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Functional Cloning, Mutagenesis and Characterization of Xylanases
Genes from Alkaliphilic *Bacillus firmus* (3/3)

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Functional Cloning, Mutagenesis and Characterization of Xylanases Genes from Alkaliphilic *Bacillus firmus*(3/3)

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¥ ‹ \ fl – *firmus* ƒ] ¿ · ¥ X ¤ " " † { › @ … ¶˘P'°⁄ »Efl ⁄ , ƒ]A*xyn10A* 'M*xyn11A* ¡A¥ B ƒ b ⁄ ¿z" – † {‡o¤ " ‡ƒ]J ¥¡"C † {‡o¤ " ⁄»Efl ⁄ , » ^ fl ⁄ ƒ]¡"z – ⁄ ƒbEfl fi ‰¡W¥ H › Ł " G ‹ ı … ÆM¥"X¥ê¥"H‹" ⁄»E fl ⁄ , »^fl ¡Cxyn10A 'Mxyn11A ⁄ » E fl ⁄ , » ^ fl "″44kDa¶ ¿ §O‹ 23kDa ¥ B ¤"⁄ » E fl ⁄ ,ƒb»^fl xylan-zymogram ‰ ƒ ¯ ØW ƒ‡ » E fl ⁄ , »¡^fl "″ ' ¡C¤ " ⁄ » E fl ⁄ , fi»^fl»"S"ƒ¿‡ J ¥ ‰ Ł § ˙ ƒ Cwxyn10A w¡C » E fl ⁄ , »^fl ƒ‡396 › i »˜† ƒ ¤ ¡ A » i˜ § ƒ»C P ¶˘P°‹ \ fl – *halodurans* " " » E fl ⁄ , »^fl« D – ` ‹ ƒ ¡ A » P ¤¥"Lfl"u⁄ 'M†E fl ⁄ ⁄, æ»^fl ß « ÆA o†{ ƒ„44 kDa " " ⁄ » E fl ⁄ ˜, »^fl – 10 s " " ⁄ » E fl ⁄ ¡Cxyn11A ⁄ » E fl ⁄ , »^fl ƒ‡210 › i »˜† Af¤ i »˜§»P¶C ˘ P ' ‹ \fl fiŁ *halodurans* "″ β-1,4-⁄ » E fl ⁄ , » ^ fl ¥ u ƒ ‡ ⁄ @ › i »"″⁄£ƒP»¡Æ»P¤⁄¥Lfl»u^ 'M† fl ⁄ æ , ß « ÆA23kDa„"″⁄ » E fl ⁄,˜ »^fl – , s 11 "″⁄, s » E fl⁄ , » fl ¡ C ƒ b¯37 « ¡ApH 4.5 ⁄ ¿11 ⁄ ¶¡¡A‡ o ¤"⁄ , »^flªƒ‡‹ ¦°¡ C ƒ b¯70 « ‡ o ¤"⁄ , »^fl·ƒ‡¶ WL80%"″» fl ‹ ¦°¡A–N‡ o ¤"⁄ , »^fl m' 62 « „ ⁄U16 ⁄ ƒ «Æ⁄·¤ªƒ‡¶ WL80%"″» fl ‹ ¦°¡C

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Abstract

Two genes encoding thermostable alkaline endo-β-1,4-xylanases, named *xyn10A* and *xyn11A*, from an alkalophilic *Bacillus firmus* were cloned and expressed in *Escherichia coli*. The *E. coli* harboring either xylanase gene showed clear zone with Congo red-clearance assay on xylan plate. Xyn10A and xyn111A enzymes have molecular weights of 44 kDa and 23 kDa, respectively, and both show xyalanse activities on xylan-zymogran. The nucleotise sequences and the deduced amino acid sequences were determined. The xyn10A xylanase gene encodes 396 amino acid residues and very similar to an alkaliphilic xylanase A from alkaliphilic *Bacillus halodurans*. From alignment of the amino acid sequence of xyn10A xylanase with those of fungal or bacterial origin, this xylanase belongs to family10 xylanase. The xym11A xyalase contains 210 amino acid residues and almost identical with an endo-β-1,4-xylanase from alkaliphilic *Bacillus halodurans* with only one amino acid difference. From alignment of the amino acid sequence of xyn11A xylanase with those of fungal or bacterial origin, this xylanase belongs to family11 xylanase. Both show enzymatic activities over the pH range of 4.5 -11.0 at 37°C. Both enzymes show over 80% enzymatic activities at 70°C and still retain over 80% enzymatic activities after 16 hours incubation at 62°C.

Keywords: Xylan, *Bacillus firmus*, xylanase, thermostability

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Xylan is the most abundant of the hemicelluloses which are heteropolysaccharides having a chain of β-1,4-linked xylopyranose residues. The complete hydrolysis of xylan requires the combined action of various enzymes such as endoxylanase (EC 3.2.1.8), exoxylanase (β-D-xylan xylohydrolase), and β-D-xylosidase (EC 3.2.1.37) etc. Xylanases randomly hydrolyze the β-1,4-glycosidic bonds of xylan to produce several xylo-oligomers. In recent years, xylanases have received attractable research interest due to their potential industrial applications. However, such applications require xylanase(s) with particular properties, the bio-bleaching of paper pulp requires a xylanase that remains active even above pH 9.0

and lacks all cellulase activity.

The bacterium used in this study, *Bacillus firmus*, was previously isolated from a wastewater treatment plant of pulp and paper industry at Bang-Pre-In at Prankornsriayuttaya province, Thailand. *Bacillus firmus* is capable of growth at pH values, ranging from 10-12. Normally growth is at temperature of 37^0C; the cultures were thermolabile at temperatures above 55^0C. Moreover, this strain produces two major extracellular xylanases, with molecular weights of 45 kDa and 23 kDa, respectively. No other hemicellulose-degrading enzyme activities were detected in the culture medium with xylan, CMC or avicel as the sole carbon source, suggesting that this strain produces xylanase mainly. The mode of action of xylanase and cellulase has been reported. It was considered that the cellulose/xylan binding domain (CBD/XBD) was an important factor in the degradation process of insoluble cellulosic materials. For xylanases the concept of substrate recognition and induction, *i.e.*, the control of transcription by the cooperative actions of an activator and a repressor, have generally been accepted. Xylanase synthesis is induced by natural xylan and other β-1,4-xylo- oligosaccharides isomers.

In this report, we describe the cloning and characterization of two major xylanases from *Bacillus firmus* bacteria. These enzymes are active over a wide range of pH and are therstable. In addition other properties presented here suggest that these xylanases could be of commercial interest.

