Process Biochemistry 49 (2014) 807–812

Contents lists available at ScienceDirect

Process Biochemistry

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

A novel biotransformation of astragalosides to astragaloside IV with the deacetylation of fungal endophyte *Penicillium canescens*

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ARTICLE INFO

Article history:
Received 25 September 2013
Received in revised form 15 February 2014
Accepted 17 February 2014
Available online 25 February 2014

Keywords:
Biotransformation
Deacetylation
Penicillium canescens
Astragalosides
Astragaloside IV

ABSTRACT

Under the deacetylation of fungal endophyte *Penicillium canescens*, which was isolated from pigeon pea, a novel and highly efficient biotransformation method of astragalosides to astragaloside IV in Radix Astragali was investigated. After single factor tests of the biotransformation procedure, the optimum biotransformation conditions were confirmed as the liquid solid ratio 20:1, the biotransformation temperature 30 °C, time 36 h and pH 7, respectively. Final content of astragaloside IV in Radix Astragali reached 7.66 ± 0.44 mg/g, which was 5.51-fold to that of untreated one and contents of astragaloside I and astragaloside II significantly decreased. The immobilized Ca-alginate gel beads with *P. canescens* could be reused at least for 13 runs. This is the first report that fungal endophyte was applied for the biotransformation of astragalosides to astragaloside IV in Radix Astragali and this novel high-efficiency biotransformation method will be an alternative to enhance the content of astragaloside IV in Radix Astragali in commercial process.

1. Introduction

Astragaloside IV (AG IV), a representative cycloartane-type triterpene glycoside, is used broadly in food and pharmaceutical fields because of its remarkable bioactivities. It is derived from dried roots of Radix Astragali such as *Astragalus membranaceus* (Fisch.) Bge. var. *mongho-licus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bunge. [1]. Modern researches have shown that AG IV demonstrates a series of pharmacological activities, such as anticancer activity [2], preventing glucose-induced podocyte apoptosis [3], immune-enhancing effect [4] and protecting against focal cerebral ischemia or reperfusion injury [5]. However, the content of AG IV is relatively low in Radix Astragali (<0.04%, w/w) [6] and its application is limited. Due to the outstanding pharmacological activities and low content of AG IV in Radix Astragali, some researchers have devoted to transforming other low bioactivity astragalosides including AG I and AG II to AG IV, such as alkaline hydrolysis and commercial enzyme hydrolysis, thus enhancing the medicinal values of Astragali Radix [7,8]. However, use of alkali may destroy the structure of target compound and use of the commercial enzymes can significantly raise the cost in commercial application. Besides, there was one attempt to enhance the content of AG IV in commercial crude astragalosides extracts by using microbial transformation of *Absidia corymbifera* AS2 bought from China Committee for Culture Collections of Microorganisms (CCCCM). However, the shortcomings were that the crude astragalosides extracts were expensive and the time of biotransformation procedure was very long (72 h) [9]. Therefore, an environmentally friendly and time-saving method to enhance the content of AG IV in Radix Astragali is urgent for the large-scale production of AG IV in commercial process.

Biotransformation can be defined as chemical reactions catalyzed by different enzymes of various microbial cells, it can be an alternative for biosynthesis of some important drug metabolites [10]. Endophytes are considered to be bacterial or fungal microorganisms that colonize healthy plant issues without causing any apparent symptoms [11]. On the basis of previous researches, endophytes can produce many bioactive metabolites. The various metabolites were widely used for biosynthesis and biodegradation [12,13]. Recently, the application of endophytes on biotransformation has also attracted people's attention. Due to the endophytes'

