Journal of Molecular Catalysis B: Enzymatic 115 (2015) 66–75

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Purification strategies and properties of a low-molecular weight xylanase and its application in agricultural waste biomass hydrolysis

Didem Sutay Kocabaş[a,*], Sevgi Güder[b], Neslihan Özben[c]

[a] Department of Food Engineering, Karamanoğlu Mehmetbey University, 70100 Karaman, Turkey
[b] Department of Chemistry, Karamanoğlu Mehmetbey University, 70100 Karaman, Turkey
[c] Department of Biology, Karamanoğlu Mehmetbey University, 70100 Karaman, Turkey

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form 22 January 2015
Accepted 22 January 2015
Available online 4 February 2015

Keywords:
Scytalidium thermophilum
Corn cob
Xylanase
Enzyme purification
Biomass hydrolysis

ABSTRACT

Xylanases are hydrolytic enzymes responsible for the depolymerization of xylan, and they are of great interest because of their wide industrial applications. Extracellular low molecular weight xylanase was purified from a submerged culture of a thermophilic fungus, *Scytalidium thermophilum* ATCC No. 16454 grown on corn cobs, and the enzyme was characterized. Among the investigated low-cost purification techniques, the highest recovery (79%) with 2.7-fold purification, was obtained using an aqueous two phase system. The maximal purity (4.3-fold) was achieved by ultrafiltration and generated a yield of 25%. A two-step chromatography procedure consisting of gel filtration and anion exchange without any additional pre-purification steps yielded *ca.* 21.8-fold xylanase purification to apparent homogeneity with 9.6% recovery as demonstrated by SDS-PAGE. This study is the first to report a complement purification procedure and the corresponding results for *S. thermophilum* low molecular weight xylanase isolation from culture medium. The enzyme has a molecular weight of 21 kDa and an isoelectric point of pH 8.6, indicating that it belongs to GH family 11. The low molecular weight of the enzyme provides the important advantage of making it easy to access in the lignocellulosic network, and it efficiently degrades hemicellulose. The optimum temperature and pH values of purified xylanase were 65 °C and 6.5, respectively. Xylanolytic activity was most stable at pH 7.0 and 40 °C. K_m and V_{max} values of xylanase on beechwood xylan were predicted as 2.4 mg xylan/ml and 168.6 IU/ml, respectively. The potential of xylanase in biomass hydrolysis was also investigated, as lignocellulosic biomass has attracted increasing attention as an important raw material for second generation biofuel production. Among the investigated commercial and lignocellulosic substrates, purified xylanase showed the maximum specificity for beechwood xylan and wheat bran. The hydrolysis of an inedible and waste lignocellulosic substrate, corn cob, by xylanase was studied. A release of reducing sugars and apparent morphological changes in the corn cob structures were observed by SEM analysis after enzymatic treatment.

1. Introduction

Maize is an important agricultural crop, and corn cobs are major by-products of maize processing industries [1]. Corn cobs are mainly considered as a waste product without any current significant application, but they are potential raw materials for heat, power, fuel and chemical production. Investigating new applications for this agricultural waste could result in cheap and sustainable industrial products [2]. Xylan is the most abundant non-cellulosic renewable polysaccharide on the earth and represents up to 30–35% of the total dry weight of land plants. Xylan is a complex molecule, mainly consisting of D-xylose as its monomeric unit linked by β-1,4-glycosidic bonds and different substituents, such as acetyl, arabinosyl and glucuronosyl residues [3,4]. The average composition of corn cobs has been reported as 32% cellulose, 35% hemicellulose, 20% lignin, 4% ash and others [5]. Due to its high hemicellulose content and wide presence, corn cobs are promising carbon sources for fermentation processes.

