after 40 days of culture. The 1-year (plant without flowers) and 3-year (plant with flowers) plants derived from in vitro cultured-plantlets, i. e., the 40-day in vitro-grown plantlets were transplanted to the pots and cultivated in a green house, then harvested in September after 1 or 3 years.

Determination of biomass and hypericin content

The adventitious roots were separated from the medium after 40 days of bioreactor culture, then the fresh weight was measured after rinsing three times with sterile water and stripping the surface moisture with a dehydrator for 2 min. The fresh roots were dried at 40 °C with a drying oven for 2 days and the dry weight was recorded.

The hypericin content was determined using the method described by Tolonen et al. (2003) with modifications. The dry adventitious roots were ground into fine powder in a grinder. The powdered material (0.4 g) was extracted with 8 ml of high-performance liquid chromatography (HPLC)-grade methanol for 60 min in an ultrasonic bath at 35 °C. The extracting solution was filtered with a 0.45-μm filter (PVDF syringe filter, Beijing, China) before injection for HPLC analysis. Hypericin was quantified by an HPLC system (Agilent 1100, Agilent Technologies, USA)

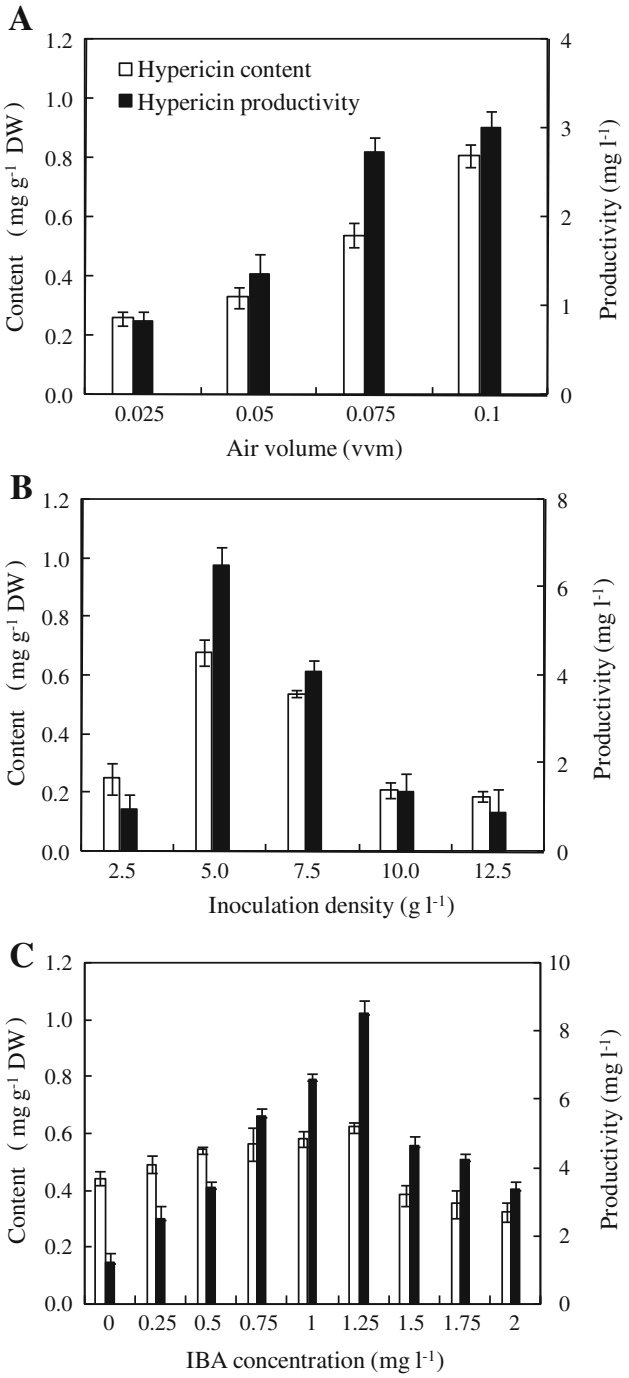

Fig. 1 Effect of air volume, inoculation density, and IBA concentration on hypericin content and productivity after 30 days of adventitious root culture in bioreactors. *Bars* mean ± SE ($n = 3$)

equipped with a 5 C_{18}-AR-II column (particle size 5 μm, 250 × 4.6 mm) maintained at 35 °C. Fractions were eluted with 80 % HPLC-grade acetonitrile in 5 mM 20 % ammonium acetate, the flow rate of eluent was 1 ml min^{-1} and the total run time was 15 min. The injection volume was 10 μl. Hypericin was detected at 284 nm. The

hypericin standards were obtained from MUST Biotechnology Co., Ltd. (Chengdu, China). Measurements of all the sample materials were integrated by comparison with an external standard calibration curve. The retention time (t_R) for hypericin was 4.15 min.

