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Molecular Cloning and the Nucleotide Sequence of a *Bacillus* sp. KK-1 β-Xylosidase Gene

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Abstract A gene coding for β-xylosidase from thermophilic xylanolytic *Bacillus* sp. KK-1 was cloned into *Escherichia coli* using plasmid pBR322. Recombinant plasmid DNAs were isloated from *E. coli* clones which were capable of hydrolyzing 4-methylumbelliferyl-β-D-xylopyranoside. Restriction analysis showed the DNAs to share a common insert DNA. Xylo-oligosaccharides, including xylotriose, xylotetraose, xylopentaose, and xylobiose were hydrolyzed to form xylose as an end product by cell-free extracts of the *E. coli* clones, confirming that the cloned gene from strain KK-1 is a β-xylosidase gene. The β-xylosidase gene of strain KK-1 designated as *xylB* was completely sequenced. The *xylB* gene consisted of an open reading frame of 1,602 nucleotides encoding a polypeptide of 533 amino acid residues, and a TGA stop codon. The 3' flanking region contained one stem-loop structure which may be involved in transcriptional termination. The deduced amino acid sequence of the KK-1 β-xylosidase was highly homologous to the β-xylosidases of *Bacillus subtilis* and *Bacillus pumilus*, but it showed no similarity to a thermostable β-xylosidase from *Bacillus stearothermophilus*.

Key words: *Bacillus*, β-xylosidase gene, cloning, nucleotide sequence

Xylan, a highly branched β-1,4-linked D-xylose polymer, is a major component of both the forest and the agricultural biomass, such as grain straw, corn cobs, and grasses [23]. The characteristic backbone of xylan can be hydrolyzed to xylose by both β-1,4-xylanase and β-xylosidase. Xylanase degrades internal xylosidic linkages on the xylan backbone to form xylo-oligosaccharides, and β-xylosidase releases xylosidic residues by an endwise attack on the short xylo-oligosaccharides. Xylose, an enzymatic hydrolyzate of xylan, is regarded as a fermentative sugar for production of valuable products, including xylitol, xyluose, and ethanol.

β-Xylosidases and xylanases are found in many xylanolytic microorganisms [22]. Although many fungal β-xylosidases have been characterized, only a few bacterial β-xylosidases have been purified and characterized from *B. stearothermophilus* [13], *B. pumilus* [9], *Clostridium acetobutylicum* [11], *Thermomonospora fusca* [3], and *Thermoanaerobacter ethanolicus* [19]. However, the β-xylosidase of *B. pumilis* has been studied extensively. In addition, β-xylosidase genes have been cloned and characterized from *B. pumilus* [14], *B. subtilis* [4], *B. stearothermophilus* [21], *Caldocellum saccharolyticum* [12], and *Bacteroides ovatus* [24].

Thermophilic *Bacillus* sp. KK-1 was isolated as a bacterial strain which produces a thermostable cellulase-free xylanase comparable with that of *B. stearothermophilus* [10]. Recently, it was found by cloning the xylanase gene into *E. coli* that KK-1 secretes another xylanase active at 40°C (unpublished data). In a cell-free extract of KK-1 at least two β-xylosidases were also detected with high level activities at approximately 40° and 70°C, respectively. It is of interest that KK-1 produces two different enzyme sets of β-xylosidase and xylanase relating to the optimum reaction temperature. This paper describes the molecular cloning and sequencing of a KK-1 β-xylosidase gene which encodes the thermolabile enzyme.

Materials and Methods

Chemicals, Enzymes and Isotopes

Restriction endonucleases, DNA polymerase (Klenow fragment), T4 DNA ligase and RNase were obtained from

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Boehringer Mannheim and were used as recommended by the manufacturer. Sequenase, a modified T7 DNA polymerase, was obtained from U.S. Biochemical Corp. [α-^{35}S] dATP (1,000 Ci/mmol) was purchased from Amersham Corp. 4-Methylumbelliferyl β-D-xylopyranoside (MUX), *p*-nitrophenyl-β-D-xylopyranoside (pNPX), ampicillin, and agarose were purchased from Sigma Chemical Co. Bacterial medium was obtained from Difco.

