Industrial Crops and Products 41 (2013) 57–63

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Cluster analysis of ginseng tissue cultures, dynamic change of growth, total saponins, specific oxygen uptake rate in bioreactor and immuno-regulative effect of ginseng adventitious root

Juan Wang[a,b], Shuli Man[b,1], Wenyuan Gao[a,b,*], Liming Zhang[b], Luqi Huang[c]

[a] Tianjin Key Laboratory for Modern Drug Delivery and High Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China
[b] Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, China
[c] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

ARTICLE INFO

Article history:
Received 13 February 2012
Received in revised form 1 April 2012
Accepted 5 April 2012

Keywords:
Panax ginseng
Adventitious root
Ginsenoside
HPLC–ESI-MS^n
Bioreactor
Immunoregulation

ABSTRACT

In order to identify ginsenoside composition in native ginseng, cell, hairy root and adventitious root, the ESI-MS^n analysis was performed. Rg_1, Re, Ro, Malonyl-Rb_1, Rb_1, Rc, Rb_2 and Rd were identified from ginseng tissue cultures on the basis of LC–MS^n analysis. The content of total saponins in adventitious root was much higher than that in other tissue cultures. Cluster analysis showed that the quality of adventitious root was mostly similar with native ginseng. During the 5 l bioreactor culture of ginseng adventitious root, the maximum dry weight and total saponin were achieved on day 40 and 30, respectively. Specific oxygen uptake rate (SOUR) increased quickly within the first 10 days. After day 10, SOUR showed a sharp drop and was almost zero after 20 days. Delayed-type hypersensitivity reaction to dinitrofluorobenzene (DNFB) and the proliferation of splenocytes from mice in response to concanavalin A (ConA) were used to evaluate the effects of native ginseng root (NGR) and ginseng adventitious root (GAR) on cellular immune response. As the result, GAR possessed a similar cellular immuno-regulation as NGR had.

1. Introduction

Panax ginseng C.A. Meyer, which belongs to the Araliaceae family, is a famous and widely used herb. It has been used since ancient times as an antioxidant, blood circulation promoter, anti-diabetic agent, pain relief treatment, anti-cancer drug and immune system stimulant (Zhong et al., 1996; Liu and Zhong, 1998). The saponins, known as ginsenosides, are widely believed to be the major bioactive compounds of ginseng. Ginsenosides are divided into three groups based on their structure, the Rb group (protopanaxadiol including Ra_1, Ra_2, Rb_1, Rb_2, Rb_3, Rc, Rd, Rg_3, Rh_2 and others), Rg group (protopanaxatriols including Rg_1, Rg_2, Re, Rf, Rh_1 and others) and the Ro group (oleanolic acid).

In recent years, ginseng has been increasingly used as a health tonic formulated into a variety of commercial health products, including ginseng capsules, soups, drinks and cosmetics, which are marketed in the Asian as well as many other countries around the world (Paek et al., 2005). However, natural sources of wild ginseng, which grows in Korea, China, Russia and Japan, have been overexploited and are therefore very limited. The current supply of ginseng mainly depends on field cultivation, which is an extremely long-lasting and labor-intensive process. Furthermore, intensive replanting of ginseng will lead to replant disease, where a second planting made in the same ground will often fail (Gao et al., 2003). As a result, tissue and organ culture have been exploited as a biotechnological alternative for more efficient and controllable production of ginseng and its active metabolites.

In the cultivation of plant cells as well as organ cultures, the bioreactor culture system offers many advantages over classical tissue culture because the culture conditions in the bioreactor can be optimized by real-time manipulation of temperature, pH, concentration of oxygen, carbon dioxide and nutrients in the medium. World-wide bioreactor production has increased more than 2-fold in volume and value during the past decade and now supplies 1/3 of bioactive secondary metabolites consumed worldwide (Sivakumar et al., 2005a).

Until now, there are no reports regarding the identification of ginsenoside composition in *P. ginseng* tissue cultures. In order to

Abbreviations: MS, Murashige and Skoog; 2,4-D, 2,4-dichlorophenoxyacetic acid; KT, kinetin; IBA, indole-3-butric acid; t_R, retention times; BTBB, balloon-type bubble bioreactor; vvm, volumes of gas per a bioreactor volume; SOUR, specific oxygen uptake rate; OUR, oxygen uptake rate; DW, dry weight; NGR, native ginseng root; GAR, ginseng adventitious root; DNFB, dinitrofluorobenzene; FBS, fetal bovine serum; ConA, concanavalin A.

* Corresponding author at: Tianjin Key Laboratory for Modern Drug Delivery and High Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China. Tel.: +86 22 87401895; fax: +86 22 87401895.
E-mail addresses: biochemgao@163.com, biochemgao@hotmail.com (W. Gao).
[1] Shuli Man is the co-first author.

0926-6690/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2012.04.005

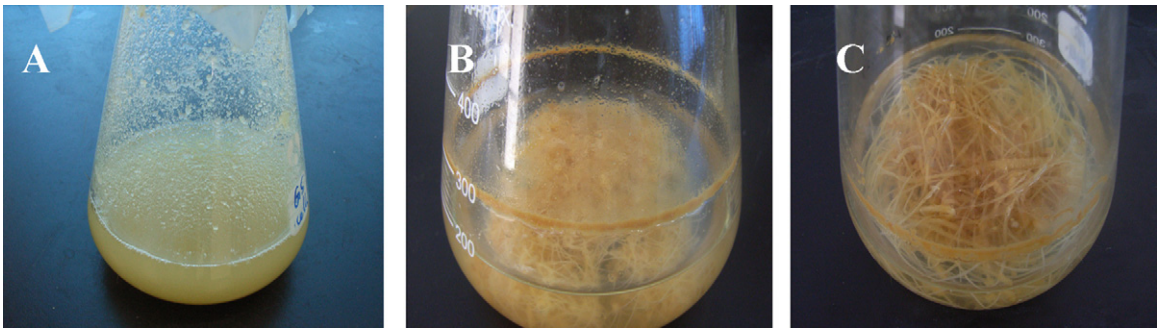

Fig. 1. Three tissue cultures of ginseng (A) cells; (B) adventitious roots; and (C) hairy roots.

