International Journal of Biological Macromolecules xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Review

Characterization of a novel psychrophilic and halophilic β-1, 3-xylanase from deep-sea bacterium, *Flammeovirga pacifica* strain WPAGA1

Zheng-Wen Cai [a], Hui-Hua Ge [a], Zhi-Wei Yi [a,b], Run-Ying Zeng [b], Guang-Ya Zhang [a,*]

[a] Department of Biotechnology and Bioengineering, Huaqiao University, Xiamen 361021, Fujian, PR China
[b] State Key Laboratory Breeding Base of Marine Genetic Resource, Third Institute of Oceanography, Xiamen, Fujian 361005, PR China

ARTICLE INFO

Article history:
Received 9 April 2018
Received in revised form 1 July 2018
Accepted 13 July 2018
Available online xxxx

Keywords:
Deep-sea bacterium
Flammeovirga pacifica strain WPAGA1
β-1, 3-Xylanase
Psychrophilic
Halophilic
Enzyme mining

ABSTRACT

β-1, 3-Xylanase is one of the most important hydrolytic enzymes to prepare oligosaccharides as functional foods in seaweed industry. However, less than five β-1, 3-xylanases have been experimentally expressed and characterized; moreover, none of them is psychrophilic and salt tolerant. Here, we mined a novel β-1, 3-xylanase (Xyl512) from the genome of the deep-sea bacterium *Flammeovirga pacifica* strain WPAGA1 and biochemically characterized it in detail. The Xyl512 did not contain any carbohydrate-binding module; the catalytic domain of it belonged to the glycoside hydrolase family 26. The optimum temperature and pH of the purified β-1, 3-xylanase was 20 °C and pH 7.0 in the condition of no NaCl. However, they shifted to 30 °C and 7.5 with 1.5 mol/L NaCl, respectively. In this condition (1.5 mol/L NaCl), the overall activity was 2-fold as high as that without NaCl. Based on the residue interactions and the electrostatic surfaces, we addressed the possible mechanism of its adaption to low temperature and relative high NaCl concentration. The Xyl512 showed significantly reduced numbers of hydrogen bonds leading to a more flexible structure, which is likely to be responsible for its cold adaptation. While the negatively charged surface may contribute to its salt tolerance. The β-1, 3-xylanase we identified here was the first reported psychrophilic and halophilic one with functionally characterized. It could make new contributions to exploring and studying the β-1, 3-xylanase for further associated investigations.

© 2018 Elsevier B.V. All rights reserved.

Contents

1. Introduction . . . 0
2. Materials and methods . . . 0
   2.1. β-1, 3-Xylan and β-1, 3-xylooligosaccharides preparation . . . 0
   2.2. Plasmid construction . . . 0
   2.3. Expression and purification of the recombinant Xyl512 . . . 0
   2.4. Enzyme activity assay . . . 0
   2.5. Characterization of the recombinant Xyl512 . . . 0
   2.6. TLC analysis of the products hydrolyzed by Xyl512 . . . 0
   2.7. Sequence and structural analysis . . . 0
3. Results . . . 0
   3.1. Expression and purification of the recombinant Xyl512 . . . 0
   3.2. Characterization of the recombinant Xyl512 . . . 0
   3.3. Sequence and structural analysis of Xyl512 . . . 0
4. Discussion . . . 0
Acknowledgments . . . 0
Conflict of interest . . . 0
Appendix A. Supplementary data . . . 0
References . . . 0

* Corresponding author.
E-mail address: zhgyghh@hqu.edu.cn (G.-Y. Zhang).

https://doi.org/10.1016/j.ijbiomac.2018.07.090
0141-8130/© 2018 Elsevier B.V. All rights reserved.

Please cite this article as: Z.-W. Cai, et al., Characterization of a novel psychrophilic and halophilic β-1, 3-xylanase from deep-sea bacterium, *Flammeovirga pacifica* strain WPAGA1..., (2018), https://doi.org/10.1016/j.ijbiomac.2018.07.090

ARTICLE IN PRESS

1. Introduction

The seaweeds contribute about 50% of the global net primary production and have a high potential for biofuels and other material resources [1]. In addition, many algae cultivated for consumption are rich in active polysaccharide good for human health. Such as anticlotting, antioxidation, antiviral, anticoagulant, antitumor, resistance to bacteria inflammation, reduction of lipid accumulation and so on [2–7]. β-1, 3-Xylan, a homopolysaccharide of β-1, 3-linked D-xylopyranose units [8], which is one of the essential feedstocks hydrolyzed for the active polysaccharide. It was mainly found in the cell walls of red algae (*Porphyra* and *Bangia* spp.) and green algae (*Caulerpa*, *Bryopsis*, and *Udotea* spp.) [9]. Similar to other biomass materials, such as starch, cellulose and β-1, 4-xylan, β-1, 3-xylan-containing algal biomass could also be a source for the ethanol fermentation [10].