Bacterial Strains, Plasmids and Media

Bacillus sp. KK-1 [10] was used as the source of the gene coding for β-xylosidase. *E. coli* XL-1 blue (*supE*44 *hsdR*17 *recA*1 *endA*1 *gyrA*46 *relA*1 *thi lac*$^-$ F'[*proAB*$^+$ *lacI*^{q} *lacZ*ΔM15 Tn*10*(*tet*^{r})]) was used as a host for cloning experiments. Plasmids pUC19 and pBR322 were used for all cloning and sequencing experiments.

Construction of a *Bacillus* sp. KK-1 Genomic Library

The standard procedures of Sambrook *et al.* [17] were used for DNA manipulation. Chromosomal DNA was isolated from *Bacillus* sp. KK-1 cells which were grown exponentially in LB medium according to the preparative method described by Rodriquez and Tait [15]. Fifty micrograms of purified *Bacillus* sp. chromosomal DNA was partially digested with *Sau*3AI. DNA fragments ranging from 2 to 10 kb were then isolated by sucrose gradient centrifugation for 20 h at 25,000 rpm in a Beckman SW40 rotor. The *Sau*3AI-generated chromosomal DNA fragments were ligated to *Bam*HI-digested, dephosphorylated pBR322. The ligation mixture was used to transform *E. coli* cells by the electroporation method [7].

DNA Sequencing

The DNA fragments generated by restriction endonucleases were introduced into pUC19. Both strands of the subcloned fragments were completely sequenced using the dideoxy-chain termination method [18] with a Sequenase version 2.0 Kit (USB Corp.) and double-stranded DNAs as templates. A reverse primer from Phamacia was used for sequencing double-stranded DNAs.

Assay of Enzyme Activity

β-Xylosidase activity was assayed spectrophotometrically by measuring the release of *p*-nitrophenol (pNP) from pNPX. Reaction mixtures of 0.5 ml containing 1 mM pNPX in a 20 mM sodium phosphate buffer, pH 6.5, were incubated at 40°C for 15 min. The reaction was stopped by adding 1 ml of 1 M sodium carbonate. The absorbance was measured at 400 nm. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol of p-nitrophenol per min under the assay conditions. Protein concentrations were determined by the Bradford method [5].

Thin-layer Chromatography

Reaction mixtures containing xylo-oligosaccharides were incubated at 40°C for complete hydrolysis. The reaction mixtures were then boiled for 10 minutes and centrifuged. The samples (approx. 60 μg) were spotted on a silica gel-precoated thin layer plate (15×20 cm, Merck Kiesegel, $60F_{254}$), and developed at room temperature with metanol, chloroform, acetic acid, and water (10 : 10 : 3 : 1, v/v). For detection of carbohydrates a mixture consisting of 0.5 ml of *p*-anisaldehyde, 0.5 ml of concentrated H_2SO_4, and a few drops of glacial acetate in 9 ml of 95% ethanol was used as a spray reagent.

RESULTS AND DISCUSSION

Cloning of the β-Xylosidase Gene from *Bacillus* sp. KK-1

The genomic library of *Bacillus* sp. KK-1 was constructed in *E. coli* XL1-Blue using pBR322 as a cloning vector. Approximately 10,000 transformants were transferred to LB agar plates for overnight incubation. Soft agar (0.7% w/v) containing 0.2 mM MUX was overlayed on the agar plates for screening *E. coli* clones which exhibited β-xylosidase activity. After incubation for 2 h at 40°C, 43 colonies capable of hydrolyzing MUX were selected by observing intense fluorescence at 360 nm due to the liberated 4-methylumbelliferone (Fig. 1). β-Xylosidases produced by all *E. coli* clones were found to be active at approximately 40°C [6], while *Bacillus* sp. KK-1 produced at least two types of β-xylosidase active at 40° and 70°C, respectively (data not shown). The recombinant plasmids were isolated from these *E. coli*

