APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1997, p. 661–664 Vol. 63, No. 2
0099-2240/97/$04.00+0

Cloning and Sequence Analysis of Genes Encoding Xylanases and Acetyl Xylan Esterase from *Streptomyces thermoviolaceus* OPC-520

HIROSHI TSUJIBO,[1]* TAKAHITO OHTSUKI,[1] TAKASHI IIO,[1] ISAO YAMAZAKI,[1] KATSUSHIRO MIYAMOTO,[1] MASANORI SUGIYAMA,[2] AND YOSHIHIKO INAMORI[1]

Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-11,[1] and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734,[2] Japan

Received 2 July 1996/Accepted 3 December 1996

Three genes encoding two types of xylanases (STX-I and STX-II) and an acetyl xylan esterase (STX-III) from *Streptomyces thermoviolaceus* OPC-520 were cloned, and their DNA sequences were determined. The nucleotide sequences showed that genes *stx-II* and *stx-III* were clustered on the genome. The *stx-I*, *stx-II*, and *stx-III* genes encoded deduced proteins of 51, 35.2, and 34.3 kDa, respectively. STX-I and STX-II bound to both insoluble xylan and crystalline cellulose (Avicel). Alignment of the deduced amino acid sequences encoded by *stx-I*, *stx-II*, and *stx-III* demonstrated that the three enzymes contain two functional domains, a catalytic domain and a substrate-binding domain. The catalytic domains of STX-I and STX-II showed high sequence homology to several xylanases which belong to families F and G, respectively, and that of STX-III showed striking homology with an acetyl xylan esterase from *S. lividans*, nodulation proteins of *Rhizobium* sp., and chitin deacetylase of *Mucor rouxii*. In the C-terminal region of STX-I, there were three reiterated amino acid sequences starting from C-L-D, and the repeats were homologous to those found in xylanase A from *S. lividans*, coagulation factor G subunit α from the horseshoe crab, *Rarobacter faecitabidus* protease I, β-1,3-glucanase from *Oerskovia xanthineolytica*, and the ricin B chain. However, the repeats did not show sequence similarity to any of the nine known families of cellulose-binding domains (CBDs). On the other hand, STX-II and STX-III contained identical family II CBDs in their C-terminal regions.

Xylan, the major hemicellulose component of the plant cell wall, consists of a β-1,4-linked D-xylose polymer which commonly contains side branches of arabinosyl, glucuronosyl, acetyl, uronyl, and mannosyl residues. The most abundant native forms are the acetylated xylans and arabinoxylans found in hardwood and softwood, respectively (8). In view of the structural complexity of xylan, the synergistic actions of a series of enzymes are necessary to hydrolyze xylan into assimilable sugars. Numerous bacteria and fungi grow on xylan as a carbon source by using a variety of enzymes, such as endoxylanases (EC 3.2.1.8), β-xylosidases (EC 3.2.1.37), α-glucuronidases, α-arabinofuranosidases (EC 3.2.1.55), and esterases (EC 3.1.1.6). Among xylan-degrading enzymes, studies have been most actively performed on xylanases because these enzymes have several uses and potential applications, including biopulping and biobleaching in the pulp and paper industry, bioconversion of lignocellulose material to fermentative products, and clarification of juice.

Streptomyces thermoviolaceus OPC-520 is a thermophilic actinomycete that excretes two types of thermostable xylanases, designated STX-I and STX-II, into growth medium in the presence of xylan. We have reported the purification, properties, and partial amino acid sequences of these enzymes (34). To unveil the mechanism of the xylan-degrading system of this strain and to elucidate the structure and the molecular architecture of xylanases, we cloned the two genes encoding STX-I and STX-II. In this report, we describe the nucleotide sequences, the domain structures, and the xylan- and Avicel-binding abilities of the native and cloned enzymes. The nucleotide sequence showed that downstream of the STX-II-encoding gene is a gene encoding an acetyl xylan esterase (STX-III).

* Corresponding author. Mailing address: Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-11, Japan. Phone: (81-726) 90-1057. Fax: (81-726) 90-1057. E-mail: tsujibo@oysun01.oups.ac.jp.

