Saudi Journal of Biological Sciences (2014) 21, 185–190

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Proteomic analysis of differentially expressed proteins induced by salicylic acid in suspension-cultured ginseng cells

Jiaman Sun [a,b], Junfan Fu [a,*], Rujun Zhou [a]

[a] Department of Plant Protection, Shenyang Agriculture University, Shenyang 110161, China
[b] Guangxi Crop Genetic Improvement and Biotechnology Key Lab, Nanning 530007, China

Received 7 April 2013; revised 21 September 2013; accepted 29 September 2013
Available online 9 October 2013

KEYWORDS
Ginseng cells;
Salicylic acid;
Proteome

Abstract In this study, optimized 2-DE sample preparation methodologies were established for suspension-cultured ginseng cells. Three commonly used protein extraction methods (Trichloroacetic acid-acetone, urea/thiourea and phenol extraction method) were evaluated for proteomic analysis of suspension cultures of ginseng. A comparative analysis of suspension-cultured ginseng cells proteome induced by salicylic acid (SA) was reported. The results demonstrated that phenol extraction method was the best method based on protein extraction efficiency and the good quality of 2-DE patterns for suspension-cultured ginseng cells. Fifteen differentially expressed proteins induced by salicylic acid in suspension-cultured ginseng cells were identified by MALDI-TOF-MS. These identified proteins were involved in defense and stress response, energy metabolism, signal transduction/transcription, protein synthesis and metabolism, and photosynthesis. Chaperonin 60, related to defense responses, was more abundant in suspension-cultured ginseng cells after application of SA. Vacuolar ATPase subunit B was newly induced in SA treatment.

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer) is widely cultivated as a medicinal herb in northeast China (Wang, 2001). The ginseng dried roots have been widely used as a traditional medicine since ancient times because of its stimulative and tonic properties (Ali et al., 2006). Plant cell culture is an important plant biotechnology tool for the growth of plant material. With increasing demand for ginseng worldwide, plant cell culture is necessary for the growth of ginseng (Ali et al., 2007). As is known, salicylic acid (SA) is one of the key components activating resistance pathways (Vlot et al., 2009). Exogenous application of SA could not only induce antioxidant enzyme activities and formation of pathogenesis-related proteins, but also stimulates the expression of defense genes in many plants (Hwang et al., 1997; Lu and Chen, 2005; Wen et al., 2005; Fernandes et al., 2006; Thulke and Conrath, 1998; Murphy

* Corresponding author. Address: Dongling Road No. 120, Shenyang 110161, China. Tel.: +86 24 88492892; fax: +86 24 88487148.
E-mail address: fujunfan@163.com (J. Fu).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1319-562X © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.sjbs.2013.09.011

et al., 2000; Rajjou et al., 2006). Many studies focused on the effect of SA on enhancing plants resistance. However, little research paid attention to the proteome of suspension-cultured ginseng cells induced by SA.

In this study, we established the suspension culture system of ginseng and made a comparison among three protein extraction methods to find out the best method of protein extraction for 2-DE analysis in suspension-cultured ginseng cells. Moreover, the differentially expressed proteins induced by SA in suspension-cultured ginseng cells were analyzed and identified by MALDI-TOF-MS.

2. Materials and methods

2.1. Chemicals

IPG gel strips, IPG buffer, urea, thiourea, CHAPS, iodoacetamide, DTT, acrylamide, and TBP were obtained from Bio-Rad (USA). Salicylic acid and other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2. Preparation of suspension-cultured ginseng cells and SA treatment

Two-year-old fresh ginseng roots were washed clean and then sterilized in 75% ethanol for 2 min followed by 7 min in 0.1% $HgCl_2$. The surface-sterilized root segments were placed on solid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing a full complement of salts and vitamins (Rahman and Punja, 2005). Kinetin (KT) (0.2 mg/L) and 2,4-dichlorophenoxyacetic acid (2,4-D) (3 mg/L) were used as the growth regulators. Cultures were maintained at 25 ± 2 °C for 4 weeks for callus induction (Ali et al., 2006). Suspension cultures were initiated as described by Punja et al. (2004). The actively proliferating suspension cultures were treated with 1 mM SA and harvested for 48 h after treatment. Suspension-cultured ginseng cells treated with deionized water were used as the control. These treated samples were frozen in liquid nitrogen and stored at −80 °C until protein extraction.