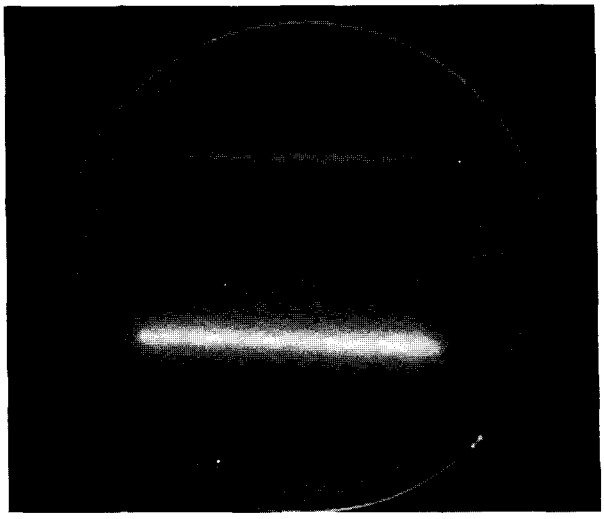

Fig. 1. The MUX phenotypes of the *E. coli* XL-1 blue cells harboring plasmids pBR322 (A) and pBX45 (B).

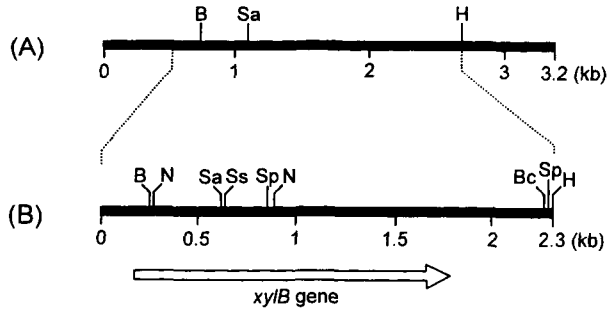

Fig. 2. Restriction endonuclease map of the pBX45 insert DNA.

(A) restriction map of the 3.2-kb insert DNA. (B) restriction map of the sequenced 2.3-kb DNA. Below the restriction map the open bar denotes the 1,602 nucleotides corresponding to the *xylB* gene with the arrow indicating the direction of transcription. Restriction site abbreviations are as follows: B, *Bam*HI; Bc, *Bcl*I; H, *Hin*dIII; N, *Nde*I; Sa, *Sal*I; Ss, *Ssp*I; Sp, *Sph*I.

clones and analyzed with various restriction enzymes. Restriction analysis indicated that the clones harbored the same gene since a commom chromosomal fragment existed in all insert DNAs of the recombinant plasmids. The smallest recombinant plasmid, named pBX45, contained a 3.2-kb chromosomal DNA which included the β-xylosidase gene of *Bacillus* sp. KK-1. A physical map of plasmid pBX45 is shown in Fig. 2.

β-Xylosidase Produced by *E. coli* Harboring Plasmid pBX45

To discriminate the gene product of pBX45 from xylanase, xylobiose and short xylo-oligosacchrides were used as substrates for enzyme reactions. Hydrolyzed products of xylo-oligosaccharides with a crude extract of *E. coli* (pBX 45) were analyzed by thin-layer chromatography (Fig. 3). The gene product catalyzed the complete hydrolysis of short xylo-oligosaccharides including xylotriose, xylotetraose, and xylopentaose, as well as xylobiose to xylose as the ultimate end product. From this result the gene product was categorized as a β-xylosidase since xylanase is known to be incapable of completely catalyzing xylo-oligosacchrides and xyobiose to xylose.

The activity of the β-xylosidase produced by *E. coli* (pBX45) was measured using pNPX as a substrate. A cell-free extract of *E. coli* (pBX45) had β-xylosidase activity with 0.7 U/mg of protein while *E. coli* (pBR322) did not show any β-xylosidase activity.

