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SHORT COMMUNICATION

Paenibacillus sp. strain HC1 xylanases responsible for degradation of rice bran hemicellulose

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Summary

Paenibacillus sp. strain HC1 is the first bacterium capable of growing on rice bran hemicellulose as a sole carbon source. Two xylanases (Xyl-I and -II) were purified from the bacterial culture fluid and enzymatically characterized. Xyl-I and -II showed monomer forms with molecular masses of 30 and 18 kDa, respectively, and were most active at around pH 5.0 and 45 °C. Xylooligosaccharides were degraded to xylobiose and xylose by Xyl-I, but not by Xyl-II, suggesting that Xyl-I plays an important role in complete depolymerization of xylan. Both enzymes acted endolytically on rice bran hemicellulose, indicating that Xyl-I and -II contribute to the structure determination and practical use of the polysaccharide, an unutilized biomass in technology.

Introduction

Rice bran (RB) shows various physiological functions such as nutritional, antioxidative, hypocholesterolemic, and antitumor factors (Sheetharamaiah and Chabdrasekhara, 1988; Saunders, 1990; Akihisa et al., 2000; Qureshi et al., 2000; Iqbal et al., 2003; Nam et al., 2005), however, most of the RB produced during rice milling process is discarded. The utilization of RB is impractical due to the presence of hemicellulose, and the removal of hemicellulose included in RB is thus expected for the effective use of RB. RB hemicellulose is a heteropolysaccharide consisting of xylan as a backbone and several sugars such as arabinose, galactose, and glucose as side chains (Harada et al., 2005).

Xylanase is a glycoside hydrolase catalyzing the endolytical hydrolysis of 1,4-β-D-xylosidic linkages in xylan. In recent years, the enzyme has been demonstrated to have potential applications in diverse industries such as food, feed, and chemistry (Saha, 2003; Collins et al., 2005). The enzyme is typically used for improvement of maceration and

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juice clarification in food industry and for biobleaching of kraft pulps in the pulp industry. Therefore, a large number of xylanases have been found and characterized from bacteria, algae, fungi, protozoa, gastropods, and anthropods (Prade, 1996). Studies on xylanases acting on RB hemicellulose have, however, been limited due to the complex structure of the heteropolysaccharide. The complete structure of RB hemicellulose with biological activities remains unclear. Polysaccharide structures are usually determined by spectrophotometry, infrared spectrometry, NMR, X-ray crystallography, etc. Enzymes acting on polysaccharide are also useful for determining its structure due to their high substrate specificity. Since we have recently reported the isolation and characterization of *Paenibacillus* sp. strain HC1 assimilating RB hemicelulose, this paper deals with the identification of the bacterial xylanases responsible for degradation of RB hemicellulose. These enzymes will undoubtedly contribute to the structure determination of the polysaccharide, and facilitate utilization of the common polysaccharide xylan as well as RB.

Materials and methods

Materials

RB produced from unpolished Japanese rice during the milling process was used in this study (Harada et al., 2005). Hemicellulose was prepared from RB after lipids and lignins were removed with organic solvents and extracted with sodium hydroxide as described elsewhere (Siegel, 1968). DEAE Toyopearl 650M was purchased from Tosoh Co., Tokyo, Japan. Mono S HR 5/5 and HiLoad™ 16/60 Superdex 75pg were purchased from Amersham Biosciences, Uppsala, Sweden. Silica gel 60/Kieselguhr F_{254} thin-layer chromatography (TLC) plates were purchased from E. Merck, Darmstadt, Germany. Oat spelt xylan was purchased from Nacalai Tesque Co., Kyoto, Japan. Xylose, xylobiose, xylotriose, and xylooligosacharides were purchased from Wako Pure Chemicals Co., Osaka, Japan. Beechwood and birchwood xylans were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Microorganism and culture condition

To purify xylanases from strain HC1, the bacterium was aerobically cultured at 30 °C for 48 h in liquid 3 l of LB medium (Sambrook et al., 1989) containing 0.2% RB hemicellulose (1.5 l/culture flask) with a reciprocal agitation at 100.

Enzyme and protein assays

Xylanase was assayed at 30 °C in 50 mM sodium phosphate (pH 7.0) using 0.5% (w/v) birchwood xylan as a substrate, and the enzyme activity was determined by measuring reducing sugars released from the substrate by the Somogyi–Nelson method (Somogyi, 1952). One unit of the enzyme activity was defined as the amount of enzyme required to release 1 μmol of product from the substrate per minute. Protein was determined by the method of Bradford (Bradford, 1976), with bovine serum albumin as a standard.

Purification of xylanses from the culture fluid of strain HC1

Unless otherwise specified, all operations were done at 0–4 °C. After cultivation, the strain HC1 cells were removed by centrifugation at 13,000*g* for 20 min. The resultant supernatant was used as an extracellular enzyme source for further purification.

Proteins in the extracellular fraction were precipitated with ammonium sulfate (80% saturation), dissolved in 50 ml of 20 mM potassium phosphate buffer (KPB) (pH 7.0) and dialyzed against the same buffer. The dialyzate was loaded to a DEAE-Toyopearl 650M column (4.2 × 38 cm) previously equilibrated with 20 mM KPB (pH 7.0). The enzymes were eluted with 20 mM KPB (pH 7.0) (500 ml), and 5 ml fractions were collected every 3 min. Active fractions (flow-through) were collected and dialyzed against 10 mM KPB (pH 6.0). The dialyzate was applied to a Mono S HR 5/5 column previously equilibrated with 10 mM KPB (pH 6.0). The enzymes were eluted with a linear gradient of NaCl (0–0.5 M) in 10 mM KPB (pH 6.0) (100 ml), and 1 ml fractions were collected every 1 min. Two active peaks were observed. One containing a single protein was used as the purified xylanase I (Xyl-I), while, in the case of the other one (xylanase II, Xyl-II), the further purification was required. Fractions containing Xyl-II were collected and then concentrated by ultrafiltration (Ultrafree-MC Centrifugal Filter Units, Millipore, Billerica, MA, USA). The enzyme solution was applied to a HiLoad™ 16/60 Superdex 75pg column previously equilibrated with 20 mM Tris–HCl (pH 7.5) containing 0.15 M NaCl. Xyl-II was eluted with the same buffer, and 3 ml fractions were collected every 3 min. Active fractions were collected and used as the purified Xyl-II.