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Biotransformation of saponins to astragaloside IV from Radix Astragali by immobilized *Aspergillus niger*

Cai-Yun Chen [b], Yu-Jie Fu [a,c,*], Yuan-Gang Zu [a,c], Wei Wang [a,c], Fan-Song Mu [a,c], Meng Luo [a,c], Chun-Ying Li [a,c], Cheng-Bo Gu [a,c], Chun-Jian Zhao [a,c]

[a] State Engineering Laboratory of Bio-Resource Eco-Utilization, Northeast Forestry University, Harbin 150040, PR China
[b] College of Pharmacy, Binzhou Medical College, Yantai 264003, People's Republic of China
[c] Engineering Research Center of Forest Bio-Preparation, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China

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ABSTRACT

Eight different strains of yeasts, *Aspergillus niger*, *Aspergillus oryzae* and *White-rot fungus* were tested for biotransformation of saponins to astragaloside IV in potato medium with Radix Astragali. Among these strains, immobilized cells of *A. niger* M85 exhibited the best performance on the biotransformation of saponins to astragaloside IV from Radix Astragali. After the biotransformation with immobilized *A. niger* M85, the contents of astragaloside IV and total astragalosides in Radix Astragali increased 10.7-fold (2.326 mg/g) and 8.6-fold (2.56 mg/g) to the solvent extraction sample, respectively. Optimization was done at incubation period 5 days, the amount of Radix Astragali powder 2 g, medium volume 50 mL/g, incubation temperature 30 °C and initial pH 6.0. The immobilized *A. niger* M85 can be reused seven times and retained 71% of its residual activity. The *A. niger* M85 was found to be a highly efficient producer of astragaloside IV.

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1. Introduction

Radix Astragali, known as Huangqi in China, is derived from the dried roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bunge. It is one of the oldest and most commonly used plants in traditional Chinese medicine and healthy food supplements. It was reported to have immunostimulant, hepatoprotective, antidiabetic, analgesic, expectorant, antioxidant and antiviral effects and was applied for the treatment of nephritis, diabetes, albuminuria, hypertension, cirrhosis, cancer, etc. (Cho and Leung, 2007; Li et al., 2011; Huang et al., 2009; Li et al., 2001; Gui et al., 2006). Traditionally, Radix Astragali functions as a major herb in various herbal decoctions either alone or together with other crude drugs for oral administration. Nowadays, various Radix Astragali preparations are commercially available, such as oral solution, injection and concentrated granule. In a word, Radix Astragali is attracting more and more attention not only as a Traditional Chinese Medicine component but also as a dietary supplement due to its low toxicity, fewer or even no complications, higher pharmacological activity as well as nourishing tonic (Xue and Roy, 2003).

Chemical investigations and pharmacological studies have demonstrated that triterpene saponins, isoflavonoids and polysaccharides in Radix Astragali are major beneficial compounds responsible for their bioactivities (Qi et al., 2006). Chemical structures of saponins in Radix Astragali are shown in Table 1 and they can transform into each other in certain conditions (Kitagawa et al., 1983). Astragaloside IV (AG IV), a natural triterpene saponin known as the "marker compounds" of *Huangqi* injections, has been reported to have comprehensive pharmacological actions. Many reports have shown that AG IV can be used to improve heart function, strengthen the heart contraction, improve the skin blood circulation and nutrition, and protect the liver against glycogen reduction to inhibit liver fibrosis (Liu et al., 2009; Chen et al., 2011; Chen et al., 2008; Wang et al., 2009; Zhang et al., 2006). However, the content of AG IV in Radix Astragali is relatively low (less than 0.04%, w/w), and the isolation of AG IV from natural products is extremely difficult and costly (Song et al., 2008).

Microbial fermentation has been widely applied in biotechnology fields, such as biotransformation, bioremediation and biofuels (Núñez and Lema, 1987; Arai et al., 2010; Ye et al., 2010). This technique is more economical and environment-friendly. It can decrease the organic solvent consumption, improve the production yield and easily be carried out in a large-scale industrial process. Several microorganisms, like bacteria, yeast and fungal species, have been reported to enhance production of fermented

* Corresponding author at: State Engineering Laboratory of Bio-Resource Eco-Utilization, Northeast Forestry University, Harbin 150040, PR China.
Tel./fax: +86 451 82190535.
E-mail address: yujie_fu2002@yahoo.com (Y.-J. Fu).

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Table 1
Chemical structures of relevant saponins in Radix Astragali. Glu: Glucose.

Number	Name of compounds	Structure	R
1	Astragaloside I		R_1=Ac, R_2=Ac, R_3=H, R_4=Glu, R_5=H
2	Astragaloside II		R_1=Ac, R_2=H, R_3=H, R_4=Glu, R_5=H
3	Astragaloside III		R_1=Glu, R_2=H, R_3=H, R_4=H, R_5=H
4	Astragaloside IV		R_1=H, R_2=H, R_3=H, R_4=Glu, R_5=H
5	Astragaloside V		R_1=Glu, R_2=H, R_3=H, R_4=H, R_5= Glu
6	Astragaloside VI		R_1=Glu, R_2=H, R_3=H, R_4=Glu, R_5=H
7	Astragaloside VII		R_1=H, R_2=H, R_3=H, R_4=Glu, R_5=Glu
8	Astramembrannin II		R_1=H, R_2=H, R_3=H, R_4=H, R_5=H
9	Isoastragaloside I		R_1=Ac, R_2=H, R_3=Ac, R_4=Glu, R_5=H
10	Isoastragaloside II		R_1=H, R_2=Ac, R_3=H, R_4=Glu, R_5=H
11	Acetyl-astragaloside I		R_1=Ac, R_2=Ac, R_3=Ac, R_4=Glu, R_5=H