The hypericin productivity of adventitious roots was calculated as [harvested DW (g) × hypericin content (mg g^{-1} DW)]/medium volume (l).

Experimental design and data analysis

All experiments were independently replicated three times and the data expressed as the mean value for each experiment. The bars indicate the standard error (SE) of the mean for each replicate.

Results and discussion

The effect of air volume, inoculation density and IBA concentration on hypericin content and productivity.

A suitable air supply inside a bioreactor is an important factor. Air volume promoted the hypericin production of adventitious roots. Hypericin content increased with increasing air volume (ranged from 0.025 to 0.1 vvm) and the highest hypericin content (0.91 mg g^{-1} DW) was observed at 0.1 vvm air volume (Fig. 1a). The growth of adventitious roots was promoted with increasing air volume ranging from 0.025 to 0.075 vvm, but inhibited at 0.1 vvm (data not shown). Hypericin productivity was related to both adventitious root biomass and hypericin content, and the highest hypericin productivity (3.0 mg l^{-1}) was tested at 0.1 vvm. Air can agitate the mixture of medium and cultures thoroughly, thereby providing dissolved oxygen for metabolic activities (Lee et al. 2006; Ahmed et al. 2008), but higher air volumes inhibit root growth because of sheer stress (Lee et al. 2006) or direct oxygen toxicity due to a high level of dissolved oxygen (Schlatmann et al. 2004; Thanh et al. 2006). Cui et al. (2011) achieved the optimal production of chlorogenic acid, total phenolics and total flavonoids of *H. perforatum* adventitious roots at 0.1 vvm. Our study found 0.1 vvm was also appropriate for hypericin production. Thus, 0.1 vvm air volume is suggested for metabolite production of *H. perforatum* adventitious roots during bioreactor culture.

Inoculation density significantly affected hypericin accumulation; the maximum hypericin content and productivity were obtained when 5 g l^{-1} FW of adventitious roots was used as inoculum (Fig. 1b). When inoculation density was lower than 5 g l^{-1} FW (2.5 g l^{-1} inoculation density), low hypericin content (0.25 mg g^{-1} DW) and productivity (0.99 mg l^{-1}) was measured. Inoculation densities higher than 5 g l^{-1} FW inhibited hypericin production. At 12.5 g l^{-1} inoculation density, only 0.19 mg g^{-1} DW of hypericin content and 0.88 g l^{-1} of hypericin productivity were determined. Low inoculation densities wasted space, extended culture time and reduced hypericin production. High inoculation densities resulted in poor growth because the adventitious roots rapidly reached their stationary phase, which limited the availability of nutrients and oxygen as well as affected the production of secondary metabolites (Min et al. 2007; Cui et al. 2010). This finding was also observed in other plant species during adventitious root culture (Jeong et al. 2009; Lee et al. 2006). In addition, the effect of inoculation density on *H. perforatum* was also verified by Cui et al. (2011); the greatest increment of chlorogenic acid, phenolics, flavonoids and polysaccharides content occurred at an inoculation density of 3 g l^{-1}. This result differed with our study in which 5 g l^{-1} inoculation density favored hypericin production. Consequently, during bioreactor culture of adventitious roots, a suitable inoculation density should be selected according to plant species, bioreactor size, working volume, target compounds, etc. 5 g l^{-1} of inoculation density was considered optimal for hypericin production of *H. perforatum* in a 5-l balloon-type airlift bioreactor with 4-l working volume.

