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# 嗜碱芽孢杆菌 C-125 木糖苷酶基因的表达与酶特征鉴定

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**摘 要:** 嗜碱芽孢杆菌(*Bacillus halodurans*)C-125 菌株的基因组中, 一个编码木糖苷酶的基因(BH1068)被克隆并在大肠杆菌中获得高效表达。通过全面分析纯化蛋白, 确证了它的木糖苷酶功能。该酶在 pH 4~9 的范围内保持稳定, 最适 pH 值为中性, 有较宽的最适温度(35°C~45°C), 且能在 45°C 范围内保持稳定。这些特性使得该酶可在较为宽广的条件下对木聚糖进行酶促降解。该酶对人工合成底物对硝基苯-β-木糖苷(*p*-nitrophenyl-β-xylose, pNPX)的比活力为 174 mU/mg 蛋白质, 且木糖对其反馈抑制较弱(抑制常数 $K_i$ 为 300 mmol/L)。结果显示该酶是活性较高且较耐木糖抑制的细菌源木糖苷酶。该酶与商品化的木聚糖酶一起水解山毛榉木聚糖(Beechwood xylan)时显示了增效作用, 且水解率可获 40%。该酶最适 pH 为中性, 对木糖耐受等特性与大多数来源于真菌、最适 pH 为酸性、对木糖敏感的木糖苷酶将有较好的互补。结果表明该酶在木聚糖或含木聚糖多糖的单糖化过程可能发挥重要作用。

**关键词:** 木糖苷酶, 嗜碱芽孢杆菌, 木聚糖, 木糖

# Expression and characterization of a xylosidase (*Bxyl*) from *Bacillus halodurans* C-125

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**Abstract:** A xylosidase gene, labeled as BH1068 in genome of *Bacillus halodurans* C-125, was successfully cloned and overexpressed in *Escherichia coli* JM109. The purified enzyme was thoroughly characterized and its xylosidase function was unambiguously confirmed. It has maximum activities in neutral condition and is stable over a wide range of pH (4.5–9.0). The enzyme has a broad temperature optimal (35°C–45°C) and is quite stable at temperature up to 45°C. The unique pH and temperature profiles of the enzyme should allow a wide range of xylanolytic operational conditions. With high specific activity of 174 mU/mg protein for its artificial substrate (*p*-nitrophenyl-β-xylose) and low xylose inhibition (inhibitor constant $K_i$ = 300 mmol/L), this enzyme is among the most active and high tolerant bacterial xylosidase to xylose inhibition. Its high synergy with commercial xylanase has been demonstrated with beechwood xylan hydrolysis, achieving a hydrolysis yield of 40%. Its neutral pH optimal and high tolerance to product inhibition complements well with its fungal counterparts that are only optimal at acidic pH and susceptible to xylose inhibition. In conclusion, this enzyme has high potential in the saccharification of xylan and xylan-containing polysaccharides.

**Keywords**: xylosidase, *Bacillus halodurans*, xylan, xylose

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Xylan is the second most abundant natural polysaccharides and a rich renewable resource for production of biofuels and biochemicals (such as xylitol). Unlike cellulose, xylan is a complex polymer, with heavy substitution by arabinosyl, acetyl, uronyl, mannosyl, and glucosyl side chains [1]. Consequently, a complete saccharification of xylan requires concerted actions of several hemicellulases, among which xylanase and xylosidase act on the xylan backbone and are considered major players in the saccharification process. Xylanolytic microbial cells, both bacteria and fungi, produce xylan-degrading enzymes that are potentially useful in industrial applications[1-4]. Alkaliphilic bacteria, which thrive at a unique niche environment, are often tapped as a source of enzymes optimal at high pH. Alkaliphilic *Bacillus halodurans* C-125 was discovered as a potent xylanase producer[5]. Its recently sequenced genome reveals 11 genes encoding xylan-degrading enzymes [6]. Subsequent cloning and expression led to the discovery of a novel exo-oligoxylanase that releases xylose from reducing end[7]. Three xylosidases were predicted to exist in this organism. Based upon their sequence similarities, BH 1867 and BH3683 are both classified as GH 43 enzymes whereas BH1068 is classified as family-39 xylosidase. Wagschal K *et al* experimentally confirmed β-xylosidase activities of BH1068 which fused with C-terminal 6His tag[8], however the properties of the enzyme, especially its hydrolysis of artificial substrates, are not consist with previous report[9], and the potential industrial application on xylan degrading of this enzyme is not evaluated as well. In this paper, we undertook the cloning and overexpression of BH1068 with N-terminal His tag. The recombinant enzyme, named Bxyl, was re-characterized and its synergy with commercial xylanase was also demonstrated with beech wood xylan.

