Bioresource Technology 130 (2013) 161–167

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Characterization of three novel thermophilic xylanases from *Humicola insolens* Y1 with application potentials in the brewing industry

Yanlong Du [a,b,1], Pengjun Shi [a,1], Huoqing Huang [a], Xiu Zhang [b], Huiying Luo [a], Yaru Wang [a], Bin Yao [a,*]

[a] *Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China*
[b] *College of Biological Science and Engineering, Bei Fang University of Nationalities, Yinchuan 750021, PR China*

HIGHLIGHTS

- ▶ One xylanase and three GH10 xylanase genes were identified in *Humicola insolens* Y1.
- ▶ The genes had identities of <83% to known fungal xylanases and $\leqslant$38% to each other.
- ▶ The natural xylanase was identical to one of the deduced proteins in sequences.
- ▶ The natural and recombinant xylanases had similar enzyme properties.
- ▶ Recombinant xylanase combination showed better mashing performance than Ultraflo.

ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form 8 December 2012
Accepted 10 December 2012
Available online 20 December 2012

Keywords:
Humicola insolens Y1
Xylanase
Thermophilic
Heterologous expression
Brewing industry

ABSTRACT

Three xylanase genes (*xynA*, *xynB*, *xynC*) of glycosyl hydrolase family 10 were identified in *Humicola insolens* Y1. The deduced protein sequences showed the highest identity of $\leqslant$83% to known fungal xylanases and of $\leqslant$38% with each other. Recombinant XynA–C produced in *Pichia pastoris* showed optimal activities at pH 6.0–7.0 and at high temperature (70–80 °C), and exhibited good stability over a broad pH range and temperatures at 60 °C. The gene *xynC* produced by *H. insolens* Y1 (named XynW) was similar in enzyme properties with XynC expressed by *Pichia*. XynA exhibited better alkaline adaptation and thermostability, and had higher catalytic efficiency and wider substrate specificity. Under simulated mashing conditions, addition of XynA–C showed better performance on filtration acceleration (37.4%) and viscosity reduction (13.5%) than Ultraflo from Novozyme. Thus the three xylanases represent good candidates for application in the brewing industry.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Plant cell wall consists mainly of cellulose, hemicellulose, lignin and pectin (Polizeli et al., 2005; Prade, 1996). Xylan as the major component of hemicellulose is composed of a backbone of β-1,4-linked D-xylopyranosyl residues and side chains of different substituents. The complete breakdown of xylan requires a variety of hydrolytic enzymes, including two backbone-hydrolyzing enzymes endo-β-1,4-xylanase and β-D-xylosidase, and five debranching enzymes α-L-arabinofuranosidase, α-D-glucuronidase, acetylxylan esterase, and feruloyl or coumaroyl esterase (Chávez et al., 2006). Among them, endo-β-1,4-xylanase is the crucial enzyme in xylan deg- radation.

Enzymatic hydrolysis of xylan has become attractive due to its biotechnological applications in the food, animal feed, waste treatment, ethanol production, textile, and pulp and paper industries (Collins et al., 2005). For commercial purposes, many xylanases have been highly expressed in heterologous systems, such as *Escherichia coli*, *Bacillus* spp. and *Pichia pastoris* (Prade, 1996; Jhamb and Sahoo, 2012). The most widely used xylanases are from the fungal genera of *Trichoderma*, *Aspergillus* and *Penicillium*, and these enzymes are generally highly active over a temperature range of 40–60 °C (Ahmed et al., 2009). At these temperatures, complete saccharification of biomass polysaccharides requires a long reaction time with high contamination risks (Berka et al., 2011). Thus high-temperature active xylanases are necessary to enhance the mass transfer and reduce the substrate viscosity (Margaritis and Merchant, 1986).

Thermophilic *Humicola* spp. are well-known microbial sources for their capacity to produce xylanases (Anand and Vithayathil,

* Corresponding author. Address: Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, No. 12 Zhongguancun South Street, Beijing 100081, PR China. Tel.: +86 10 82106053; fax: +86 10 82106054.
E-mail addresses: binyao@caas.net.cn, yaobin@mail.caas.net.cn (B. Yao).
[1] These authors contributed equally to this work.

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.12.067