⁄T¡B‹ ª¤s⁄Ł"k

A. Construction of genomic libraries of *Bacillus firmus*: The chromosomal DNA of *Bacillus firmus* was purified and digested partially with *Eco*RI or *Hin*dIII and the 1-4 kb and larger DNA bands were isolated. Then ligated the purified DNA fragments into *Eco*RI- or *Hin*dIII-linearized pUC18, respectively, and transformed into *E. coli* XL10-Gold by electroporation. The tansformants were selected on ampicillin LB plate and collected as genomic libraries. These two genomic libraries are using for the functional cloning of xylanase genes.

B. Functional cloning of xylanases genes: To identify the xylanases genes, *E. coli* cells containing the genomic libraries of *Bacillus* sp. strain K-1 will be grew on 0.5% oat spelts xylan-LB agar plates at 37^oC. The colonies harboring xylanase activity will show clear zones on the plates. The xylanase activity-positive colonies will be picked and re-confirmed by the xylan-Congo red clearance plate assay.

C. Nucleotide sequence analysis: The nucleotide sequences of the insert DNA will be analyzed for open reading frames coding for xylanases, putative –35 and –10 promoter elements and ribosome binding sites. The two deduced amino acid sequences of xylanases genes would be confirmed by the presence of the N-terminal amino acid sequences shown in "preliminary data" following signal peptides. By homology comparisons of the deduced amino acid sequences of the *firmus* xylanases genes with those of other endoxylanases complied in the GenBank/EMBL Data Bank will reveal which family, 10 or 11, these two xylanases belong to.

D. Xylanase assay: The assay mixture consisted of 40 µl of crude enzyme solution and 160 µl of a 0.5% birchwood xylan suspension in 100 mM Na_2CO_3-$NaHCO_3$ buffer (pH 9.0). The reaction was incubated at 37°C for 10 min and 0.4 ml of DNS reagent (1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, 1% sodium hydroxide) was added to stop the reaction, then boiled for 5 min. The absorbance at 500 nm was measured after adding 2.4 ml of water.

E. Effect of pH on activity of xylanases: The pH values of various reaction solution were adjusted with 100 mM of following buffer systems: acetate buffer (pH 4.0-5.5), phosphate buffer (pH 6.0-6.5), Tris-HCl buffer (pH 7.0-9.0), and glycine-NaOH buffer (pH 10-12.0). The substrate, 0.5% birchwood xylan in various pH buffer, was incubated with crude enzyme solution for 10 min at 37°C and 0.4 ml of DNS reagent was added to stop the reaction, and then boiled for 5 min. The absorbance at 500 nm was measured after adding 2.4 ml of water.

F. Effect of temperature on activity of xylanases: The substrate, 0.5% birchwood xylan in pH 7 or 9 buffer, was incubated with crude xylanase solution for 10 min at various temperature and 0.4 ml of DNS reagent was added to stop the reaction, and then boiled for 5 min. The absorbance at 500 nm was measured after adding 2.4 ml of water.

G. Thermostability of xylanases: The crude xylanase solutions were preincubated at 62°C or 72°C for indicated period of time, then added 160 µl of a 0.5% birchwood xylan suspension in 100 mM buffer (pH 7.0). The reaction was incubated at 37°C for 10 min and 0.4 ml of DNS reagent (1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, 1% sodium hydroxide) was added to stop the reaction, then boiled for 5 min. The absorbance at 500 nm was measured after adding 2.4 ml of water.

H. Zymogram analysis for xylanase activity: Samples were subjected to

electrophoresis on a SDS-PAGE containing 0.1% xylan. After electrophoresis, the gel was washed three times for 30 min at 4°C in 100 mM Na_2CO_3-$NaHCO_3$ buffer (pH 9.0) containing 25% isopropanol for the first two washes to remove SDS, then incubated in the same buffer for 10 min at 37°C. The zymogram was prepared by soaking the gel in 0.1% Congo red solution for 15 min at room temperature, then washed with 1 M NaCl and introduced 0.5% acetic acid to expose two xylanase active bands that contrasted the dark background.

¥|¡B †"G»P Q‰

A. Cloning of two xylanase genes from *Bacillus fimus*: The *E. coli* harboring the genomic library of *Bacillus firmus* was used for the functional cloning of xylanase genes. The colonies grew on LB-ampicillin agar plates were picked and re-grew on xylan agar plate. The Congo red clearance assay was used to identify the clones with xylanase activity on xylan plate, the positive ones showed clear zones around colonies. The nucleotide sequences of two clones with clear zones were sequenced and presented in Figure 1 (xyn11A) and 3 (xyn10A), respectively. Two sets of 5'- and 3'-primers corresponding to the N- and C-terminal of xyn10A and xyn11A xylanase genes of *Bacillus firmus* were synthesized. The PCR reaction was performed with genomic DNA of *Bacillus fimus* as template; the obtained two xylanase DNA fragments were cloned into PCR cloning vectors. Both groups of *E. coli* colonies showed clear spots implied functional activities of xylanase. The *E. coli* harboring plasmid containing 44 kDa xylanase gene (xyn10A) showed bigger clear zone than that of 23 kDa xylanase gene (xyn11A). Both *E. coli* extracts also showed xylanase activities on a xylan-zymogram gel with the protein sizes of the corresponding molecular weights of 23 and 44 kDa.

B. Nucleotide sequences and deduced amino acid sequences of xylanase genes: The nucleotide sequences of both genes were determined. Analyzed the sequence of DNA fragment containing 23 kDa xylanase gene identified an open reading frame (ORF). Search for homology by screening the GenBank database revealed that the 630 bp ORF encoded a family 11 xylanase sequence. The alignment of the 23 kDa xylanase with those of 7 family 11 xylanases revealed a significant identity (over 73%). There is only amino acid residue difference between this *Bacillus firmus* xylanase and an endo-β-1,4-xylanase from alkaliphilic *Bacillus halodurans*, Val *v.s.* Ile of residue 169 (Fig. 2). Therefore, we named this 23 kDa xylanase as xyn11A xylanase. Analyzed the sequence of DNA fragment containing 44 kDa

xylanase gene identified two open reading frames (ORFs). Search for homology by screening the GenBank database revealed that the 1188 bp ORF encoded a family 10 xylanase sequence. Therefore, we named this 44 kDa xylanase as xyn10A xylanase. The alignment of this xyn10A xylanase with those of 5 family 10 xylanases revealed a significant identity (over 43%). It showed 97% identity in amino acid sequence with the alkaline xylanase A from *Bacillus halodurans* (Fig.4). The other ORF was on the anti-sense strand in the upstream of the xyn10A xylanase encoded for a two-component response regulator gene (Fig. 3). Both nucleotide sequences have been submitted to GenBank with accession numbers of AY376352 and AY376353 (see references 2 and 3).