## 1 Materials and methods

### 1.1 Materials

Molecular biology reagents were purchased from various commercial sources. Plasmid Mini kit and DNA gel extraction kit were purchased from Fermentas and Qiagen respectively; Primers for gene amplification were synthesized from Invitrogen; DNA restriction enzymes and DNA ligase were products of Promega, DNA amplification kit was obtained from BioRad. Chemicals used in this study were purchased from Sigma-Aldrich and Fisher Scientific unless otherwise indicated. *E. coli* expression vector pQE80L (Qiagen product) was used for cloning and expression. *Bacillus halodurans* C-125 genomic DNA was obtained from ATCC. *E coli* JM109 strain used as host for *Bxyl* expression was taken from our lab's strain collection.

### 1.2 Construction of pQEBXyl, a *Bxyl* expression vector

The gene of β-xylosidase (*Bxyl*) was amplified from *Bacillus halodurans* C-125 genomic DNA with the following primers: Bxyl P1 (5'-TAA***GGATCC***ATGGC AATGAAAACAGTAGTTGTAAATGATC-3' cotaining *Bam*H I site (bold and italic)) and Bxyl P2 (5'-TTGTT GTA***GTCGAC***ATACGAAGGAATCAGCCGATCA-3') with *Sal* I site (bold and italic). The primers were designed according to BH1068 sequence (GenBank Accession No. NC-002570 region: 1149950–1151458). The 1.5 kb PCR amplified product, being digested by *Bam*H I and *Sal* I, was inserted into the same sites of pQE80L (Qiagen) to give pQEBXyl for expression of Bxyl protein with N-terminal fusion of HHHHHHGSMA and C-terminal fused VDLQPSLIS. The plasmid map of the constructed pQEBxyl in this study is shown in Fig. 1.

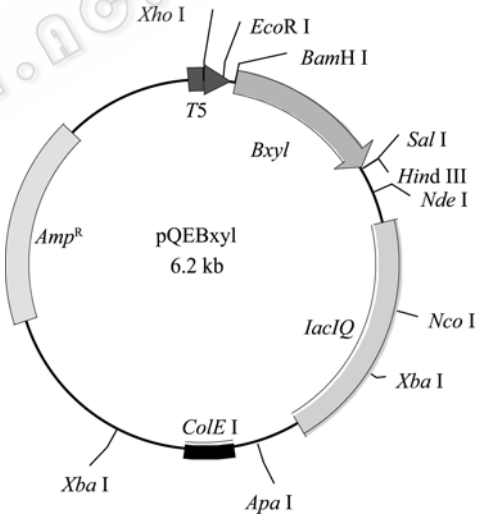


Fig. 1 The map of pQEBxyl. *AmpR*: ampicillin resistanct gene; *T5*: phage T5 promoter; *Bxyl*: β-xylosidase gene (BH1068); *IacIQ*: *LacIQ* repressor gene; *ColE* I: *colE* I origin of replication.

### 1.3 Expression of Bxyl protein in *E. coli* JM109

Inoculums of JM109/pQEBxyl were prepared by growing the cells at 37ºC in a cultural tube with 3 mL of LB. After collection by centrifugation, cells were diluted 100 times with freshly prepared LB medium supplemented with 100 mg/L ampicillin. Cells were incubated again at 37ºC until $OD_{600}$ reached 0.4–0.6, upon which IPTG was added to a final concentration

of 0.5 mmol/L. Cells were incubated at 30ºC for additional 4–6 hours, and the induced cells were collected by centrifugation for enzyme activity assay, enzyme purification and xylan hydrolysis.

### 1.4 Purification of recombinant xylosidase

Induced cells collected as above were re-suspended in a sodium phosphate buffer (50 mmol/L, pH 8.0), containing 0.3 mol/L NaCl and 10 mmol/L imidazole, to a final concentration of 0.2 g (wet cell)/mL. Lysozyme and protease inhibitor, phenylmethylsulfonyl fluoride (PMSF), were added into the cell suspension to a final concentration of 1 mg/mL and 2 mmol/L, respectively. After incubation on ice for 30 min with 150 r/min shaking speed, the cells were sonicated. The supernatant of cell-free extract obtained by centrifugation was loaded onto a nickel-ntrilotriacetic acid-agarose (Ni-NTA) (Sigma product) column. The column was washed with the same buffer containing 20 mmol/L imidazole until a constant absorption at 280 nm was reached. The absorbed enzyme was eluted with the same buffer containing 250 mmol/L imidazole. The fraction containing the recombinant protein was desalted by dialysis against phosphate buffered saline (PBS) buffer (10 mmol/L, pH 7.0). Fractions containing the enzyme were collected for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and other enzymatic characterization studies. In this work, Protein concentrations were determined by Bradford method [10].

