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# The Complete Nucleotide Sequence of Alkalophilic *Bacillus* sp. K-17 β-Xylosidase Gene

**CHUN, HYO-KON, HAK-RYONG KO, YUNG-HEE KHO[1], AND NACK-KIE SUNG***

*Department of Food Science and Technology, Gyeongsang National University, Chinju 660-701, Korea*
*[1]Genetic Engineering Research Institute, KIST, P. O. Box 17, Taeduck Science Town, Taejon 305-606, Korea*

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**The complete nucleotide sequence of alkalophilic *Bacillus* sp. K-17 β-xylosidase gene and its flanking regions were established. A 1263-bp of an open reading frame for β-xylosidase was observed. The molecular weight (50, 521 dalton), deduced from the nucleotide sequence of β-xylosidase gene, agreed with the result obtained by SDS-polyacrylamide gel electrophoresis of the purified enzyme (51,000 dalton). The Shine-Dalgarno sequence, 5'-GAGGAGG-3', was found 8 bp upstream of the initiation codon ATG. The −10 sequence (TAAAAT) in the promoter region for β-xylosidase gene was similar to the consensus sequence for *Bacillus subtilis* RNA polymerase, whereas the −35 sequence (TCGATCA) different from all the known −35 regions in the promoter for *Bacillus subtilis* RNA polymerase.**

It is necessary to construct a high-yield xylan-degradation system to make good use of the biomass of plant xylan. For this purpose a xylanase high producer, alkalophilic *Bacillus* sp. K-17 was isolated (1). In this bacterium the degradation of xylan to xylo-oligosaccharide is catalized by xylanases, xylanase I and II, followed by hydrolysis to xylose by β-xylosidase (2). To study this xylan-degrading system in alkalophilic *Bacillus* sp. K-17 at molecular level, xylanase and β-xylosidase genes were cloned (3-5). Xylanase genes of other *Bacillus* species, such as *B. pumilus* IPO (6), *B. subtilis* (7), *B. polymixa* (8) and an alkalophilic *Bacillus* sp. (9) have been cloned. β-Xylosidase gene of *B. pumilus* IPO (10) have been cloned. Our interest in the alkalophilic *Bacillus* sp. K-17 xylan degrading enzyme is focused on the structure and expression of these two genes. In this experiment we describe the complete nucleotide sequence of the β-xylosidase gene and its flanking regions.

## MATERIALS AND METHODS

### Enzymes and Chemicals

5-Brome-4-chloro-3-indoyl-β-D-galactoside (X-gal), isopropyl-thiogalactoside (IPTG), sodium dodecyl sulfate, ethidium bromide, agarose, acrylamide, bisacrylamide, urea and TEMED were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (α-$^{35}$S) dAPT was obtained from Amersham International (Amersham). T4 DNA ligase and all restriction enzymes were purchased from Takara Shuzo Co. (Japan) and Kosco (Korea). All other chemicals were of standard reagent grade.

### Bacterial Strains and Vectors

*E. coli* JM109 (*rec*A1, *end*A1, *gyr*A96, *thi*, *hsd*R17, *sup*E44, *rel*A1, (*lac-pro*AB), F, *tra*D36, *pro*AB, *lac*IqZ M15) was used as the host strain for all transformation and transfection done in this experiment. Phages M13 mp.18 and mp.19 were used as a vector for DNA sequencing. Plasmid pAK208, consisting of pUC19 and a 1.4 kb alkalophilic *Bacillus* sp. K-17 DNA fragment encoding the β-xylosidase gene, have been described (4).

### Media and Growth Condition

*E. coli* strain was grown routinely at 37℃ in LB medium (10 g Bactotryptone, 5 g yeast extract and 5 g NaCl per 1 *l* of distilled water, pH 7.0). Ampicillin (100 μg/m*l*) was added in the medium when *E. coli* carried plasmid. MacConkey agar was used as a selective medium. Minimal medium for *E. coli* culture was M9 medium (11).

*Corresponding author
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**Isolation of Phage DNAs**

Bacteriophage M13 single stranded DNAs were rapidly prepared following procedure described by Messing (12). M13 replicative form DNAs were isolated by the alkaline lysis method (13).

