A multidomain xylanase from a *Bacillus* sp. with a region homologous to thermostabilizing domains of thermophilic enzymes

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The gene *xynC* encoding xylanase C from *Bacillus* sp. BP-23 was cloned and expressed in *Escherichia coli*. The nucleotide sequence of a 3538 bp DNA fragment containing *xynC* gene was determined, revealing an open reading frame of 3258 bp that encodes a protein of 120567 Da. A comparison of the deduced amino acid sequence of xylanase C with known *β*-glycanase sequences showed that the encoded enzyme is a modular protein containing three different domains. The central region of the enzyme is the catalytic domain, which shows high homology to family 10 xylanases. A domain homologous to family IX cellulose-binding domains is located in the C-terminal region of xylanase C, whilst the N-terminal region of the enzyme shows homology to thermostabilizing domains found in several thermophilic enzymes. Xylanase C showed an activity profile similar to that of enzymes from mesophilic micro-organisms. Maximum activity was found at 45 °C, and the enzyme was only stable at 55 °C or lower temperatures. Xylotetraose, xylotriose, xylobiose and xylose were the main products from birchwood xylan hydrolysis, whilst the enzyme showed increasing activity on xylo-oligosaccharides of increasing length, indicating that the cloned enzyme is an endoxylanase. A deletion derivative of xylanase C, lacking the region homologous to thermostabilizing domains, was constructed. The truncated enzyme showed a lower optimum temperature for activity than the full-length enzyme, 35 °C instead of 45 °C, and a reduced thermal stability that resulted in a complete inactivation of the enzyme after 2 h incubation at 55 °C.

Keywords: xylanase, multidomain, thermostabilizing domain, *Bacillus*

INTRODUCTION

Biodegradation of xylan, a major component of plant cell walls, requires the action of several enzymes, among which xylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) play a key role. Xylanases from several micro-organisms, including fungi and bacteria, have been characterized and cloned. Sequencing of these genes has revealed that many of the encoded enzymes have a modular structure containing a catalytic domain joined to one or more ancillary domains with different functions (Gilkes *et al.*, 1991; Tomme *et al.*, 1995). The

most common non-catalytic domains in modular xylanases are cellulose-binding domains, suggested to promote accessibility to xylan, which occurs in spatial proximity to cellulose in natural substrates (Black *et al.*, 1997). Analysis of sequences of xylanases from thermophilic micro-organisms has identified other conserved sequences that have been proposed to act as thermostabilizing domains (Fontes *et al.*, 1995). Removal of these domains decreases the optimum temperature and thermostability of the modular enzymes (Lee *et al.*, 1993; Winterhalter *et al.*, 1995; Zverlov *et al.*, 1996). However, a region with an homologous sequence has recently been found in a xylanase from a mesophilic organism that does not show noticeable thermal stability (Clarke *et al.*, 1996). Clarke *et al.* (1996) have suggested a more general role for the regions previously regarded

as thermostabilizing domains, and have proposed that these regions could act as stabilizers of enzymes against a range of factors including thermal or pH denaturation.

Members of the genus *Bacillus* produce many different xylanases, several of which have been cloned and characterized. Although the xylanases of most xylanolytic bacteria have been found to possess many domains, sequencing of *Bacillus* xylanases has shown that they are single-domain enzymes. Only a xylanase from *Bacillus polymyxa* has been shown to be a modular enzyme, containing a catalytic domain and a region homologous to cellulose-binding domains (Gosalbes *et al.*, 1991).

The mesophilic strain *Bacillus* sp. BP-23 was previously isolated from a rice field (Blanco & Pastor, 1993). It shows a multiple β-glycanase system, several of which have been cloned and characterized (Blanco *et al.*, 1995, 1996, 1998). In this article we describe the cloning and sequencing of the gene encoding xylanase C from *Bacillus* sp. BP-23 and the characterization of the enzyme.