Hypericin content and productivity increased as IBA concentration increased from 0 to 1.25 mg l^{-1}; the highest hypericin content and productivity appeared at 1.25 mg l^{-1} IBA (Fig. 1c). IBA concentrations higher than 1.25 mg l^{-1} adversely affected adventitious root growth and hypericin content. The optimum concentration of plant growth regulators is critical in controlling the accumulation of secondary metabolites. Auxin is a root-inducing agent critical to the formation of adventitious roots, whereas IBA affects metabolite accumulation. A previous study revealed that 7.0 mg l^{-1} IBA was the optimum concentration for promoting both cell growth and saponin production during cell suspension culture of *Panax ginseng* (Lian et al. 2002). Baque et al. (2010) found that the biomass and secondary metabolite accumulation of *Morinda citrifolia* adventitious roots could reach maximum values at 5.0 mg l^{-1} IBA. These results demonstrated that the optimum IBA concentration for cell and organ growth varied per plant species. In the present study, 1.25 mg l^{-1} IBA is favored for the hypericin production of adventitious roots in bioreactors.

Kinetic changes in hypericin content and productivity during culture period

To confirm the suitable culture period for efficient production of hypericin during the bioreactor culture of adventitious roots, the changes in hypericin content and

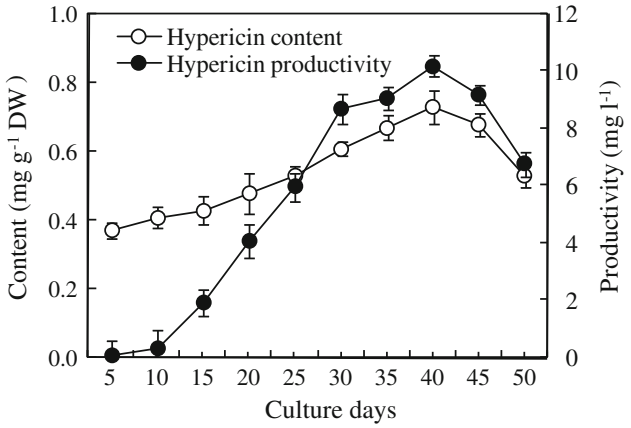

Fig. 2 Changes in hypericin content and productivity during 50 days of adventitious root culture in bioreactors. *Bars* mean ± SE ($n = 3$)

productivity were studied. A similar change pattern of hypericin content and productivity was observed. Hypericin content and productivity were both low after the initial 5 days of culture. An exponential phase was observed from 5 to 30 days, followed by a slow growth phase from 30 to 40 days and a declining phase thereafter (Fig. 2). Hypericin content (0.73 mg g^{-1} DW) and productivity (10.19 mg l^{-1}) reached their maximum values after 40 days of culture.

Biomass accumulation and hypericin production in response to different MeJA concentrations

Exogenous MeJA effectively induces secondary metabolites in medicinal plants. MeJA can modulate a plant defense reaction to enhance secondary metabolism in plant cell and root cultures. In the present study, the effect of MeJA concentration on biomass accumulation and hypericin production was studied. Fresh weight and dry weight of *H. perforatum* adventitious roots were suppressed in the culture medium supplemented with MeJA. The highest fresh (147.9 g l^{-1}) and dry weight (13.4 g l^{-1}) of adventitious roots was observed in the group without MeJA, and both the fresh and dry weight of adventitious roots decreased with increasing MeJA concentrations (Figs. 3, 4a). At 350 or 400 μM MeJA, the roots were short, and the color was slightly dark, with fresh and dry weight of only approximately 90.0 and 9.0 g l^{-1}, respectively.

MeJA promoted the hypericin production of *H. perforatum*. The hypericin content and productivity increased with increasing MeJA concentrations (ranging from 0 μM to 350 μM). The maximum hypericin content (1.61 mg g^{-1} DW) and productivity (15.57 mg l^{-1}) were achieved at 350 μM MeJA (Fig. 4b). However, excess MeJA (400 μM) slightly decreased hypericin production. MeJA is a plant hormone involved in regulating plant response to environmental stress through the modulation of gene expression. Exogenously applied MeJA induced biosynthesis of many secondary metabolites (Chen et al. 2007). Modern bioreactor culture systems provide a more advanced technology to produce higher secondary metabolites from plant cell, tissue or organ using artificial nutrients with MeJA. Yu et al. (2002) found that the ginsenoside content was obviously enhanced by the addition of 100 μM MeJA during adventitious root culture of *Panax ginseng*; Donnez et al. (2011) examined that 0.2 mM MeJA was optimal for the efficient production and high accumulation of resveratrol in grape cell; Shohael et al. (2008) indicated that the accumulation of eleutherosides and chlorogenic acid in the somatic embryo of *Eleutherococcus sessiliflorus* were promoted when 200 μM MeJA was supplied to the culture medium. Our study found that 350 μM MeJA efficiently elicited the hypericin synthesis of *H. perforatum* adventitious roots. Therefore, it can be assumed that the presence of MeJA might be beneficial in triggering the expression of biosynthetic genes in active compounds during plant cell, tissue or organ culture, which results in an increase of metabolite production.

