J Ginseng Res 40 (2016) 121–126

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Production of ginsenoside F1 using commercial enzyme Cellulase KN

Yu Wang [1,2], Kang-Duk Choi [3], Hongshan Yu [1], Fengxie Jin [1,**], Wan-Taek Im [2,3,*]

[1] College of Biotechnology, Dalian Polytechnic University, Ganjingzi-qu, Dalian, PR China
[2] Department of Biotechnology, Hankyong National University, Anseong, Korea
[3] Genomic Informatics Center, Graduate School of Future Convergence Technology, Hankyong National University, Anseong, Korea

ARTICLE INFO

Article history:
Received 27 July 2014
Received in Revised form
8 June 2015
Accepted 15 June 2015
Available online 19 June 2015

Keywords:
biotransformation
Cellulase KN
ginsenoside
ginsenoside F1
mass production

ABSTRACT

Background: Ginsenoside F1, a pharmaceutical component of ginseng, is known to have antiaging, antioxidant, anticancer, and keratinocyte protective effects. However, the usage of ginsenoside F1 is restricted owing to the small amount found in Korean ginseng.
Methods: To enhance the production of ginsenoside F1 as a 10 g unit with high specificity, yield, and purity, an enzymatic bioconversion method was developed to adopt the commercial enzyme Cellulase KN from *Aspergillus niger* with food grade, which has ginsenoside-transforming ability. The proposed optimum reaction conditions of Cellulase KN were pH 5.0 and 50°C.
Results: Cellulase KN could effectively transform the ginsenosides Re and Rg1 into F1. A scaled-up biotransformation reaction was performed in a 10 L jar fermenter at pH 5.0 and 50°C for 48 h with protopanaxatriol-type ginsenoside mixture (at a concentration of 10 mg/mL) from ginseng roots. Finally, 13.0 g of F1 was produced from 50 g of protopanaxatriol-type ginsenoside mixture with 91.5 ± 1.1% chromatographic purity.
Conclusion: The results suggest that this enzymatic method could be exploited usefully for the preparation of ginsenoside F1 to be used in cosmetic, functional food, and pharmaceutical industries.

1. Introduction

Ginseng, a well-known medicinal plant, has not only been used as a traditional medicine in East Asia for thousands of years, but has also gained recognition in the past decade in the West for its beneficial uses [1–3]. Many previous reports have shown that ginseng has extensive pharmacological and therapeutic utility. Ginsenosides, the major components of ginseng, appear to be responsible for the principal biological and pharmacological activities underpinning anticancer, antitumor, antiallergic, anti-inflammatory, and antioxidant effects [4–10]. Based on the structure of the aglycon and the dammarane skeleton, ginsenosides can be categorized as protopanaxadiol, protopanaxatriol (PPT), and oleanane saponins [11]. The PPT-type ginsenosides are further identified by the number of sugar moieties and the position of a glycosidic bond at the aglycon C6 and C20. After oral intake of ginseng, the major ginsenosides are hydrolyzed into the more active minor ginsenosides through human intestinal digestion, and are further absorbed into the blood. Therefore, conversion of the major ginsenosides, which account for > 80% of the total ginsenosides, to the highly active minor ginsenosides may have a significant impact on the pharmaceutical industry [4,5].

The minor ginsenoside F1 is present in low concentrations in Korean ginseng. It can be processed through deglycosylation by intestinal microflora from the major ginsenosides Re and Rg1; it is absorbed into the blood, where it exerts an estrogenic effect [12]. Ginsenoside F1 exhibited anticancer effect, showing strong suppression of B16 cell proliferation [13]. Furthermore, ginsenoside F1 shows antiaging and antioxidant effects, and exhibits competitive inhibition of the activity of CYP3A4 and weaker inhibition of the activity of CYP2D6 [14,15].

* Corresponding author. Department of Biotechnology, Hankyong National University, 327 Chungang-no Anseong, Kyonggi, 456-749, Korea.
** Corresponding author. College of Biotechnology, Dalian Polytechnic University, Qinggong-yuan Number 1, Ganjingzi-qu, Dalian 116034, PR China.
E-mail addresses: fxjin@dlpu.edu.cn (F. Jin), wandra@hknu.ac.kr (W.-T. Im).

1226-8453/$ – see front matter
http://dx.doi.org/10.1016/j.jgr.2015.06.003

Ginsenoside F1 can be produced using microbes or through enzymatic processes. One example is *Fusarium moniliforme* var. *sublutinas*, which has β-glucosidase activity and has shown the ability to convert ginsenoside Rg1 into F1 [16]. Another example is commercially available naringinase from *Penicillium decumbens* [17], which has the ability to hydrolyze glucose and the rhamnose moieties at the C6 position to produce ginsenoside F1 from Re and Rg1. However, in that study, the researchers conducted only a simple enzyme presentation, without further scale-up or process engineering. Although several PPT-type ginsenoside-hydrolyzing recombinant enzymes have been constructed [18–22], the majority of these have the ability to hydrolyze the glucose moiety at the C20 position of aglycon to produce Rg2(*S*) or Rh1(*S*) from Re or Rg1, respectively, rather than the glucose moiety at the C6 position of aglycon. Among these, two enzymes were reported to have hydrolyzing activity for the glucose moiety at the C6 position of PPT aglycon to produce ginsenoside F1 [19,21]. However, the researchers conducted only a simple enzyme characterization, without further scale-up or process engineering.

The experiment in this study was designed to overcome the above disadvantages and meet the industrial demand for mass production of ginsenoside F1 as a food-grade health supplement. From several commercial enzymes capable of transforming ginsenosides, we selected Cellulase KN on the basis of its high activity for transforming ginsenosides Re and Rg1 into ginsenoside F1. Treatment of a PPT-type ginsenoside mixture (PPTGM) composed of Re and Rg1 mainly with Cellulase KN followed by purification yielded 10 gram-scale F1 with high purity. This is the first report of 10 g-scale production of high-purity F1 by the application of commercial enzymes to a crude substrate.