Nucleotide Sequence of the *xylB* Gene

The complete nucleotide sequence of the *xylB* gene and its flanking regions in pBX45 was determined (Fig. 4). The nucleotide sequence revealed that the open reading frame (ORF) was composed of 1,602 base pairs coding for a polypeptide of 533 amino acid residues and terminated at nucleotide position 1,840 with the stop codon TGA. The

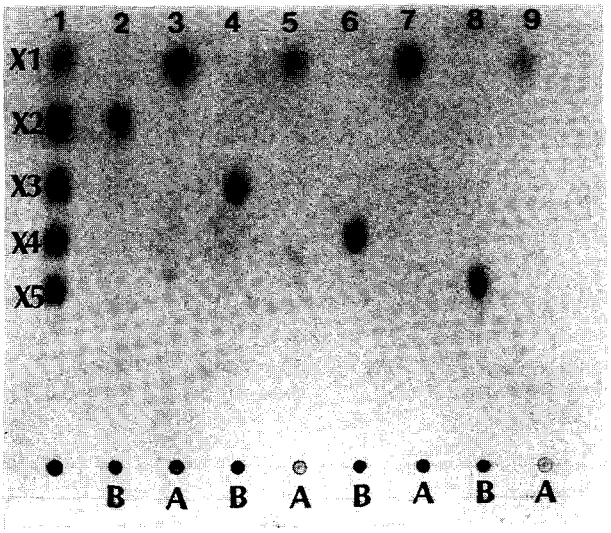

Fig. 3. Thin-layer chromatogram of the reaction products of xylobiose and xylo-oligosaccharides.

Lane 1 shows the mixture of xylo-oligosaccharides containing xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), and xylopentaose (X5). β-Xylosidase hydrolyzates: X2, lanes 2 and 3; X3, lanes 4 and 5; X4, lanes 6 and 7; X5, lane 8 and 9, before enzyme reaction (B, at the bottom) and after the reaction (A, at the bottom).

translational initiation site of *xylB* in the ORF was confirmed by the N-teminal amino acid sequence of purified β-xylosidase (Met-Lys-Ile-Ile-Asn-Val) [6], in agreement with the predicted amino acid sequence.

The codon utilization pattern for the *xylB* gene resembled those of the weakly expressed *E. coli* genes [8], showing a bias in the utilization of the codons as follows; TTT > TTC (Phe), ATT > ATC (Ile), CCC > CCT (Pro), GGC > GGT (Gly), TAT > TAC (Tyr), and AAT > AAC (Asn). In addition, the *xylB* gene had a total G/C content of 43.7% while the G/C content was 41.5% at the third base of the codon. The *xylB* gene did not show any preference for G or C residues at the third base of the codons, similar to the weakly expressed *E. coli* genes.

The putative promoter region was detected in nucleotide positions 87~92 and 111~116, approximately 130 bases upstream from the ATG start condon. The putative ribosome binding site, an AGGAGG sequence element, was seven bases upstream from the ATG start codon at nucleotide position 228~233. In the 3'-flanking region of *xylB* gene there was one palindromic region from 14 nucleotides to 34 nucleotides downstream of the TAA stop codon. Its free energy was -25.4 kcal/mol. This element could be involved in rho-independent termination of *xylB* gene transcription [16].

To determine whether the cloned fragment of plasmid pBX45 hybridizes to a specific region of the *Bacillus* sp. KK-1 genome, Southern blot analysis [20] was performed with a *Hin*dIII and *Bam*HI-generated 2.0-kb insert

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1                                                       GCTATGCCGCAGGCCAGGTCCAAACACCTGAAGCGTTGACAG   42