METHODS

Bacterial strains and plasmids. *Bacillus* sp. strain BP-23 was isolated from soil from a rice field in Ebro river's delta (Blanco & Pastor, 1993) and used as the source of chromosomal DNA. The strain was grown as described previously (Blanco & Pastor, 1993). *Escherichia coli* 5K (Godessart *et al.*, 1988) was cultivated at 37 °C in LB medium and was used as the recipient strain for recombinant plasmids. Plasmid pBR322 (Boehringer Mannheim) was used as the cloning vector. *E. coli* BL21 (Grodberg & Dunn, 1988) was used as a host for analysing the gene products of *xynC* and its truncated derivative.

Nucleic acid manipulations. Chromosomal DNA from *Bacillus* sp. strain BP-23 was prepared as described by Dubnau & Davidoff-Abelson (1971). Plasmid DNA was purified by the alkaline lysis procedure (Sambrook *et al.*, 1989). Restriction nucleases and ligase were purchased from Boehringer Mannheim and used in accordance with the manufacturer's specifications. DNA was partially digested with *Sau*3AI; fragments of 4–7 kb were isolated from a 0·8 % (w/v) agarose gel by electroelution and ligated to *Bam*HI-digested pBR322. The resulting molecules were introduced into *E. coli* 5K cells by transformation according to the method of Hanahan (1983) and Ap^r Tc^s colonies were selected. Southern blot analysis was performed as described by Sambrook *et al.* (1989).

The sequences of both strands of *xynC*, contained in plasmid pX31, were determined by automated fluorescence sequencing with an ABI PRISM dye terminator cycle sequencing ready reaction mix (Perkin Elmer) in a 377 Perkin Elmer DNA sequencer.

The construction of the truncated derivative of xylanase C was performed by PCR amplification (DyNAzyme DNA Polymerase; Finnzymes Oy) of the DNA region encoding the catalytic and cellulose-binding domains of xylanase C (nucleotides 1253–3425 of the sequenced region) and in-frame ligation to a DNA fragment encoding the 63 N-terminal residues of xylanase C including the signal sequence (nucleotides 1–347). The resulting plasmid was named pX31ΔTD.

Enzyme assays. Screening of the gene library for recombinant bacteria producing xylanase was performed on agar LB plates supplemented with 0·1 % (w/v) Remazol Brilliant Blue xylan (Sigma).

Cells from *E. coli* BL21/pX31 cultures were disrupted by sonication and the lysates obtained were clarified by centrifugation. Xylanase activity was assayed by measuring the amount of reducing sugar released from birchwood xylan (Sigma) using the method of Nelson & Somogyi (Spiro, 1966). The assay mixture contained 0·5 % (w/v) birchwood xylan in a final volume of 0·1 ml of 50 mM acetate buffer pH 5. The mixture was incubated at 45 °C for 10 min. Colour development was measured at 520 nm. One unit of enzymic activity was defined as the amount of enzyme that releases 1 μmol reducing sugar equivalent min^{-1} under the assay conditions described.

To study the effect of temperature on xylanase activity of full-length enzyme and its truncated derivative, the same assay conditions described above were used but incubation was at different temperatures. For the study of thermostability, xylanase samples (7 μg μl^{-1}) were pre-incubated in 50 mM Tris/HCl pH 7·0 at temperatures ranging from 50 °C to 60 °C, and residual activity was determined under the standard assay conditions.

Gel electrophoresis and zymograms. SDS-PAGE was performed in 7 % (w/v) gels essentially as described by Laemmli (1970). For detection of xylanase activity, 0·2 % (w/v) birchwood xylan was included in the gels before polymerization. Samples were heated for 10 min at 45 °C in sample buffer before being applied to gels. After electrophoresis, gels were soaked in 2·5 % (w/v) Triton X-100 for 30 min, washed in 50 mM acetate buffer pH 5·0 for 30 min and incubated at 45 °C for 2 h in the same buffer. Gels were stained with 0·1 % (w/v) Congo red for 15 min and washed with 1 M NaCl until xylanase bands became visible. Gels were then immersed in 5 % (w/w) acetic acid, in which the background turned dark blue, and photographed.

IEF was performed with a Pharmacia PhastSystem unit. Gels with a pH range of 3·0–9·0 (Pharmacia) were used. For zymogram analysis, IEF gels were overlaid with agarose gels containing 0·5 % birchwood xylan and incubated at 45 °C for 30 min. Agarose gels were then stained with Congo red and washed with NaCl.