**Transformation and Transfection**

Transformation of *E. coli* was done as described previously (11). Transfection by M13 RF DNA was carried out as follows; A 0.1 ml of *E. coli* JM109 competent cells was mixed with ligated DNA, incubated on ice for 30 min, and followed by heat shock at 42℃ for 60 sec. To 3 ml of 0.7% top agar preheated at 48℃, 200 μl of the fresh *E. coli* JM109 grown at 37℃ and the transformed cells were added, and mixed briefly. If needed, 10 μl of 100 mM IPTG and 50 μl of X-gal (2% w/v) were added. The above mixture was overlayed on the LB agar plate and incubated overnight at 37℃.

**Sequencing Strategy**

A deletion plasmid pAK208 was previously constructed by ligation of *Eco*RI-*Xba*I fragment and pUC19 plasmid (4). *E. coli* transformants harboring pAK208 were ampicillin resistant and β-xylosidase positive, and formed white colonies on MacConkey agar. The nucleotide sequence of 1.4 kb chromosomal DNA insert was determined by strategy outlined in Fig. 1. The specific fragments generated by treatment of various restriction enzymes were firstly ligated into the multi-cloning site of the pUC 19 plasmid. Specific DNA fragments were subcloned again into the same enzyme digested phages M13 mp18 and M13 mp19, respectively. Single-strand phage DNAs isolated from the clone harboring these phages. Nucleotide sequence analysis was performed using single stranded phage DNA as a template and the entire sequence was determined from both strands.

**DNA Sequence Determination**

DNA sequencing was performed to determine the nucleotide sequence of *Bacillus* sp. K-17 β-xylosidase gene by the dideoxy chain termination of Sanger *et al.* (14) with a DNA sequencing kit obtained from New England BioLabs (15).

## RESULTS AND DISCUSSION

**Nucleotide Sequence of β-Xylosidase Gene**

The final sequence of 1,425 bp covering the entire β-xylosidase gene and its flanking regions is shown in Fig. 2. Assignment of the open reading frame relies on the molecular weight of the purified enzyme. Open reading frame (1,263 bp) commencing ATG start codon, encoding amino acid residues and a TGA stop codon was found in this nucleotide sequence. The β-xylosidase was deduced to consist of 419 amino acid residues, corresponding to a molecular weight of 50,521, whih agrees with the molecular weight of purified β-xylosidase of alkalophilic *Bacillus* sp. K-17 strain estimated by the method of SDS-polyacrylamide gel electrophoresis (51,000). The ribosome binding sequence, 5'-GAGGAGG-3', complementary to the 3' end of 16S rRNA of *B. subtilis* (16) was observed 8 bp upstream of initiation codon, ATG. Candidates for promotor sequences, the −35 and −10 regions, were found 48 bp upstream (TCGATCA) and 29 bp upstream (TAAAAT) from the initiation codon, respectively. However, they are different from the consensus sequences, TTGACA and TATAAT of *B. subtilis* (17), and the possibility remains that the true promoter is located upstream of the 5'-end determined here. Harwood *et al.* (18) reported that the chloroamphenicol acetyltransferase gene of *B. pumilus* started translation by TTG instead of ATG. Such a novel initia-

**Table 1. Amino acid composition of *Bacillus* sp. K-17 β-xylosidase deduced from DNA sequence (Molecular weight: 50,521 Da.)**

| | | | | | |
|---|---|---|---|---|---|
| Ala | 27 | Gly | 11 | Pro | 20 |
| Asn | 16 | His | 11 | Ser | 33 |
| Asp | 25 | Ile | 29 | Thr | 33 |
| Arg | 41 | Leu | 30 | Trp | 4 |
| Cys | 14 | Lys | 13 | Tyr | 23 |
| Gln | 13 | Met | 11 | Val | 32 |
| Glu | 19 | Phe | 14 | Total | 419 |