C. Characterization of the xylanases activities:

1) *The pH stability of the xylanases extract*- Both xylanases showed >50 % of their optimal activities over a wide pH range of 4.5-11.0 at 37°C. The xyn10A xylanase was slightly more alkaline resistant than the xyn11A enzyme. With this broad pH optimum, these two xylanases are satisfactory for the criteria in the pulp and paper industry.
2) *Effect of temperature on activity of xylanases*- Both xylanases showed >50 % of their optimal activities over a wide temperature range of 37-70°C in pH 7.0 buffer.
3) *Thermostability of xylanases*- Both xylanases protein still showed over 80% enzymatic activity even after 16-hour incubation at 62°C.

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We cloned two xylanases with molecular weights of 44 kDa (xyn10A) and 23 kDa (xyn11A)from the genome of *Bacillus firmus* and expressed both xylanase gene products in *E. coli.* In the past year, we have sequenced the nucleotide sequences of these two xylanase genes and their flanking regions and characterized the physical properties and the enzymatic activity toward xylan of these two xylanases. Both xylanase proteins are alkalophilic and thermostable and suitable for pulp industry. All the progress and expected results are in the pace of our proposal. A manuscript entitled "Purification and characterization of two cellulase free xylanases from an alkolophilic *Bacillus firmus*" has been published in *Enzyme and Microbial Technology* (see reference 1). Another manuscript entitled "Isolation of an extracellular serine protease from an alkalophilic *Bacillus firmus*" also is in well preparation. This paper described the isolation and characterization of the 14.5 kDa protease secreted by this

bacterium during our preparation of xylanase sample. As we known, this protease was the smallest serine protease that had been reported so far. The DNA libraries of this bacterium were constructed and functional cloning of these two xylanases is being in hot pursuit.

We also in the process of preparing a manuscript entitled "Cloning and characterization of two thermostable xylanase from an alkalophilic *Bacillus firmus*". This manuscript describes the result of this funding year. The nucleotide sequences of this finding have already been submitted to GenBank (http://www.ncbi.nlm.nih.gov/, see references 2 and 3). Overall, we had a fruitful progress with three manuscripts and one of them has already been published.

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[1] **Tseng, M.-J.**, Yap, M.-N., Ratanakhanokchai, K., Kyu, K. L., & Chen, S.-T. (2002) Purification and characterization of two cellulase free xylanases from an alkaliphilic *Bacillus firmus*. *Enzyme Microbial Technology*, 30,590-595.

[2] **Tseng, M.-J.**, Chang, P. & Tsai, C.-L. (2003) *Bacillus firmus* BH0898-like protein and xylanase 11A genes, complete cds. GenBank accession number: AY376352.

[3] **Tseng, M.-J.**, Chang, P. & Tsai, C.-L. (2003) *Bacillus firmus* BH2133-like protein gene, partial cds; and xylanase 10A and BH2119-like protein genes, complete cds. GenBank accession number: AY376353.

Fig. 1. Nucleotide sequence of the *Bacillus firmus* xyn11A xylanase gene (23 kDa xylanase) and deduced amino acid sequence of xyn11A xylanase gene protein. The ribosome binding sequence (RBS) is boxed. The symbol "***" designates stop codon.

```
1
GATCAGGCTACAAAGTGAAAACCATTCAACCCGTGGATATGTTCCCGTGGACAGCGC
AGGTGGAGAGTTG
71
TTCACTACTTGTTTATGAAGGGAAATAAATAAAATAACTTGATCAGAAGGTATCTTT
TTTGTGTATACGT
141
ATAACTAGACGGACGTTGTCAAGTTGATGGTCAAAATCACGAGACGCCCGCGGGAAA
AGCAAGAGCTGAA
211
GATCCATCGGGGTGTTTTCCCCCGATTAGCTGAAACCTTGCCCGCGGCAAGCGAGTG
GTTTTTGAAACAA
281
TCAATCCCATATAAAAATAAGAGTGGCACTCATCGTCGGTGAGTGTCACTCTTAGTC
CCAGCCTATTCTT
351
GTTTAAAGGAAGTTAAGATTTGCATTAACCCCTTTAGCAGAATGAGTTGAGAAGTGG
ACAGGATTACGGT
421
AATAAATAATTCACTACTTAAAATAGAGAATGGAGAGCTATAATGGCTGATGGTAAA
GAACATAAATACA
BH0898                                RBS
MetAlaAspGlyLysGluHisLysTyrA
                                              1
491
GAATGGTTTTTGGTTGGGAAGATGATAACGGAATTGGTGAAATGTTAATCCAGCAAA
TTATAAATGGAGA

rgMetValPheGlyTrpGluAspAspAsnGlyIleGlyGluMetLeuIleGlnGlnI
leIleAsnGlyGl
      10                        20                          30
561
AAAGACAGCGACATGTGCTCCGAAAGAAGAGTATTCAGAGCAAGAATTGCAAGAAAC
GTACGAACCAGTT

uLysThrAlaThrCysAlaProLysGluGluTyrSerGluGlnGluLeuGlnGluTh
rTyrGluProVal
                        40                              50
631
GGTGAGCTCGTTACGGTATTCGATAAAAATGGCAATGCCAGATGTACGGTTAGGCTA
CTAGAAGTATTTG

GlyGluLeuValThrValPheAspLysAsnGlyAsnAlaArgCysThrValArgLeu
LeuGluValPheG
              60                          70
701
AAACGACGTTCGGCAATCCAGATTTAAGACTTGTACGAGGTGAAGGGAATGGCGACA
ATGTTTGTAAGTT

luThrThrPheGlyAsnProAspLeuArgLeuValArgGlyGluGlyAsnGlyAspA
snValCysLysPh
```

```
      80                          90
100
771
TCAAGAGGACCATAGGATAGCTTGGAAAGATATTGGTGTTGACTTAAGAGATGATAC
CGTTTTAATTGTC

eGlnGluAspHisArgIleAlaTrpLysAspIleGlyValAspLeuArgAspAspTh
rValLeuIleVal
                        110                         120
841
GAACTATTTGAATTGGTAGAAGGTTGATATTTGTGCTTTGCATAAGGGGTTCGTGAG
TTAAAAAATTTCA
      GluLeuPheGluLeuValGluGly***
               130       134
911
ATCTAACCTTAAATGAAGAGGTCTCATTCAGAGACCTCTTCATTTATTCCTATTACC
TAATCTATTTTTA
981
ATATAATAAATAAAAACAAAATCAAAATATGGAAAAAATCTTAATATTGATAAAATT
TGCAATGTTTGCG
1051

ATTTCGTACTATAATCTCCAAATGAAGGAGGTGATTGTATGAAGTTCCCATAATATGATAAAAGGTAAA

T

1121

GTGGACTGAATCAAGAAATCGACAACAAATGTGTAGATAAGTAGTACGATAAAAATTTTGAGGAGGACG


    RBS

1191

AATCATGTTTAAGTTCGTTACGAAAGTTTTGACGGTAGTAATTGCAGCTACAATTAGTTTTTGTTTGAG

T
```

