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A Novel pH-Stable, Bifunctional Xylanase Isolated from a Deep-Sea Microorganism, *Demequina* sp. JK4

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A genomic library was constructed to clone a xylanase gene (*Mxyn10*) from *Demequina* sp. JK4 isolated from a deep sea. *Mxyn10* encoded a 471 residue protein with a calculated molecular mass of 49 kDa. This protein showed the highest sequence identity (70%) with the xylanase from *Streptomyces lividans*. Mxyn10 contains a catalytic domain that belongs to the glycoside hydrolase family 10 (GH10) and a carbohydrate-binding module (CBM) belonging to family 2. The optimum pH and temperature for enzymatic activity were pH 5.5 and 55°C, respectively. Mxyn10 exhibited good pH stability, remaining stable after treatment with buffers ranging from pH 3.5 to 10.0. The protein was not significantly affected by a variety of chemical reagents, including some compounds that usually inhibit the activity of other related enzymes. In addition, Mxyn10 showed activity on cellulose. These properties mark Mxyn10 as a potential enzyme for industrial application and saccharification processes essential for bioethanol production.

Keywords: *Demequina* sp. JK4, xylanase, pH-stable, bifunctional, carbohydrate-binding module

Xylan is a major component of hemicellulose and is composed of homopolymeric backbones of β-D-1,4-linked xylopyranose units substituted with *O*-acetyl, α-L-arabinofuranosyl and α-D-glucuronyl residues [27]. Besides cellulose, xylan is the second most abundant renewable polysaccharide consisting of approximately one-third of all renewable organic carbons on earth [20].

Xylanases are a series of glycosidases (*O*-glycoside hydrolases, E.C. 3.2.1.x), which catalyze the endohydrolysis of 1,4-β-xylosidic linkages into short xylooligosaccharides or xyloses [4]. These enzymes include endo-β-1,4-xylanase (E.C. 3.2.1.8) and β-xylosidase (E.C. 3.2.1.37), both of which hydrolyze the xylan backbone, whereas acetyl xylan esterases (E.C. 3.1.1.72), α-L-arabinofuranosidase (E.C. 3.2.1.39) and ferulic or *p*-coumaric acid esterase (E.C. 3.2.1.73) are debranching-type enzymes [19]. Based on the amino acid sequences similarities and hydrophobic cluster analysis of their catalytic domains, glycoside hydrolases have been grouped into families (refer to http://www.cazy.org/fam/acc_GH.html). Xylanases are normally reported as being confined to families 10 and 11. Enzymes with xylanase activity are also found in other families, including 5, 7, 8, 16, 26, 43, 52, and 62 [4]. Xylanases have attracted particular attention owing to widespread applications in the animal feed, textile, bioethanol, waste treatment, and paper industries.

A large number of xylanases have been found in a variety of microorganisms, some of which have been cloned and characterized. However, there are only a few reports on marine bacteria and fungi producing xylanases. In this vast and varied living environment, there is the possibility that a number of microorganisms are able to produce enzymes with particular favorable characteristics that enable their adaptation to extreme environments. To satisfy industrial needs, xylanases that are active over a wide range of temperatures and pHs are of particular interest because they can be introduced more freely at different stages of the bleaching xylosidic line without the need of cost changes in temperature and pH. Consequently, this has raised interest in the research and application of exploiting particular microorganisms and enzymes from the marine environment. Recently, a thermostable xylanase gene from a deep-sea thermophilic *Geobacillus* sp. was cloned and expressed in *Escherichia coli*. This isolated enzyme was active up to 90°C [29].

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In this paper, we report a novel xylanase gene cloned from *Demequina* sp. JK4 isolated from the deep sea. The isolated xylanase exhibited some distinct enzymatic properties desirable for industrial applications.

Materials and Methods

Bacterial Strains, Plasmids, and Media

Demequina sp. JK4 was isolated from the deep sea where the temperature is 25°C, and was supplied by the Marine Culture Collection of China (MCCC 1A02937). *E. coli* DH5α and BL21 (DE3) strains were used as the host cells for DNA manipulation and protein expression, respectively. Plasmid pUC19 was used for the plasmid preparations and gene cloning, whereas the pET28 vector was used for the production of the xylanase.