### 1.5 Enzyme assay

Xylanase activity was determined in a sodium phosphate buffer (50 mmol/L, pH 6.0) containing 1.0% (*W*/*V*) boiled oat spelt xylan (Sigma) and an appropriately diluted enzyme solution. After 30 min incubation at 50°C, the reducing sugar liberated in the reaction mixture was measured by the dinitrosalicyclic acid (DNS) method [11]. One unit of xylanase activity was defined as the amount of enzyme that produces 1 μmol reducing sugar in the reaction mixture per minute. Recombinant β-xylosidase activity was measured by using *p*-nitrophenyl-β-xylose (pNPX) (Sigma) as a substrate. Hydrolysis rate was determined by the release of *p*-nitrophenol. The reaction was carried out in a mixture containing 20 μL pNPX stock solution (20 mmol/L in 50% ethanol), 780 μL sodium phosphate buffer (50 mmol/L, pH 7.0), and 100 μL appropriately diluted enzyme solution at 40°C for 10 min, The reaction was stopped by adding 100 μL 0.5 mol/L sodium hydroxide, and released *p*-nitro- phenol was measured by the absorbance at 410 nm. One unit of enzyme activities was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per minute.

### 1.6 Effects of pH and temperature on xylosidase activity

For pH effects, enzymatic activity was measured under standard condition as described in section 1.5 except under different pHs. The buffer systems used were glysine HCl (pH 2.5–3.0), sodium citrate (pH 3.5–6.0), sodium phosphate (pH 6.0–8.0), glycine NaOH (pH 8.0–9.5) and sodium bicarbonate (pH 10.0–11.0), the concentration of each buffer which was used in all experiments is 50 mmol/L. The pH stability was measured by analyzing enzyme activities after incubating the enzyme at a desired pH at 4°C for 12 hours. The optimal temperature was determined by measuring enzyme activity under the same conditions as describe in section 1.5 except temperature varied from 25°C–55°C. The thermal stability was determined by measuring the residual enzyme activity after incubating the enzyme at a desired temperature (25°C–55°C) for 30 minutes.

### 1.7 Kinetic analysis of xylosidase and its product (xylose) inhibition effects

The apparent kinetic parameters of recombinant β-xylosidase were determined by formation of product (*p*-nitrophenol), which was measured by the 410 nm absorption in a sodium phosphate buffer (pH 7.0) at 40°C. The kinetic parameters were estimated by regressing the experimental data (substrate concentration range, 0.33–4 mmol/L) with the Lineweaver-Burk plot. The product inhibition was carried out under identical conditions as described above except with addition of xylose to concentrations from 0.1 mol/L to 0.5 mol/L.

### 1.8 Enzymatic hydrolysis of beech wood xylan

To investigate the effectiveness of recombinant β-xylosidase on natural substrates, beech wood xylan (2.0% (*W*/*V*), Sigma) in sodium phosphate buffer (50 mmol/L, pH 7.0) was incubated with either xylosidase or xylanase, or their combination with 250 r/min shaking speed at 45°C for a total 24 h reaction time. The doses of xylan-degrading enzymes were 25 U/g-xylan for recombinant β-xylosidase and 100 μ/g xylan for xylanases (Multifect xylanase of Genencor) or xylanase from *Thermomyces lanuginosus* (Sigma product) respectively. After 1, 2, 4, 8, 12, and 24 h reaction, the reducing sugars of hydrolysates were analyzed by the dinitrosalicyclic acid (DNS) method to determine the degree of hydrolysis [11].