Xylanse

```
MetPheLysPheValThrLysValLeuThrValValIleAlaAlaThrIleSerPheCysLeuSer
      1                          10                          20
1261
GCAGTACCGGCAAGTGCCAATACCTATTGGCAATATTGGACCGATGGTGGTGGAACA
GTAAATGCTACAA

AlaValProAlaSerAlaAsnThrTyrTrpGlnTyrTrpThrAspGlyGlyGlyThr
ValAsnAlaThrA
                         30                          40
1331
ATGGACCTGGTGGAAATTACAGTGTGACATGGAGAGATACAGGGAACTTTGTTGTCG
GTAAAGGCTGGGA

snGlyProGlyGlyAsnTyrSerValThrTrpArgAspThrGlyAsnPheValValG
lyLysGlyTrpGl
               50                              60
1401
AATCGGTTCACCAAATCGAACGATCCATTACAATGCTGGTGTCTGGGAACCGTCTGG
AAATGGATATTTG
```

```
uIleGlySerProAsnArgThrIleHisTyrAsnAlaGlyValTrpGluProSerGl
yAsnGlyTyrLeu
        70                          80
       90
1471
ACTCTCTATGGGTGGACAAGGAATCAGCTCATAGAATATTATGTCGTTGATAATTGG
GGAACTTACAGAC

ThrLeuTyrGlyTrpThrArgAsnGlnLeuIleGluTyrTyrValValAspAsnTrp
GlyThrTyrArgP
                      100                         110
1541
CTACTGGAACCCATCGAGGCACCGTTGTCAGTGATGGGGGAACATATGACATCTATA
CGACTATGCGATA

roThrGlyThrHisArgGlyThrValValSerAspGlyGlyThrTyrAspIleTyrT
hrThrMetArgTy
              120                         130
1611
CAATGCACCTTCCATTGATGGCACACAAACGTTCCAACAGTTTTGGAGTGTGAGGCA
ATCGAAGAGACCG

rAsnAlaProSerIleAspGlyThrGlnThrPheGlnGlnPheTrpSerValArgGl
nSerLysArgPro
       140                         150
160
1681
ACTGGAAATAACGTTAGCGTTACGTTTAGCAACCACGTGAATGCGTGGAGAAATGCA
GGAATGAATCTGG

ThrGlyAsnAsnValSerValThrPheSerAsnHisValAsnAlaTrpArgAsnAla
GlyMetAsnLeuG
                      170                         180
1751
GAAGTAGTTGGTCTTACCAGGTATTAGCAACAGAAGGCTATCAAAGTAGCGGGAGAT
CGAATGTAACGGT

lySerSerTrpSerTyrGlnValLeuAlaThrGluGlyTyrGlnSerSerGlyArgS
erAsnValThrVa
              190                         200
1821
CTGGTAGAACGAGAAAGATAAAGTCAAATTTCTGAATATTTAAAAATAAATCTATTG
TTGTGACTTCGAA
      lTrp***
       210
1891
CTTAAGATTTACTCATTAAGAAGAATGAAGCGGAGCGGTCAGGATCTCGAGCGAGGA
CAACCTTTTATCC
1961
GAAAAATGGCTATCGTCCTATTGGACAAGCCAATGGCCGTTCCTTCCTTTTTTATTACGCAGGACCCAA
A
2031   CATAAGCGAGTGATAGGGCATTGGCATCTATCACTCATCCTATTCGTCTAAAGGAGATC
```

Fig. 2. Alignment of the amino acid sequence of 23 kDa (xyn11A) xylanase with those of family 11 xylanases of fungal or bacterial origin. Ba. fi., endo-β-1,4-xylanase from *Bacillus firmus* (this study); Ba. ha., endo-β-1,4-xylanase from *Bacillus halodurans* (NC_002570); Ba. st., endo-β-1,4-xylanase from *Bacillus stearothermophilus* (U15985); Ae. pu., xylanase I precursor from *Aeromonas punctata* (D32065); Pa. sp., xylanase A from *Paenibacillus* sp. KCTC8848P (AF195421); Ba. sp., endo-xylanase from *Bacillus* sp. NBL420 (AF441773); Ba. su., xylanase from *Bacillus subtilis* (Z34519); Ba. ci., endo-1,4-xylanase from *Bacillus circulans* (AF490980). The amino acid residues identical to these of *Bacillus firmus* are shaded. The Glu residues corresponding to Ba. fi. xylanase Glu 104 and 196, essential to the catalytic activity, are marked by (¡¶) and (¡„), respectively.

```
Ba. fi. 1
MFKFVTKVLTVVIAATISFCLSAVPASANT--YWQYWTDGGGTVNATNGPGGNYSVTWRDTGNFVVGKG
WEIGSPNR 75
Ba. ha. 1
MFKFVTKVLTVVIAATISFCLSAVPASANT--YWQYWTDGGGTVNATNGPGGNYSVTWRDTGNFVVGKG
WEIGSPNR 75
Ba. st. 1
M-KLKKKMLTLLLTASMSFGLFGATSSAAT-DYWQYWTDGGGMVNAVNGPGGNYSVTWQNTGNFVVGKG
WTVGSPNR 75
Ae. pu. 1
MFKFGKKLMTVVLAASMSFGVFAATSSAAT-DYWQNWTDGGGTVNAVNGSGGNYSVSWQNTGNFVVGKG
WTYGTPNR 76
Pa. sp. 1
MFKSSKKLLTVVLAASMSFGFFASTSNAAT-DYWQNWTDGGGTVNAVNGSGGNYSVTWKNSGNFVVGKG
WTTGSPDR 76
Ba. sp. 1
MFKFKRNFLVGLTAALMSISLFSATASAASPDYWQNWTDGGGTVNAVNGPGGNYSVNWSNTGNFVVGKG
WTTGSPSR 77
Ba. su. 1
MFKFKKNFLVGLSAALMSISLFPATASAASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKG
WTTGSPFR 77
Ba. ci. 1
MFKFKKNFLVGLSAALMSISLFSATASAASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKG
WTTGSPFR 77

Ba. fi. 76
TIHYNAGVWEPSGNGYLTLYGWTRNQLIEYYVVDNWGTYRPTGTHRGTVVSDGGTYDIYTTMRYNAPSI
DGTQ-TF 150
Ba. ha. 76
TIHYNAGVWEPSGNGYLTLYGWTRNQLIEYYVVDNWGTYRPTGTHRGTVVSDGGTYDIYTTMRYNAPSI
DGTQ-TF 150
Ba. st. 76
VINYNAGIWEPSGNGYLTLYGWTRNALIEYYVVDSWGTYRPTGNYKGTVNSDGGTYDIYTTMRYNAPSI
DGTQ-TF 150
Ae. pu. 77
VVNYNAGVFAPSGNGYLTFYGWTRNALIEYYVVDSWGTYRPTGTYKGTVNSDGGTYDIYTTMRYNAPSI
DGTQ-TF 151
Pa. sp. 77
TINYNAGVWAPSGNGYLALYGWTRNSLIEYYVVDSWGTYRPTGTYKGTVISDGGTYDIYTTMRYDAPSI
```