Demequina sp. JK4 was cultivated in Luria-Bertani (LB) medium with 3% (w/v) NaCl, whereas the *E. coli* was grown in LB medium or on LB agar plates. Ampicillin (100 μg/ml) or kanamycin (50 μg/ml) was added when required. To screen for xylanase activity, xylan minimal medium (1% oat spelt xylan, 0.2% NaCl, 0.2% K_2HPO_4, 0.03% $MgSO_4$, 0.02% $CaCl_2$, 0.1% yeast extract, and 1.5% agar, pH 7.0) was used.

Materials and Chemicals

Oat spelt and birchwood xylan, carboxymethyl cellulose, locust bean gum, and Avicel were purchased from Sigma (U.S.A.). Restriction endonucleases, T4 DNA ligase, *pfu* DNA polymerase, and GC buffer were purchased from Takara (Japan). AxyPrep DNA purification kits were purchased from Axygen (U.S.A.). All the other chemicals were of analytical grade.

DNA Manipulation

All the standard recombinant DNA techniques, including plasmid extraction, restriction endonuclease digestion, DNA ligation, and PCR were performed as described by Sambrook and Russell [23].

Construction of the Genomic Library

Chromosomal DNA was isolated from *Demequina* sp. JK4 and partially digested with Sau3AI. DNA fragments (5 to 8 kb) were purified using an AxyPrep DNA purification kit and ligated to BamHI-digested and dephosphorized pUC19 using T4 DNA ligase. The plasmids were subsequently transformed into *E. coli* DH5α cells using a heat-shock process. The transformants were spread onto LB plates containing 5-bromo-4-chloro-3-indolyl-D-galactopyranoside (X-Gal) and isopropyl-1-thio-β-D-galactoside (IPTG) for blue-white screening. White colonies appearing within 24 h were picked and transferred onto xylan minimal medium plates (1% xylan was used as the sole carbon source) and screened for their enzyme activity using a Congo red staining method [26]. The clones with clear halo-forming activities were selected as xylanase-positive colonies and used for further analysis.

Gene Sequencing and Analysis

DNA sequencing was performed by the Beijing Genomics Institute (BGI). The sequence data were analyzed using the Softberry Gene Finding tool (http://linux1.softberry.com/berry.phtml). The presence of a signal peptide in the deduced amino acid sequence was predicted by SingalP (http://www.expasy.org/). Protein functional analysis was performed with InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/). The DNA and protein sequence alignments were carried out with the Blastn and Blastx programs (http://www.ncbi.nlm.nih.gov/BLAST/), respectively. Multiple sequence alignments were performed using the ClustalW program.

Purification of Mxyn10 from the Inclusion Bodies

In order to express Mxyn10, the *Mxyn10* gene without the sequence encoding the signal region was amplified by PCR using the plasmid of the positive clone as the template. The primers, CXF (CATGGATCCGCCGGCTCGACGCTCCAGG) containing a BamHI restriction site (underlined) and CXR (CATGAATTCTCAGGCCGTGCAGGTCAGCG) containing an EcoRI restriction site (underlined), were designed to facilitate in-frame cloning into the pET28 expression vector to generate pET28-*Mxyn10*.

E. coli DE3 cells carrying pET28-*Mxyn10* were grown at 37°C in LB medium containing ampicillin (100 μg/ml) to an OD_{600} of 0.6. Subsequently, IPTG was added to a final concentration of 0.2 mM. After induction, cells were grown for a further 6 h at 28°C (to an OD_{600} of 1.5). Cells were harvested and resuspended in lysis buffer (0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 7.0; 5.0% Triton X-100, pH 7.5) and disrupted using a French Press [14]. The pellet containing the inclusion bodies was washed with wash buffer (0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 7.5) 3 times to remove residual Triton X-100. Finally, the insoluble fraction was collected from the 1 l culture broth and solubilized in 25 ml of dissolving buffer (0.1 M NaH_2PO_4, 0.01 M Tris-HCl; 8 M urea, pH 7.5). After stirring at 4°C for 2 h, the remaining insoluble material was removed by centrifugation.

The soluble fraction was loaded onto a Ni^{2+}–NTA column equilibrated with dissolving buffer and left at 25°C for 2 h. To elute the unbound proteins, the column was washed successively with wash buffer (0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 7.5) containing gradually decreasing concentrations of urea and increasing concentrations of imidazole to enhance refolding of the denatured protein as well as to remove nonspecifically bound proteins [21]. Finally, the Mxyn10 protein was eluted in an elution buffer (0.1 M NaH_2PO_4, 0.01 M Tris-HCl, 1 M urea, 250 mM imidazole, pH 7.5) and dialyzed overnight to completely remove the urea and imidazole. The quantification of the protein was determined by the method of Bradford using the Bradford reagent (Sigma). Bovine serum albumin (BSA) was used as a standard.

