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# Purification and characterisation of a major xylanase with cellulase and transferase activities from *Fusarium oxysporum*

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**Abstract**

A major xylanase from *Fusarium oxysporum* was purified to homogeneity by gel filtration, affinity, and ion-exchange chromatographies. It has a molecular mass of 60.2 kDa and pI of 6.6 and was optimally active at pH 7.4 and at 50 °C. The enzyme was stable over the pH range 5.8–8.2 at 40 °C for 24 h and lost 45% of its original activity at pH 9.0 under the identical conditions. The enzyme rapidly hydrolysed xylans from oat spelts (husks) and birchwood, but the activities on carboxymethylcellulose (CMC), filter paper, and Avicel were very low. Determination of $k_{cat}/K_m$ revealed that the enzyme hydrolysed oat spelts and birchwood xylans, 15–30 times more efficiently than CMC. In a 24 h incubation, at pH 7.0 and 9.0, the enzyme hydrolysed oat spelts and birchwood xylans by 75 and 65%, respectively. However, at pH 7.0, the enzyme released almost equal amounts of xylose and xylobiose from both xylans, whereas at pH 9.0, the concentration of xylobiose was twice as muchi as that of xylose and xylotriose. Xylanase attacked

Abbreviations: CMC, carboxymethylcellulose; DNS, dinitrosalicylic acid; HPLC, high pressure liquid chromatography; IEF–PAGE, isoelectric focusing–polyacrylamide gel electrophoresis; MeUmbGlc, 4-methylumbelliferyl β-D-glucoside; $MeUmb(Glc)_n$ ($n = 2–6$), 4-methylumbelliferyl cello-oligosaccharides; *p*NP, *p*-nitrophenol; *p*NPG, *p*-nitrophenyl β-D-glucopyranoside; *p*NPX, *p*-nitrophenyl β-D-xylopyranoside; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

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preferentially the internal glycosidic bonds of xylo- and 4-methylumbelliferyl cello-oligosaccharides [MeUmb(Glc)$_n$]. The enzyme catalysed transglycosylation reaction with xylotriose, xylotetraose, and xylopentaose as donors and 4-methylumbelliferyl β-D-glucoside (MeUmbGlc) as an acceptor. © 1996 Elsevier Science Ltd.

*Keywords:* Xylanase; Hemicellulose

## 1. Introduction

Agricultural and forestry biomass which consists of 20–35% of hemicellulose serves as a second major renewable source of energy on earth [1,2]. Xylans, the most abundant hemicellulose, are heteropolysaccharides having a main chain of 1,4-β-D-xylanopyranose, substituted with α-L-arabinofuranose, acetic acid, 4-*O*-methyl α-D-glucuronic acid, ferulic (4-hydroxy-3-methoxycinnamic), and *p*-coumaric (4-hydroxycinnamic) acids [3]. The sugars and sugar acids are attached through glycosidic linkages while acids are linked by ester linkages [4]. The type and extent of substitution varies depending on the origin and the type of tissue from which xylan is isolated [5,6]. The complete hydrolysis of xylan requires the concerted action of endoxylanase (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8), exoxylanase (β-D-xylan xylohydrolase), β-D-xylosidase (β-D-xyloside xylohydrolase, EC 3.2.1.37), α-L-arabino-furanosidase (α-L-arabinofuranoside arabinohydrolase, EC 3.2.1.55) α-D-glucuronidase, acetylxylan esterase, and phenolic acid esterases [7,8].

Recently, xylanases and xylan-debranching enzymes have attracted considerable research interest because of their potential industrial applications [2,9]. The use of xylanases has been shown to (a) improve the quality and texture of bakery products [2], (b) reduce the amount of chlorine required for bleaching of paper pulp [9], and (c) increase the nutritive quality of poultry diet [2]. However, such applications require xylanase(s) with special properties. For example, the bio-bleaching of paper pulp requires a xylanase, active either at or above pH 9.0 and having no cellulase activity [9]. On the other hand, xylanase together with xylan-debranching enzymes are believed to be essential to improve the nutritive quality of bakery products and animal feed [2]. In the present paper, we describe the purification and characterisation of a major xylanase from *F. oxysporum*, which is active over a wide range of pH and retains more than 55% of its original activity for 24 h at pH 9.0 and at 40 °C. These and other properties presented here suggest that this xylanase could be of commercial interest.