43   GGATATTATTAACGACAACTGTAGTTCCGGTTATTTTCCTTATATTGGCTATGATCGACATTAATTTTTACAATTTAAGCGATGAAAAATATGCAAAAG   141
                                                  -35                     -10
142  TCGTACGTGAATTAGAGAATAGAGATAAAGTGACTTGGACTCTATGAAGAATTTCAGTGGAAATATACATTTTAAAGACAACCGTGAGGAGGAATGAAG   240
                                                                                             SD
241  ATG AAA ATT ATC AAT CCG GTG CTT AAA GGT TTC AAC CCG GAT CCA TCC ATA TGT AGA GTA GGG GAA GAT TAT TAT   315
1    MET Lys Ile Ile Asn Pro Val Leu Lys Gly Phe Asn Pro Asp Pro Ser Ile Cys Arg Val Gly Glu Asp Tyr Tyr    25

316  ATC GCA GTA TCC ACC TTC GAA TGG TTT CCA GGA GTG CAA ATC CAT CAT TCA AAA GAT TTG GTG AAT TGG CGT TTA   390
26   Ile Ala Val Ser Thr Phe Glu Trp Phe Pro Gly Val Gln Ile His His Ser Lys Asp Leu Val Asn Trp Arg Leu    50

391  ATT GCA CAT CCG TTG CAG AGG GTT TCA CAA TTG GAT ATG AAA GGG AAT CCC GAC TCA GGC GGT GTA TGG GCG CCT   465
51   Ile Ala His Pro Leu Gln Arg Val Ser Gln Leu Asp MET Lys Gly Asn Pro Asp Ser Gly Gly Val Trp Ala Pro    75

466  TGT CTA AGT TAT AGT GAA GGA AAG TTT TGG CTT ATA TAT ACA GAT GTA AAA GTT GTG GAC GGA GCC TGG AAA GAC   540
76   Cys Leu Ser Tyr Ser Glu Gly Lys Phe Trp Leu Ile Tyr Thr Asp Val Lys Val Val Asp Gly Ala Trp Lys Asp   100

541  TGT CAT AAC TAT TTA GTT ACT TGC GAA ACC GTC GAT GGG GAT TGG GGG GAG CCG ATT CAA CTA AAT AGT TCC GGC   615
101  Cys His Asn Tyr Leu Val Thr Cys Glu Thr Val Asp Gly Asp Trp Gly Glu Pro Ile Gln Leu Asn Ser Ser Gly   125

616  TTC GAT GCT TCA TTG TTC CAT GAT GTC GAC GGA AGA AAA TAT TTG TTA AAT ATG CTG TGG GAT CAC CGG ATC GGC   690
126  Phe Asp Ala Ser Leu Phe His Asp Val Asp Gly Arg Lys Tyr Leu Leu Asn MET Leu Trp Asp His Arg Ile Gly   150

691  CGC CAT TCA TTC GGA GGC ATT GTG ATG CAG GAA TTT TCC GTG CGC GAA AAA AGG CTT ATC CAT CAA CCG AAA ATC   765
151  Arg His Ser Phe Gly Gly Ile Val MET Gln Glu Phe Ser Val Arg Glu Lys Arg Leu Ile His Gln Pro Lys Ile   175

766  ATT TTT GAA GGC ACA GAT ATT AAA CTG ACT GAA GCG CCG CAC CTT TAT CAT ATC GGG GAT TAT TAC TAT TTG TTG   840
176  Ile Phe Glu Gly Thr Asp Ile Lys Leu Thr Glu Ala Pro His Leu Tyr His Ile Gly Asp Tyr Tyr Tyr Leu Leu   200

841  ACA GCG GAA GGC GGG ACC CGT TAC GAG CAT GCC GCC ACA ATT GCC CGT TCA AAA CAT ATC GAA GGA CCA TAT GAG   915
201  Thr Ala Glu Gly Gly Thr Arg Tyr Glu His Ala Ala Thr Ile Ala Arg Ser Lys His Ile Glu Gly Pro Tyr Glu   225

916  ATT CAC CCC GAC AAT CCA ATT TTA ACG TCT TGG CAC GAA CCT CGG AAT CCG TTG CAG AAA TGC GGA CAT GCA TCA   990
226  Ile His Pro Asp Asn Pro Ile Leu Thr Ser Trp His Glu Pro Arg Asn Pro Leu Gln Lys Cys Gly His Ala Ser   250