SDS–PAGE and Zymogram Analysis

The molecular mass and purity of the extracted proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The zymogram analysis was performed according to the method of Beguin [2] with slight modifications. Briefly, the recombinant protein was electrophoretically applied to a 10% native PAGE containing 0.1% xylan. After electrophoresis, the gel was incubated in 0.2 M HAc–NaAc (pH 5.5) for 30 min at 40°C and stained using the Congo red method.

Enzyme Assays

As described by Miller [17], the xylanase activity was determined by measuring the release of the reducing sugar from birchwood xylan using a 3,5-dinitrosalicylic acid (DNS) reagent. One unit of xylanase activity was defined as the amount of the enzyme that generated 1 μmole of the reducing sugar from xylan per minute

(xylose was used as the standard). To determine the optimum temperature and pH for the purified xylanase activity, the reaction mixture containing 10 μl of diluted enzyme solution and 90-μl suspension of 1% birchwood xylan was incubated for 30 min at different temperatures (10–90°C) and at different pH values by using 0.2 M HAc–NaAc (pH 3.0–8.0) and 0.05 M glycine–NaOH (pH 8.0–11.0) buffers. Thermal stability studies were carried out by incubating the enzyme over a 20 to 80°C range for 1 h and measuring the remaining activity under optimum conditions. For pH stability, relative activity was determined at the optimum conditions after the enzyme had been incubated without substrate in different pH buffers ranging between 3 and 11 at 25°C for 2 h. The effects of different metal ions and chemical reagents on the enzyme activity were assessed under optimum assay conditions with the reaction mixtures containing 1 mM additional reagents.

Substrate specificity was determined by replacing 1% birchwood xylan with 1% barley glucan (Sigma), 1% oat spelts xylan (Sigma), 1% CMC-Na (Sigma), 1% Avicel (Sigma), 1% laminarin (Sigma), and 1% locust bean gum (Fluka), and measuring the hydrolysis rate of the enzyme in the presence of each substrate under the same conditions. The hydrolysis rate for birchwood xylan was standardized at 100%.

Kinetic Analysis

To determine the kinetic parameters, the enzymatic activity at the optimum conditions was assayed using a range of substrate concentrations from 0.5 to 15 mg/ml. The data were plotted according to the Lineweaver–Burk method.

Nucleotide Sequence Accession Number

The nucleotide sequence of the xylanase gene (*Mxyn10*) from *Demequina* sp. JK4 has been deposited in the GenBank database under Accession No. FJ601705.

RESULTS

Cloning and Sequencing of a Xylanase Gene

A total of 8,000 transformants containing genomic inserts were screened for xylanase activity by the Congo red method. In total, two clones expressing xylanase activity were detected, which both carried the same 4.9-kb fragment. After sequencing and analysis of this fragment, one complete open reading frame (ORF) consisting of 1,416 bp was found. The overall G+C content of the ORF was 70.7%. The ORF encoded a 471-residue protein with a calculated molecular mass of 49 kDa. A typical signal peptide sequence and cleavage site between Ala35 and Ala36 were located at the N terminus. As such, the mature polypeptide consisted of 436 residues with a calculated mass of 46 kDa. Further analysis revealed the presence of a potential Shine–Dalgarno ribosome-binding site sequence, 5'-AGGAGG-3', 10 bp upstream of the ATG start codon.

Sequence Analysis

The deduced amino acid sequence of the ORF was aligned with available protein sequences from GenBank and SWISSPROT databases as well as the literature. The sequence showed extensive similarity to endo-1, 4-β-xylanase belonging to GH10. Furthermore, this protein had a high molecular mass, which was a typical feature of the GH10 family [4]. Based on the analysis above, we concluded that this enzyme belongs to the the GH10 family and denominated it Mxyn10. Mxyn10 had the highest identity (70%) with the xylanase (SlXLNA) from *Streptomyces lividans* [24]. Otherwise, the enzyme shared 67% identity with Tfxyn from *Thermopolyspora flexuosa* [12], 63% identity with the xylanase (CFX) from *Cellulomonas fimi* [10], 44% with Xyl24 from *Gibberella zeae* (AAT84258), and 42% with Xyl4 from *Cochliobolus carbonum* (AAT49296).