### 1.9 Sugar analysis of xylan hydrolysates

Each enzymatic hydrolysate of beech wood xylan,

after 24 h reaction, was analyzed by High Performance Anion-Exchange Chromatography (HPAEC) using a DIONEX system equipped with an ED50 electro-chemical detector (Sunnyvale, CA US). Monosaccharides and oligosaccharides were separated on a CarboPac PA-20 column (Dionex). Detection was through pulsed amperometry (waveform: $t$ = 0.41 s, $p$ = –2.00 V; $t$ = 0.42 s, $p$ = –2.00 V; $t$ = 0.43 s, $p$ = 0.60 V; $t$ = 0.44 s, $p$ = –0.10 V; $t$ = 0.50 s, $p$ = –0.10 V). The mobile phase consisted of degassed solution A containing 100 mmol/L sodium hydroxide and solution B containing 500 mmol/L sodium acetate plus 100 mmol/L sodium hydroxide. The mobile phase was pressurized with inert gas (He) to prevent interference of airborne carbon dioxide. A flow rate of 0.5 mL/min was used. The following linear gradient was used: $t$ = 0 min, 100:0 (A:B); $t$ = 30 min, 30:70; $t$ = 35 min, 30:70; $t$ = 45 min, 100:0; $t$ = 55 min, 100:0.

## 2 Results and discussion

### 2.1 Cloning and expression of Bxyl

*Bxyl* was previously predicted to be a putative β-xylosidase belonging to family 39 of glycoside hydrolases[12-13], and its β-xylosidase activity was experimentally confirmed by Wagschal K *et al*[8-9]. Its sequence alignment (Fig. 2) with four other family 39 enzymes from *Thermoanaerobacterium saccharolyticum* (I), Alpha-L-iduronidase precursor from *Canis familiaris* (II), β-xylosidase from *Caldicellulosiruptor saccharolyticus* (III) and Alpha-L-iduronidase precursor from *Mus musculus* (IV) showed that its 502 amino acids polypeptide contains a conserved region, VXKWXFE XWNEPNL (X denotes a less conserved amino acid) (Fig. 2). The *Bxyl* gene, franked by genes encoding D-mannonate oxidoreductase (BH1067) and Mn catalase (BH1069), appears to have its own promoter and transcription terminator. The promoter region has a conserved *Bacillus*-16 region (TRTG, R being either G or A)[14], but no conserved −10 and −35 regions can be found. The gene has a Shine-Dalgarno (SD) sequence (AGGAGG) located 9 bases upstream from its start codon (ATG). The transcriptional terminator is a typical hairpin structure with high GC content followed by a seven continuous Ts. It contains no signal peptide and therefore is expected to be an intracellular enzyme. The coding region of *Bxyl* was successfully amplified using PCR and inserted into an *E. coli* expression vector pQE80L (Qiagen), resulting in an expression vector pQEBxyl (Fig. 1). The plasmid was

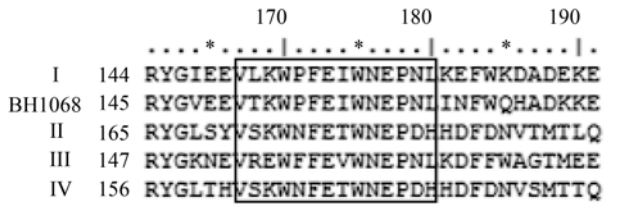


Fig. 2 Alignment of Bxyl with other four family 39 enzymes identifies a conserved region (boxed). I: β-D-Xylosidase from *Thermoanaerobacterium saccharolyticum*; II: Alpha-L- iduronidase precursor from *Canis familiaris*; III: β-xylosidase from *Caldicellulosiruptor saccharolyticus*; IV: Alpha-L-iduronidase precursor from *Mus musculus*.

transformed into *E. coli* JM109. When 450 mL cultivated cells of *E. coli* JM109/pQEBxyl were induced by IPTG, we obtained 45 mg purified enzyme from total 913 mg protein of cell extracts by purification of affinity chromatography of nickel-nitrilotriactic acid Agarose (Table 1). This indicates the recombinant enzyme is highly expressed because the enzyme reaches about 5% of total soluble protein. High expression of the enzyme was also evidenced by using SDS-PAGE analysis. The prominent band, which located between 50 kD and 75 kD of standards protein molecular weight (MW) marker, with apparent MW about 61 kD, corresponds to the predicated molecular weight of recombinant *Bxyl* (Fig. 3). The apparent MW is consist with previous report of Wagschal K[8], the apparent MW value of its homo-tetramer enzyme was 235 kD. The crude enzyme, prepared after cell lysis (by sonication), was analyzed for xylosidase activity using *p*-nitrophenyl-β-xylose (pNPX) as substrate. The activity, based upon the release rate of the chromophore, *p*-nitrophenol, was estimated to be 11 U from 450 mL shaker-flask cultivation or 24 U/L (Table 1).