Xylose can be converted to single-cell proteins and chemical fuels by microbial cells, which serve as the cheapest method of chemical production [6]. Due to the complex structure of xylan, the synergistic action of different hemicellulase enzymes, such as endo-xylanase (E.C.3.2.1.8), β-xylosidase (E.C.3.2.1.37), α-glucuronosidase (E.C.3.2.1.139), α-arabinofuranosidase

* Corresponding author at: Karamanoğlu Mehmetbey University, Food Engineering Department, Yunus Emre Campus, 70100 Karaman, Turkey.
Tel.: +90 0338 2262000x5008; fax: +90 0338 2262214.
E-mail address: didemkocabas@kmu.edu.tr (D. Sutay Kocabaş).

http://dx.doi.org/10.1016/j.molcatb.2015.01.012
1381-1177/

(E.C.3.2.1.55), acetylxylan esterase (E.C.3.1.1.72), ferulic acid esterase (E.C.3.1.1.73) and *p*-coumaric esterase (E.C.3.1.1.-) are required for complete hydrolysis. Endoxylanases, which randomly cleave the backbone of xylan, are one of the most important enzymes in this cooperatively acting enzyme group. Generally, xylanases are classified into two main groups: the GH family 10, characterized by high molecular weights (>30 kDa) with acidic pI values, and GH family 11, characterized by low molecular weights (<30 kDa) and basic pI values. Xylanases belonging to families 5, 7, 8, 26 and 43 have also been determined, but to a lesser extent [7–11]. Recently, exo-xylanases (E.C.3.2.1.37) with the potential to increase xylose yield when used in combination with endo-xylanases, have been reported [12].

Bacteria and fungi are the main microbial xylanase producers [13]. Fungal systems produce higher levels of xylanase activities compared to yeasts and bacteria, and because filamentous fungi produce extracellular xylanase, fungal xylanases are favourable at industrial scales. Xylanases have a wide range of applications related to animal feed, food and drinks, textiles, pharmaceutical and chemical industries, pulp and paper production and biorefinery [14,15]. Many industrial xylanases are produced by mesophiles, but xylanases from thermophilic sources may be of tremendous utility in some biotechnological processes due to their robust nature [16]. Thermophilic fungi are known to be important actors in mushroom composting in terms of mycelium growth and quality. *Scytalidium thermophilum* plays a leading role in mushroom composting by decreasing the ammonia concentration, immobilizing the nutrients necessary for the mushroom and showing a growth-promoting effect on mushroom mycelia [17]. In addition, low molecular weight xylanases are desirable in some industrial applications, such as the pretreatment of Kraft cooked pulp in the pulp and paper industry, where only low molecular xylanases can establish pores and penetrate into the re-precipitated xylan network for effective hydrolysis [18,19].

Parallel to the increasing usage of enzymes in industry, efficient and low-cost techniques must be employed for their purification because recovery and purification are the most expensive steps in industrial enzyme production processes [20]. Recently, we studied the statistical optimization of extracellular xylanase production from a thermophilic fungus, *S. thermophilum* using corn cob as the carbon source. We also demonstrated enzymatic xylooligosaccharide production from lignocellulosic agricultural wastes [21]. As a continuation of this work, in the current study, purification strategies of *S. thermophilum* xylanase were investigated, and *S. thermophilum* xylanase was biochemically characterized for industrial and analytical purposes. Low-cost techniques, including ammonium sulphate precipitation, aqueous two phase system (ATPS) and ultrafiltration (UF) were employed. To achieve a high level of purity, a two-step column chromatography technique, including gel filtration and anion exchange chromatography, was developed. This is the first report of the complete purification of *S. thermophilum* low molecular weight xylanase from culture medium, which contains a lignocellulosic waste (corn cob) as the carbon source. Xylanase was also tested as the catalyst for biomass hydrolysis with the aim of simple sugar production, a potential raw material for second generation fuel production.