MATERIALS AND METHODS

Bacterial strains and plasmids. *S. thermoviolaceus* OPC-520 was isolated from decayed wood (34) and used as the source of chromosomal DNA. This strain was grown as described previously (34). *Escherichia coli* JM109 and *Streptomyces griseus* PSR2 were used as the recipient strains for recombinant plasmids. *E. coli* JM109 was grown at 37°C on Luria-Bertani medium. *S. griseus* was grown at 27°C on GMP medium (9). Plasmids pUC18, pUC19, M13mp18, and M13mp19 were used as cloning vectors for *E. coli*. Plasmid pIJ702 was used as a cloning vector for *S. griseus* PSR2.

Recombinant DNA technique. *S. thermoviolaceus* chromosomal DNA was isolated by the method of Hopwood et al. (9). Plasmid DNA from *E. coli* was purified with the Qiagen kit (Qiagen Inc., Chatsworth, Calif.) or by the alkaline lysis procedure (25). Purification of plasmid DNA from *S. griseus* PSR2 was performed by the method of Kieser (13). Restriction endonucleases were purchased from Toyobo (Tokyo, Japan) or New England BioLabs, Inc., and used in accordance with the manufacturer's specifications. DNA ligation was carried out by the Ligation Pack (Nippon Gene, Inc., Tokyo, Japan). Chromosomal DNA was partially digested with *Bam*HI or *Sph*I and electrophoresed on a 0.6% agarose gel in 0.089 M Tris–0.089 M boric acid–2 mM EDTA (pH 8.0). Each fragment in the range of 3 to 10 kb was excised from the gel and purified with a Sephaglas BandPrep kit (Pharmacia). Fragments were ligated into the dephosphorylated *Bam*HI or *Sph*I site of pUC18 or pIJ702, respectively. These ligation mixtures were incubated at 4°C overnight, and one of the two recombinant plasmids (pUC18) was inserted into competent *E. coli* JM109, and the other (pIJ702) was inserted into protoplasts of *S. griseus* PSR2. Transformation of *E. coli* and *S. griseus* PSR2 protoplast transformation were performed as described by Sambrook et al. (25) and Hopwood et al. (9), respectively. For screening of xylanase-producing clones, plates on which *E. coli* or *S. griseus* clones had been grown were overlaid with 5 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 0.5% agar and 3% oat spelt xylan (Fluka, Tokyo, Japan). These plates were then incubated at 50°C for several hours or overnight. Colonies forming clear halos indicated putative clones containing hybrid plasmids with genomic inserts coding for xylanase activity. Transformants containing only pUC18 or pIJ702 did not form clear halos on the plates.

Nucleotide sequencing. DNA sequencing was performed by the dideoxy-chain termination method (26) with a Thermo Sequenase fluorescence-labelled primer

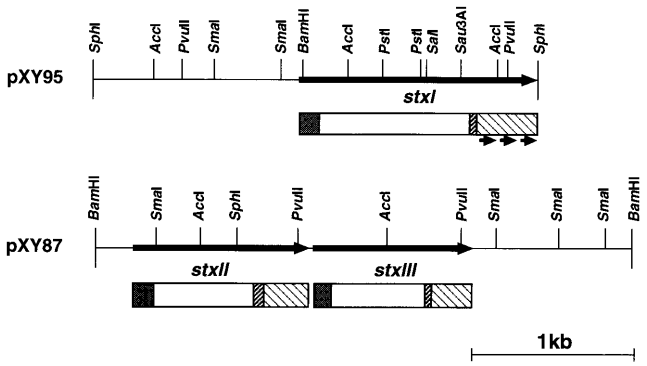

FIG. 1. Restriction maps of pXY95 and pXY87 and domain structures of STX-I, STX-II, and STX-III. The plasmids isolated from two different libraries, which expressed xylanase activity, contain a 2.7-kb *Sph*I fragment and a 3.3-kb *Bam*HI fragment of genomic DNA from *S. thermoviolaceus* OPC-520. The long solid arrows indicate the coding sequences of STX-I, STX-II, and STX-III. The short solid arrows indicate the repeated sequences. ▬, signal peptide; ▭, catalytic domain; ▨, linker; ▧, substrate-binding domain.

cycle sequencing kit (Amersham International plc.) as specified by the manufacturer. DNA fragments were analyzed on a DNA sequencer (Hitachi SQ3000).