**Table 1 Xylosidase purification**

| Purification stage | Total activities (mU) | Total protein (mg) | Specific activities (mU/mg) | Purification (fold) | Yield (%) |
|---|---|---|---|---|---|
| Cell free extract | 11003.3 | 913 | 12.1 | 1.0 | 100 |
| Ni-NTA -Agarose | 8099.7 | 50 | 173.6 | 14.4 | 73.6 |
| Dialysis | 7811.1 | 45 | 172.9 | 1.0 | 70 |

450 mL IPTG(0.5 mmol/L) induced JM109/pQEBxyl culture was used for enzyme purification; 1 mmol/L pNPX in 50 mmol/L sodium phosphate buffer (pH 7.0) was used for enzyme assay; protein concentration was estimated by Bradford methods with BSA as standard; purification folds were estimated from specific activities of enzyme and yield was calculated from total recovered activities.

### 2.2 Purification of the recombinant xylosidase

The purification of the recombinant xylosidase was facilitated by the N-terminal His-tag. A single step of affinity chromatography using nickel-nitrilotriacetic acid Agarose purified the enzyme to its homogeneity (Fig. 3), resulting in a purification fold of 14 (Table 1) and the purification yield of 70%. The enzyme's specific activity, which appears to be higher than most bacterial xylosidase and lower than its fungal counterpart [15-16], is about 174 mU/mg protein. Subsequent characterization was carried out with the purified enzyme.

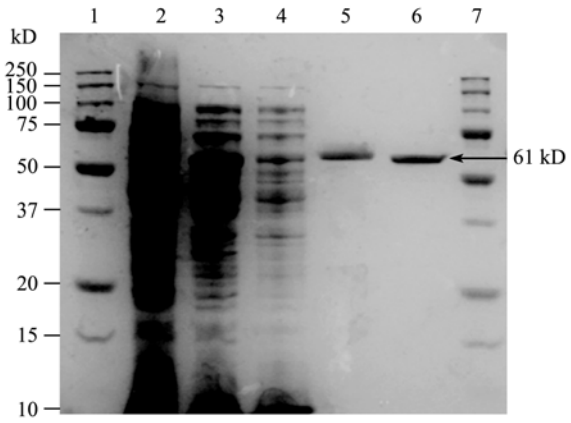


Fig. 3 SDS-PAGE analysis of recombinant xylosidase. 1, 7: molecular weight standard; 2: cell lysate from control cells with empty vector, JM109/pQE80L; 3: cell lysate of JM109/pQEBxyl; 4: Ni NTA Agrose flow through; 5: imidazole eluent before dialysis; 6: imidazole eluent after dialysis.

### 2.3 Characterization of the recombinant xylosidase

#### 2.3.1 *Substrate specificity*

Xylosidase is sometimes found bifunctional or even multifunctional[1,17]. Smaali previously reported that BH1068 (His) 6 is unable to cleave *p*-NP-araF and weakly hydrolyzes *p*-NP-β-D-glucopyranoside[8], which is contradiction to the report that BH1068 with (His) 6 can cleave the arabinofuranosyl, Arabinopyranosyl[9]. Thus, the substrate specificity of our recombinant enzyme was investigated with chromogenic substrates, *2*-Nitrophenyl-β-D-galactopyranoside, *p*-Nitrophenyl-L-α-arabinofranoside, Nitrophenyl-β-D-mannopyranoside and *p*-Nitrophenyl-β-D-glucopyranoside. Our results, which are more consistent with reports of Wagschal K *et al*, showed that very low activities for *p*-nitrophenyl-L-α-arabinofur-anoside and *p*-Nitrophenyl-β-D-glucopyra noside, and nearly negligible activity was found with other artificial substrates, indicating a high specificity of this recombinant xylosidase (Table 2).

#### 2.3.2 *Enzyme kinetics*

The enzyme kinetics was analyzed with pNPX as substrate in the concentration range of 0.33–4 mmol/L in the absence and presence of xylose inhibitor at varying concentrations (0.1–0.5 mol/L). Lineweaver-Burk plot showed a competitive inhibition by xylose (Fig. 4). $K_m$ and $V_{max}$ were estimated to be 1.9 mmol/L (pNPX) and 0.65 μmol/min/mg proteins, and its inhibitor constant ($K_i$) for xylose was estimated to be 300 mmol/L or so (Fig. 4). Fungal xylosidase is typically very susceptible to xylose inhibition, with $K_i$ on the order of 1 mmol/L[16]. Bacteria xylosidase fares better but varies considerably depending on the enzyme source (20–650 mmol/L)[15]. Therefore, this enzyme is one of the most xylose tolerant enzymes. This property should make this enzyme useful in saccharification of xylan-containing polysaccharides.