Binding assay. Cell extracts from *E. coli* BL21/pX31 were mixed with Avicel (Merck) at 2·5 % (w/v) final concentration in 50 mM Tris/HCl pH 7·0 and kept at 4 °C with gentle shaking for 1 h. Samples were then centrifuged and supernatants collected. Pellets were placed in a Pasteur pipette, washed several times with the same buffer and adsorbed material was eluted with 0·2 M glucose. Regenerated cellulose was obtained as described by Wood (1988).

TLC. Silica gel plates were used (Merck). The solvent used was chloroform/acetic acid/water (6:7:1, by vol.). Oligosaccharides were detected by spraying the plates with an ethanol/concentrated sulfuric acid mixture (95:5, v/v).

RESULTS

Cloning of xylanase-C-encoding gene

A gene library from *Bacillus* sp. BP-23 was constructed as described in Methods and screened for xylanase activity on LB agar plates containing Remazol Brilliant
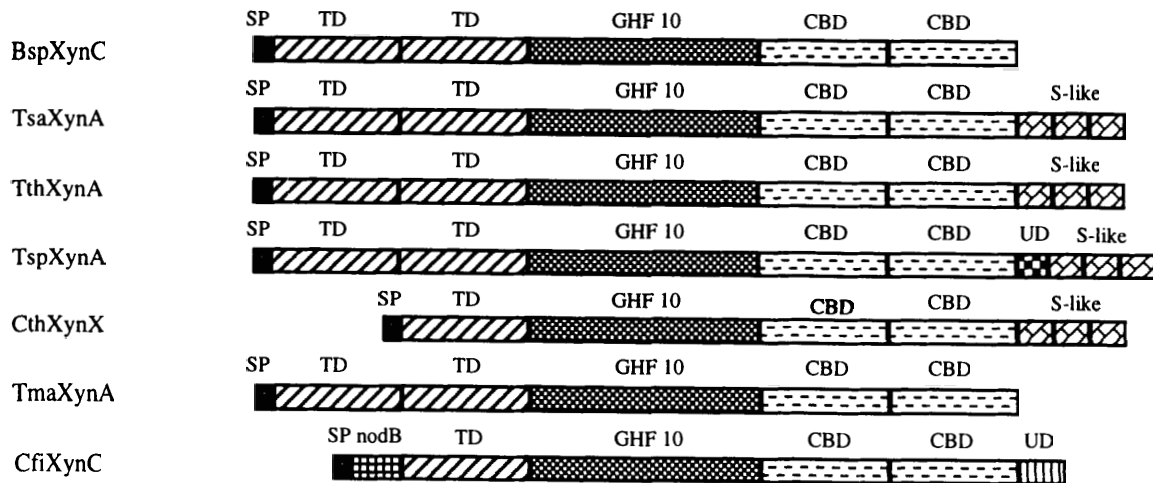
Fig. 1. Modular structure of xylanase C and homologous enzymes. Structures of *Bacillus* sp. BP-23 XynC (BspXynC), *Thermoan. saccharolyticum* XynA (TsaXynA), *Thermoan. thermosulfurigenes* XynA (TthXynA), *Thermoan.* JW/SL-YS 485 XynA (TspXynA), *Cl. thermocellum* XynX (CthXynX), *Thermot. maritima* XynA (TmaXynA) and *C. fimi* XynC (CfiXynC). Boxes representing domains with similar function are filled with the same pattern. SP, signal peptide; TD, thermostabilizing domain; GFH 10, glycosyl hydrolase family 10 catalytic domain; CBD, cellulose-binding domain; S-like, S-layer-like domain; nodB, domain homologous to NodB proteins of *Rhizobium* spp.; UD, domain with unknown function.

Blue xylan. Three recombinant bacterial colonies of the 2000 tested showed a clear halo of xylan hydrolysis and were selected. These three carried an identical recombinant plasmid, pX31, with a DNA insert of 5·2 kb.

Hybridization analysis confirmed that the DNA insert in pX31 belonged to *Bacillus* sp. BP-23. A 2·7 kb *Bam*HI–*Eco*RV fragment of pX31 used as a probe hybridized to DNA bands from *Bacillus* sp. BP-23 and pX31 doubly digested with *Eco*RI and *Eco*RV, whilst no hybridization was detected to *E. coli* chromosomal DNA or pBR322 (data not shown).