991  ATT GTG GAA ACG CAT ACA GGT GAG TGG TAT TTA GCT CAC TTA ACC GGG CGT CCT ATT CAT CCT GCT GAT GAC TCG  1065
251  Ile Val Glu Thr His Thr Gly Glu Trp Tyr Leu Ala His Leu Thr Gly Arg Pro Ile His Pro Ala Asp Asp Ser   275

1066 GTT ATT CAC CAA AGA GGA TAT TGT CCT TTG GGA AGA GAA ACC GCG ATT CAA AAA TTA GAG TGG AAA GAC GGC TGG  1140
276  Val Ile His Gln Arg Gly Tyr Cys Pro Leu Gly Arg Glu Thr Ala Ile Gln Lys Leu Glu Trp Lys Asp Gly Trp   300

1141 CCT TAT GTG GCA GGC GGA AAA GAG GGG AGG GTG GAG GTG GAA GCG CCC CGC ATA TCT GAA ACC AAG TTT CCA TCG  1215
301  Pro Tyr Val Ala Gly Gly Lys Glu Gly Arg Val Glu Val Glu Ala Pro Arg Ile Ser Glu Thr Lys Phe Pro Ser   325

1216 ACA TAT CAG GAA GTC GAT CAA TTT GCT GAT CGG ACA TTA AAT ATG AAC TTT CAG ACA TTG CGG ATT CCC TTT ACG  1290
326  Thr Tyr Gln Glu Val Asp Gln Phe Ala Asp Arg Thr Leu Asn MET Asn Phe Gln Thr Leu Arg Ile Pro Phe Thr   350

1291 AAA GAA TTA GGA TCA TTG ACT GAA AGG CCG AAT CAT TTA CGT TTA TAT GGA CGT GAA TCA CTA ACC TCT ACA TTT  1365
351  Lys Glu Leu Gly Ser Leu Thr Glu Arg Pro Asn His Leu Arg Leu Tyr Gly Arg Glu Ser Leu Thr Ser Thr Phe   375

1366 ACC CAG GCG TTT GTA GCC AGG CGT TGG CAA AGC CTC CGT TTT ACA GCG GAG ACT GCT GTT GAT TTC AAC CCG GAG  1440
376  Thr Gln Ala Phe Val Ala Arg Arg Trp Gln Ser Leu Arg Phe Thr Ala Glu Thr Ala Val Asp Phe Asn Pro Glu   400

1441 ACC TTT CAG CAA GCC GCG GGA CTC GTG AAT TAC TAC AAC ACC GAA AAC TGG ACG GCT CTT CAA GTG ACT TAT GAT  1515
401  Thr Phe Gln Gln Ala Ala Gly Leu Val Asn Tyr Tyr Asn Thr Glu Asn Trp Thr Ala Leu Gln Val Thr Tyr Asp   425

1516 GAA GAC CTT GGG CGC ATT TTG GAT GTA ACG ATA TGC GAT AAT TTT ACA TTC TCA CAG CCG ATA AAA GAC AAA ATT  1590
426  Glu Asp Leu Gly Arg Ile Leu Asp Val Thr Ile Cys Asp Asn Phe Thr Phe Ser Gln Pro Ile Lys Asp Lys Ile   450

1591 GTC ATC CCG CGG GAC ATA CAA TAT GTT TAT TTA AGA GTA AAT GTC GAA TGG GAA ACA TAT TAT TAC TCC TAT TCT  1665
451  Val Ile Pro Arg Asp Ile Gln Tyr Val Tyr Leu Arg Val Asn Val Glu Trp Glu Thr Tyr Tyr Tyr Ser Tyr Ser   475

1666 TTT AAT AAA AAA GAC TGG TAT AAA ATT GAG ATC CCG TTG GAA TCT AAA AAA CTG TCA GAT GAT TAT GTG CGC GGA  1740
476  Phe Asn Lys Lys Asp Trp Tyr Lys Ile Glu Ile Pro Leu Glu Ser Lys Lys Leu Ser Asp Asp Tyr Val Arg Gly   500