extracts (Maragkoudakis et al., 2010; Arifin et al., 2011). In the previous research, Ye et al. (2011) reported that *Absidia corymbifera* AS2 can transform crude astragalosides containing 26% astragalosides to AG IV and this strain enhanced AG IV production approximately four fold. However, the shortages of biotransformation by *A. corymbifera* AS2 are obvious. *A. corymbifera* is a pathogenic fungus to human (Tiong et al., 2006) so it is not suitable for medicine and health production. Moreover, its biotransformation efficiency for AG IV production is relatively lower with the higher cost and limited biotransformation yield. Numerous biotechnological processes are facilitated by immobilization technology. Calcium alginate is considered to be the most common immobilization matrix. It is a readily available non-toxic biological material and an ideal immobilization matrix for bio-molecules and microorganisms (Oliveira et al., 2011). Therefore, in the present study, calcium alginate was used to immobilize the selected strain cells in order to produce enhanced AG IV. Moreover, cell immobilization is a very appealing approach because it offers a number of unique advantages over free-cell fermentation, such as easy product isolation, reusability of the immobilized cell beads, prevention of washout, reduced risk of contamination and operational stability (Tsekova et al., 2010). Furthermore, using the immobilized technology, a dense cell culture can be established, leading to the synthesis of complicated bio-products with the multi-enzyme catalysis.

Thus, in the present study, the purpose was to screen a more effective microorganism and set up a convenient biotransformation method to improve the content of AG IV from Radix Astragali. Various parameters in the biotransformation process such as incubation period, the amount of Radix Astragali powder, medium volume, incubation temperature and pH were optimized. Up to now, this is the first report on the biotransformation of saponins to AG IV with immobilized cell from Radix Astragali.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile and methanol used were of HPLC grade (J & K Chemical Ltd., China). Formic acid of HPLC grade was purchased from Dima Technology INC. (USA). Ethanol, ethyl acetate, n-butanol, acetic acid and perchloric acid obtained from Tianjin Chemical Reagents Co. (Tianjin, China) were of analytical grade. Vanillin and oieanolic acid standard were purchased from Beijing Chemicals and Reagents Co. (Beijing, China). Distilled deionized water was supplied using a Milli-Q water purification system (Millipore, MA, USA). AG IV (≥98.0%) was purchased from Fluka (Switzerland). All solutions and samples prepared for LC-MS/MS were filtered through 0.45 μm nylon membranes (Millipore, MA, USA) before injecting.

2.2. Preparation of raw material

Dried roots of *A. membranaceus* var. *mongholicus* (Bunge) Hsiao were purchased from the medicinal materials market of Harbin, China, and authenticated by Prof. Shao-Quan Nie. The dried roots were pulverized in a disintegrator (HX-200 A, Yongkang Hardware and Medical Instrument Plant, China) to obtain a fine powder. Two gramsg of Radix Astragali root powder was extracted under optimum microwave-assisted extraction (MAE) conditions with 80% ethanol solution, ratio of solid/liquid of 1:25 (g/mL), at an extraction temperature of 70 °C, with three extraction cycles, each for 5 min under microwave irradiation power of 700 W (Yan et al., 2010). Then, the filtrates were combined and analyzed as the solvent extraction sample or concentrated to dryness on a rotary evaporator (RE-52AA, Shanghai Huxi Instrument, Shanghai, China) at 50 °C. The residues were dissolved in 10 mL of deionized water for further use.

2.3. Microorganism and preparation of inoculum

The strains of yeasts, *Aspergillus* niger, *Aspergillus oryzae* and *White-rot fungus*, namely yeast DQY-1, JB, *A. niger* 3.3148, *A. niger* M85, *A. oryzae* 3.302, *A. oryzae* Y29, *White-rot fungus* 5.776 and F-9, were obtained from laboratory stocks. They were maintained on potato dextrose agar (PDA) plates at 4 °C and periodically subcultured. Inoculum preparation (spore suspension) was done according to the suggested method (Alam et al., 2008). Each strain was first cultured on PDA plates at 30 °C for 5 days. Then, plates containing fungal spores were washed with sterile water to obtain a spore suspension. The spore count of the suspension was counted using a Neubauer hematocytometer and the inoculum was adjusted to 1×10^7 spores/mL.

2.4. Screening of the potential strain and medium

For yeasts, *A. niger, A. oryzae and White-rot fungus* strains, 20% potato extract with 2% glucose medium (M_1), 2% sucrose medium (M_2) or no sugar medium (M_3) were used in this study. 100 mL of medium and two grams Radix Astragali powder or extract (prepared Section 2.2) were taken into 250 mL Erlenmeyer flasks and autoclaved at 121 °C for 30 min. After cooling at room temperature, 10 mL of the prepared spore suspension was inoculated under aseptic conditions and incubated at 30 °C for 4 days on

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