## 2. Experimental

*Materials and methods.—F. oxysporum*, strain F3, isolated from cumin [10] was used. Ultrogel AcA 54 and DEAE-Trisacryl M were from Jones Chromatography and Pharmacia, respectively. The *p*-aminobenzyl-1-thio-β-D-cellobioside coupled to Sepharose 4B was prepared as described [11]. Avicel was purchased from Fluka while

xylo-oligosaccharides were from Megazyme, Australia. 4-Methylumbelliferyl cello-oligosaccharides [MeUmb(Glc)$_n$] ($n$ = 2–6) were prepared as described [12]. Birchwood and oat spelts glucurono-xylans were from either Sigma (X-0502) or Rotec (Roth 7500). All the remaining chemicals were AnalaR grade and from either Sigma or BDH.

*Production of xylanase.*—*F. oxysporum* was grown in a 20 L bioreactor for 4 days at 30 °C, pH 6.0 using 17 L culture medium [13] containing 2% (w/v) alkali-treated cellulose 123 (Schleicher and Schüll). The culture broth was filtered (GF/A), concentrated (10-fold) using a flash evaporator (38 °C) and desalted on a PD-10 column (Sephadex G-25), before freeze drying.

*Purification of xylanase.*—*(a) Gel filtration.* A 48.2 mg protein, in 5 mL of 10 mM sodium acetate, pH 5.0, was applied on to an Ultrogel AcA 54 column (90 × 1.5 cm), equilibrated, and eluted with the same buffer (30 mL h$^{-1}$). Fractions (2 mL) were assayed for xylanase, endoglucanase, and protein ($A_{280}$). Active xylanase fractions were pooled and concentrated (20-fold) using PM-10 membrane.

*(b) Affinity chromatography.* Sample from step (a) was dialysed (100 mM sodium acetate buffer, pH 5.0 containing 0.01% $NaN_3$, 0.1 M glucose, and 10 mM glucono-δ-lactone) and applied on to *p*-aminobenzyl-1-thio-β-D-cellobioside coupled to Sepharose 4B column (1 × 10 cm). The column was equilibrated and eluted with the same buffer. Two mL fractions were collected (30 mL h$^{-1}$) and pooled according to $A_{280}$. Pooled samples were dialysed and assayed for xylanase and endoglucanase.

*(c) Ion-exchange chromatography.* Xylanase from step (b; 8 mL, 24.6 mg) was concentrated (4-fold; PM-10 membrane), dialysed (100 mM imidazole buffer, pH 7.0), and applied on to a DEAE–Trisacryl M column (18 × 16 cm), equilibrated with the same buffer. The column was washed with two bed volumes of the above buffer. The bound xylanase was eluted using a linear (0 to 1 M) NaCl gradient in 80 mL imidazole buffer, pH 7.0, at a flow rate of 1 mL min$^{-1}$. Four mL fractions were collected and assayed for endoglucanase and xylanase activities.

*Biochemical characterisation of purified xylanase.*—*(a) Determination of purity and molecular mass.* These were determined by SDS–PAGE as described [14].

*(b) Determination of isoelectric point (pI).* The IEF–PAGE was performed using Mini IEF cell (Bio-Rad) and a 5% polyacrylamide gel containing ampholytes covering the pH range 5.5–8.0 (Bio-Rad IEF manual).

*(c) Protein determination.* This was determined by bicinchoninic acid method according to Pierce instruction manual No. 23235X using bovine serum albumin as a standard. The $A_{280}$ was used to monitor the protein in column eluents.

*(d) Enzyme assays.* Xylanase was assayed using 1.8 mL of 1.0% (w/v) birchwood xylan (Sigma) in 50 mM sodium acetate buffer, pH 6.0 and 0.2 mL of suitably diluted enzyme at 50 °C for 5 min as described [15]. The reducing sugar released was determined as described [16]. Xylosidase was assayed using 5 mM *p*-nitrophenyl β-D-xylopyranoside (*p*NPX) in 50 mM sodium acetate buffer, pH 6.0 at 50 °C for 15 min as described [17]. Endoglucanase and exoglucanase were assayed using low viscosity CMC (Sigma, 60 mg) and Avicel (20 mg; 50 μ) at pH 6.0 as described [18,19].