EGQKTTF 152
Ba. sp. 78
TINYNAGVWAPNGNGYLALYGWTRAPLI**E**YYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSI
DGEKTTF 153
Ba. su. 78
TINYNAGVWAPNGNGYLTLYGWTRSPLI**E**YYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSI
DGDRTTF 153
Ba. ci. 78
TINYNAGVWAPNGNGYLTLYGWTRSPLI**E**YYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSI
DGDRTTF 153

¡¶

Ba. fi. 151
QQFWSVRQSKRPTGNNVSVTFSNHVNAWRNAGMNLGSSWSYQVLAT**E**GYQSSGRSNVTVW 210
Ba. ha. 151
QQFWSVRQSKRPTGNNVSITFSNHVNAWRNAGMNLGSSWSYQVLAT**E**GYQSSGRSNVTVW 210
Ba. st. 151
QQFWSVRQSKRPTGSNVSITFSNHVNAWRSKGMNLGSSWAYQVLAT**E**GYQSSGRSNVTVW 210
Ae. pu. 152
PQYWSVRQSKRPTGVNSTITFSNHVNAWPSKGMYLGNSWSYQVMAT**E**GYQSSGNANVTVW 211
Pa. sp. 153
IQYWSVRQTKRPTGGNSTITFSNHVKAWARQGMHLGNNWSYQVLAT**E**GYQSSGSSNVTVW 212
Ba. sp. 154
TQYWSVRQTKRPTGSNAKITFSNHVRAWKSHGMNLGSIWSYQVLAT**E**GYQSSGSSNVTVW 213
Ba. su. 154
TQYWSVRQSKRPTGSNATITFSNHVNAWKSHGMNLGSNWAYQVMAT**E**GYQSSGSSNVTVW 213
Ba. ci. 154
TQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMAT**E**GYQSSGSSNVTVW 213

¡„

Fig. 3. Nucleotide sequence of the *Bacillus firmus* xyn11A gene (44 kDa xylanase) and deduced amino acid sequence of xyn11A gene protein. The ribosome binding sequence (RBS) is boxed. The symbol "***" designates stop codon.

```
1
     GATCTTGCTATAAATCTCACTTATATAATTTTTTACAGTACCTTCCGTAATATAAAGCTTTTCG
     GAAATT

    IleLysSerTyrIleGluSerIleTyrAsnLysValThrGlyGluThrIleTyrLeuLysGluSe
    rIleL
                  200                             190
71
TTTTTATTCGTCAATCTTTGGGCTAAAAGGGAGGCGATTTGTCGTTCCCGCTCAGAAAAATGCAACCCT
T

ysLysAsnThrLeuArgGlnAlaLeuLeuSerAlaIleGlnArgGluArgGluSerPheHisLeuGlyG
l
        180                             170
160
141
CTTTTTTTAACTTTTCAATTTGTAAATCTACTTCAGTCAGCTCATTATATTTAGAGAGCTGTTTGGCGA
G

uLysLysLeuLysGluIleGlnLeuAspValGluThrLeuGluAsnTyrLysSerLeuGlnLysAlaLe
u
                               150                                 140
211
CTTAACGGCAATGGAGTTAGGAATTAACATCTGTCCGTCAACCGATTGACGTATGGAATAGATCAGTTG
G

LysValAlaIleSerAsnProIleLeuMetGlnGlyAspValSerGlnArgIleSerTyrIleLeuGln
A
                  130                             120
281
TCATAATTTAAATCTTTCAACAAAAAACCATTAGCTCCACCGACCAAACTTTCAATAATGTACTCATCA
T

spTyrAsnLeuAspLysLeuLeuPheGlyAsnAlaGlyGlyValLeuSerGluIleIleTyrGluAspA
s
        110                             100
    90
351
CTTCAAACGTTGTTAACATTAATACGTTAATGTGGGGATAAATTCTCTTGACTACCTTTAAACATTCAA
T

pGluPheThrThrLeuMetLeuValAsnIleHisProTyrIleArgLysValValLysLeuCysGluIl
e
                               80                                  70
421
CCCATTCATCATAGGCATTTGAATGTCCATTAAGATCACATGGGGAAGAAGGGAGGGGATTTGATCCAA
C

GlyAsnMetMetProMetGlnIleAspMetLeuIleValHisProLeuLeuSerProIleGlnAspLeu
V
                  60                              50
491
ACCTCCTTTCCGTTCTTTGCTAAACCGGTCACATTCATATCGTCTTCAAGATCGATGATCGTCTTCAGG
C

alGluLysGlyAsnLysAlaLeuGlyThrValAsnMetAspAspGluLeuAspIleIleThrLysLeuG
l
        40                              30
    20
561
```