As with most xylanases in family 10 [7, 22], the xylanase from *Demequina* sp. JK4 constituted a catalytic module (CM) from positions 36 to 339 and a carbohydrate-binding module (CBM; 91 residues) at the C-terminus. A 39-residue linker between the two domains existed. Among all the 43 families of CBMs grouped according to the primary structure similarities [8], the CBM of Mxyn10 belonged to family 2. The catalytic module of Mxyn10 xylanase had the highest identity (78%) with CFX from *Cellulomonas fimi* and showed 73% identity with Sl XLNA from *Streptomyces lividans*. The CBM of Mxyn10 showed the highest identity (49%) with the CBM of CelA classified to GH9 from *Thermobispora bispora* (P26414). Moreover, it showed 48% identity with the CBM of a mannosidase from *Thermobifida fusca* YX [15] and 39% identity with the CBM of a chitinase (YP_001134266) from *Mycobacterium gilvum* PYR-GCK. Both these enzymes belong to the GH5 family. However, Mxyn10 had only 38% identity with the CBM of CFX whose CM showed the highest identity with Mxyn10. All the four CBMs mentioned above that shared identity with Mxyn10 belong to family 2. Multiple sequence alignments were conducted using ClustalW (Fig. 1). In 1994, the three-dimensional structure of the catalytic domain of Sl XLNA from *Streptomyces lividans* was solved by X-ray crystallography to a resolution of 2.6 Å and represents the first family 10 enzyme structure to be reported [5]. According to the crystallography, the catalytic domain of Sl XLNA consists of $(\alpha/\beta)_8$-barrels, and Glu128 and Glu236 were found to be the catalytic amino acids. Based on the alignment, Glu163 and Glu271 are the most likely residues located in the catalytic site of Mxyn10.

Enzyme Expression and Purification

Mxyn10 without the coding sequence of its predicted signal peptide was cloned into the pET28 vector and expressed in *E. coli* BL21 (DE3). Upon induction, high expression levels of the protein were observed and the majority of the expressed Mxyn10 was produced in inclusion bodies. Urea (8 M) effectively solubilized approximately half of the total amount of inclusion bodies. After renaturation and purification with the Ni^{2+}–NTA column and dialysis, the active conformation of Mxyn10 was obtained.

