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Characterization of a cellulase-free, neutral xylanase from *Thermomyces lanuginosus* CBS 288.54 and its biobleaching effect on wheat straw pulp

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Abstract

A xylanase purified from the thermophilic fungus *Thermomyces lanuginosus* CBS 288.54 was characterized and its potential application in wheat straw pulp biobleaching was evaluated. Xylanase was purified 33.6-fold to homogeneity with a recovery yield of 21.5%. It appeared as a single protein band on SDS-PAGE gel with a molecular mass of approx. 26.2 kDa. The purified xylanase had a neutral optimum pH ranging from pH 7.0 to pH 7.5, and it was also stable over pH 6.5–10.0. The optimal temperature of the xylanase was 70–75 °C and it was stable up to 65 °C. The purified xylanase was found to be not glycosylated. The xylanase was highly specific towards xylan, but did not exhibit other enzyme activity. Apparent K_m values of the xylanase for birchwood, beechwood, soluble oat-spelt and insoluble oat-spelt xylans were 4.0, 4.7, 2.0 and 23.4 mg ml^{-1}, respectively. The potential application of the xylanase was further evaluated in biobleaching of wheat straw pulp. The brightness of bleached pulps from the xylanase pretreated wheat straw pulp was 1.8–7.79% ISO higher than that of the control, and showed slightly lower tensile index and breaking length than the control. Although chlorine consumption was reduced by 28.3% during bleaching, the xylanase pretreated pulp (15 U g^{-1} pulp) still maintained its brightness at the control level. Besides, pretreatment of pulp with the xylanase was also effective at an alkaline pH as high as pH 10.0.

Abbreviations: CAPS: (cyclohexylamino)-1-propanesulphonic acid; CHES: 2-(cyclohexylamino)ethanesulfonic acid; CMC: carboxymethylcellulose; DNS: dinitrosalicylic acid; EDTA: ethylenediaminetetraacetic acid; MES: 2-(*N*-Morpholino)ethane sulfonic acid; MOPS: 3-(*N*-morpholino)-propane sulphonic acid; *p*NP: *p*-nitrophenyl; TLC: thin-layer chromatography; X2: xylobiose; X3: xylotriose

Keywords: Biobleaching; Characterization; *Thermomyces lanuginosus*; Wheat straw pulp; Xylanase

1. Introduction

Recently, interest in xylanase (1,4-β-D-xylan xylanohydrolase, EC3.2.1.8) has increased markedly due to its huge potential industrial applications. The use of cellulase-free and thermostable xylanases is particularly important in the pulping and bleaching processes (Subramaniyan and Prema, 2002). Viikari et al. (1986) first reported that treating pulps with hemicellulases can reduce chlorine bleaching requirements and other investigators have subsequently confirmed this result (Clarke et al., 1997; Srinivasan and Rele, 1999). For the purpose of biobleaching, the enzymes must be stable and active both at high temperature and in alkaline condition (Srinivasan and Rele, 1999).

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Fungal xylanases of *Aspergillus* sp., *Trichoderma* sp., and bacterial xylanases of *Bacillus* sp., *Streptomyces* sp., and *Clostridium* sp. have been studied intensively (Subramaniyan and Prema, 2002). Commercial xylanases are typically produced by mesophilic filamentous fungi such as *Trichoderma reesei* and *Aspergillus niger*. However, these xylanases may not be sufficiently thermostable for processes where enzymes need to remain active at higher temperatures to have a competitive advantage (Shobhit et al., 2001; Techapun et al., 2003). Several strains of the thermophilic *Thermomyces lanuginosus* produce cellulase-free xylanases which are active and stable at elevated temperatures (Singh et al., 2000a). Therefore, the xylanase of *T. lanuginosus* may be suitable for such high temperature processes. Xylanases from several *T. lanuginosus* strains have exhibited promising results when applied as a bleaching agent to kraft and sulfite pulp produced from sugar cane bagasse, *Eucalyptus* and beech (Haarhoff et al., 1999; Bissoon et al., 2002). The enzymes also significantly reduced the amount of bleaching chemicals required to attain the desired brightness (Madlala et al., 2001).