*(e) Effect of pH and temperature on activity and stability of xylanase.* The effect of pH was measured using the following buffers: 100 mM sodium acetate, pH 4.0–6.0; 100

mM potassium phosphate, pH 6.0–7.0, and 100 mM Tris–HCl, pH 7.5–9.0. The stability at different pH was determined using the above buffers at 40 °C for 24 h. The effect of temperature (35–75 °C) on xylanase activity was determined using 100 mM potassium phosphate buffer, pH 7.0, while the thermostability was measured using the same buffer, between 30–70 °C for 1 h.

*(f) Determination of substrate specificity.* This was determined by measuring the reducing sugar released from xylan (1%; w/v), Avicel (1%; w/v), filter paper (1%; w/v), CMC (1%; w/v), laminarin (1%; w/v), lichenan (1%; w/v), and *p*NP released from *p*NPX (2 mM) and *p*NPG (2 mM).

*(g) Determination of kinetic constants using xylan and CMC.* The initial activities towards birchwood and oat spelts xylans and CMC were determined using varying concentrations of substrate (0–60 mg). From the reciprocal plot of initial activities versus substrate concentration, the $K_m$ and $k_{cat}$ were obtained.

*(h) Determination of binding of xylanase on to xylan and avicel.* The insoluble xylan was prepared from larchwood xylan according to the method of Irwin et al. [20]. A 50 $\mu$g protein was incubated with 2 mg of either Avicel or insoluble xylan in 0.5 mL of 100 mM potassium phosphate buffer, pH 6.0 at 4 °C for 1 h. Samples were centrifuged and the xylanase activity in the supernatant was measured.

*(i) Hydrolysis of xylans and xylo-oligosaccharides.* Reaction mixtures (1 mL) containing 100 $\mu$g of enzyme and 20 mg of either oat spelts or birchwood xylan in 100 mM, either potassium phosphate buffer, pH 7.0 or Tris–HCl buffer, pH 9.0 were incubated at 40 °C for 20 h. Reaction mixtures (1 mL) containing the same amount of xylanase in 100 mM sodium acetate buffer, pH 6.0, and 20 mM of either xylobiose, xylotriose, xylotetraose or xylopentaose were incubated at 40 °C for 1 h. A 50 $\mu$L sample was withdrawn from the xylan reaction mixtures at different time intervals and the products were analysed using Waters HPLC system and $NH_2$–Spherisorb column (25 × 4 mm) with acetonitrile: water (70:30) as a mobile phase. Products released from xylo-oligosaccharide were analysed after 1 h of incubation. Xylose, xylobiose, xylotriose, xylotetraose, and xylopentaose were identified using Waters refractive index detector (RI 401) and quantified using standard sugars.

*(j) Hydrolysis of MeUmb(Glc)$_n$.* A reaction mixture containing 0.9 mL of 2 mM MeUmb(Glc)$_n$ ($n$ = 2–6) in 20 mM sodium acetate buffer, pH 6.0, and 0.1 mL xylanase (10 $\mu$g) was incubated at 25 °C for 1 h. A 25 $\mu$L sample from each reaction mixture was withdrawn at 10 min intervals and mixed with 25 $\mu$L of HPLC grade acetonitrile. A 20 $\mu$L sample from the above mixture was analyzed using a Waters HPLC system as described above. Hydrolysis products released from MeUmb(Glc)$_n$ were detected at 313 nm using Waters UV detector (Model 440) and quantified using MeUmb, MeUmbGlc, and MeUmb(Glc)$_n$ as standards. The bond cleavage frequency of MeUmb(Glc)$_n$ was analysed as described [21].

*(k) Measurement of transferase activity.* This was determined using, xylotriose, xylotetraose, and xylopentaose as donors and MeUmbGlc as an acceptor. Two mL buffer (100 mM sodium acetate, pH 6.0) containing either xylotriose, xylotetraose or xylopentaose (20 mM), MeUmbGlc (1 mM), and xylanase (100 $\mu$g) was incubated at 30 °C for 2 h. A 50 $\mu$L sample withdrawn after 1 and 2 h was analysed by HPLC as described above.

## 3. Results

*Production of cellulase and hemicellulase components by* F.oxysporum.—*F. oxysporum*, when cultured on alkali treated cellulose, produced endoglucanase, exoglucanase, β-glucosidase, xylanase, and β-xylosidase. The highest endoglucanase, exoglucanase, β-glucosidase, xylanase, and β-xylosidase activities in the culture filtrates were 5.0, 0.02, 0.9, 5.2, and 0.5 units $mg^{-1}$ protein, respectively.