2.3. Protein extraction

To find out an ideal method of protein extraction for 2-DE analysis in suspension-cultured ginseng cells, three protein extraction methods (trichloroacetic acid (TCA)-acetone, urea/thiourea, and phenol extraction method) were investigated.

2.3.1. TCA-acetone precipitation

TCA-acetone precipitation was performed according to the protocol described by Kim et al. (2003). The suspension-cultured ginseng cells were ground to a fine powder with a pestle in liquid nitrogen. The powder was incubated in a sample buffer I (0.3% SDS, 50 mM Tris–HCl pH 8.0, 200 mM DTT) at 100 °C for 10 min, and then transferred onto ice and incubated with 0.1 volume of sample buffer II (RNase A 0.25 mg/mL, DNase I 1 mg/mL, 50 mM Tris–HCl pH 8.0, 50 mM $MgCl_2$) for 10 min. After centrifugation at 13,000*g* for 30 min, the supernatant was precipitated with acetone and incubated overnight at −20 °C. After centrifugation (13,000*g*, 30 min), the pellet was washed three times with ice-cold 80% (v/v) acetone and lyophilized.

2.3.2. Urea/thiourea method

Total protein extraction was performed according to the protocol described by Rabilloud (1998) with minor modification. Briefly, the suspension-cultured cells were ground to a fine powder and homogenized with extraction buffer (8 M urea, 2 M thiourea, 2 mM disodium EDTA salt, 4% (w/v) CHAPS, 65 mM DDT, 2% (v/v) ampholyte (pH 3–10), and 1% TBP) by vortexing for 3 h at 30 °C, followed by centrifugation (13,000*g*) for 30 min. The supernatant was precipitated with ice-cold acetone and incubated overnight at −20 °C. The precipitated proteins were washed with cold 80% (v/v) acetone three times and lyophilized.

2.3.3. Phenol extraction method

The method was manipulated from the protocol described by Carpentier et al. (2005) with some modifications. The suspension-cultured ginseng cells were ground to a fine powder and extracted with extraction buffer (0.7 M sucrose, 0.5 M Tris–HCl, pH 8.0, 10 mM disodium EDTA salt, 4 mM ascorbic acid, 2% (v/v) 2-ME, and 1% isopropanol) on ice, incubated at 4 °C for 30 min. The homogenate was vortexed with ice-cold Tris-buffered phenol (pH 8.0), and the upper phenol phase was mixed with ice-cold 0.1 M ammonium acetate in methanol, vortexed briefly and incubated at −20 °C overnight before centrifuging at 13,000*g* for 30 min. The pellet was washed with cold 80% (v/v) acetone, lyophilized and stored at −80 °C until use.

2.3.4. 2-Dimensional electrophoresis analysis

The protein concentration was determined according to Bradford's method (1976) after resuspended in a solubilization buffer (9 M urea, 2 M thiourea, 2% (w/v) CHAPS, 50 mM DTT, 1% tributylphosphine (TBP) and 2% (v/v) Bio-Lyte 3/10 Bio-Rad ampholytes). IEF was carried out using 17 cm pH 5–8 linear IPG strips in a PROTEAN IEF Cell (Bio-Rad). The loading sample volume used was 350 μl of protein extract, corresponding to a protein amount of 0.8 mg per strip. Focusing was carried out in 4 steps (250 V for 3 h, 500 V for 3 h, 1000 V for 1 h, and 10,000 V for 9 h). SDS–PAGE was performed using 12% polyacrylamide gels and runs at 5 mA/gel for 30 min and then at 60 mA/gel for 6 h in a PROTEIN II electrophoresis kit (Bio-Rad). The experiment was performed three times.

Coomassie brilliant blue (CBB) R-350 (Sigma, USA) was used to stain the 2-DE gels after electrophoresis. Images of the 2-DE gels were scanned using an Image Scanner (PowerLook 2100XL, UMAX). The image analysis of the gels was performed using PDQuest™ 2-D Analysis Software 8.0 (Bio-Rad). Only spots with an abundance ratio of at least two were considered as the differentially expressed proteins. The selected protein spots were subjected to identification by MALDI-TOF-MS.

2.4. In-gel digestion and protein identification

Protein spots were excised from the CBB-stained gels, washed with 100 mM NH_4HCO_3/30% CAN solution, and then dried in a vacuum concentrator. The proteins were digested in-gel