Thermostable β-Glucosidase (BglH) from Bacillus licheniformis KCTC 1918: Purification and Characterization

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1. Introduction

β-Glucosidases are the hydrolytic enzymes as well as diglucosides and oligosaccharides. For most enzymatic hydrolysis processes, β-1-4-endoglucanase and β-1-4-exoglucanase catalyze the random hydrolysis of cellulose to produce cellobiose, which is hydrolyzed to glucose by β-glucosidase to give glucose (Goyal et al., 2001 Yoshida et al., 2004). Furthermore, β-glucosidase regulates the enzymatic hydrolysis process by cellobiose, and is often inhibitory to endo- and exoglucanases activity (Bhat and Bhat, 1997 Harhangi HR et al., 2002). Therefore, the complete degradation of cellulose requires the synergistic action of all these three enzymes. The present report describes the purification and characterization of family 1 β-glucosidase, BglH of B. licheniformis.

2. Methods

2.1. PCR and cloning

B. licheniformis (KCTC1918) was obtained from the Korea Research Institute of Bioscience and Biotechnology (KIRIB). β-Glucosidase gene encoding mature protein was amplified from total genomic DNA by using primers 1 (CTG CAG ATG ACT GAA CAA ACG AAA AAG) with Pst1 recognition site and 2 (CTC GAG TCA CAA ACT CTC GCC ATT CG) with Xho1 recognition site. The PCR parameter consisted of 50 cycles of denaturation at 94 °C, annealing at 52 °C, and extension at 72 °C. The recovered structural gene was ligated in previously Xcm digested pBSK- vector.

2.2. Purification of β-Glucosidase

The recombinant Escherichia coli cells were cultivated by using a rotary platform at 37 °C in Luria-bertani broth (LB) medium (400 ml). Cultivation was continued overnight, after which E. coli cells were harvested by centrifugation (3,500 g, 20 min, 4 °C). Purification was accomplished by binding specificity of crude extracted cell lysate by Ni-NTA agarose slurry. For the final purification step, the active fractions were concentrated by using ultrafiltration (Amicon Ultra-15) with centrifugation (3,500 g, 40 min, 4 °C). The fractions with β-glucosidase activity eluted as a single protein peak and the purity of the enzyme was assessed by SDS-PAGE.

2.3. Assay of β-Glucosidase

β-Glucosidase was determined by measuring p-nitrophenyl release from the p-nitrophenyl β-D-glucopyranoside (Kwon et al., 1992). The specific activity was obtained by pNPG as substrate at 47 °C. The enzyme mixture containing 100 mM pNPG in citrate-phosphate buffer (pH 6.0) was incubated with enzyme for 90 min in a total volume of 1 ml. The reaction was stopped by the addition of 1 ml of 1 M Na2CO3 (pH 12), while the amount of pNP release was determined by measuring absorbance at 405 nm.

2.4. Characterization of β-Glucosidase

2.4.1. SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was prepared. Prestained Protein Marker (Biolabs) were used to determine the subunit molecular weight of the enzyme. After electrophoresis, proteins were stained by Coomassie Brilliant blue R 250
2.4.2. Effect of pH and temperature stability

The optimum temperature of the enzyme was obtained by determining activity on pNPG between 32 °C and 52 °C. The optimum pH was determined by measuring activity between pH 4.0 and 8.0 using citrate-phosphate buffer at 47 °C. Thermostability was measured by preincubating the enzyme in pH 6.0 citrate-phosphate buffer for 0–120 h, while residual activity was measured with pNPG.

3. Results

3.1. Sequence analysis and protein purification of β-glucosidase (BglH)

The analyzed data of β-glucosidase from B. licheniformis was cloned and sequenced (Fig. 1). The PCR product of β-glucosidase(BglH) has 1410 bp as shown in Fig. 2a. The purity and molecular mass of the enzyme was evaluated, the fraction was analyzed by SDS-PAGE as shown in Fig. 2b. The molecular mass of single protein band was 53.4 kDa. The BglH protein sequence of B. licheniformis showed 92% and 88% homology with B. subtilis and B. pumilus, respectively.

3.2. Effect of pH and temperature on β-glucosidase activity

Its optimal pH was observed at pH 6.0 in same buffer (Fig 3A). Enzyme activity with increasing reaction to temperature peaked at 47 °C while the temperature optimum at pH 6.0 was 47 °C(Fig. 3B). The optimal β-glucosidase pH was measured at pH 6.0 at a temperature on 47 °C. The enzyme retained 100% of its original activity, at the condition described, for 24 h (Fig. 4) and 49.4% relative activity was retained at pH 6.0 after 96h of incubation at 47 °C. According to previous reports, half-life times of Bacillus β-glucosidases were not longer. About 80% of the activity of β-glucosidases from B. circulans and B. subtilis remained after incubatingthe enzyme at its optimal temperature for 15 min and 2 h, respectively (Paavilainen et al., 1993 Kuo and Lee, 2008). Among the Bacillus β-glucosidases, the unique property of BglH of B. licheniformis is its longest half-life.

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