Comparison of hypericin contents from different sources

The hypericin content of adventitious roots reached 1.65 mg g^{-1} DW, which was higher than that of in vitro

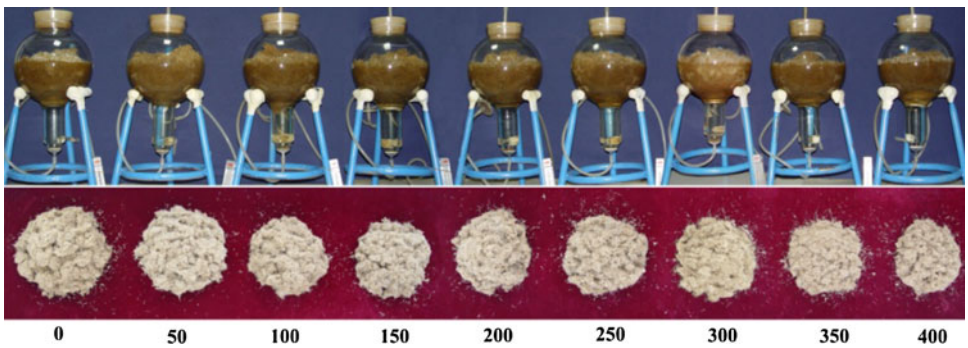

Fig. 3 Effect of MeJA concentration on adventitious root growth after 40 days of bioreactor culture

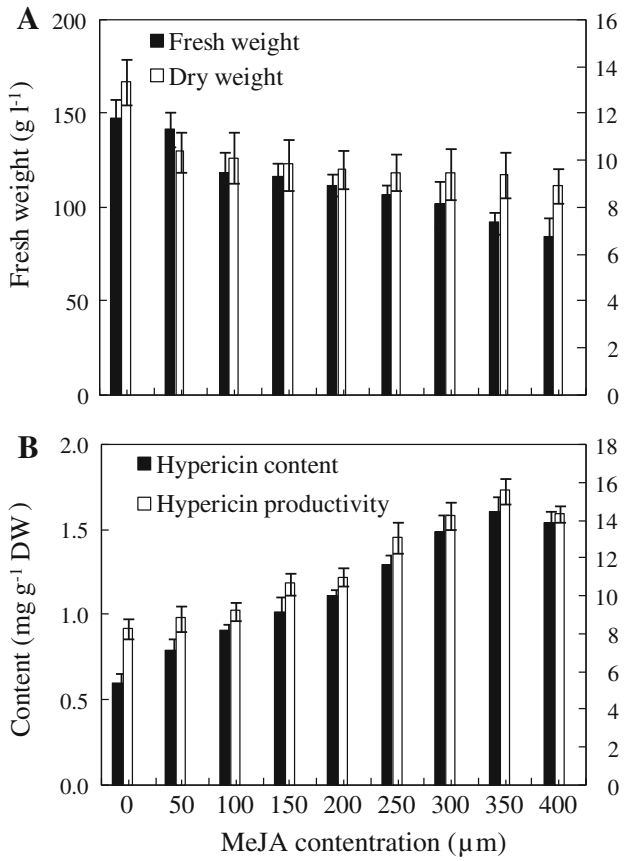

Fig. 4 Effect of MeJA concentration on biomass accumulation and hypericin production after 40 days of adventitious root culture in bioreactors. *Bars* mean ± SE ($n = 3$)

Table 1 Comparison of hypericin content in bioreactor-grown adventitious roots with in vitro-grown plantlets, 1- and 3-year field-grown plants