CTTCCCTCATAAGGGTTTGGTCATCGGCAATCAAAACATTTATTAATTCCATAGTATTCATCCTTTCTA
T
yGluArgMetLeuThrGlnAspAspAlaIleLeuValAsnIleLeuGluMet RBS
BH2133
10 1
631
TAAATAACTAGCCCTATTTCTTATTTTGCTATATTCCATTAATGGTATAAATTTTTAATATTTGGATGA
T
701
AAAGAGGCTTTCGAAAGAGATATTCGACTTGGTTAAAAAATCCCTTTTTTATGTATTGAGTAATTAGAA
T
771
CTGCTCATAAGAGTTCCTCCTATCTAAACCTATACAATAAAATTTAATAATATATTACAATTTTGTAAA
G
841
GAACATGGCATTATTTTGAGATAAATGTCATAAAAAAGTAGAACTAGAATGTCTGCTGTGCAATGGTTT
G
911
TTTGTCATTCTATACACTCTATTCCATTTATACGATAATGAACGAATAGTGTGGGTCTGGAGAGGAAAA
G
981
GGAGCGTAGCAGCAGTGAAGTACCTGCTTCACAAGAGTGTAGTTCAATTTTAAATATAAACCTAGTTTG
G
1051
TTATAATAAAAAATGCTTTCATCTTGTTGATGAAAAAGGCATAAAGCATGTACTTAGGACTAATTGATC
T
1121
TTAGGACAACTAAAATAGAACTAAACACAAGTTTTAATTACTATAACATTCGGATTATTTTGAATAAAC
T
1191
TAGTTTGTTCACTGGATCAACTAAGTTTATTGTTGCTATAATGGGGTTATAAGTAGCCAGTAGGTAAAA
G
1261
ATTCAATTTTTTCACAATTTTATGAAAGCCCTTTCTTTTATCGACAGCACCCACTCTATAGAAAGGAGG
T
1331
TTACTTAAAGCCCTATTTTTTGCATTTTACTTTGCTACGAAAGGAGAATTTGTGATGATTACACTTTTT
A
Xyn10A RBS
MetIleThrLeuPheL
1
1401
AAAAGCCTTTTGTTGCTGGACTAGCGATCTCTTTATTAGTTGGAGGGGGGCTAGGCAATGTAGCTGCTG
C
ysLysProPheValAlaGlyLeuAlaIleSerLeuLeuValGlyGlyGlyLeuGlyAsnValA
laAlaAl
10 20
1471
TCAAGGAGGACCACCAAAATCTGGAGTCTTTGGAGAAAATCAAAAAAGAAATGATCAGCCTTTTGCATG
G
aGlnGlyGlyProProLysSerGlyValPheGlyGluAsnGlnLysArgAsnAspGlnProPh
eAlaTrp
30 40
50
1541
CAAGTTGCTTCTCTTTCTGAGCGATATCAAGAGCAGTTTGATATTGGAGCTGCGGTTGAGCCCTATCAA
T
GlnValAlaSerLeuSerGluArgTyrGlnGluGlnPheGluIleGlyAlaAlaValGluPro
TyrGlnL
60 70
1611
TAGAAGGAAGACAAGCCCAAATTTTAAAGCATCATTATAACAGCCTTGTGGCGGAAAATGCAATGAAAC
C
euGluGlyArgGlnAlaGlnIleLeuLysHisHisTyrAsnSerLeuValAlaGluAsnAlaM
etLysPr
80 90
1681
TGTATCACTCCAGCCAAGAGAAGGTGAGTGGAACTGGGAAGGCGCTGACAAAATTGTGGAGTTTGCCCG
C

```
      oValSerLeuGlnProArgGluGlyGluTrpAsnTrpGluGlyAlaAspLysIleValGluPh
eAlaArg
       100                             110
120
1751
AAACATAACATGGAGCTTCGCTTCCACACACTCGTTTGGCATAGCCAAGTACCAGAATGGTTTTTCATC
G
      LysHisAsnMetGluLeuArgPheHisThrLeuValTrpHisSerGlnValProGluTrpPhe
PheIleA
                              130                             140
1821
ATGAAAATGGCAATCGGATGGTTGATGAAACCGATCCAGAAAAACGTAAAGCGAATAAACAATTGTTAT
T
      spGluAsnGlyAsnArgMetValAspGluThrAspProGluLysArgLysAlaAsnLysGlnL
euLeuLe
                  150                             160
1891
GGAGCGAATGGAAAACCATATTAAAACGGTTGTTGAACGTTATAAAGATGATGTGACTTCATGGGATGT
G
      uGluArgMetGluAsnHisIleLysThrValValGluArgTyrLysAspAspValThrSerTr
pAspVal
       170                             180
190

1961
GTGAATGAAGTTATTGATGATGGCGGGGGCCTCCGTGAATCAGAATGGTATCAAATAACAGGCACTGAC
T
      ValAsnGluValIleAspAspGlyGlyGlyLeuArgGluSerGluTrpTyrGlnIleThrGly
ThrAspT
                              200                             210
2031
ACATTAAGGTAGCTTTTGAAACTGCAAGAAAATATGGTGGTGAAGAGGCAAAGCTGTACATTAATGATT
A
      yrIleLysValAlaPheGluThrAlaArgLysTyrGlyGlyGluGluAlaLysLeuTyrIleA
snAspTy
                  220                             230
2101
CAACACCGAAGTACCTTCTAAAAGAGATGACCTTTACAACCTGGTGAAAGACTTATTAGAGCAAGGAGT
A
      rAsnThrGluValProSerLysArgAspAspLeuTyrAsnLeuValLysAspLeuLeuGluGl
nGlyVal
       240                             250
260
2171
CCAATTGACGGGGTAGGACATCAGTCTCATATCCAAATCGGCTGGCCTTCCATTGAAGATACAAGAGCT
T
      ProIleAspGlyValGlyHisGlnSerHisIleGlnIleGlyTrpProSerIleGluAspThr
ArgAlaS
                              270                             280
2241
CTTTTGAAAAGTTTACGAGTTTAGGATTAGACAACCAAGTAACTGAACTAGACATGAGTCTTTATGGCT
G
      erPheGluLysPheThrSerLeuGlyLeuAspAsnGlnValThrGluLeuAspMetSerLeuT
yrGlyTr
                  290                             300