**Table 2 Substrate specificity of Bxyl**

| Substrates | Relative activities (%) |
|---|---|
| *p*-nitrophenyl-β-xylopyranoside | 100 |
| *2*-nitrophenyl-β-D-galactopyranoside | 0.4 |
| *p*-nitrophenyl -L-α-arabinofuranoside | 3.4 |
| *p*-nitrophenyl β-D-mannopyranoside | 0.3 |
| *p*-nitrophenyl- β-D-glucopyranoside | 0.9 |

Activities with listed substrate was determined in sodium phosphate buffer (50 mmol/L, pH 7.0) with a final substrate concentration of 1 mmol/L, data listed in this table are means of 3 independent determinations.

#### 2.3.3 *pH and temperature optimal and stability*

The optimal pH and temperature were investigated. The enzyme shows a strong dependence on pH, with its activity peaked at pH 7.0 (Fig. 5). This is expected since it is predicted an intracellular enzyme. The enzyme exhibited excellent pH stability over a wide range of pH 4.5 to 9.0, but stability decreased dramatically out of this range (Fig. 5A). Since fungal xylosidases typically have an acidic pH optimal (such as pH 3.5) and a narrow pH

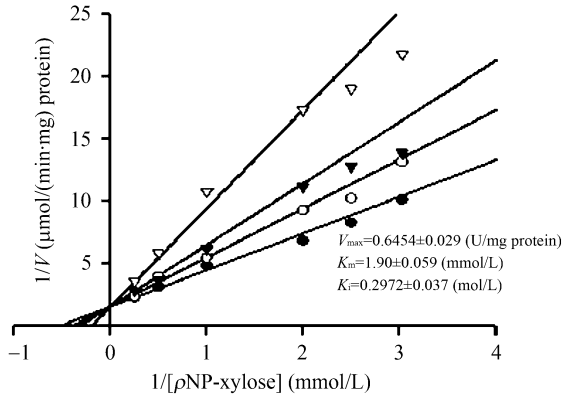


**Fig. 4** Lineweaver-Burk plot of the kinetic study of recombinant β-xylosidase, data in this figure are means of three independent determinations ●: 0 mol/L xylose; ○: 0.1 mol/L xylose; ▼: 0.2 mol/L xylose; ▽: 0.5 mol/L xylose.

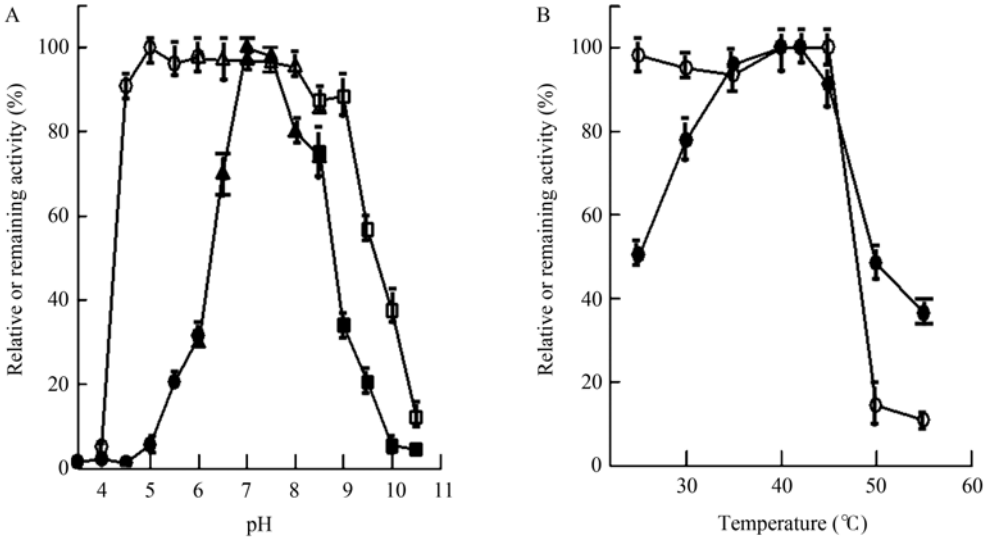


Fig. 5 Effects of pH (A) and temperature (B) on enzyme activities (close) and stability (open). ●, ○: 50 mmol/L sodium citrate buffer (pH 3.5–6); ▲, Δ: 50 mmol/L sodium phosphate buffer (pH 6-8); ■, □: 50 mmol/L Glycine-NaOH buffer (pH 8.5-10.5), data in this figure are means of 3 independent measurements.