select an alternative of native ginseng, the ginsenoside composition in *P. ginseng* tissue cultures was investigated. To provide a theoretical reference for commercial-scale culture of ginseng adventitious root that could be more productive in commercial health products, we conducted studies on 5 l balloon-type bubble bioreactor (BTBB) culture of ginseng adventitious root. In addition, immunoregulative effect of ginseng adventitious roots is also discussed for real application.

2. Materials and methods

2.1. Induction and proliferation of callus

Callus was induced as reported by Huang et al. (2010a). Roots of *P. ginseng* (5-year-old) obtained from Zuo Jia Institute, Chinese Academy of Agricultural Sciences, Jilin, China were collected, washed in water and blotted dry. After spraying the roots with 70% ethanol (20110812, Rionlon, China), the internal tissue of the roots was cut into 5 mm sections and inoculated onto Murashige and Skoog (MS) (1962) media containing 2.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) (D7299, Sigma, USA), 0.5 mg l^{-1} kinetin (KT) (K3378, Sigma, USA), 30 g l^{-1} sucrose and 7 g l^{-1} agar. Each Petri dish (9 cm in diameter and 1.5 cm in height) was sealed and placed at 23 ± 2 °C in the dark. After 4 weeks, induced calli were subcultured to the same medium every 4 weeks.

2.2. Shake flask culture of suspension cells

Suspension cells were initiated from callus according to Langhansova et al. (2005), with some modifications. 10 g fresh weight of cells were cultivated in 0.5 l conical flasks with 200 ml MS medium supplemented with 2.0 mg l^{-1} 2,4-D, 0.5 mg l^{-1} KT and 30 g l^{-1} sucrose. Suspension cells were cultured on a rotary shaker (HNY200B, Honour, China) (120 rpm) at 23 ± 2 °C. Subculture was routinely conducted at 3 weeks interval (Fig. 1A).

2.3. Induction and proliferation of hairy roots

Hairy roots were induced from the plant roots after infection with *Agrobacterium rhizogenes* strain A4 as reported by Mallol et al. (2001). Sterilized root discs were wounded with a sterile needle loaded with *A. rhizogenes* suspension grown in liquid YEB medium (Vervliet et al., 1975) for 24 h at 28 ± 2 °C on a rotary shaker at 100 rpm. The inoculated root discs were placed on MS solid medium, After 2 days of cocultivation, the explants were transferred to the fresh medium containing 500 mg l^{-1} cefotaxime in order to eliminate the bacteria. After cultivation for one or two months, roots started to appear at the infection sites and, in order to obtain the root lines, single roots were picked off and placed onto new media together with 500 mg l^{-1} cefotaxime, until the bacteria were eliminated. Hairy roots with no bacterial contamination were transferred to hormone-free MS liquid medium and subcultured every 4 weeks in fresh medium (Fig. 1C).

2.4. Induction and proliferation of adventitious roots

Adventitious roots were initiated from callus according to Huang et al. (2010a). Calli were inoculated onto MS solid media containing 5.0 mg l^{-1} indole-3-butric acid (IBA) (15386, Sigma, USA), 0.1 mg l^{-1} KT, 30 g l^{-1} sucrose and 7 g l^{-1} agar. After 4 weeks of culture, adventitious roots were induced. Separated roots were transferred to liquid 3/4 strength MS medium supplemented with 5.0 mg l^{-1} IBA and 4% sucrose. 2 g fresh weight of adventitious roots were cultivated in 0.5 l conical flask containing 200 ml same liquid medium on a rotary shaker (120 rpm) at 23 ± 2 °C and subcultured every 4 weeks (Fig. 1B).

2.5. Bioreactor culture of adventitious roots

Bioreactor cultures were initiated by inoculating 15 g fresh weight of adventitious roots into a 5 l BTBB containing 3 l 3/4 strength MS liquid medium supplemented with 5.0 mg l^{-1} IBA and 4% sucrose. The air volume was adjusted to a constant flow rate of 0.1–0.2 volumes of gas per a bioreactor volume (vvm) during BTBB cultivation as previously described by Jeong et al. (2006). Samples were taken on day 0, 10, 20, 30 and 40 to determine the dry weight, total ginsenoside content and specific oxygen uptaker rate (SOUR). Each experiment was repeated at least 3 times.

2.6. Experimental mouse

Male Kun-Ming mice (China), weighing 23–28 g were used in all experiments. Animals were obtained from the Institute of Laboratory Animal of Chinese Academy of Medical Science, Beijing, China. The animals were fed with a rodent standard diet with free access water ad libitum and were housed in rooms maintained at 25 ± 1 °C with a 12 h light/dark cycle following international recommendations. The Animal Ethics Committees of the Faculty of Medicine, approved all experimental protocols, in accordance with 'Principles of Laboratory Animal Care and Use in Research' (Ministry of Health, Beijing, China).

2.7. Experimental groups

The mice below were all randomly divided into 3 groups:

- The non-treated group (control group) received an oral administration of 10 ml of 0.9% sodium chloride (E11071502, Tiancheng, China) per kg of body weight every day for 6 days.
- NGR group received an intragastric administration of 200 mg of NGR in 5 ml of 0.9% sodium chloride per kg of body weight every day for 6 days.