2311
GCCACCGACAGGGGCCTATACCTCTTATGACGACATTCCAGAAGAGCTTTTTCAAGCTCAAGCAGACCG
T
      pProProThrGlyAlaTyrThrSerTyrAspAspIleProGluGluLeuPheGlnAlaGlnAl
aAspArg
       310                             320
330
2381
TATGATCAGCTATTTGAGTTATATGAAGAATTAAGCGCTACTATCAGTAGTGTAACCTTCTGGGGAATT
G
      TyrAspGlnLeuPheGluLeuTyrGluGluLeuSerAlaThrIleSerSerValThrPheTrp
GlyIleA
                              340                             350
2451
```

```
CTGATAACCATACATGGCTTGATGACCGCGCTAGAGAGTACAATAATGGAGTAGGGGTCGATGCACCAT
T
      laAspAsnHisThrTrpLeuAspAspArgAlaArgGluTyrAsnAsnGlyValGlyValAspA
laProPh
                360                             370
2521
TGTATTTGATCACAACTATCGAGTGAAGCCTGCTTACTGGGGAATTATTGATTAATTTGAAGCTACTCA
A
      eValPheAspHisAsnTyrArgValLysProAlaTyrTrpGlyIleIleAsp***
       380                           390               396
2591
TCGATAGTCTAGCAACGAGAGGCTGGGACAAAACTAGCCAAACGTAATAAAAAGGAGCTGCCTCAAAGG
T
2661
CTATCTTTTAAGGCAGCTTTTCTGATTCGTTCGACATCGCGACTTAGCGGACACTTTCACCACAGGCAC
A
2731
AGTGCAACAACGACTCACGCGCACTTTGTTTGCATTCGTCGTGTTTTCTTTGCCGTGGGCAACGCTCCA
G
2801
CTTTCTCGAGAACCCGACTCTCGGCGATCTTCCGCTGTTGCTTTTCCCGCAGGAGTCACCGCCTGTCGC
A
2871
CGTTCGAATCTCTTTAGCACAGGAAAGAACATACAAAAAAATCGCCCGATTGTTTAAAGAATAGCTATT
C
2941
CGTCCGTTGAAGATCTCCCTGTCATTCAGATACTTTTGCTTTGGCTGAGAAAATCTTGAAACTGTTTAT
G
3011
GGCGTTCTTCGGTTTGTTTTCGTTTTGAACAAAGCACTCACGGAGAAGGTAATGGTAGAAGGTTTTTCA
C
3081  CTACGGATAGCGAAAAGTAAATAATGACACCGTATGAGAAAGGGAGGAAATATTATGAGCTAT
      GAAATCT
BH2119                                                  RBS
MetSerTyrGluIleL
                                                                    1
3151  TAACATTAGCAGCCTATCGGGCAATCGGATTAAAATGGGAGGGAGCCTTTTCTGAAATTGTCC
      CCGATTT
      euThrLeuAlaAlaTyrArgAlaIleGlyLeuLysTrpGluGlyAlaPheSerGluIleValP
      roAspLe
                10                                  20
3221  AAAAAACGTCATTCAACAAATGGAAGGTCGTGCCGATGAATTAGAGCATAGAATCAATCCTAA
      CGTTCAA
      uLysAsnValIleGlnGlnMetGluGlyArgAlaAspGluLeuGluHisArgIleAsnProAs
      nValGln
        30                              40
      50
3291  TTAGGTCTCTCCTATCATACCATAGAAAATGGATTCGCACATTATGCTGTATATGAAGTGAGT
      GAGGAGC
      LeuGlyLeuSerTyrHisThrIleGluAsnGlyPheAlaHisTyrAlaValTyrGluValSer
      GluGluG
                            60                                  70
3361  AGGAGATTCCTGATGGGATGATTGAAATAAGGGTTCCTGAATGGACGTATGTAAAGACAACAC
      ATAACAA
      lnGluIleProAspGlyMetIleGluIleArgValProGluTrpThrTyrValLysThrThrH
      isAsnLy
                80                                  90
3431  AGGAGAAGATATCCAAAAGACTTATCAGGACTTACTTCAATGGTTATTTGATAGTGATTATAC
      CGTATTT
      sGlyGluAspIleGlnLysThrTyrGlnAspLeuLeuGlnTrpLeuPheAspSerAspTyrTh
      rValPhe
       100                              110
    120
3501  AGAGAAGATGGCGTAGATTACTATGATCCTTATATGCCAATTAAACATGAACATTATCCAGTT
      GATCGTG
      ArgGluAspGlyValAspTyrTyrAspProTyrMetProIleLysHisGluHisTyrProVal
      AspArgA
                            130                                 140
3571  ATCCGAATGATCCGCATTTTGATATTTATATACCGATTGTAAAAAAATAATGTTTTGGCGATT
```

```
      ACGAAAC
      spProAsnAspProHisPheAspIleTyrIleProIleValLysLys***
                150                             160
3641  AACATAGGCAGATTGGTTTTCAAGGTTGTTTATTTGCCATACTCGTAAGCTCGATACAATTCT
      AGGTGGT
3711
TGGAGGAGGGGCTTCTTGAGGAGGATTGAATGATAGCAACACCCATGATAAAGAAAAGAATGACAAAGT
G
3781
CTGCTATCGATAAGGTCATGAAGGTGGGTTGCCTACCTACAGCTTTTAACGAGAGACCTATCTCCGCAA
A
3851
GTAAGGCGTCAATTGTTCGCCTGGTTGAGGAAGGAAAGTAATTCTCTACGGCTGTTGACGTTCATTTTG
G
3921  CAAAGATAGATTTCAAGTGATC
```