1741 GGA GGA TTC TTC ACC GGA GCT TTT GTG GGA ATG CAA TGC CAA GAT ACA AGC GGA GCA CAT CAA CAT GCC GAT TTT  1815
501  Gly Gly Phe Phe Thr Gly Ala Phe Val Gly MET Gln Cys Gln Asp Thr Ser Gly Ala His Gln His Ala Asp Phe   525

1816 GAT TAC TTT ATG TAT AAA GAG CTA TGA GTCAAAAAAACAGCGCCCCCGACAATCGGGGGCGGTTTAAATCCGTTTTCGTCCCTTCCAGTT  1905
526  Asp Tyr Phe MET Tyr Lys Glu Leu TER                ------>    <------                                  533

1906 TTAATGCTCCTTTCTTCCCTCGGTTTATTTTAAAAGTTGTCATCCTCGCTCGCTTAACGGGTAAGTGAAACCCGGAAGGGGCAGATGCATGATAAAACCT 2005
2006 TTCAAATTAAACTTCATTACTAAACAATGATTCCGGAAGGTGTTCATTAATGAACATCCATCGCTTGATTGCCGATTGAATTTGAAAGTTGCCCCAACTT 2105
6106 ATAATAAACACATGTTCATTAGTTGATGAGTGTTCAGAATGGCAAAGGAGGTTTTGCGATGAAGCAATCCGTTTGACTTTTCCAGCGAGCCATCCTCAAG 2205
2206 CTCCATGTCGCTTTCTAACCAAAACCTTTGATCAAACGCAGGAAAACGAACCTGGGCATGCGTGAACAGGGCTGAAAGCTT                 2287
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Fig. 4. Nucleotide sequence of the *Bacillus* sp. KK-1 β-xylosidase gene and the deduced amino acid sequence.

The deduced amino acid sequence is given in the three letter codon below the nucleotide. The ATG start codon was verified by comparison with the underlined N-terminus amino acid residues of purified β-xylosidase. The putative ribosome-binding site (SD) and promoter sequences (-10 and -35) are underlined. The inverted repeat is underlined by horizontal arrows. Numbers at the end of each line correspond to the nucleotide position.

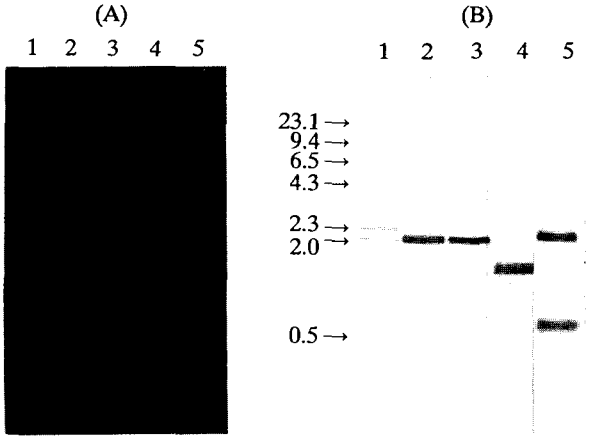

Fig. 5. Southern hybridization analysis of *Bacillus* sp. KK-1 chromosomal DNA.

The restriction enzyme digests of chromosomal DNA were analyzed on 0.8% agarose gel (A), and hybridized with a DNA probe (B). Lane 1, λ-DNA fragments digested with *Hin*dIII as size makers. Lane 2, A *Hin*dIII and *Bam*HI-generated insert fragment of pBX45 as a DNA probe. Chromosomal DNA was digested with *Hin*dIII and *Bam*HI (lane 3), *Sph*I (lane 4), and *Nde*I (lane 5).

fragment of pBX45 which included the structural *xylB* gene in nucleotide position 279~2,273 as a DNA probe (Fig. 5). When the chromosomal DNA of strain KK-1 was completely digested with *Hin*dIII and *Bam*HI the DNA probe was hybridized to only one fragement, co-migrating with the *Hin*dIII and *Bam*HI-generated 2.0-kb insert fragment of pBX45. Digestion with *Sph*I, for which the *xylB* gene had two sites at positions 864 and 2,253, resulted in two hydridization bands. Digestion with *Nde*I, for which the *xylB* gene also had two sites at 288 and 905, resulted in two hybridization bands. These bands were expected based on the restriction patterns of the *xylB* gene. No unexpected hybridization bands were detected. From these results the *xylB* gene has no homology to the thermostable β-xylosidase gene of strain KK-1.