Different materials	Hypericin content (mg g^{-1} DW)
Bioreactor-grown adventitious roots	1.68 ± 0.065
In vitro-grown plantlets	0.15 ± 0.033
1-year field-grown plants	0.27 ± 0.051
3-year field-grown plants	2.41 ± 0.062

Data represent mean values ($n = 3$) with standard error

plantlets (0.15 mg g^{-1} DW) and 1-year field-grown plants (0.27 mg g^{-1} DW), but lower than 3-year field-grown plants (2.41 mg g^{-1} DW) (Table 1). In general, medicinal plants require long-term cultivation, the biosynthesis of metabolites varies in different seasons and the accumulation quantity of the secondary metabolite is often unstable (Zobayed and Saxena 2004). Hypericin in the field-grown plants of *H. perforatum* during the full flowering stage also showed instability and the contents varied from 1.06 to 2.70 mg g^{-1} DW (Bagdonaitė et al. 2010). In the present study, we obtained 1.65 mg g^{-1} DW of hypericin from adventitious roots in a short term (40 days); thus we could conclude that bioreactor application on adventitious root culture of *H. perforatum* provides an alternative for economical industrial hypericin production.

Conclusion

MS medium supplemented with 1.25 mg l^{-1} IBA and 30 g l^{-1} sucrose at 0.1 vvm air volume and 5.0 g l^{-1} inoculation density is efficient for hypericin production in the bioreactor culture of *H. perforatum* adventitious roots. Moreover, a suitable MeJA concentration (350 μM) can enhance hypericin content and productivity. Some other chemical or biotic elicitors should be used in further studies that aim at increasing the hypericin content of adventitious roots.

Author contribution All authors contributed extensively to the work presented in this paper (several factors affect hypericin production of *Hypericum perforatum* during adventitious root culture in airlift bioreactors). Song-Quan Wu, Xiao-Kun Yu, Mei-Lan Lian and So-Young Park designed the experiments and wrote the paper under the guidance of Xuan-Chun Piao.

Acknowledgments This research was supported by the National Science Foundation of China (81160497 and 31260182). We thank Chungbuk National University for providing experimental materials.

References

Ahmed S, Hahn EJ, Paek KY (2008) Aeration volume and photosynthetic photon flux affect cell growth and secondary metabolite contents in bioreactor cultures of *Morinda citrifolia*. J Plant Biol 51:209–212

Bagdonaitė E, Mártonfi P, Repčák M, Labokas J (2010) Variation in the contents of pseudohypericin and hypericin in *Hypericum perforatum* from *Lithuania*. Biochem Syst Ecol 38:634–640

Baque MA, Hahn EJ, Paek KY (2010) Growth, secondary metabolite production and antioxidant enzyme response of *Morinda citrifolia* adventitious root as affected by auxin and cytokinin. Plant Biotechnol Rep 4:109–116

Büter B, Orlacchio C, Soldati A, Berger K (1998) Significance of genetic and environmental aspects in the field cultivation of *Hypericum perforatum*. Planta Med Nat Prod Med Plant Res 64:431–437

Butterweck V (2003) Mechanism of action of St John's wort in depression: what is known? CNS Drugs 17:539–562

Chakrabarty D, Hahn EJ, Yoon YJ, Paek KY (2003) Micropropagation of apple rootstock M.9 EMLA using bioreactor. J Hortic Sci Biotechnol 78:605–609

Chen LR, Chen YJ, Lee CY, Lin TY (2007) MeJA-induced transcriptional changes in adventitious roots of *Bupleurum kaoi*. Plant Sci 173:12–24

Cui XH, Chakrabarty D, Lee EJ, Paek KY (2010) Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. Biores Technol 101:4708–4716

Cui XH, Murthy HN, Jin YX, Yim YH, Kim JY, Paek KY (2011) Production of adventitious root biomass and secondary metabolites of *Hypericum perforatum* L. in a balloon type airlift reactor. Biores Technol 102:10072–10079

Donnez D, Kim K-H, Antoine S, Conreux A, De Luca V, Jeandet P, Clement C, Courot E (2011) Bioproduction of resveratrol and viniferins by an elicited grapevine cell culture in a 2 L stirred bioreactor. Proc Biochem 46:1056–1062