Fig. 4. Alignment of the amino acid sequence of xylanase A (44 kDa xylanase) with those of family 10 xylanases of fungal or bacterial origin. Ba. fi., xylanase A from *Bacillus firmus* (this study); Ba. ha., alkaline xylanase A from *Bacillus halodurans* (NC_002570); Ba. sp., alkaline thermostable endoxylanase ftom *Bacillus* sp. NG-27 (AF015445); Ba. st., endo-1,4-β-xylanase T-6 from *Bacillus stearothermophilus* (AF098273); Cl. st. thermostable celloxylanase from *Clostridium stercorarium* (D12504); Ca. sa., xylanase precursor from *Caldicellulosiruptor saccharolyticus* (M34459). The amino acid residues identical to these of *Bacillus firmus* are shaded. The Glu residue corresponding to Ba. fi. xylanase Glu 301, essential to the catalytic activity, is marked by (¡¶).

```
Ba. fi. 1
      MITLFKKPFVAGLAISLLVGGGIGNVAAA---QGGPPKSGVFGENQKRND--QPFAWQVASLS
      ERYQEQFDIGAAVE 72
Ba. ha. 1
      MITLFRKPFVAGLAISLLVGGGIGNVAAA---QGGPPKSGVFGENEKRND--QPFAWQVASLS
      ERYQEQFDIGAAVE 72
Ba. sp. 1
      MLKTLRKPFIAGLALSLLLTGGASSVFAQGNGQAGPPKGGIFKEGEKGNGNVQPFAWQVASLA
      DRYEESFDIGAAVE 77
Ba. st. 1
      MRNVVRKPLTIGLALTLLLPMGMT------------ATSAKNADSYAKKP--HISALNAPQLD
      QRYKNEFTIGAAVE 63
Cl. st. 1
MNKFLNKKW-----SLILTMGGIFLMATLSLIFA--------TGKKAFND--QTSAEDIPSLAEAFRDYFPIGAAIE 62
Ca. sa. 1
MRCLIVCENLEMLNL-----------------------------------------SLAKTYKDYFKIGAAVT 32

Ba. fi. 73
PYQL-EGRQAQILKHHYNSLVAENAMKPVSLQPREGEWNWEGADKIVEFARKHN-MELRFHTLVWHSQVPEWFFIDE 147
Ba. ha. 73
PYQL-EGRQAQILKHHYNSLVAENAMKPESLQPREGEWNWEGADKIVEFARKHN-MELRFHTLVWHSQVPEWFFIDE 147
Ba. sp. 78
PHQL-NGRQGKVLKHHYNSIVAENAMKPISLQPEEGVFTWDGADAIVEFARKNN-MNLRFHTLVWHNQVPDWFFLDE 152
Ba. st. 64
PYQLQNEKDVQMLKRHFNSIVAENVMKPISIQPEEGKFNFEQADRIVKFA-KANGMDIRFHTLVWHSQVPQWFFLDK 139
Cl. st. 63
PGYT-TGQIAELYKKHVNMLVAENAMKPASLQPTEGNFQWADADRIVQFA-KENGMELRFHTLVWHNQTPTGFSLDK 137
Ca. sa. 33
AKDL-EGVHRDILLKHFNSLTPENAMKFENIHPEEQRYNFEEVARIKEFAIK-NDMKLRGHTFVWHNQTPGWVFLDK 107

Ba. fi. 148
NGNRMVDETDPEKRKANKQLLLERMENHIKTVVERYKDDVTSWDVVNEVIDDG----GGLRESEWYQITGTDYIKV 219
Ba. ha. 148
DGNRMVDETDPDKREANKQLLLERMENHIKTVVERYKDDVTSWDVVNEVIDDG----GGLRESEWYQITGTDYIKV 219
Ba. sp. 153
EGNPMVEETNEAKRQANKELLLERLETHIKTVVERYKDDVTAWDVVNEVVDDGTPNERGLRESVWYQITGDEYIRV 228
Ba. st. 140
EGKPMVNETDPVKREQNKQLLLKRLETHIKTIVERYKDDIKYWDVVNEVVGD----DGKLRNSPWYQIAGIDYIKV 211
Cl. st. 138
EGKPMVEETDPQKREENRKLLLQRLENYIRAVVLRYKDDIKSWDVVNEVIE--PNDPGGMRNSPWYQITGTEYIEV 211
Ca. sa. 108
NG-----------EEASKELVIERLREHIKTLCERYKDVVYAWDVVNEAVED--KTEKLLRESNWRKIIGDDYIKI 170

Ba. fi. 220
AFETARKYGGEEAKLYINDYNTEVPSKRDDLYNLVKDLLEQGVPIDGVGHQSHIQIGWPS--IEDTRASFEKFTSL 293
```

```
Ba. ha. 220
AFETARKYGGEEAKLYINDYNTEVPSKRDDLYNLVKDLLEQGVPIDGVGHQSHIQIGWPS--IEDTRASFEKFTSL 293
Ba. sp. 229
AFETARKYAGEDAKLFINDYNTEVTPKRDHLYNLVQDLLADGVPIDGVGHQAHIQIDWPT--IDEIRTSMEMFAGL 302
Ba. st. 212
AFQAARKYGGDNIKLYMNDYNTEVEPKRTALYNLVKQLKEEGVPIDGIGHQSHIQIGWPS--EAEIEKTINMFAAL 285
Cl. st. 212
AFRATREAGGSDIKLYINDYNTDDPVKRDILYELVKNLLEKGVPIDGVGHQTHIDIYNPP--VERIIESIKKFAGL 285
Ca. sa. 171
AFEIAREYAG-DAKLFYNDYNNEMPYKLEKTYKVLKELLERGTPIDGIGIQAHWNI-WDKNLVSNLKKAIEVYASL 244

Ba. fi. 294
GLDNQVTELDMSLYGWPPTGAYTSYDDIPEELFQAQADRYDQLFELYEELSATISSVTFWGIADNHTWLDDRAREY 369
Ba. ha. 294
GLDNQVTELDMSLYGWPPTGAYTSYDDIPAELLQAQADRYDQLFELYEELAADISSVTFWGIADNHTWLDGRAREY 369
Ba. sp. 303
GLDNQVTELDVSLYGWPPRPAFPTYDAIPQERFQAQADRYNQLFELYEELDADLSSVTFWGIADNHTWLDDRAREY 378
Ba. st. 286
GLDNQITELDVSMYGWPPR-AYPTYDAIPKQKFLDQAARYDRLFKLYEKLSDKISNVTFWGIADNHTWLDSRADVY 360
Cl. st. 286
GLDNIITELDMSIYSWNDRSDYG--DSIPDYILTLQAKRYQELFDALKENKDIVSAVVFWGISDKYSWLNGFPVKR 359
Ca. sa. 245
GLEIHITELDISVFEF--EDKRTDLFEPTPEMLELQAKVYEDVFAVFREYKDVITSVTLWGISDRHTWKDNFPVKG 318
                ¡¶

Ba. fi. 370  ------------------NNGVGVDAPFVFDHNYRVKPAYWGIID       396
Ba. ha. 370  ------------------NNGVGIDAPFVFDHNYRVKPAYWRIID       396
Ba. sp. 379  ------------------NDGVGKDAPFVFDPNYRVKPAFWRIID       405
Ba. st. 361  YDANGNVVVDPNAPYAKVEKGKGKDAPFVFGPDYKVKPAYWAIIDHK     407
Cl. st. 360  ------------------TN-----APLLFDRNFMPKPAFWAIVDPSRLRE 387
Ca. sa. 319  ----------------------RKDWPLLFDVNGKPKEALYRILRF      342
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