Enzyme assay. Xylanase activity was measured by determining the reducing sugar released from oat spelt xylan as described previously (34). Protein was assayed by the method of Lowry et al. (17) with bovine serum albumin as the standard. A xylanase-positive clone of *E. coli*, designated pXY87, was grown in LB medium containing ampicillin (100 μg/ml). *E. coli* carrying pXY87 was cultured to the early stationary phase at 37°C with vigorous shaking. The cells were collected by centrifugation, and the periplasmic fraction containing xylanase activity was prepared. A xylanase-positive clone of *S. griseus*, designated pXY95, was grown in GMP medium containing 50 μg of thiostrepton (Sigma) per ml for 3 days, and the filtrate was used as crude xylanase. These enzymes (STX-I and STX-II) were purified as described previously (34).

Binding assay. Xylanase binding assays and preparation of insoluble xylan were performed by the method of Irwin et al. (10). Binding assays were carried out by adding 2 μg of purified xylanases to various amounts of Avicel (Merck, Darmstadt, Germany) or insoluble xylan (Fluka, Tokyo, Japan) in 1.0 ml of 50 mM Tris-HCl buffer (pH 8.0) in 1.5-ml microcentrifuge tubes. Samples were placed on ice for 1 h and then centrifuged. The xylanase in the supernatant was measured, and the activity lost from the supernatant was assumed to be the activity bound.

Other procedures. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and amino-terminal acid sequencing were performed as described before (34).

Nucleotide sequence accession numbers. The nucleotide sequence data reported here will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession numbers D85896 (*stx-I*), D85897 (*stx-II*), and D85898 (*stx-III*).

RESULTS AND DISCUSSION

Cloning and nucleotide sequences of xylanase genes. Four and one xylanase-positive clones were isolated by the substrate overlay method from *E. coli* and *S. griseus* transformants, respectively. The four xylanase-positive clones from *E. coli* transformants contained the same 3.3-kb *Bam*HI insert, and the xylanase-positive clone from *S. griseus* contained a 2.7-kb *Sph*I insert, based on restriction maps. Restriction maps of pXY87 and pXY95 are shown in Fig. 1. The nucleotide sequences of both strands of the genomic inserts containing the *stx-I* and *stx-II* genes were determined. Analysis of the sequence of the genomic insert present in pXY95 revealed the presence of a complete open reading frame, *stx-I* (bp 121 to 1548), coding for a 476-amino-acid protein with a calculated molecular weight of 51,093. The value without the signal peptide corresponds to the 47,000 obtained for the native protein by SDS-PAGE. The N-terminal sequences of native and cloned STX-I coincided precisely with the sequence starting from alanine residue 41 of the deduced amino acid sequence encoded by *stx-I*, indicating that cleavage of the signal peptide occurred between alanine

STX-I	82	**TAENEMK**	88	119	RGHTLAWHSQQPYW	132	163	**WDVVNE**	168
SL-A	83	**TAENEMK**	89	120	RGHTLAWHSQQPGW	133	164	**WDVVNE**	169
TA	40	**TPENRMK**	46	77	YGHTLVWWSQLPPW	90	108	**WDVVNE**	113
CT-Z	557	**VCENEMK**	563	594	RGHTLIWHNQNPSW	607	640	**WDVANE**	645
PF-A	305	**TAENIMK**	311	343	HALVWHPSYQLPNW	356	386	**WDVVNE**	391
								*	
STX-I	205	**AKLCYNDYN**	213	238	**PI**DCVG**F**QSH	247	274	IT**E**LD-**I**	279
SL-A	206	**AKLCYNDYN**	214	239	**PI**DCVG**F**QSH	248	275	IT**E**LD-**I**	280
TA	133	**AKLYINDYN**	141	166	**PI**IGIG**N**QTA	173	221	IG**E**-D**Y**I	226
CT-Z	682	**ALLFYNDYN**	690	714	**PI**DGVG**F**QCH	723	752	FT**E**ID-**I**	757
PF-A	438	**AELYYNDFN**	446	470	**PI**DGVG**F**QMH	479	508	IT**E**LDV-	513