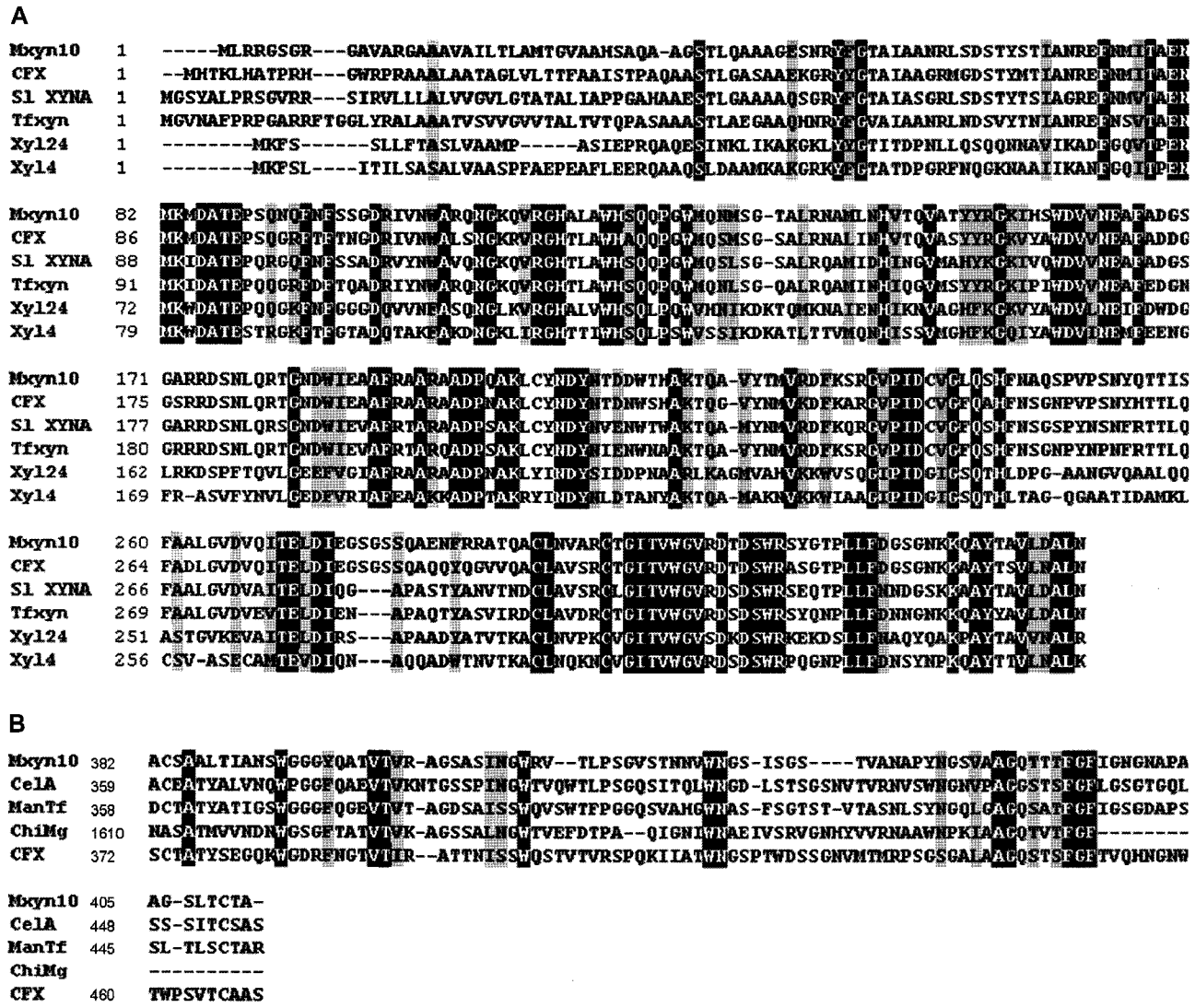

Fig. 1. Multiple sequence alignments.

A. Amino acid alignment of the catalytic module of Mxyn10 with similar xylanases from the GH10 family using the ClustalW program. Identical amino acids conserved in all proteins are shaded. Mxyn10 was cloned from *Demequina* sp. JK4 in this study. CFX, Sl XYNA, Tfxyn, Xyl24, and Xyl4 were obtained from *Cellulomonas fimi*, *Streptomyces lividans*, *Thermopolyspora flexuosa*, *Gibberella zeae,* and *Cochliobolus carbonum*, respectively. **B.** Amino acid alignment of the carbohydrate-binding module of Mxyn10 with similar CBMs from the CBM2 family using the ClustalW program. Identical amino acids conserved in all proteins are shaded. Mxyn10, ManTf, ChiMg, CelA, and CFX indicate the CBM of Mxyn10 from *Demequina* sp. JK4, the CBM of CelA from *Thermobispora bispora*, the CBM of a mannosidase from *Thermobifida fusca* YX, and the CBM of a chitinase from *Mycobacterium gilvum* PYR-GCK, respectively.

SDS–PAGE analysis revealed that the recombinant Mxyn10 was purified to homogeneity, with approximately 46 kDa in size. This molecular mass corresponds to the calculated mass. A clear zone was produced on the zymogram, thus confirming that the purified protein was a xylanase (Fig. 2).

Enzyme Characterization

The pH and temperature dependence of the activity of Mxyn10 is shown in Fig. 3. Mxyn10 exhibited maximum activity at 55°C, with 50% activity at 45°C and 60°C. The pH optimum was found to be 5.5. However, the enzyme showed substantial activity across a broad pH range, with >50% of the maximum activity observed between pH values 5.0 to 7.5. The xylanase activity remained stable between 20 and 50°C, and 80% of the relative activity was retained at 60°C. The relative activities of the enzyme following preincubation in pH values ranging from 5.0 to 10.5 were essentially equivalent to the activity measured for the untreated enzyme sample.

