Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 1252–1258
BIORESOURCE TECHNOLOGY

Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32

Suchita Ninawe [a,b], Mukesh Kapoor [a], Ramesh Chander Kuhad [a,*]

[a] *Lignocellulose Biotechnology Laboratory, Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India*
[b] *Department of Biotechnology, Ministry of Science and Technology, Government of India, CGO Complex, Block 2, 7th Floor, Lodi Road, New Delhi 110 003, India*

Received 4 January 2007; received in revised form 10 February 2007; accepted 11 February 2007
Available online 3 April 2007

Abstract

Streptomyces cyaneus SN32 was used in this study to produce extracellular xylanase, an important industrial enzyme used in pulp and paper industry. The enzyme was purified to homogeneity by ammonium sulfate precipitation followed by anion exchange chromatography using DEAE–Sepharose column, with 43.0% yield. The enzyme was found to be a monomer of 20.5 kDa as determined by SDS gel electrophoresis and has a pI of 8.5. The optimum pH and temperature for purified xylanase activity was 6.0 and 60–65 °C, respectively. The half-lives of xylanase at 50 and 65 °C were approximately 200 and 50 min, respectively. The xylanase exhibited K_m and V_{max} values of 11.1 mg/ml and 45.45 μmol/min/mg. The 15 residue N-terminal sequence of the enzyme was found to be 87% identical up to that of endoxylanases from *Steptomyces* sp. Based on the zymogram analysis, sequence similarity and other characteristics, it is proposed that the purified enzyme from *S. cyaneus* SN32 is an endoxylanase and belongs to Group1 xylanases (low molecular weight – basic proteins). The purified enzyme was stable for more than 20 week at 4 °C. Easy purification from the fermentation broth and its high stability will be highly useful for industrial application of this endoxylanase.

Keywords: *Streptomyces cyaneus*; Purification; Xylanase; Endoxylanase; N-terminal sequence

1. Introduction

Xylan, a major structural component of plant cell walls and the most abundant renewable hemicellulose, constitutes 20–40% of total plant biomass. Therefore, hydrolysis of xylan becomes an important step towards proper utilization of abundantly available lignocellulosic material in nature (Kuhad and Singh, 1993; Kuhad et al., 1997; Beg et al., 2001; Polizeli et al., 2005). Chemical hydrolysis of xylan applied extensively by the industries, though faster, is accompanied with the formation of toxic compounds and is hazardous to the environment (Beg et al., 2001). Xylan hydrolysis using enzymes such as xylanases provides a viable alternative to chemical hydrolysis as it is highly specific in nature apart from being an environment friendly process (Bajpai, 1997; Kuhad et al., 1997). The continuous effort in this direction has stimulated basic and applied research on microbial hemicellulases and has not only produced significant scientific knowledge, but also revealed their enormous biotechnological potential as food additives in poultry, in wheat flour for improving dough handling and the quality of baked products, for extraction of coffee, plant oils, and starch, in the improvement of nutritional properties of agricultural silage and grain feed, in combination with pectinase and cellulase for clarification of fruit juices and recovery of fermentable sugars from hemicelluloses and production of xylo-oligosaccharides (Bedford and Classen, 1992; Kuhad et al., 1997; Beg et al., 2001; Kapoor and Kuhad, 2007). Special attention has been given to their use in the pulp and paper industry for bleaching purposes,

* Corresponding author. Tel.: +91 11 24112062; fax: +91 11 24115270, +91 11 688 5270.
E-mail address: kuhad@hotmail.com (R.C. Kuhad).

0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.02.016

resulting in a decrease of chlorine utilization and consequently lowering environmental impact (Viikari et al., 1994; Bajpai, 2004; Ninawe and Kuhad, 2006; Kapoor et al., 2007). A purified xylanase is the pre-requisite for various biochemical studies needed to be done for better understanding of the xylanolytic system (Beg et al., 2001; Polizeli et al., 2005).

Streptomyces cyaneus SN32 was reported previously to be a good producer of xylanases when grown on lignocellulosic material and xylan as the substrates (Ninawe and Kuhad, 2005). The most significant effect of a cellulase-free crude enzyme preparation from *S. cyaneus* SN32 on pulp bleaching was on the delignification of kraft pulp and reduction of the pulp viscosity (Ninawe and Kuhad, 2006). In this present work, we describe the purification and characterization of a new xylanase from *S. cyaneus* SN32.