stability range, this enzyme could be advantageously used in applications requiring neutral or higher pH xylanlytic condition. As shown in Fig. 5B, this enzyme exhibits a broad temperature optimal. The enzyme activity varies little in the range of 35°C–45°C. This broad optimal temperature should offer some flexibility in the design of a bioprocess. The purified enzyme was observed to be quite stable. The activity can maintain at 4°C for several weeks, and about 80% of its activity was retained at room temperature for nearly a week. Further studies were carried out to evaluate the utility of this enzyme in xylan saccharification. Using beech wood xylan as substrate, hydrolysis was carried out with recombinant xylosidase, commercial xylanases, or combination. Because the recombinant enzyme was stable up to 45°C but lost its activity at temperature higher than 50°C (Fig. 5), a reasonable range of xylanlytic operation with pH range of 6.5–8.5 (retaining at least 70% of its optimal activity) and temperature window of 35 to 45°C (retaining at least 90% of its optimal activity) was used.

**2.3.4** ***Application of β-xylosidase in xylan saccharification and its synergy with commercial xylanase***

Resulting enzymatic hydrolysate was monitored by DNS method, and resulting carbohydrates were analyzed by using a Dionex system, respectively. When recombinant xylosidase (25 U/g xylan) was used alone, very little xylose (about 4% of all available xylose) was produced (Fig. 6). Commercial xylanases, Multifect xylanase (Genencor International) and *Thermomyces lanuginosus* xylanase (Sigma) (each at 100 U/g xylan) were also tested for their ability to hydrolyze beech wood xylan. Both commercial enzymes were highly active, and the reaction was fast, reaching maximal yield within 1 hour. *Thermomyces lanuginosus* xylanase reached higher hydrolysis yield (27%) than that of Multifect xylanase (18%) (Fig. 6). When recombinant β-xylosidase was combined with either commercial xylanase, the extent of xylan hydrolysis was significantly increased and the final yields reached were 37% (with Multifect xylanase) and 42% (with *Thermomyces lanuginosus* xylanase). The synergistic factor, defined as the ratio of yield obtained with both enzymes to the sum of that with individual enzymes, were 1.7 and 1.4 for Multifect xylanase and *T. lanuginosus* xylanase, respectively. The resulting hydrolysate from enzyme hydrolysis, after 24 hours of reaction, was analyzed using the Dionex carbohydrate analysis system. As shown in Fig. 7, the hydrolysate was dominated by xylose and xylobiose, with the former being the most abundant sugar in the mixture. A comparison of sugar profiles after xylanase treatment showed that Genencor enzyme generates a mixture of more complicated oligomers than Sigma counterpart. Addition of xylosidase significantly reduced the complexity of the resulting mixture in both cases, suggesting a high synergy of xylosidase with commercial xylanases (Fig. 7). The highest xylose (product) concentration obtained was 8.4 g/L (or 56 mmol/L), well below the concentration inhibitive to the xylosidase (300 mmol/L). Therefore, the cessation of the reaction cannot be attributed to the product inhibition to xylosidase. Other

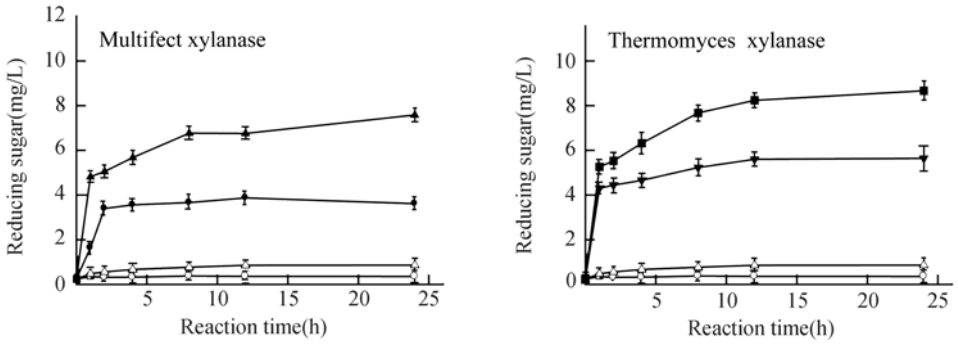


Fig. 6 Enzymatic hydrolysis of beech wood xylan by recombinant β-xylosidase and commercial xylanase.○: no enzyme; △: β-xylosidase; ●: Multifect xylanase; ▲: Multifect xylanase plus β-xylosidase; ▼: *Thermomyces* xylanase; ■: *Thermomyces* xylanase plus β-xylosidase.