Sequencing of the encoded gene

The DNA sequence of pX31 showed an open reading frame of 3258 bp encoding a protein of 1086 amino acids with a predicted molecular mass of 120567 Da. The open reading frame starts with GTG as initiation codon. This start codon is frequently found in *Bacillus* genes and in some instances it has been correlated with translation efficiency (Vellanoweth, 1993). Seven nucleotides upstream of the start codon there is a GGAGG sequence, resembling ribosome-binding sites (Shine–Dalgarno sequences). The deduced amino acid sequence shows an N-terminal region of 31 amino acids with the features of a typical *Bacillus* signal peptide (Nagarajan, 1993). It contains three positively charged residues at its N-terminal region, followed by a hydrophobic span of 17 amino acids. The hydrophobic core is followed by a β-turn-favouring residue (Gly) and a hydrophilic span with the sequence ASAA, resembling those recognized by bacterial signal peptidase (von Heijne, 1986), which would cleave the bond between the two last alanine residues (positions 31 and 32).

The deduced amino acid sequence of *xynC* was compared to β-glycanase sequences contained in the SWISS-PROT and EMBL databases. Alignment by standard computerized methods revealed that xylanase C is a modular protein containing three different domains: an N-terminal domain homologous to sequences found in enzymes from several thermophilic micro-organisms, a family 10 catalytic domain located in the central region of the enzyme and a C-terminal cellulose-binding domain (Fig. 1). The catalytic domain, residues 367–717, shows high homology to catalytic domains of family 10 β-glycanases (Gilkes *et al.*, 1991). The highest homology (53% identity) was found with XynA from *Thermoanaerobacterium saccharolyticum* (Lee *et al.*, 1993), whilst XynX from *Clostridium thermocellum* (SWISS-PROT accession no. P38535), XynA from *Thermoanaerobacterium thermosulfurigenes* (Matuschek *et al.*, 1996), XynA from *Thermoanaerobacterium* JW/SL-YS 485 (Liu *et al.*, 1996) and XynB from *Clostridium stercorarium* (Fukumura *et al.*, 1995) showed 52, 52, 48 and 47% identity, respectively. *Bacillus* sp. BP-23 xylanase C catalytic domain contains eight amino acid sequences shown to be conserved in xylanases of family 10 (Baba *et al.*, 1994; Fukumura *et al.*, 1995). Among the conserved amino acids, xylanase C shows the two Glu residues, Glu-502 and Glu-620, proposed to be the catalytic acid–base and nucleophile residues in family 10 xylanases (Harris *et al.*, 1994; Dominguez *et al.*, 1995).