FIG. 2. Comparison of the amino acid sequence of the catalytic domain of STX-I with those of family F xylanases. The conserved regions of family F xylanases are aligned with the sequence of STX-I. The amino acid residues conserved in all of the sequences are in boldface. The active sites of family F xylanases are marked by asterisks. STX-I, xylanase I from *S. thermoviolaceus*; SL-A, xylanase A from *S. lividans* (29); TA, xylanase from *T. aurantiacus* (32); CT-Z, xylanase Z from *C. thermocellum* (6); PF-A, xylanase A from *P. fluorescens* subsp. *cellulosa* (7).

residues 40 and 41. Analysis of the nucleotide sequence of pXY87 showed two open reading frames of 1,005 (*stx-II*) and 993 (*stx-III*) bp which were transcribed from the same strand and separated by 37 bp. The open reading frames of *stx-II* and *stx-III* encoded a protein of 335 amino acids with a molecular weight of 35,278 and a protein of 331 amino acids with a molecular weight of 34,388, respectively. The N-terminal sequences of native and cloned STX-II were in perfect agreement with the deduced amino acid sequence starting from aspartic acid residue 41, indicating that the cleavage site was after alanine residue 40. Accessory enzymes like acetyl xylan esterase, α-L-arabinofuranosidase, and α-D-glucuronidase are essential for complete depolymerization of xylan. However, the number of reports on genes encoding these enzymes is low in comparison with that of reports on xylanase genes. When 4-methylumbelliferyl acetate or acetylated oat spelt xylan was the substrate, acetyl xylan esterase activity was detected in the crude preparations from *S. thermoviolaceus* and *E. coli*(pXY87) (data not shown). Although purification, characterization, and N-terminal sequencing of acetyl xylan esterase (STX-III) have not been performed, it is presumable that the putative site of cleavage lies between alanine residues 37 and 38, which is compatible with the −3, −1 rule of von Heijne (35). In addition, the signal sequence is also characteristic of actinomycete signal sequences in both size and composition (22). Analysis of the nucleotide sequence revealed potential ribosome-binding sites, GGAG, of three genes located upstream of each initiation codon, ATG. The putative −10 and −35 regions of *stx-I* and *stx-III* could not be identified upstream of the Shine-Dalgarno sequence; however, an A+T-rich region, characteristic of a promoter sequence, was found upstream of the ribosome-binding site of *stx-II*. This region contained partial inverted and direct repeats which overlapped the putative −35 RNA polymerase-binding site. Delic et al. reported that the same 12-bp direct repeats within the promoter regions of *Streptomyces* chitinases (Chi63 and Chi35) may be involved in repression and induction of this entire class of catabolite-controlled genes (2). Furthermore, all of the *Thermomonospora fusca* cellulase genes (E1, E2, E4, and E5) contain a 14-base inverted repeat within the A+T-rich region that precedes the translational start codon of each gene (11, 15), and the inverted repeat in the E5 gene was identified as a binding site for a protein involved in induction (16). STX-I and STX-II are induced by xylan and strongly repressed by glucose. Therefore, the promoter region of *stx-II* may interact with a DNA sequence-specific binding protein that regulates induction.

Sequence homology with other enzymes. The deduced amino acid sequences encoded by *stx-I*, *stx-II*, and *stx-III* were compared with available protein sequences from the GenBank and