T. lanuginosus strains are particularly attractive producers of thermostable xylanases from the industrial point of view, due to the fact that they excrete high-level of cellulase-free xylanases into medium (Singh et al., 2003). Efficient production of xylanolytic enzymes from *T. lanuginosus* has been described in numerous studies (Alam et al., 1994; Hoq and Deckwer, 1995; Puchart et al., 1999; Singh et al., 2000b). However, until the present study, the properties and application of endoxylanases from *T. lanuginosus* strains have not been investigated in detail (Singh et al., 2003; Xiong et al., 2004). Furthermore, most of the published studies on xylanase bleaching have focused on either hardwood or softwood pulps, but investigation on non-woody materials, particularly wheat straw is scarce (Clarke et al., 1997; Jiménez et al., 1999; Srinivasan and Rele, 1999; Zhao et al., 2002; Roncero et al., 2003). The main purpose of the present study is to investigate the biochemical and catalytic properties of a purified xylanase from *T. lanuginosus* CBS 288.54 and to establish its relationship with the characterized xylanases. The potential application of this thermostable xylanase in biobleaching as well as its ability to reduce the use of chlorine during bleaching of wheat straw pulp were also studied. To the best of our knowledge, this is the first report on the characterization of this xylanase and application of *T. lanuginosus* xylanase in biobleaching of wheat straw pulp.

2. Methods

2.1. Materials

The Sephacryl S-200 HR and Q-Sepharose Fast Flow were from Pharmacia (Pharmacia, Uppsala, Sweden). Econo-Pac High Capacity Ion Exchange Cartridges (Econo-Pac High Q) were from Bio-Rad (USA). Birchwood xylan, beechwood xylan, oat-spelt xylan, laminarin, lichenan, konjac gum, locust bean gum and carboxymethylcellulose (low viscosity) were purchased from Sigma (St. Louis, USA). Artificial substrates (*p*-nitrophenyl derivatives) such as *p*NP-β-D-xylopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-β-D-cellobioside, *p*NP-α-L-arabinofuranoside and *p*NP-β-D-mannopyranoside were also from Sigma.All other chemicals were of analytical grade. The unbleached wheat straw (*Triticum sativum*) NaOH-AQ pulp was supplied by China National Pulp and Paper Research Institute (Beijing, China). Wheat straw was cooked using the following condition: temperature 150 °C, time 60 min, NaOH concentration 14.0% (w/v), AQ concentration 0.025% (w/v) and liquid/solid ratio 6. Pulps were thoroughly washed with deionized water to remove soluble reducing sugars and any free soluble residual lignin before they were used. The wheat straw pulp had a kappa number of 21.92 and a brightness of 39.3% ISO. Corncob xylan was prepared according to the method of Kusakabe et al. (1977).

2.2. Microorganism and growth conditions

Thermomyces lanuginosus CBS 288.54 was obtained from the Centraalbuteau voor Schimmelculturen (under the number 288.54). Stock cultures were maintained using potato dextrose-agar (PDA) at 4 °C and were transferred every 6–7 weeks. PDA plates were incubated at 40 °C for 4–5 days and stored at 4 °C until use.

For xylanase production, the basal medium of flask culture contained ($g\,l^{-1}$): corncob xylan, 20; yeast extract, 10; tryptone, 10; $(NH_4)_2SO_4$, 2; $MgSO_4 \cdot 7H_2O$, 0.3; $FeSO_4$, 0.3; $CaCl_2$, 0.3. The initial pH of the medium was adjusted to 7.0 and not further controlled thereafter. Shake flasks, prepared in triplicate with each containing 100 ml of the medium in 300 ml Erlenmeyer flasks, were inoculated with an agar block (1 cm^2) from a 5-day-old plate culture and incubated at 150 rpm for 7 days at 50 °C.

2.3. Determination of enzyme activities and protein

Xylanase activity was assayed according to Bailey et al. (1992). Reaction mixture containing 0.9 ml of 1.0% (w/v) birchwood xylan and 0.1 ml of suitably diluted enzyme solution was incubated in 0.05 $mol\,l^{-1}$, pH 7.0 Tris-HCl buffer at 50 °C for 10 min. The reaction was stopped by adding 1 ml of 1.0% (w/v) DNS (dinitrosalicylic acid). Amount of reducing sugar liberated was determined by the DNS method using xylose (Sigma) as the standard (Miller, 1959). One unit of xylanase activity was defined as the amount of enzyme that produced 1 μ mol of xylose equivalent per minute.