The C-terminal region of xylanase C contains a duplicated sequence (residues 718–898 and 899–1086) showing high homology to cellulose-binding domains of XynX from *Cl. thermocellum*, XynA from *Thermoan. saccharolyticum*, XynA from *Thermotoga maritima*
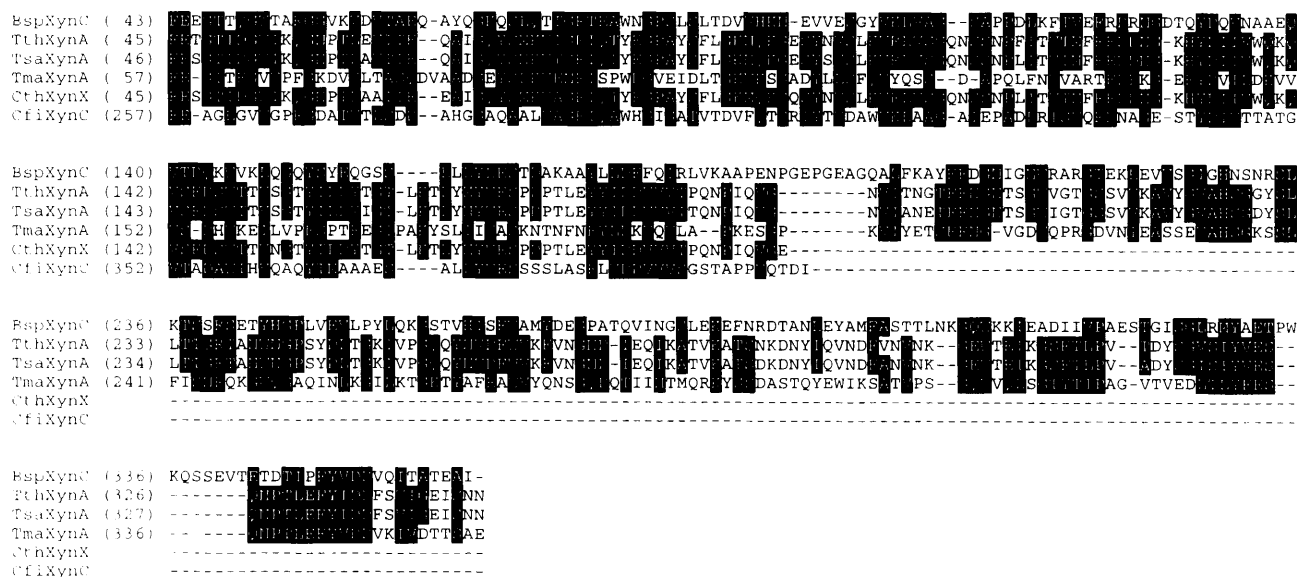
Fig. 2. Amino acid alignment of thermostabilizing domain sequences of several xylanases. Alignment was performed with the CLUSTAL W program. Numbering of the amino acids starts at the N termini of the proteins. Gaps are indicated by dashes. Amino acids identical or conserved in at least four (region from amino acids 43 to 191 of BspXynC) or three (region from amino acids 192 to 363 of BspXynC) of the sequences aligned are shown in black boxes. The sequences shown are: *Bacillus* sp. BP-23 XynC (BspXynC), *Thermoan. thermosulfurigenes* XynA (TthXynA), *Thermoan. saccharolyticum* XynA (TsaXynA), *Thermot. maritima* XynA (TmaXynA), *Cl. thermocellum* XynX (CthXynX) and *C. fimi* XynC (CfiXynC).

(Winterhalter *et al.*, 1995) and XynC from *Cellulomonas fimi* (Clarke *et al.*, 1996). They form a recent family of cellulose-binding domains named type IX (Tomme *et al.*, 1995).

The N-terminal region of xylanase C contains two duplicated sequences (residues 32–191 and 192–366) showing high homology to regions found in β-glycanases from several thermophilic micro-organisms. The duplicated region shows 30% identity with residues 45–346 of XynA from *Thermoan. thermosulfurigenes*, 28% identity with residues 46–347 of XynA from *Thermoan. saccharolyticum* and 26% identity with residues 57–356 of XynA from *Thermot. maritima*, whilst 35% identity was found between the first stretch of the duplicated sequence of mature xylanase C (residues 32–191) and residues 45–195 of XynX from *Cl. thermocellum* (Fig. 2). These sequences have been referred to as thermostabilizing domains by Fontes *et al.* (1995). Most of the enzymes mentioned, including xylanase C, contain two copies in tandem of this domain at the N terminus of the enzyme, whilst XynX from *Cl. thermocellum* shows only one copy of the domain.

Analysis of the XynC sequence failed to reveal any obvious linker sequence such as the serine- or threonine/proline-rich sequences found in many multi-domain β-glycanases.

Characterization of xylanase C

Plasmid pX31 was introduced into *E. coli* BL21 and the transformed strain was used for further experiments. Cell extracts from *E. coli* BL21/pX31 showed high

Table 1. Substrate specificity of xylanase C

Substrate	Specific activity [mU (mg protein)$^{-1}$]
Birchwood xylan	78·72
Oat spelt xylan	52·55
CMC	0·27
Avicel	4·46
Lichenan	0·20
Laminarin	0·19
Arabinogalactan	ND
Polygalacturonate	0·20
p-Nitrophenyl β-xylopyranoside	1·49

ND, not detected.

hydrolytic activity on birchwood and oat spelt xylan, whilst low activity was found on Avicel, CMC and *p*-nitrophenyl β-xylopyranoside (Table 1).