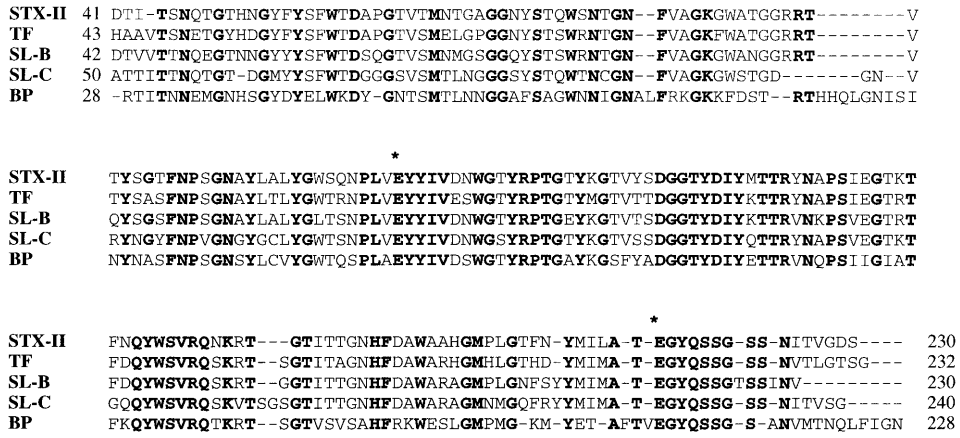

FIG. 3. Comparison of the amino acid sequence of the catalytic domain of STX-II with those of family G xylanases. The amino acid residues conserved in all of the sequences are in boldface. The active sites of family G xylanases are marked by asterisks. STX-II, xylanase II from *S. thermoviolaceus*; TF, xylanase from *T. fusca* (10); SL-B and -C, xylanases B and C, respectively, from *S. lividans* (29); BP, xylanase from *B. pumilus* (4).

EMBL databases, as well as those from the literature. As a result of computer analysis, it was assumed that these three enzymes contain two functional domains, a catalytic domain and a substrate-binding domain (Fig. 1). STX-I showed high sequence homology to several xylanases (6, 7, 29, 32) which belong to family F, according to Gilkes et al. (5). As shown in Fig. 2, the six conserved regions were found in the predicted amino acid sequences of the genes for family F xylanases. Two glutamic acids essential for the activity of *S. lividans* xylanase A (21) were found in the conserved regions. In the C-terminal region of the enzyme, there were three reiterated amino acid sequences starting from C-L-D, and between the N-terminal region and the C-terminal region was a small glycine-, serine-, and proline-rich linker sequence. The three tandem repeats showed sequence homology with the repeats found in xylanase A from *S. lividans* (29), coagulation factor G subunit α from the horseshoe crab (27), *Rarobacter faecitabidus* protease I (31), β-1,3-glucanase from *Oerskovia xanthineolytica* (30), and the ricin B chain (14). Cellulose-binding domains (CBDs) are grouped into nine different families based on sequence similarities (33). However, the repeats did not show sequence similarity to all of the CBDs in these nine families. To evaluate the precise role of the repeated sequences in xylan degradation, expression of the gene for the putative CBD and its function remain to be examined. The catalytic domain of STX-II showed extensive homology to xylanases (4, 10, 29) classified in family G by Gilkes et al. (5), and in addition, two glutamic acid residues essential for catalysis in *B. pumilus* xylanase (1) were conserved in all of the enzymes shown in Fig. 3. The deduced amino acid sequence of STX-III revealed no similarities to the reported acetyl xylan esterases of *Caldocellum saccharolyticum* (18), *Pseudomonas fluorescens* subsp. *cellulosa* (3), and *Trichoderma reesei* (19); however, the protein sequence of STX-III showed striking homology to the acetyl xylan esterase from *S. lividans* (28). Furthermore, the catalytic domain of STX-III was homologous to nodulation proteins of *Rhizobium* sp. (24), chitin deacetylase of *Mucor rouxii* (12), and polysaccharide deacetylase of *B. stearothermophilus* (23) (Fig. 4). The amino acid sequences of the C termini of STX-II (amino acid residues 250 to 332) and STX-III (amino acid residues 246 to 328) revealed similarity to each other (98% identical). These regions (83 residues long) showed high sequence homology to family II CBDs found in cellulases, xylanases, and chitinases. From the alignment of these binding domains, several amino acid residues were found to be well conserved in CBDs. These results indicate that the C-terminal regions of STX-II and STX-III belong in the CBD family II proposed by Tomme et al. (33). All of them belonging to the group contain a cysteine residue at the their N and C termini, and aromatic, aliphatic, and hydroxyamino acids at the same or similar locations within their primary structures. The extensive glycine-rich regions occurring in a few β-1,4-glycanases, such as XynD from *C. fimi*