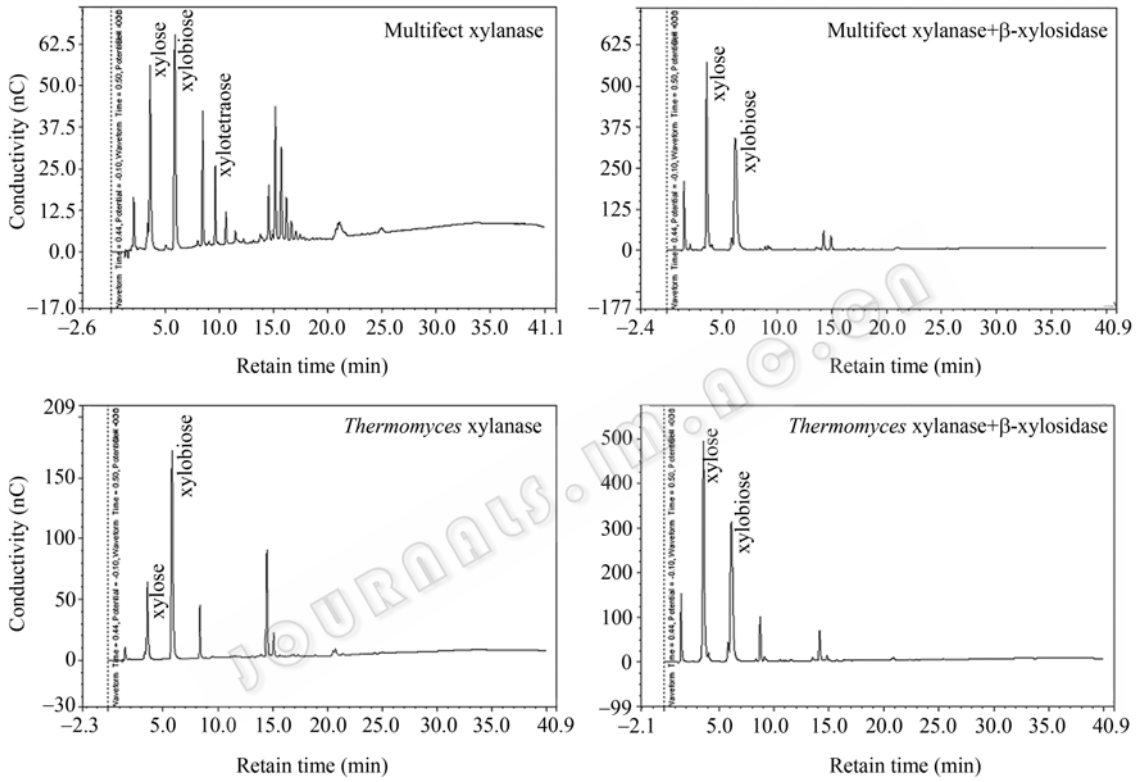


Fig. 7 Sugar profiles of 24 hour enzymatic hydrolysate of beech wood xylan.

factors such as the needing of other accessory enzymes for side chain removal may be the key factors to increase the yield of hydrolysis. It is interesting to note that although the recombinant xylosidase has much lower specific activity than fungal xylosidase, when used in combination with commercial xylanase, the hydrolysis yield achieved was similar to that with fungal xylosidase (40%)[14]. This indicated that xylosidase activity is not the limiting factor.

## 3 Conclusions

The gene encoding the xylosidase, *Bxyl* from *Bacillus halodurans* C-125, was successfully cloned and overexpressed in *E. coli* JM109. The recombinant enzyme possesses several favorable characteristics, which should make the enzyme useful in xylan and lignocellulose saccharification. Importantly, its tolerance to xylose inhibition is one of the highest. Together with its high synergy with commercial xylanases, its readily availability through *E. coli* overexpression and high specific activity make it a xylosidase of choice in the hemicellulase cocktail for biomass processing. The stability of the enzyme, with respecting to both pH and temperature, offers additional advantages in terms of process flexibility by providing a wide range of

operational conditions. Furthermore, the near neutral pH optimal complements well with fungal enzymes that are only optimal at acidic pH. In conclusion, this enzyme has high potential in the application of saccharification of xylan and xylan-containing polysaccharides

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