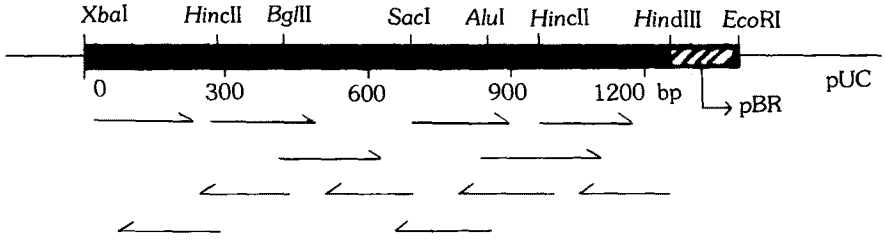


**Fig. 1. Restriction map of the region surrounding the pAK208-encoded β-xylosidase gene from alkalophilic *Bacillus* sp. K-17 and sequencing strategies.**

Restriction sites used in sequencing are presented. Arrows indicate the direction and extent of DNA sequencing analysis.

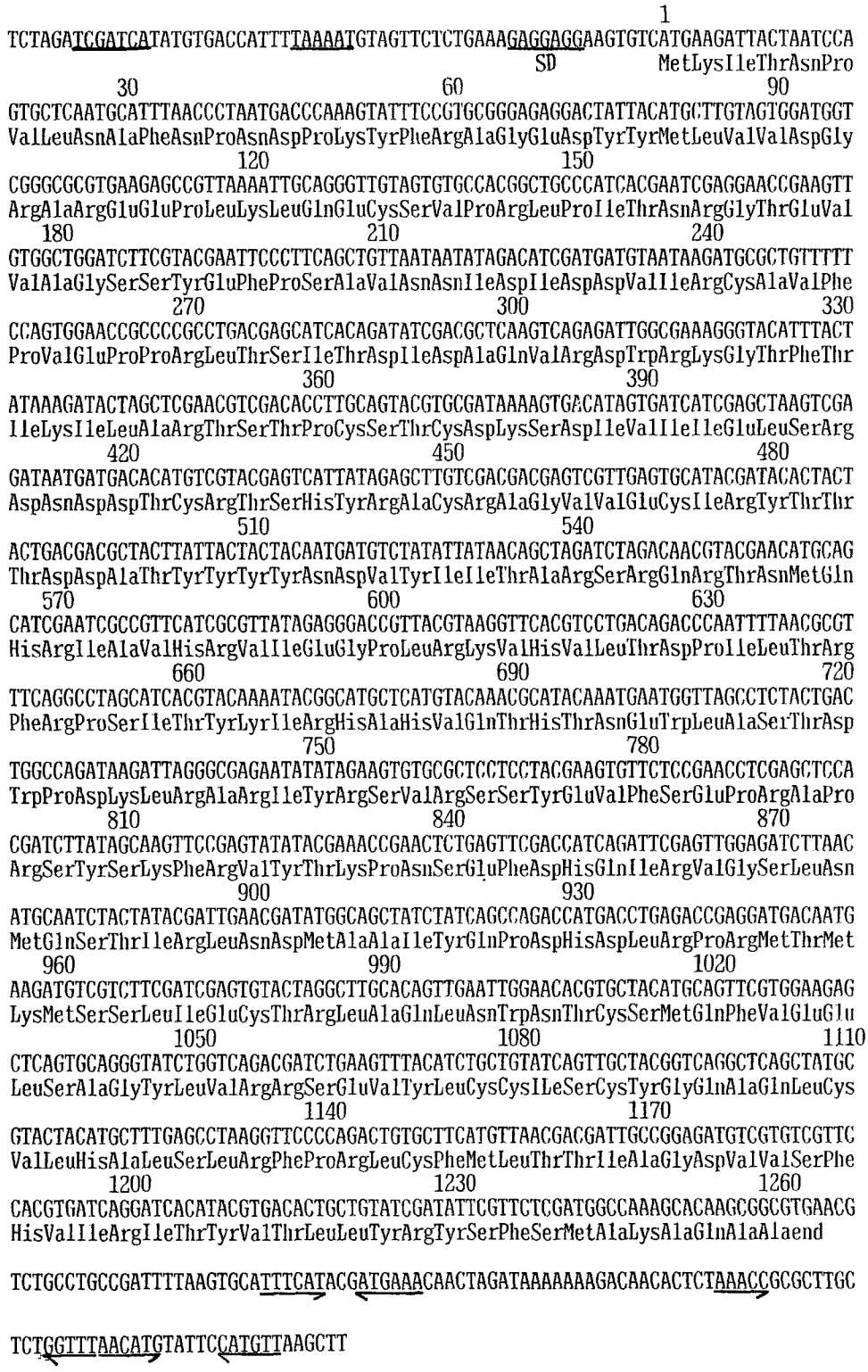


**Fig.2. The complete nucleotide sequence and amino acid sequence for *Bacillus* sp. K-17 β-xylosidase.** The Shine-Dalgarno sequences are indicated by SD. The putative transcription terminator sequences are indicated by arrows under the nucleotide sequences.

**Table 2. Codon utilization of *Bacillus* sp. K-17 β-xylosidase gene**

| Amino acids | No. | Codon | Amino acids | No. | Codon | Amino acids | No. | Codon | Amino acids | No. | Codon |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phe | 3 | UUU | Ser | 10 | UCU | Tyr | 12 | UAU | Cys | 5 | UGU |
| | 11 | UUC | | 3 | UCC | | 11 | UAC | | 9 | UGC |
| Leu | 5 | UUA | | 2 | UCA | Och | 0 | UAA | Opl | 1 | UGA |
| | 4 | UUG | | 11 | UCG | Amb | 0 | UAG | Trp | 4 | UGG |
| | 4 | CUU | Pro | 5 | CCU | His | 9 | CAU | Arg | 8 | CGU |
| | 19 | CUC | | 3 | CCC | | 2 | CAC | | 6 | CGC |
| | 6 | CUA | | 7 | CCA | Gln | 5 | CAA | | 6 | CGA |
| | 9 | CUG | | 5 | CCG | | 8 | CAG | | 3 | CGG |