β-1, 3-Xylanases (EC 3.2.1.32) are the hydrolytic enzymes that act on the hydrolysis of β-1, 3-xylan. So far, only five β-1, 3-xylanases have been expressed and biochemically characterized. They came from *Alcaligenes* sp. strain XY-234 [11,12], *Vibrio* sp. strain XY-214 [13,14], *Vibrio* sp. strain AX-4 [15] *Thermotoga neapolitana* strain DSM 4359 [16] and *Pseudomonas vesicularis* MA103 [17], respectively. β-1, 3-Xylanases usually contain a catalytic module and none or several carbohydrate-binding modules (CBMs) by sequence analysis in the Carbohydrate Active Enzymes database (CAZy: http://www.cazy.org/) [18]. It is an effective tool to prepare oligosaccharides from β-1, 3-xylan. The oligosaccharides with different molecular weights and bonds have various activities and could be the important ingredients in some functional foods. In addition, it can also combine with other glycosidases to prepare algae protoplasts, making it possible for the cell fusion and gene manipulation of algae [19,20]. Moreover, the algae have been considered an edible food in Asia for thousands of years [8]. As the oligosaccharides produced from β-1, 3-xylan at low temperature can be more stable and will not devitalize, thus the mining of psychrophilic and halophilic β-1, 3-xylanase could meet the demands of low temperature and high concentration of salt. This can also be beneficial for industrial production from the marine pickling products.

Flammeovirga pacifica strain WPAGA1, which was a Gram-negative bacterium isolated from the marine sediment of the Western Pacific (CM3MC04-1 157°24′31″E, 19°30′30″N) by *in situ* enrichment using *Gracilaria lemaneiformis* as the substrate [21]. It could grow well with the temperature ranging from 4 °C to 42 °C. By mining the translated genomic sequences of *Flammeovirga pacifica* strain WPAGA1 (Accession: PRJNA263711) [22], we identified a possible novel β-1, 3-xylanase (hereafter referred as Xyl512) based on the protein sequence analysis.

In this study, we synthesized a new gene coding the possible novel β-1, 3-xylanase (Xyl512), cloned it to the vector and transformed it into *E. coli* BL21 (DE3) for expression. The purified β-1, 3-xylanase had an optimum temperature of 20 °C without NaCl. However, it shifted to 30 °C in the present of 1.5 mol/L NaCl. When the concentration of NaCl increased to 3 mol/L, the activity was still 1.2-fold of that without NaCl. The β-1, 3-xylanase can operate efficiently at low temperature and relative high salt condition, which is suitable for many low temperatures processes, especially in food and nutraceutical industries [23,24]. To our present knowledge, this is the first report on a psychrophilic and salt-tolerant β-1, 3-xylanase from deep-sea bacterium with biochemical characteristics in detail.

2. Materials and methods

2.1. β-1, 3-Xylan and β-1, 3-xylooligosaccharides preparation

β-1, 3-Xylan was prepared from green algae, *Caulerpa racemosa* var. *laete-virens* by using the method of Iriki et al. [9], the glycol β-1, 3-xylan was prepared according to Yamaura et al. [25]. The total concentration of sugar was measured by the phenol sulfuric acid method [26] and quantified using D-xylose as the standard. The total sugar content of the polysaccharide was above 95%. Partial hydrolysis of β-1, 3-xylan with TFA (Trifluoroacetic acid) was carried out as follows [27]: a reaction mixture consisting of 1 g of β-1, 3-xylan in 20 mL of 1 mol/L TFA was incubated at 70 °C for 3 h. After that, the residual insoluble β-1, 3-xylan was removed by centrifugation at 25 °C (12,000 ×*g*, 10 min), and then the supernatant was neutralized by adding 1 mol/L NaOH. All the other reagents were of the highest grade commercially available.