2. Materials and methods

2.1. Microbial strain and culture conditions

The culture conditions of *S. thermophilum* (type culture *Humicola insolens*, ATCC No. 16454) were as described previously with slight modifications [22]. It was cultivated on YpSs agar plates containing 4.0 (g/l) yeast extract, 1.0 (g/l) K_2HPO_4, 0.5 (g/l) $MgSO_4{\cdot}7H_2O$, 15.0 (g/l) soluble starch and 20.0 (g/l) agar in distilled water [23]. The main culture was composed of 20 (g/l) corn cob particles (<2 mm sizes), 4 (g/l) yeast extract, 1 (g/l) K_2HPO_4, 0.5 (g/l) $MgSO_4{\cdot}7H_2O$, and 0.1 (g/l) $CuSO_4{\cdot}5H_2O$. On the 5th day of fermentation, the growth medium was filtered through Whatman no. 1 filter paper and centrifuged at 10,000 × *g* for 7 min. The supernatant was used as the crude enzyme solution.

2.2. Analytical methods

S. thermophilum xylanase activity was determined using the DNS method by measuring reduced sugar release caused by xylan degradation [24]. Beechwood xylan at a concentration of 1% (w/v) in 50 mM pH 7.0 sodium phosphate buffer was used as the substrate [25]. The reaction mixture consisted of 10 ml substrate and 1 ml of suitably diluted enzyme. The reaction was carried out at 60 °C for 4 min, and samples were taken at 1 min intervals. To stop the reaction, samples were immediately mixed with DNS reagent and boiled in hot water for 5 min for color development. Absorbance was monitored at 540 nm by a spectrophotometer (Hach, USA). Enzyme activity was determined using the initial reaction rate and the xylose standard curve (20–160 μg/ml). One unit of enzyme activity (IU/ml) was defined as the amount of enzyme that releases 1 μmol of reducing sugar at 60 °C and pH 7.0 per minute. Protein concentration was determined by the Bradford method [26] with bovine serum albumin (BSA) used as the standard protein. After each enzyme purification step, specific activities, yields and purification folds were calculated.

2.3. Xylanase purification

2.3.1. Ammonium sulphate precipitation

Proteins in 100 ml of crude enzyme solution were precipitated at 4 °C using a saturated ammonium sulphate solution to reach a final concentration ranging from of 20 to 80% (w/v). For example, for a 50% final concentration, the 100 ml of crude enzyme was mixed with 100 ml of saturated ammonium sulphate solution. Ammonium sulphate solution was slowly added to the crude enzyme while stirring. Precipitation was performed by 10 h of gentle stirring at 4 °C. Proteins were collected and centrifuged at 11,000 × *g* for 10 min. The supernatant solution was separated from the pellet, and the precipitate was dissolved in 50 mM pH 7.0 sodium phosphate buffer for further xylanase activity and protein content analysis.

2.3.2. Aqueous two phase system (ATPS)

ATPS was performed using Triton X-114 (TX-114), a non-ionic detergent with a low cloud point of 23 °C, to obtain a final concentration range of 2–20% (v/v). For each TX-114 concentration, the total volume of the mixture was adjusted to 20 ml. Eight millilitres of crude enzyme solution was mixed with 50 mM pH 7.0 sodium phosphate buffer (at a definite volume depending on TX-114 concentration), and TX-114 was slowly mixed in to avoid bubble formation and to reach final concentrations of 2, 5, 10 and 20% (v/v). The mixture compositions are given in Table 1. The mixtures were incubated in a water bath at 37 °C for 24 h. Phase separation was achieved by centrifugation at 25 °C at 11,000 × *g* for 60 min. The bottom (TX-114) and top (water) phases were separated, the volumes were recorded and the phases were analyzed for xylanase activity and protein content.

2.3.3. Ultrafiltration (UF)

UF was carried out in a 50 ml (Millipore-Amicon, USA) stirred cell using regenerated cellulose membranes (Millipore-Amicon, USA) at room temperature. Before UF, crude extract was filtered through Whatmann No. 1 filter paper and centrifuged at 14,000 × *g*