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http://dx.doi.org/10.1016/j.procbio.2014.02.014
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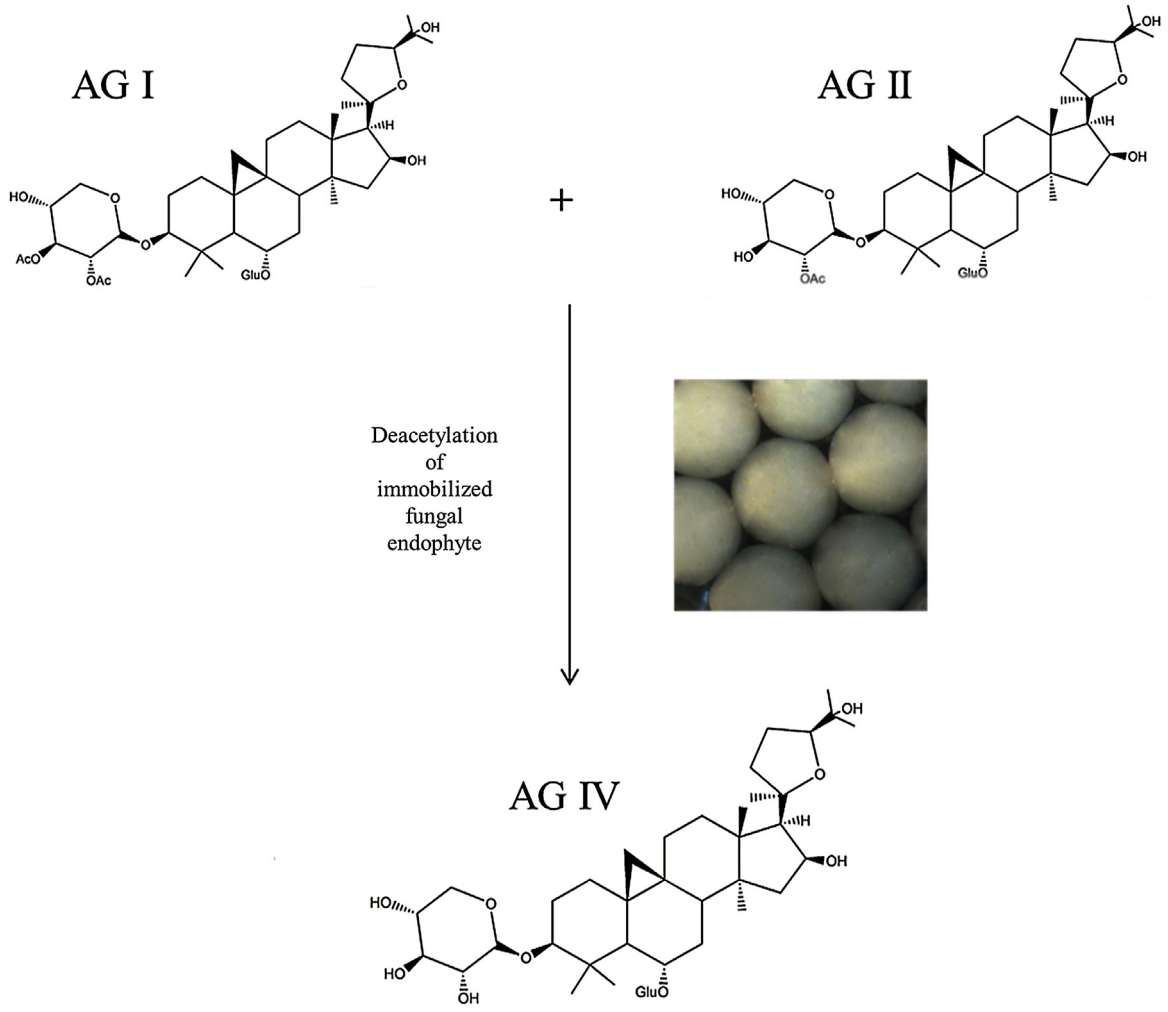

Fig. 1. The equation of the biotransformation of astragalosides to astragaloside IV with immobilized fungal endophyte (Glu: glucose).

special living environment and long term co-evolutionary with their hosts, the endophytes have developed a specific lifestyle to maintain a stable symbiosis and they can produce various extracellular enzymes for secondary metabolites biosynthesis to resist kinds of stress from environment, insects, microorganism, etc. Therefore, endophytes have been used for some complex reactions to obtain more active compounds instead of traditional chemical methods [14]. The application of endophytes on biotransformation of region- and stereoselective production was a feasible strategy to obtain more active substances; they have been used in natural compounds conversion such as thioridazine, flavans and tetrahydrofuran lignan [15–17]. Chemical structure analysis of astragalosides suggests that astragalosides can be transformed to AG IV if the acetyl groups are released (Fig. 1). There are only a few reports about deacetylation microorganisms for chitin deacetylation; however, there is almost no report about deacetylation effect by fungal endophytes [18]. Therefore, it will be of great importance and significance in searching for an effective fungal endophyte with deacetylation function that can be applied for the biotransformation of astragalosides to AG IV. As microorganisms can produce continuous effective enzymes, immobilization of microorganism used for biotransformation procedures has recently been an efficient method. Moreover, it can improve the stability of microorganism cells. Environmental friendliness, high substrate tolerance, reusability and easy separation from the interaction substrates are all the benefits of immobilization method [19].

Based on our previous screening tests, a special fungal endophyte *Penicillium canescens* isolated from pigeon pea was found to have strong deacetylation ability, therefore, the biotransformation of astragalosides to astragaloside IV with the deacetylation of fungal endophyte *P. canescens* was investigated in the present study. To the best of our knowledge, fungal endophyte with deacetylation function was first applied for biotransformation of astragalosides to astragaloside IV. The present study aimed to enhance the content of AG IV in Radix Astragali by immobilized fungal endophytes cells. The optimization of biotransformation conditions was conducted by single factor tests of the biotransformation procedure. This strategy will provide an alternative to enhance the content of AG IV in Radix Astragali and make it possible to the large-scale production of AG IV in commercial process.

2. Materials and methods

2.1. Materials

AG I and AG II (≥98.0%) were purchased from ChromaDex (Santa Ana, USA), AG IV and Ginsenoside Rg1 (≥95.0%) were bought from Fluka (Switzerland). Ginsenoside Rg1 was used as the internal standard (IS). In our previous research [20], *P. canescens* was firstly isolated from pigeon pea (*Cajanus cajan* [L.] Millsp.) and identified based on ITS sequences (Fig. 2). Sequence data has been submitted to GenBank under the accession number JQ356543