Zymographic analysis of SDS-polyacrylamide gels of cell extracts from *E. coli* BL21/pX31 showed two xylanase activity bands of 125 and 82 kDa not found in extracts from *E. coli* BL21/pBR322 (Fig. 3). The apparent molecular mass of the higher size band is in agreement with the predicted molecular mass of xylanase C (120567 Da). The smaller band could correspond to a proteolytic product from the xylanase. The difference in mobility of both xylanase bands corresponds to a molecular mass difference of 43 kDa. This could be due to the removal of the cellulose-binding
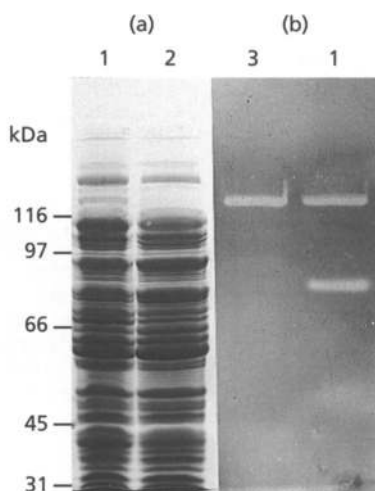
Fig. 3. Electrophoretic analysis of xylanase C. (a) Coomassie blue stained SDS polyacrylamide gel. (b) Zymogram of an SDS polyacrylamide gel. Lanes: 1, cell extracts from *E. coli* BL21/pX31; 2, cell extracts from *E. coli* BL21/pBR322; 3, Avicel-adsorbed proteins from cell extracts from *E. coli* BL21/pX31. The position of molecular mass marker proteins is indicated.

Fig. 4. Thin-layer chromatograms of xylan and xylo-oligosaccharide hydrolysis products. Cell extracts from *E. coli* BL21/pX31 were incubated at 37 °C with 0·5% (w/v) xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4) or birchwood xylan (X_n) in acetate buffer pH 5. Samples were taken at time 0 (lanes 0) and 3 h (lanes 1), and the products of hydrolysis were analysed. Lane M contains size markers of xylose (X_1), X_2, X_3 and X_4 (MegaZyme).

domain that showed a deduced molecular mass of 42704 Da. In fact, cellulose-binding assays showed that the 125 kDa band species bound to Avicel whilst the 85 kDa band species did not (Fig. 3). The 125 kDa band species also bound bacterial microcrystalline and re-generated cellulose but did not bind insoluble xylan (data not shown).

Analysis of *E. coli* BL21/pX31 extracts by IEF showed a main xylanase band of pI 7·8 and two less intense bands showing pI of 4·7 and 5·8. The appearance of three xylanase bands in IEF gels, instead of two bands as detected in SDS gels, could be the result of micro-heterogeneity of the enzyme, a phenomenon widely reported for β-glycanases (Matte & Forsberg, 1992).

The effect of pH on the activity of xylanase C was determined. The cloned enzyme was most active at pH 5 and retained at least 50% of its maximum activity between pH 4·5 and 8·0. The enzyme was not active at pH values below 4·5, whilst at least 35% of maximum activity was found in the pH range 8·5–11·0. An analysis of the effect of temperature on enzyme activity showed that the optimum temperature was 45 °C.

Hydrolysis products of enzyme action were analysed by TLC. Birchwood xylan was incubated with the enzyme and samples were taken after different periods of incubation and analysed. After 3 h incubation, the main products of hydrolysis were xylose, xylobiose, xylotriose, xylotetraose and longer xylo-oligomers (Fig. 4). When incubation was continued up to 24 h, a similar hydrolysis pattern was found. Analysis of the mode of action of the enzyme on xylo-oligosaccharides showed that the enzyme had little activity on xylobiose and xylotriose, whereas xylotetraose was nearly completely
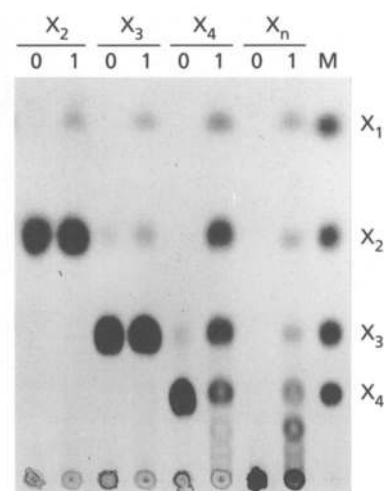
degraded. Xylo-oligosaccharides of higher degreee of polymerization than the starting substrate were produced by the action of the xylanase C on xylotetraose, indicating that the enzyme has transxylosidase activity. The increasing activity of xylanase C on oligomers of increasing length and its mode of action on xylan indicate that the enzyme cloned is an endoxylanase.