Hadjur C, Richard MJ, Parat MO, Jardon P, Favier A (2008) Photodynamic effects of hypericin on lipid peroxidation and antioxidant status in melanoma cells. Photochem Photobiol 64:375–381

Jeong JA, Wu CH, Murthy HN, Hahn EJ, Paek KY (2009) Application of an airlift bioreactor system for the production of adventitious root biomass and caffeic acid derivatives of *Echinacea purpurea*. Biotechnol Bioproc Eng 14:91–98

Kirakosyan A, Hayashi H, Inoue K, Charchoglyan A, Vardapetyan H (2000) Stimulation of the production of hypericins by mannan in *Hypericum perforatum* shoot cultures. Phytochemistry 53:345–348

Kornfeld A, Kaufman PB, Lu CR, Gibson DM, Bolling SF, Warber SL, Chang SC, Kirakosyan A (2007) The production of hypericins in two selected *Hypericum perforatum* shoot cultures is related to differences in black gland structure. Plant Physiol Biochem 45:24–32

Lawvere S, Mahoney MC (2005) St. John's wort. Am Fam Phys 72:2249

Lee EJ, Mobin M, Hahn EJ, Paek KY (2006) Effects of sucrose, inoculum density, auxins, and aeration volume on cell growth of *Gymnema sylvestre*. J Plant Biol 49:427–431

Lian ML, Chakrabarty D, Paek KY (2002) Effect of plant growth regulators and medium composition on cell growth and saponin production during cell-suspension culture of mountain ginseng (*Panax ginseng* C.A. Mayer). J Plant Biol 45:201–206

Linde K (2009) St. John's wort-an overview. Forsch Komplementärmed/Res Complem Med 16:146–155

Meruelo D, Lavie G, Lavie D (1988) Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. Proc Nat Acad Sci 85:5230–5234

Min JY, Jung HY, Kang SM, Kim YD, Kang YM, Park DJ, Prasad DT, Choi MS (2007) Production of tropane alkaloids by small-scale bubble column bioreactor cultures of *Scopolia parviflora* adventitious roots. Biores Technol 98:1748–1753

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Murthy HN, Hahn EJ, Paek KY (2008) Adventitious roots and secondary metabolism. Chin J Biotechnol 24:711–716

Schlatmann JE, Nuutila AM, Van Gulik WM, Ten Hoopen HJG, Verpoorte R, Heijnen JJ (2004) Scaleup of ajmalicine production by plant cell cultures of *Catharanthus roseus*. Biotechnol Bioeng 41:253–262

Shohael AM, Murthy HN, Hahn EJ, Lee HL, Paek KY (2008) Increased eleutheroside production in *Eleutherococcus sessiliflorus* embryogenic suspension cultures with methyl jasmonate treatment. Biochem Eng J 38:270–273

Sirvent T, Gibson D (2002) Induction of hypericins and hyperforin in *Hypericum perforatum* L. in response to biotic and chemical elicitors. Physiol Mol Plant Pathol 60:311–320

Southwell IA, Campbell MH (1991) Hypericin in different varieties of *Hypericum perforatum* in Australia. Phytochemistry 30:475–478

Thanh NT, Murthy HN, Yu KW, Seung JC, Hahn EJ, Paek KY (2006) Effect of oxygen supply on cell growth and saponin production in bioreactor cultures of *Panax ginseng*. J Plant Physiol 163:1337

Tolonen A, Hohtola A, Jalonen J (2003) Fast high-performance liquid chromatographic analysis of naphthodianthrones and phloroglucinols from *Hypericum perforatum* extracts. Phytochem Anal 14:306–309

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1:13–25

Walker TS, Pal Bais H, Vivanco JM (2002) Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort). Phytochemistry 60:289–293

Yu KW, Gao WY, Hahn EJ, Paek KY (2002) Jasmonic acid improves ginsenoside accumulation in adventitious root culture of *Panax ginseng* C.A. Meyer. Biochem Eng J 11:211–215

Zobayed S, Saxena PK (2004) Production of St. John's wort plants under controlled environment for maximizing biomass and secondary metabolites. In Vitro Cell Dev Biol Plant 40:108–114