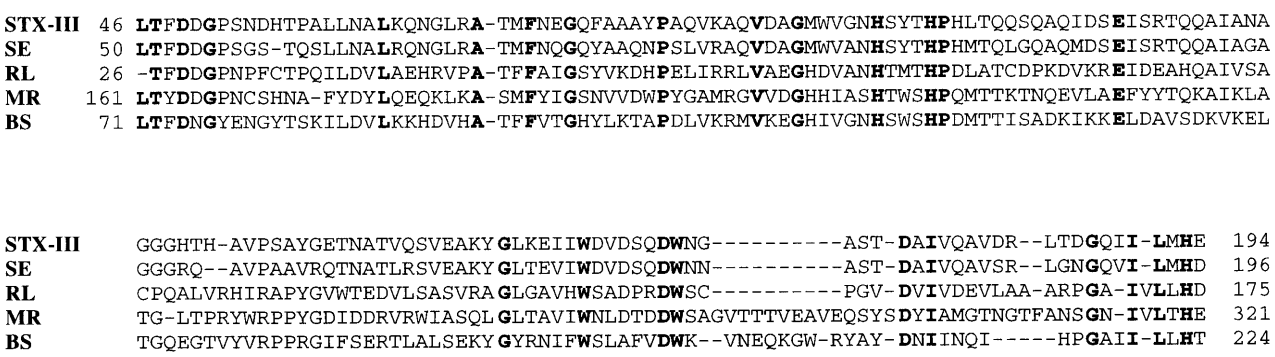

FIG. 4. Comparison of the amino acid sequence of STX-III with those of other proteins. The amino acid residues conserved in all of the sequences are in boldface. STX-III, acetyl xylan esterase from *S. thermoviolaceus*; SE, acetyl xylan esterase from *S. lividans* (28); RL, nodulation protein from *R. leguminosarum* (24); MR, chitin deacetylase from *M. rouxii* (12); BS, polysaccharide deacetylase from *B. stearothermophilus* (23).

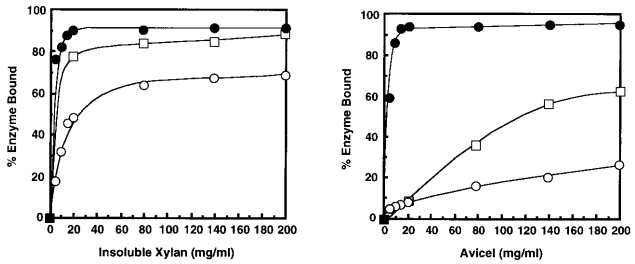

FIG. 5. Binding of STX-I and STX-II to insoluble xylan and Avicel. Symbols: ●, intact STX-I; ○, proteolyzed STX-I; □, STX-II.

(20), were found after the catalytic domains of STX-II and STX-III, which were similar to the linker or hinge region of microbial xylanases and cellulases.

Binding of STX-I and STX-II to xylan and Avicel. Cloned STX-I was purified to homogeneity from the culture filtrate of *S. griseus*(pXY95) as described previously (34). The molecular mass estimated by SDS-PAGE was about 32 kDa, and the N-terminal amino acid sequences of cloned 32-kDa STX-I and native 47-kDa STX-I were found to be identical (A-E-S-T-L-G-A-A-A-A-Q-S-G). These results indicate that 32-kDa STX-I appears to be the product of partial proteolysis of native STX-I by a protease(s) from *S. griseus*. Proteolytic STX-I showed the same specific activity as native STX-I when soluble xylan was the substrate. The abilities of native STX-I to bind to insoluble xylan and Avicel showed similar patterns (Fig. 5). On the other hand, proteolyzed STX-I bound to xylan more weakly than did native STX-I, although the enzyme almost did not bind to Avicel. These results suggest that the C-terminal repeated sequence of STX-I is essential for cellulose binding rather than xylan binding. Native STX-I and cloned STX-II bound to both xylan and Avicel, although the enzymes bound much less strongly to Avicel. As far as we know, STX-II is one of three family G xylanases that contain a substrate-binding domain. Repeated washes with low-salt buffer (50 mM Tris-HCl buffer, pH 8.0), high-salt buffer containing 1 M NaCl, or distilled water did not desorb the proteins from xylan or Avicel. The precise role of the substrate-binding domains is not understood. However, they may play an important role in targeting the enzyme to the substrate by increasing its local concentration on the plant cell wall.