2.2. Plasmid construction

The protein sequence of Xy1512 was translated from the coding gene (Gene locus = Scaffold2: 836345: 837391:+) of *Flammeovirga pacifica* strain WPAGA1 [21] by mining the genome (Accession: PRJNA263711), it has the accession number of A0A1S1YTI6 (with signal peptide) in the Uniprot databases. After deleting the signal peptides and optimizing the codon, a new gene was synthesized (*Xyl512*, GenBank accession no. MG574980) according to the protein sequence. It had 69% sequence identity with the original gene from the source bacteria. Gene synthesis and sequencing were performed by GENEWIZ (Suzhou, China) and sequentially cloned into pET 22b(+) (Takara, Dalian, China) using *Ned*I and *Hind*III as restriction sites. The plasmids harboring the Xyl512 gene was then transformed into the protein expression host *E. coli* BL21 (DE3) (SANGON, Shanghai, China).

2.3. Expression and purification of the recombinant Xyl512

The stain was cultured overnight at 37 °C with shaking at 200 rpm in the Luria–Bertani (LB) medium containing 100 μg/mL ampicillin. Then inoculated into the terrific broth (TB) medium for a continued incubation at 37 °C. When OD_{600} reached 0.5–0.6, isopropyl-β-thiogalactopyranoside (IPTG) was added to a final concentration of 0.2 mmol/L for induction at 18 °C with 160 rpm shaking for 24 h. *E. coli* cells were harvested by centrifugation at 4 °C (8000 ×*g*, 15 min). The pellets were resuspended in cold 20 mmol/L phosphate-buffered saline (PBS: $Na_2HPO_4 \cdot 12H_2O/NaH_2PO_4 \cdot 2H_2O$, pH 7.5) and disrupted on ice by an ultrasonicator, followed by centrifugation at 4 °C (12,000 ×*g*, 10 min) to remove the cell debris.

The fusion protein (His-tagged) was purified from the cleared lysate by using nickel affinity column (1.4 × 6.5 cm, Smart-Lifesciences, Changzhou, China). First, it was diluted in the binding buffer and filtered through a 0.45 μm sterilized membrane (Millipore, Co. Cork, Ireland). After loading, the column was washed by 30 mL wash buffer (20 mmol/L sodium phosphate, 500 mmol/L NaCl, 30 mmol/L imidazole, pH 7.5). Then the recombinant Xyl512 was washed with a linear gradient of 10–300 mmol/L imidazole in wash buffer. After washing, the enzymes were eluted by the dialysis bag (MWCO 8–12 kDa, Solarbio, China) with the 20 mmol/L phosphate buffer (pH 7.0) containing 5% glycerol (the elution buffer).

The molecular mass and purity of Xyl512 were analyzed by SDS-PAGE with Coomassie blue staining. The concentration of Xyl512 was determined by Bradford method with bovine serum albumin serum (BSA) as the reference standard [28]. The sequence of Xyl512 was determined with the Liquid Chromatography-Electrospray ionization-Quadrupole-Time of Flight (LC-ESI-QUAD-TOF) by Beijing Protein Innovation (Peking, China). The purified β-1, 3-xylanases were digested into fragments by trypsase before LC-ESI-QUAD-TOF.

2.4. Enzyme activity assay

The activity of the recombinant Xyl512 was measured by the 3, 5-dinitrosalicylic acid (DNS) method with xylose as the standards [29]. The reaction systems were composed of 350 μL 10 g/L glycol β-1, 3-xylan in 20 mmol/L phosphate buffer, 1.5 mol/L NaCl at pH 7.5 and 50 μL 0.276 g/L purified enzyme, then carried out at 20 °C for 15 min and stopped with 400 μL DNS reagents. The reaction mixture was then boiling at 100 °C for 5 min. After cooling on ice for 2 min, 1.6 mL deionized

Please cite this article as: Z.-W. Cai, et al., Characterization of a novel psychrophilic and halophilic β-1, 3-xylanase from deep-sea bacterium, *Flammeovirga pacifica* strain WPAGA1..., (2018), https://doi.org/10.1016/j.ijbiomac.2018.07.090

Download English Version:

https://daneshyari.com/en/article/10156917

Download Persian Version:

https://daneshyari.com/article/10156917

Daneshyari.com