Amino Acid and Nucleotide Sequence Homologies

When the amino acid sequence of a KK-1 β-xylosidase was compared with the sequences of other β-xylosidases in the NCBI data base using BLAST search program [1] the KK-1 β-xylosidase was found to be highly homologous to the β-xylosidases of *B. subtilis* and *B. pumilus* IPO among the β-xylosidases reported so far, on the basis of sequence alignments as shown in Fig. 6. The amino acid sequence of the *xylB* gene product showed 83% homology with the sequence of *B. subtilis* and 73% homology with the sequence of *B. pumilus* [25]. The nucleotide sequence of the *xylB* gene showed 78% and 70% homologies with the sequences of *B. subtilis* and *B. pumilus*, respectively.

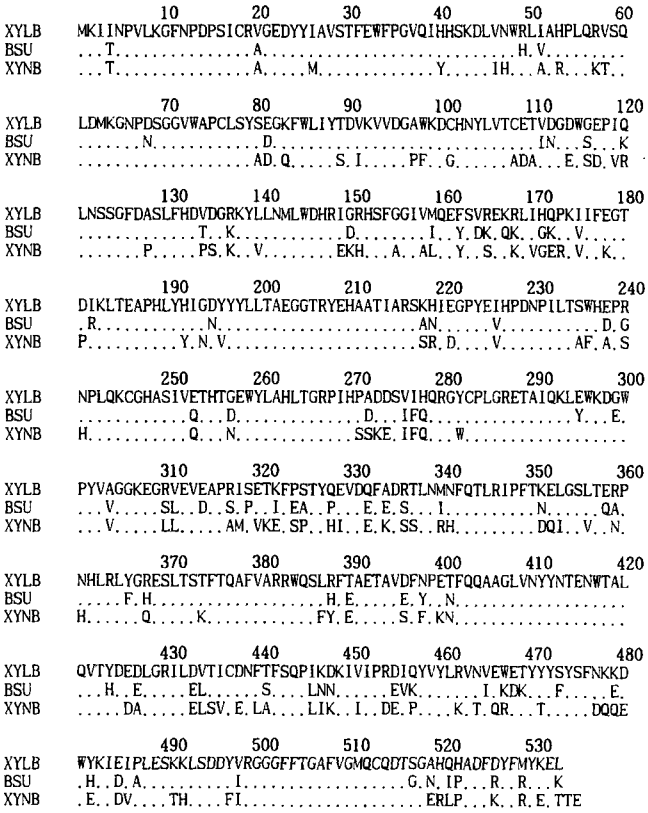

Fig. 6. Comparison of β-xylosidases from *Bacillus* sp. KK-1 (XYLB), *B. subtilis* (BSU), and *B. pumilus* (XYNB).

The amino acid sequences of the three polypeptides are shown in the one-letter code. Residues identical to the β-xylosidase amino acid sequence of strain KK-1 are depicted as dots in all other sequences. Numbers denote the amino acid position in the protein.

It is worth considering that the amino acid sequence similarities between β-xylosidases may be related to their reaction temperatures, even though the reaction properties of the *B. subtilis* β-xylosidase have not been reported. The optimum temperature for *B. pumilus* β-xylosidase was 40°C at which the *xylB* gene product was active. However, the thermolabile *xylB* gene product showed no homology with β-xylosidase of *B. stearothermophilus*, which is known to be active at 70°C [2].

Acknowledgments

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