REFERENCES

1. **Arise, A., T. Yom, I. Urabe, Y. Hata, Y. Katsube, and H. Okada.** 1993. Stabilization of xylanase by random mutagenesis. FEBS Lett. **316:**123–127.
2. **Delic, I., P. Robbins, and J. Westpheling.** 1992. Direct repeat sequences are implicated in the regulation of two *Streptomyces* chitinase promoters that are subject to carbon catabolite control. Proc. Natl. Acad. Sci. USA **89:**1885–1889.
3. **Ferreira, L. M., T. M. Wood, G. Williamson, C. Faulds, G. P. Hazlewood, G. W. Black, and H. J. Gilbert.** 1993. A modular esterase from *Pseudomonas fluorescens* subsp. *cellulosa* contains a non-catalytic cellulose-binding domain. Biochem. J. **294:**349–355.
4. **Fukasaki, E., W. Panbangred, W. Shinmyo, and H. Okada.** 1984. The complete nucleotide sequence of the xylanase gene (*xynA*) of *Bacillus pumilis*. FEBS Lett. **171:**197–201.
5. **Gilkes, N. R., B. Henrissat, D. G. Kilburn, R. C. Miller, Jr., and R. A. J. Warren.** 1991. Domains in microbial β-1,4-glycanases: sequence conservation, function, and enzyme families. Microbiol. Rev. **55:**303–315.
6. **Grepinet, O., M.-C. Chebrou, and P. Beguin.** 1988. Nucleotide sequence and deletion analysis of the xylanase gene (*xynZ*) of *Clostridium thermocellum*. J. Bacteriol. **170:**4582–4588.
7. **Hall, J., G. P. Hazlewood, N. S. Huskisson, A. J. Durrant, and H. J. Gilbert.** 1989. Conserved serine-rich sequences in xylanase and cellulase from *Pseudomonas fluorescens* subspecies *cellulosa*: internal signal sequence and unusual protein processing. Mol. Microbiol. **3:**1211–1219.
8. **Hazlewood, G. P., and H. J. Gilbert.** 1993. Molecular biology of hemicellulases, p. 103–126. *In* M. P. Coughlan and G. P. Hazlewood (ed.), Hemicellulose and hemicellulases. Portland Press, London, United Kingdom.
9. **Hopwood, D. A., M. J. Bibb, K. C. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*: a laboratory manual. The John Innes Foundation, Norwich, United Kingdom.
10. **Irwin, D., E. D. Jung, and D. B. Wilson.** 1994. Characterization and sequence of a *Thermomonospora fusca* xylanase. Appl. Environ. Microbiol. **60:**763–770.
11. **Jung, E. D., G. Lao, D. Irwin, B. K. Barr, A. Benjamin, and D. B. Wilson.** 1993. DNA sequences and expression in *Streptomyces lividans* of an exoglucanase gene and an endoglucanase gene from *Thermomonospora fusca*. Appl. Environ. Microbiol. **59:**3032–3043.
12. **Kafetzopoulos, D., G. Thireos, J. Vournakis, and V. Bouriotis.** 1993. cDNA cloning of chitin deacetylase of *Mucor rouxii*, p. 431–436. *In* R. A. A. Muzzarelli (ed.), Chitin enzymology, Ancona.
13. **Kieser, T.** 1984. Factors affecting the isolation of cccDNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12:**19–36.
14. **Lamb, F. I., L. M. Roberts, and J. M. Lord.** 1985. Nucleotide sequence of cloned cDNA coding for preproricin. Eur. J. Biochem. **148:**265–270.
15. **Lao, G., G. S. Ghangas, E. D. Jung, and D. B. Wilson.** 1991. DNA sequences of three β-1,4-endoglucanase genes from *Thermomonospora fusca*. J. Bacteriol. **173:**3397–3407.
16. **Lin, E., and D. B. Wilson.** 1988. Identification of a *celE* binding protein and its potential role in induction of the *celE* gene in *Thermomonospora fusca*. J. Bacteriol. **170:**3843–3846.
17. **Lowry, O. H., N. H. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