Activity of a truncated derivative of xylanase C

A derivative of xylanase C, in which the region homologous to thermostabilizing domains had been deleted, was constructed as described in Methods. The truncated protein, devoid of the putative thermo-stabilizing domain, contained the signal peptide fused in-frame with the C-terminal two-thirds of xylanase C, containing the catalytic and cellulose-binding domains. The influence of temperature on activity and stability of both full-length and truncated enzyme was tested (Fig. 5). The truncated xylanase showed an optimum temperature of 35 °C, 10 °C below that of the full-length enzyme. Truncated xylanase C also showed a remarkable difference in thermal stability. Whereas full-length xylanase C retained more than 77% activity after 2 h incubation at 55 °C and pH 7, the truncated derivative was completely inactivated after the same treatment. Both full-length and truncated enzymes remained stable at 50 °C in buffer at pH 7 (Fig. 5). However, when thermostability was tested at this temperature (50 °C) and pH 6 or 8·5 instead of pH 7, full-length enzyme remained stable for at least 2 h, but the truncated enzyme showed 30% inactivation under the same conditions (data not shown).
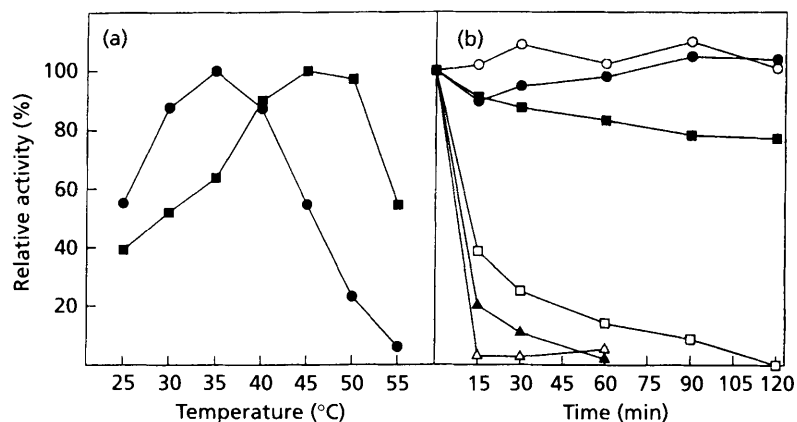
Fig. 5. Influence of temperature on xylanase C and its truncated derivative XynCΔTD. (a) Effect of temperature on xylanase activity of XynC (■) and XynCΔTD (●). (b) Thermal stability of XynC (●, ■, ▲) and XynCΔTD (○, □, △) at pH 7 and 50 °C (●, ○), 55 °C (■, □) or 60 °C (▲, △). In both (a) and (b), 100% xylanase activity was equivalent to 0·086 units (mg protein)$^{-1}$.

DISCUSSION

β-Glycanases from many micro-organisms are modular enzymes containing different independent regions or domains. The occurrence of several domains in an enzyme may be the result of mutation and domain shuffling during the course of evolution, which in turn has yielded a multiplicity of β-glycanases. *Bacillus* endoglucanases often contain additional domains to the catalytic domain. In this way, many modular endoglucanases showing cellulose-binding domains (Hansen *et al.*, 1992; Robson & Chambliss, 1986) or S-layer-like domains (Ozaki *et al.*, 1990) have been reported, although several single-domain *Bacillus* endoglucanases have also been described (Blanco *et al.*, 1998). However, whilst modular endoglucanases are common in the genus *Bacillus*, multidomain xylanases have not been generally reported. Only recently, homology studies have revealed a cellulose-binding domain in xylanase D from *B. polymyxa* (Gosalbes *et al.*, 1991), although binding of the enzyme to crystalline cellulose has not been reported. The results described here show that xylanase C from *Bacillus* sp. BP-23 binds crystalline cellulose and contains a region highly homologous to family IX cellulose binding domains, according to the classification of Tomme *et al.* (1995). The enzyme cloned, together with *B. polymyxa* XynD, would be the only xylanases of the genus *Bacillus* containing a cellulose-binding domain. Whilst the *B. polymyxa* XynD cellulose-binding domain belongs to family VI, the cellulose-binding domain of the cloned enzyme belongs to family IX. This last family mostly contains enzymes from thermophilic micro-organisms. Cellulose-binding domains of XynC from *Bacillus* sp. BP-23 and XynC from *C. fimi* (Clarke *et al.*, 1996) are the only members of this family of cellulose-binding domains from non-thermophilic micro-organisms.