The effects of metal ions and chemical regents on Mxyn10 are shown in Table 1. The activity measured without additional reagent was taken to be 100%. The results showed that the activity of Mxyn10 was not

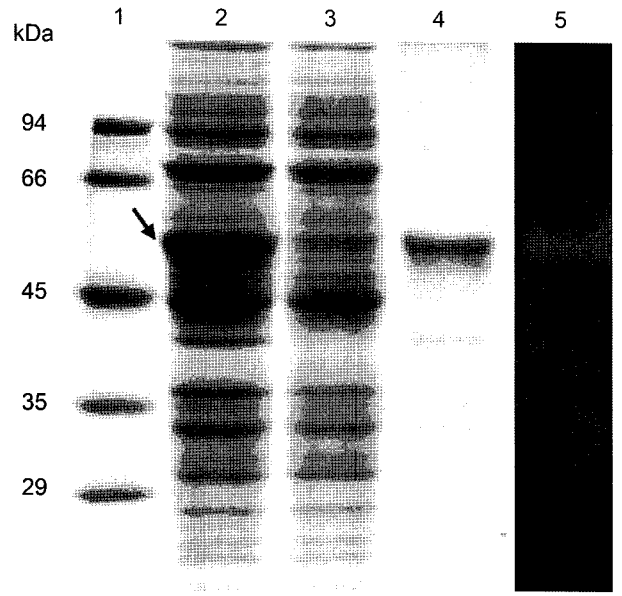

Fig. 2. 10% SDS–PAGE and zymogram analysis of the purified xylanase.

Lanes: 1, protein marker; 2, proteins expressed by the induced bacteria cells (soluble), where the arrow points to the band of the recombinant protein; 3, proteins expressed by the induced bacteria cells (insoluble); 4, purified xylanase (Mxyn10); 5, Zymogram stained with Congo red.

significantly affected by many of the reagents tested. The activity of Mxyn10 was affected only slightly by a few of the reagents, including Ca^{2+}, Hg^{2+}, EDTA, and β-mercaptoethanol. These reagents are known to inhibit other related enzymes. The coexistence of Cu^{2+}, Zn^{2+}, and Mn^{2+} led to a slight increase in activity.

As a xylanase, Mxyn10 showed maximum activity on birchwood xylan. The enzyme showed a degree of activity on oat spelts xylan. Apart from this, Mxyn10 did not hydrolyze the other polysaccharides examined, except for barley glucan and carboxymethyl cellulose in which Mxyn10 showed a limited amount of activity (Table 2).

Table 1. The effect of different metal ions and reagents on the activity of Mxyn10.

Reagent	Concentration (mM)	Relative activity (%)
Control	0	100%
Na^{+}	1	92.12±0.2
K^{+}	1	92.87±0.5
Ca^{2+}	1	84.93±0.4
Mg^{2+}	1	95.30±0.6
Cu^{2+}	1	108.42±0.5
Zn^{2+}	1	102.18±0.8
Mn^{2+}	1	101.09±0.1
Co^{2+}	1	65.27±0.3
Pb^{2+}	1	83.13±0.4
Fe^{2+}	1	88.96±0.5
Hg^{2+}	1	60.13±0.3
Dithiothreitol	1	99.10±0.4
β-Mercaptoethanol	1%	54.62±0.5
EDTA	1%	71.72±0.7

Assay was performed under optimum conditions.

Table 2. Activity of the purified xylanase on different substrates.

Substrate	Activity (IU/mg)
Birchwood xylan	8.9±0.04
Oat spelts xylan	3.7±0.02
Avicel	0
Barley glucan	0.8±0.02
Carboxymethyl cellulose	1.0±0.03
Laminarin	0
Locust bean gum	0
Starch	0

The kinetic parameters of Mxyn10 were determined on birchwood xylan at 55°C. Specifically, the K_m and V_{max} of Mxyn10 were 3.45 mg/ml and 7.7 μmol/min/mg proteins, respectively.

DISCUSSION

In this study, we described the cloning of a novel xylanase (designated Mxyn10) by constructing a genomic library from *Demequina* sp. JK4, formerly isolated from the deep sea. So far, there has been no reports of xylanases produced by the genus *Demequina*. This represents the first time a xylanase gene has been cloned from this genus.

