Bioresource Technology 129 (2013) 659–662

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Short Communication

An efficient novel glycosylation of flavonoid by β-fructosidase resistant to hydrophilic organic solvents

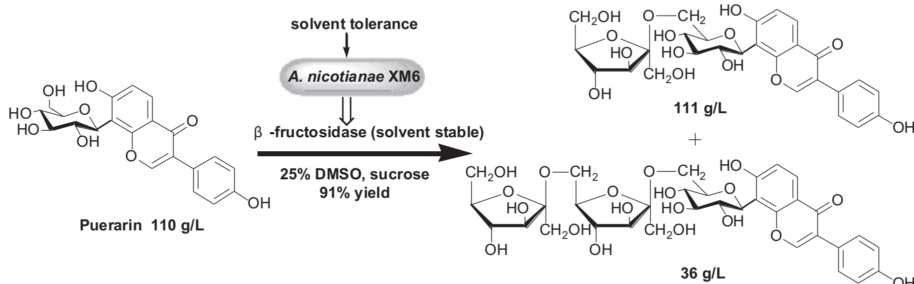
Xueming Wu [a,b], Jianlin Chu [a], Bin Wu [a], Sen Zhang [a], Bingfang He [a,*]

[a] College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, 30 Puzhunan Road, 211816 Nanjing, China
[b] Pharmaceutical College, Nanjing University of Chinese Medicine, 210023 Nanjing, China

HIGHLIGHTS

- An effective approach was developed to isolate solvent-stable glycosidase.
- The solvent-stable β-fructosidase with acceptor specificity of target substrate.
- Highly efficient synthesis of puerarin glycoside was achieved in nonaqueous media.
- The β-fructosidase did not hydrolyze almost formed product using sucrose as donor.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 September 2012
Received in revised form 4 December 2012
Accepted 7 December 2012
Available online 27 December 2012

Keywords:
Biocatalysis
Glycosylation
Solvent-stable enzyme
Flavonoid
β-fructosidase

ABSTRACT

An effective approach was successfully developed to isolate glycosidase with resistance of hydrophilic organic solvent, simultaneously with acceptor specificity of the target substrate. By this approach, an efficient solvent tolerant glycosidase producing bacterium *Arthrobacter nicotianae* XM6 was obtained. The β-fructosidase from strain XM6 shows high activity and stability in 10–25% DMSO and 10–20% methanol with 90–99% yields of puerarin glycosides. The addition of hydrophilic solvents not only greatly promoted the solubility of puerarin, but also regulated main products from multifructosyl puerarin to monofructosyl puerarin with increasing solvent concentration. Extraordinary highly efficient synthesis of puerarin glycosides (111.3 g/L of monofructosyl puerarin and 35.6 g/L of difructosyl puerarin) was attained in 25% DMSO solvent system from 110.4 g/L puerarin, which resulted a great facility for purification in large-scale process. The most novelty was that the β-fructosidase did not hydrolyze almost the newly formed glycosides using simply sucrose as donor.

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1. Introduction

Glycosylation serves to modulate the hydrophilicity, bioactivity, bioavailability, stability and chemical properties of aglycones (Gill and Valivety, 2000). However, chemical glycosidation requires glycosyl activation and involves multiple steps of protection/deprotection to control regionselectivity. This can often reduce the yield of the final glycoside (Matwiejuk and Thiem, 2011). Enzymatic glycosylation has attracted especial interest as the enzymes use unprotected aglycones, and their catalytic activity is chemo-, region-, and enantioselective (Li et al., 2004a; Rather et al., 2012). In biological systems, glycosidases and glycosyltransferases are responsible for the synthesis and catabolism of carbohydrates. However, the availability of suitable glycosyltransferases has been limited, and their use as biocatalysts is constrained by need to supply activated sugar for the glycosylation (Daines et al., 2004). The catalytic efficiency of glycosidases to use nonactivated sugars and their broad specificity for aglycones, has made these enzymes attractive in the practical synthesis of glycosides (Yu et al., 2010; Li et al., 2004a). Yet, the activity capable of glycosidases hydrolyzing reaction product usually results in low glycoside yields. The need for efficient glycoside synthesis has promoted substantial advances in the engineering of glycosidases (Hancock et al., 2006). One of the

* Corresponding author. Tel./fax: +86 25 5813 9902.
E-mail address: bingfanghe@njut.edu.cn (B. He).

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http://dx.doi.org/10.1016/j.biortech.2012.12.041