| Ile | 5 | AUU | Thr | 9 | ACU | Asn | 0 | AAU | Ser | 8 | AGU |
| | 13 | AUC | | 1 | ACC | | 7 | AAC | | 4 | AGC |
| | 11 | AUA | | 13 | ACA | Lys | 5 | AAA | Arg | 15 | AGA |
| Met | 11 | AUG | | 10 | ACG | | 8 | AAG | | 3 | AGG |
| Val | 9 | GUU | Ala | 12 | GCU | Asp | 12 | GAU | Gly | 3 | GGU |
| | 6 | GUC | | 4 | GCC | | 13 | GAC | | 0 | GGC |
| | 5 | GUA | | 2 | GCA | Glu | 10 | GAA | | 7 | GGA |
| | 12 | GUG | | 4 | GCG | | 9 | GAG | | 1 | GGG |

tion codon was also found in *Staphylococcus aureus* β-lactamase (16). In the nucleotide sequence determined here, no open reading frames started by TTG were found except those at 85 bp and 123 bp on the same frame of β-xylosidase gene. These codons and ATG at 81 bp and 94 bp might be excluded from the functional initiation codon because of the long distance from the ribosome binding site. The region downstream from termination codon TGA presents a sequence containing inverted repeats which could form stem loop structure. The amino acid composition of β-xylosidase, deduced from the DNA sequence, is shown in Table 1.

### Condon Usage

Codon utilization of β-xylosidase is presented in Table 2. There appears to be bias for codon selection, for example UUC for Phe, AAU for Asn, CAU for His, GCU for Ala, ACU for Thr, UCU for Ser, GAA for Glu, and CGA for Arg. There are 11 glycines, but GGC is not used.