Mxyn10 was found to belong to the GH10 family. The protein contains a catalytic module of GH10 and a carbohydrate-binding module of CBM2. The catalytic module of Mxyn10 xylanase had the highest identity (78%) with CFX from *Cellulomonas fimi*, whereas the carbohydrate-binding module was found to have maximal identity (49%) with the CBM of CelA of GH9 from *Thermobispora bispora*. It seemed that higher rankers of sequence similarity were different for the whole sequence, CM, and CBM, which was in accord with some other xylanases from family 10, including the XynAS9 from *Streptomyces* sp. S9 [13]. Additionally, the CBM of this xylanase also shared identity with some other CBM2s. Ligand specificity of CBMs has been found to generally reflect the substrate specificity of the catalytic module with various polysaccharides [1, 3]. As the largest prokaryotic CBM family, CBM2 contains members that bind cellulose (CBM2a) and xylan (CBM2b) [25, 28]. By mutation of a single residue, Simpson *et al.* [25] found that when an arginine was changed to a glycine, a CBM2b xylan-binding module completely lost xylan affinity, yet became a cellulose-binding module. Using sequence alignments, we identified the site that corresponded to Simpson's study. At this site, Mxyn10 and the cellulases that showed identity with Mxyn10 all contain a glycine, whereas CFX