18. **Luthi, E., D. R. Love, J. McAnulty, C. Wallance, A. Caughey, D. Saul, and P. L. Bergquist.** 1990. Cloning, sequence analysis, and expression of genes encoding xylan-degrading enzymes from the thermophile *Caldocellum saccharolyticum*. Appl. Environ. Microbiol. **56:**1017–1024.
19. **Margolles-Clark, E., M. Tenkanen, H. Soderlund, and M. Penttila.** 1996. Acetyl xylan esterase from *Trichoderma reesei* contains an active-site serine residue and a cellulose-binding domain. Eur. J. Biochem. **237:**553–560.
20. **Millward-Sadler, S. J., D. M. Poole, B. Henrissat, G. P. Hazlewood, J. H. Clarke, and H. J. Gilbert.** 1994. Evidence for a general role for high-affinity non catalytic cellulose-binding domains in microbial plant cell wall hydrolases. Mol. Microbiol. **11:**375–382.
21. **Moreau, A., M. Roberge, C. Manin, F. Shareck, D. Kluepfel, and R. Morosoli.** 1994. Identification of two acidic residues involved in the catalysis of xylanase A from *Streptomyces lividans*. Biochem. J. **302:**291–295.
22. **Perlman, D., and H. O. Halvorsson.** 1983. A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptides. J. Mol. Biol. **167:**391–409.
23. **Reaney, S. K., S. J. Bungard, and J. R. Guest.** 1993. Molecular and enzymological evidence for two classes of fumarase in *Bacillus stearothermophilus* (var. *non-diastaticus*). J. Gen. Microbiol. **139:**403–416.
24. **Rossen, L., A. W. B. Johnston, and J. A. Downie.** 1984. DNA sequence of the *Rhizobium leguminosarum* nodulation genes *nodAB* and *C* required for root hair curling. Nucleic Acids Res. **12:**9497–9508.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
27. **Seki, N., T. Muta, T. Oda, D. Iwaki, K. Kuma, T. Miyata, and S. Iwanaga.** 1994. Horseshoe crab (1,3)-β-D-glucan-sensitive coagulation factor G. J. Biol. Chem. **269:**1370–1374.
28. **Shareck, F., P. Biely, R. Morosoli, and D. Kluepfel.** 1995. Analysis of DNA flanking the *xynB* locus of *Streptomyces lividans* reveals genes encoding acetyl xylan esterase and the RNA component of ribonuclease P. Gene **153:**105–109.
29. **Shareck, F., C. Roy, M. Yaguchi, R. Morosoli, and D. Kluepfel.** 1991. Sequence of three genes specifying xylanases in *Streptomyces lividans*. Gene **107:**75–82.
30. **Shen, S.-H., P. Chretien, L. Bastien, and S. N. Slilaty.** 1991. Primary sequence of the glucanase gene from *Oerskovia xanthineolytica*. J. Biol. Chem. **266:**1058–1063.
31. **Shimoi, H., Y. Iimura, T. Obata, and M. Tadenuma.** 1992. Molecular structure of *Rarobacter faecitabidus* protease I. J. Biol. Chem. **267:**25189–25195.
32. **Srinivasa, B. R., P. J. Vithayathil, R. P. Roy, and K. R. Swaminathan.** 1990. Significance of structural homology of *Thermoascus aurantiacus* xylanase with the exoglucanase of *Cellulomonas fimi*. J. Protein Chem. **9:**337–338.
33. **Tomme, P., R. A. J. Warren, and N. R. Gilkes.** 1995. Cellulose degradation by bacteria and fungi. Adv. Microb. Physiol. **37:**1–81.
34. **Tsujibo, H., K. Miyamoto, T. Kuda, K. Minami, T. Sakamoto, T. Hasegawa, and Y. Inamori.** 1992. Purification, properties, and partial amino acid sequences of thermostable xylanases from *Streptomyces thermoviolaceus* OPC-520. Appl. Environ. Microbiol. **58:**371–375.
35. **von Heijne, G.** 1983. Patterns of amino acids near signal sequence cleavage sites. Eur. J. Biochem. **133:**17–21.