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## REFERENCES

1. **Sung, N.K., I.S. Kang, H.K. Chun, T. Akiba, and K. Horikoshi.** 1984. Xylanase produced by thermophilic alkalophilic bacteria-Isolation and some properties of *Bacillus* sp. *Kor. Appl. Microbiol. Bioeng.* **12**: 81-89.
2. **Sung, N.K., I.S. Kang, H.K. Chun, T. Akiba, and K. Horikoshi.** 1987. Purification and properties of extracellular β-xylosidase from thermophilic alkalophilic *Bacillus* sp. K-17. *Kor. J. Appl. Microbiol. Bioeng.* **15**: 267-272.
3. **Sung, N.K., H.K. Chun, K.H. Shim, I.S. Kang, and T. Akiba.** 1989. Molecular cloning and expression of a xylanase gene from alkalophilic *Bacillus* sp. K-17 in *Escherichia coli*. *Kor. J. Appl. Microbiol. Bioeng.* **17**: 178-182.
4. **Sung, N.K., H.R. Ko, Y.H. Kho, H.K. Chun, and Y.C. Chung.** 1989. Subcloning and enhanced expression of the β-xylosidase gene cloned from alkalophilic *Bacillus* sp. K-17. *Kor. J. Appl. Microbiol. Bioeng.* **17**: 283-288.
5. **Sung, N.K., H.K. Chun, D.H. Chung, K.H. Shim, and I.S. Kang.** 1989. Molecular cloning and expression of β-xylosidase gene from thermophilic alkalophilic *Bacillus* sp. K-17 into *Escherichia coli* and *Bacillus subtilis*. *Kor. J. Appl. Microbiol. Bioeng.* **17**: 436-439.
6. **Panbangred, W., E. Fukusaki, E. C. Epifanio, A. Shinmyo, and H. Okada.** 1985. Expression of a xylanase gene of *Bacillus pumilus* in *Escherichia coli* and *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* **22**: 259-264.
7. **Bernier, R. Jr., H. Driguez, and M. Desrochers.** 1983. Molecular cloning of a *Bacillus subtilis* xylanase gene in *Escherichia coli*. *Gene.* **26**: 59-65.
8. **Sandhu, J.S. and J.F. Kennedy.** 1984. Molecular cloning of *Bacillus polymyxa* (1-4)-β-D-xylanase gene in *Escherichia coli*. *Enzyme Microbiol. Technol.* **8**: 271-274.
9. **Honda, H., T. Kudo, and K. Horikoshi.** 1985. Molecular cloning and expression of the xylanase gene of alkalophilic *Bacillus* sp. strain C-125 in *Escherichia coli*. *J. Bacteriol.* **161**: 784-785.
10. **Panbangred, W., O. Kawaguchi, T. Tomita, T. Shinmyo, and H. Okada.** 1984. Isolation of two β-xylosidase genes of *Bacillus pumilus* and comparison of their gene products. *Eur. J. Biochem.* **138**: 267-273.

11. **Maniatis, T., E.F. Fritsch, and J. Sambrook.** 1982. *Molecular cloning, a laboratory manual.*, p. 88. Cold Spring Harbor Laboratory, NY.
12. **Messing, J.,** 1983. New M13 vectors for cloning. *Methods Enzymol.* **101**: 20-78.
13. **Birnboim, H.C. and J. Doly.** 1982. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res.* **7**: 1513-1523.
14. **Sanger, F., S. Nicklen, and A.R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* **74**: 5463-5467.
15. **Williams, S.A., B.E. Slakto, L.S. Moram, and S.M. Desimone.** 1986. Sequencing in the fast lane: a rapid protocol for [α-$^{35}$S] dATP DNA sequencing. *Biotechniques.* **4**: 138-147.
16. **McLaughlin, J.R., C.L. Murray, and J.C. Rabinowitz.** 1981. Unique features in the ribosome binding site sequence of the gram positive *Staphylococcus aureus* β-lactamase gene. *J. Biol. Chem.* **302**: 11283-11291.
17. **Moran, C.P. Jr., N. Lang, S.F.J. LeGrice, G. Lee, M. Stephens, A.L. Soneshein, J. Pero, and R. Losick.** 1982. Nucleotide sequences that signal the initiation of transcription and translation in *Bacillus subtilis. Mol. Gen. Genet.* **186**: 339-346.
18. **Harwood, C.R., D.M. Williams, and P.S. Lovett.** 1983. Nucleotide sequences of chloroamphenicol acethyltransferase gene of *Bacillus pumilus. Gene.* **24**: 163-169.