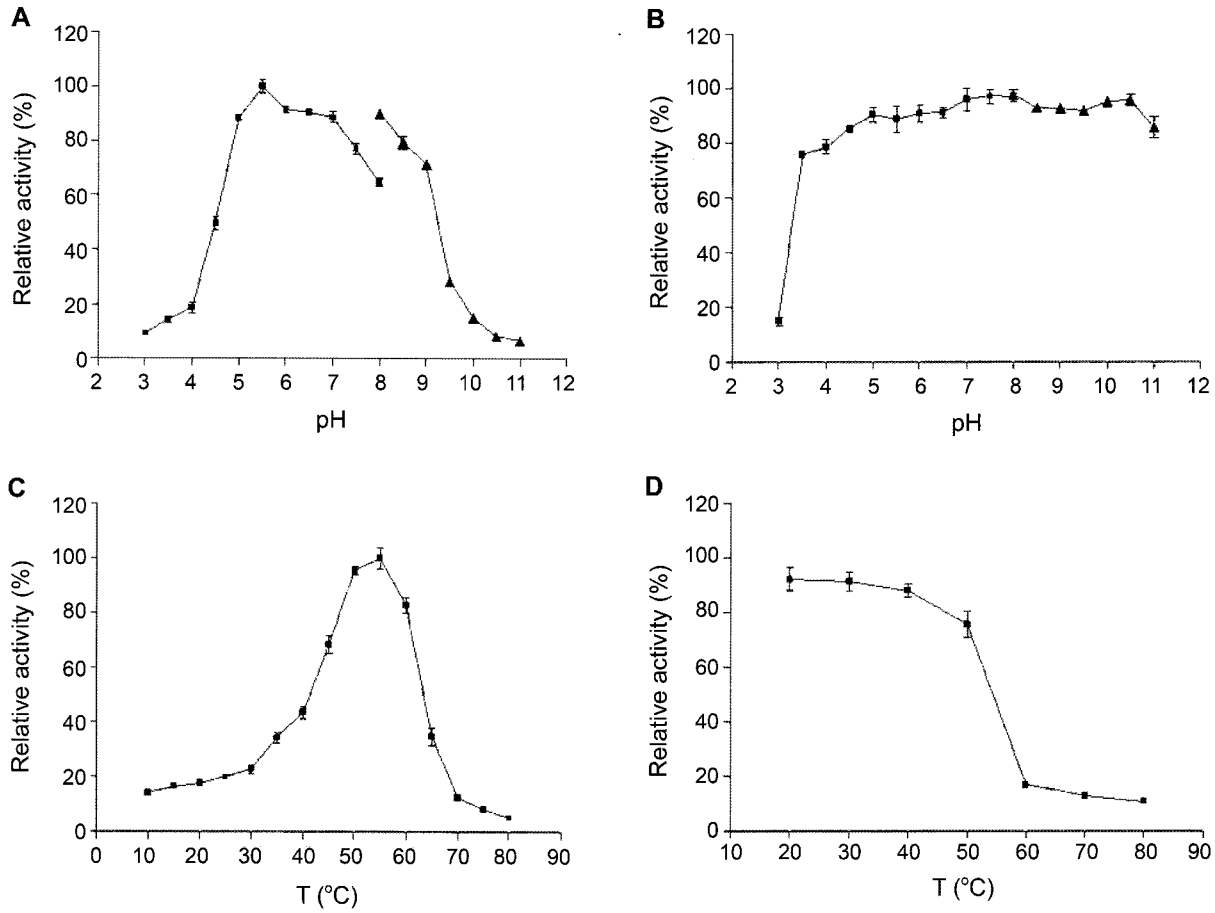

Fig. 3. The effects of pH and temperature on the activity and stability of Mxyn10.
A. The effect of pH on the activity of Mxyn10. The activity was assayed at 55°C in the pH range 3–11 with the following buffers containing 1% birchwood xylan: 0.2 M Na_2HPO_4/0.1 M citric acid for pHs 3.0–8.0 (●); 50 mM Glycine-NaOH buffer for pHs 8.0–12.0 (▲). The activity at the optimum pH was defined as 100%. **B**. The effect of pH on the stability of Mxyn10. After preincubation at 4°C for 2 h in buffers ranging from pH 3 to 11, activity was measured in Na_2HPO_4–citric acid buffer containing 1% birchwood xylan (pH 5.5) at 55°C. The activity without the pH treatment was defined as 100%. **C**. The effect of temperature on the activity of Mxyn10. The xylanase activity was measured in Na_2HPO_4–citric acid buffer containing 1% birchwood xylan (pH 5.5) at various temperatures. The activity at the optimum temperature was defined as 100%. **D**. The effect of temperature on the stability of Mxyn10. After preincubation at a range of temperatures for 60 min, the activity of the enzyme measured in Na_2HPO_4–citric acid buffer containing 1% birchwood xylan (pH 5.5) at 55°C was determined. The activity without treatment was taken as 100%. Error bars represent the standard deviation of the mean calculated for the three replicates.

has an arginine. This property indicates why Mxyn10 hydrolyzed barley glucan and carboxymethyl cellulose and showed weak activity on these two substrates.

Experimental data showed that Mxyn10 was sensitive to temperature but showed activity over a broad pH range. Under the conditions examined, the optimum temperature and pH were found to be 55°C and pH 5.5, respectively. Many other xylanases have been found to have similar temperature and pH activity maxima, including the xylanase from *Cellulomonas flavigena* [16], XynA from *Streptomyces olivaceoviridis* [30], XynA from *Paenibacillus* sp. HY-8 [10], and xylanase A from *Streptomyces lividans* [18]. However, Mxyn10 exhibited some distinct enzymatic properties. Mxyn10 was observed to be fully stable after treatment with buffers ranging from pH 3.5 to 10.0 for 2 h, which is a wider pH range when compared with the enzymes mentioned above. Acidophilic and alkaliphilic enzymes would obviously be beneficial in processes where extreme pH conditions are required, or where adjustment of the pH to neutral conditions is uneconomical and inefficient. Additionally, Mxyn10 was not affected by many of the reagents that usually inhibit many other xylanases, such as Hg^{2+}, Co^{2+}, and Pb^{2+}, which further indicates the potential wider industrial applications of Mxyn10. Consequently, Mxyn10 may be used extensively in bioconversion processes where a variety of treatments, including mineral salt, alkaline, solvent, or acidic pretreatments may be required prior to or simultaneously to enzyme treatments [4].

Furthermore, Mxyn10 showed activity on barley glucan and carboxymethyl cellulose. It has been reported that, in addition to their xylanolytic activity, some xylanases of the GH family 10 also display a range of activities against glucose-

derived substrates such as cellulose. The *Streptomyces lividans* enzyme, SI XLNA, which exhibited the highest sequence identity to Mxyn10, is primarily a xylanase with little activity against glucose-based polymers [6].

Tremendous amounts of cellulose are available from urban domestic refuse and industrial wastes. Both contribute to our pollution problems. Therefore, there is significant interest in the use of cellulosic biomass as a renewable source of energy *via* breakdown to sugars that can subsequently be converted to liquid fuel. Xylanases able to hydrolyze both xylan and cellulose could result in a more efficient and cheaper saccharification process of the agricultural residues, urban domestic refuse, and industrial wastes used for bioethanol production. Saccharification of the cellulose and hemicellulose in biomass results in sugar-rich liquid streams to further obtain a variety of products, including furfural, ethanol, and various functional biopolymers [11].

In conclusion, Mxyn10 cloned from *Demequina* sp. JK4 was found to be a novel xylanase. The enzyme was stable over a wide pH range and showed cellulose-degrading activity. This activity allows the potential application of Mxyn10 in saccharification processes essential for bioethanol production, bread making, and improving animal nutrition.

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