2. Methods

2.1. Chemicals

Commercial xylans and BSA were purchased from Sigma Chemical Co., USA. Protein marker was obtained from Amersham Pharmacia Biotech, UK. All other chemicals and materials used in the present study were of highest purity grade.

2.2. Microorganism and culture conditions

S. cyaneus SN32 was isolated from garden soil using Actinomycete Isolation Agar and maintained on Nutrient Agar Medium (Ninawe and Kuhad, 2006). The organism was identified using molecular methods (Accession no. AY232254 for 16S rRNA sequence) and is deposited with Microbial Type Culture Collection and Gene bank, Institute of Microbial Technology, Chandigarh, India (Accession no. MTCC 7060). For xylanase production, *S. cyaneus* SN32 was cultivated in 250 ml Erlenmeyer flasks containing 50 ml of optimized production medium containing wheat bran (3% w/v), peptone (1% w/v), KH_2PO_4 (0.1% w/v), $MgSO_4 \cdot 7H_2O$ (0.01% w/v); pH 9.0 under shaking (200 rpm) conditions at 42 °C for 48 h (Ninawe and Kuhad, 2005). The contents of the flasks were filtered through Whatman No. 1 filter paper and the filtrate was centrifuged at 10,000*g* for 30 min to obtain cell free culture fluid.

2.3. Purification of xylanase

The purification of xylanase (cell free supernatant) was carried out in two steps. The first step involved ammonium sulphate precipitation (0–60% saturation) of 500 ml cell free supernatant. The saturated solution was left overnight at 4 °C, centrifuged and precipitates were dissolved in 35 ml of 50 mM Tris–HCl buffer (pH 7.5). The dialyzed fraction of concentrated proteins (40 ml) was then loaded on to the anion-exchange DEAE–Sepharose column (0.9 cm × 20 cm; Pharmacia LKB Biotechnology, Uppsala, Sweden) with a with a bed volume of 40 ml was pre-equilibrated with Tris–HCl buffer. The column was operated at a flow rate of 30 ml/h. A continuous NaCl gradient (0–1 M) was applied and fractions (1.5 ml each) were collected.

2.4. Electrophoresis of protein samples

SDS–PAGE of the samples from above steps was carried out by using BIORAD electrophoresis apparatus. Stacking gel (5%) and resolving gel (12%) with prescribed compositions were used (Sambrook and Russel, 2001). Protein bands in the gel were visualized by Coomassie Brilliant Blue R-250 staining. Molecular weight of test protein(s) was compared with standard LMW protein marker (Amersham Pharmacia, Biotech, UK).

Native PAGE of the samples at different steps of purification was carried out with the aim to study the zymographic pattern of purified xylanase. The procedure followed was mostly similar to SDS–PAGE described above with few modifications. 250 µl of 1% (w/v) birchwood xylan was added to 1.75 ml of water during the preparation of gel. SDS and mercaptoethanol were not added in the buffer/tracking dye solution. The step of boiling samples during protein sample preparation was eliminated. The samples were loaded in duplicate sets. After completion of electrophoresis, the gel was cut in two halves, each with one set of samples. One half was stained with Coomassie Brilliant Blue solution to locate the position of the purified protein and the other portion was used for zymogram analysis. The gel for activity staining was incubated in 0.2 M citrate–phosphate buffer, pH 6.0 (stock solutions for 1 M buffer consist of 192 g/l of citric acid and 141.9 g/l Na_2HPO_4) for 2 min at 60 °C followed by staining the gel in Congo-Red solution (0.5% w/v Congo-Red and 5% v/v ethanol in distilled water) for 15 min. The gel was de-stained with 1 M NaCl to visualize the clearing zone of hydrolysis. The gel was further exposed to 5% acetic acid to increase the colour contrast between the hydrolysis zone and the remaining portion of the gel (Chadha et al., 1999).

Iso-electric focusing of purified xylanase was carried out using a mini 111 IEF apparatus (Amersham Pharmacia, Sweden) with ampholytes in the pH range of 3–11. Polyacrylamide gel was casted on the hydrophobic surface of the gel support film. 2 µl of each protein sample and marker were loaded on to the gel. Focusing was carried out under constant voltage conditions in a step-wise manner (100 V for 15 min, 200 V for 15 min and 450 V for 60 min). The gel was stained with Coomassie Brilliant Blue solution to locate the position of the purified xylanase protein. pI for the purified xylanase was determined with respect to pI marker proteins in the gel.

2.5. Characterization of xylanase

The optimal pH for xylanase activity was determined by using different buffers (0.2 M) ranging between 3.0 and 11.0