2. Materials and methods

2.1. Materials

Standard-grade ginsenosides, including Rg1, Re, Rh1(*S*), F1, and PPT, were purchased from Nanjing Zelang Medical Technology Co., Ltd (Nanjing, China). The PPTGM was extracted from the dried roots of 4 kg of *Panax ginseng* Meyer together with 1 kg of *Panax quinquefolius*. Five kilograms of ginseng root powder was extracted with 50 L of 70% ethanol twice. The extract was filtered through filter papers and then dried using a rotary evaporator. The resultant dried powder was dissolved in water and loaded onto a glass column (400 mm L × 100 mm D) packed with Diaion HP-20 resin (Tokyo, Mitsubishi Chemical). Free sugar molecules and unwanted hydrophilic compounds from HP-20 absorbed beads were washed with 8 column volumes of water, and finally PPT-type ginsenosides were eluted by 8 column volumes of 36% ethanol. The ethanol extracts were evaporated *in vacuo*, and the dried residue was used as the substrate ginsenoside for F1 production. According to the HPLC analysis, the PPTGM was mainly composed of Re (245 mg/g), Rg1 (203 mg/g), Rb1 (32 mg/g), Rb2, (25 mg/g), Rb3 (53 mg/g), Rd (80 mg/g), and small amounts of other ginsenosides, and was dissolved in 50mM acetate buffer, which could solubilize the PPTGM at up to 100 mg/mL [19]. HPLC-grade methanol and acetonitrile were obtained from SK Chemical Co., Ltd (Seoul, Korea). The other chemicals used in this study were of analytical grade or better.

2.2. Screening of commercial enzymes that can transform PPT-type ginsenosides

The PPTGM was diluted in 500 μL of acetate buffer (pH 5.0) as 1% (w/v) and the same volumes of commercial enzymes—Novozym 960 (Bagsvaerd, Novozyme), Viscozyme (Novozyme), Pectinex AFPL-4 (Novozyme), Pectinex Ultra SP-L (Novozyme), Fungamyl 800L (Novozyme), and Cellulase KN (0.2 g/mL, Kagawa, Kyowa Chemical)—to be mixed for subsequent testing. The mixture was shaken aerobically at 150 rpm and 50°C for 48 h. The reactants were taken at regular intervals and analyzed via TLC or HPLC after pretreatment (see "Analytic methods" section).

2.3. Biotransformation activity of PPT-type ginsenosides using Cellulase KN

The activity of Cellulase KN was examined to determine the specificity and selectivity of conversion of ginsenosides Re and Rg1 through hydrolysis of the rhamnose and glucose moieties attached at the C6 position. Enzyme solutions at a concentration of 100 mg/mL in 100mM acetate buffer (pH 5.0) were reacted with an equal volume of Re and Rg1 solution at a concentration of 1 mg/mL in 100mM acetate buffer (pH 5.0) at 50°C. The samples were taken at regular intervals and analyzed via TLC or HPLC after pretreatment (see "Analytic methods" section).

2.4. Biotransformation optimization based on concentration of the enzyme and substrate

In order to determine the optimal conditions for biotransformation of the PPTGM using Cellulase KN, the substrate concentration of PPTGM in the reaction was optimized. The final enzyme concentration was fixed at 50 mg/mL and 100 mg/mL and reacted with the PPTGM dissolved in acetate buffer solution (pH 5.0), in order to obtain the final substrate concentrations of 5 mg/mL, 10 mg/mL, and 15 mg/mL. The six optimization reactions were performed in a 2 mL Eppendorf tube with a 1 mL working volume at 200 rpm for 48 h at 50°C. The samples were taken at regular intervals and analyzed via TLC and HPLC.

2.5. Scaled-up biotransformation of PPTGM using Cellulase KN

The scaled-up biotransformation was performed in a 10 L stirred-tank reactor (Biotron GX; Hanil Science Co. Ltd, Seoul, Korea) with a 5 L working volume at 200 rpm for 48 hours. The reaction was performed under pH 5.0 at 50°C. The reaction started with a composition of 10 mg/mL of substrate ginsenoside (PPTGM; total 50 g) as the final concentration, and 500 g of Cellulase KN was added. Samples were collected at regular intervals and analyzed by HPLC in order to determine the production of ginsenoside F1 from the PPTGM.

2.6. Purification of F1

Following the 5 L reaction of the PPTGM with Cellulase KN, the mixture was cooled at 4°C and centrifuged at 4,000 rpm for 20 min (Component R; Hanil Science Co. Ltd). The biotransformed ginsenoside F1 in the supernatant and precipitate was processed separately in order to remove the enzymes, salt, and free sugars from the reaction mixture. Ginsenoside F1 in the supernatant was purified using a Biotage SNAP flash chromatography cartridge (180 × 70 φ mm; Biotage, Uppsala, Sweden) packed with 340 g of octadecylsilane (ODS; ZEOprep 60 C_{18}, 40–63 μm), based on gradient concentration elution with methanol. The precipitate was also dissolved in 3.0 L of 95% ethanol solution twice and filtered through a filter paper (Advantec, Tokyo, Japan) and evaporated *in vacuo*.

The resulting ginsenoside powder derived from the supernatant and precipitate was denoted as crude ginsenoside F1 and purified further using a Biotage SNAP flash chromatography cartridge (180 ×70 φ mm; Biotage) packed with 340 g of silica resin (230–400 mesh). The cartridge was equilibrated with chloroform, and