The N-terminal region of *Bacillus* sp. BP-23 XynC is highly homologous to regions found in several thermostable xylanases. These regions have been considered thermostabilizing domains because their removal from a thermostable enzyme gives rise to a decrease in thermal stability and in the optimun temperature for activity. This has been shown for XynY from *Cl. thermocellum*, where the deletion of this region resulted in a decrease in

optimum temperature from 75 °C to 60 °C and in a notable reduction of enzyme thermostability (Fontes *et al.*, 1995). A similar decrease in optimum temperature and thermostability has been found in XynA from *Thermoan. saccharolyticum* (Lee *et al.*, 1993), whilst a significant decrease in thermostability has been reported for XynA from *Thermot. maritima* (Winterhalter *et al.*, 1995) and XynA from *Thermotoga neapolitana* (Zverlov *et al.*, 1996).

The occurrence of thermostabilizing domains seems to be a common trait of xylanases from thermophilic bacteria, although several single-domain xylanases from thermophiles have been described that do not have thermostabilizing regions but show high thermal stability (Gibbs *et al.*, 1995; Saul *et al.*, 1995).

Truncated derivatives of *Bacillus* sp. BP-23 XynC, in which the region homologous to thermostabilizing domains had been deleted, showed a diminished thermostability and optimum temperature compared to the full-length enzyme. This indicates that the deleted region acts as a thermostabilizing domain of *Bacillus* sp. BP-23 XynC. Regions homologous to thermostabilizing domains have also been described in two other enzymes from mesophilic bacteria: xylanase D from *Ruminococcus flavefaciens* (Flint *et al.*, 1993) and xylanase C from *C. fimi* (Clarke *et al.*, 1996). Whilst no data have been reported about the thermal profile of *R. flavefaciens* XynD, xylanase C from *C. fimi*, similarly to the cloned xylanase, does not show notable thermal stability or activity at high temperatures. Clarke *et al.* (1996) suggested that the domain of *C. fimi* XynC has a more general role in the stability of the enzyme against a range of factors and can be regarded as a stabilizing domain. Our results are similar to those found for *C. fimi* XynC, although derivatives devoid of thermostabilizing domain have not been reported for the *C. fimi* enzyme. Similar to what has been suggested for *C. fimi* XynC (Clarke *et al.*, 1996), the domain characterized in *Bacillus* sp. BP-23 XynC can be considered as a stabilizing domain of the enzyme. However, the difference in stability found between full-length and truncated enzyme could also be the result of a disruption of the native structure of the enzyme, instead of a specific effect of the thermostabilizing domain. In fact, it

has been reported that a small deletion of three amino acids in the N-terminal region of xylanase B from *Dictyoglomus thermophilum* results in an important loss of stability (Morris *et al.*, 1998). The removal of the putative thermostabilizing domain of *Bacillus* sp. BP-23 XynC could change and disrupt the folded structure of the native enzyme in a non-specific way, giving rise to the decreased stability found.

The enzyme XynC reported here is, to our knowledge, the only xylanase from *Bacillus* spp. showing a multidomain structure with three independent domains. Besides the catalytic domain, it shows a family IX cellulose-binding domain and a region homologous to thermostabilizing domains. Xyn C is thus unique, being the only example of an enzyme from the genus *Bacillus* showing these kinds of domains.

ACKNOWLEDGEMENTS

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