*Purification of xylanase from* F.oxysporum.—The extracellular protein from *F. oxysporum* was initially fractionated on an Ultrogel AcA 54 column. The xylanase activity peak was separated from a low molecular mass endoglucanase, but co-eluted

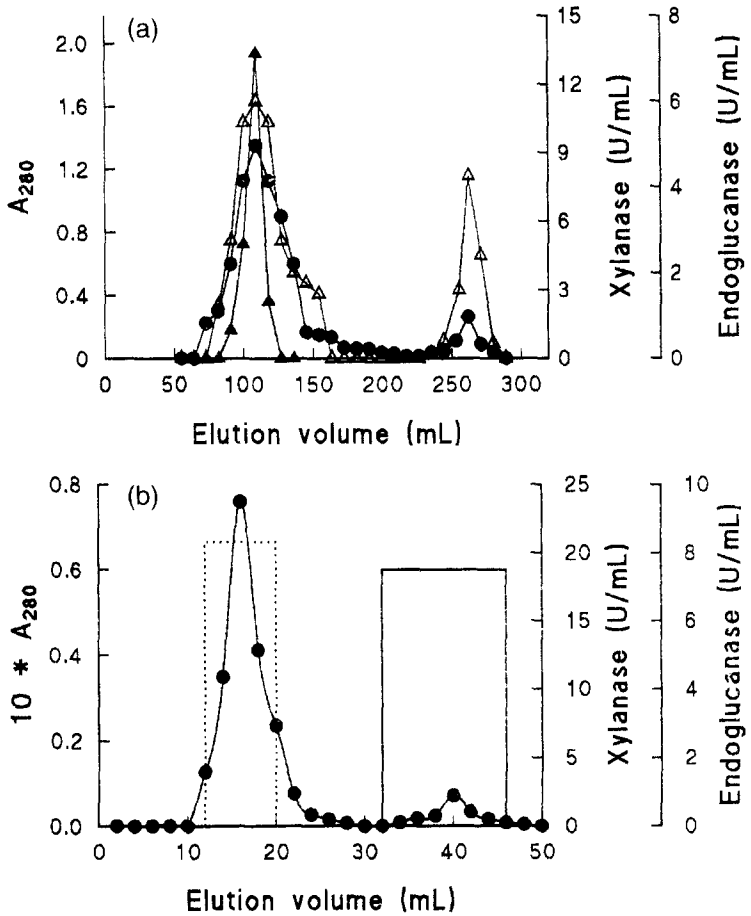


Fig. 1. Purification of a major xylanase from the culture filtrates of *F. oxysporum*. (A) Gel filtration chromatography on Ultogel AcA 54 column. ●, △ and ▲ represent protein ($A_{280}$), endoglucanase and xylanase, respectively. (B) Affinity chromatography on p-aminobenzyl-1-thio-β-D-cellobioside Sepharose 4B. ●, ———— and --- represent protein ($A_{280}$), endoglucanase and xylanase, respectively.

Table 1
Summary of the purification of a major xylanase from *F. oxysporum*

| Purification step | Total activity (U) | Total protein (mg) | Specific activity (U/mg protein) | Purification (Fold) | Recovery (%) |
|---|---|---|---|---|---|
| 1. Culture filtrate | 272 | 48.2 | 5.6 | – | 100 |
| 2. Flash evaporation | 251 | 48.2 | 5.2 | 0.93 | 92.3 |
| 3. Sephadex G-25 chromatography | 232 | 45.0 | 5.2 | 0.93 | 85.3 |
| 4. Lyophilization | 211 | 45.0 | 4.7 | 0.84 | 77.6 |
| 5. Ultogel AcA 54 chromatography | 198 | 28.3 | 7.0 | 1.25 | 72.8 |
| 6. Affinity chromatography | 170 | 24.6 | 6.9 | 1.23 | 62.5 |
| 7. Ion exchange chromatography | 145 | 20.0 | 7.3 | 1.30 | 53.3 |

One unit (U) of xylanase activity is defined as the amount of enzyme required to release one μmole of reducing sugar (xylose equivalent) per min under the assay conditions described in Materials and methods.

with a high molecular mass endoglucanase (Fig. 1A). The latter endoglucanase was subsequently separated from xylanase by affinity chromatography, using *p*-aminobenzyl-1-thio-β-D-cellobioside coupled to Sepharose 4B (Fig. 1B). The xylanase was subsequently purified by DEAE–Trisacryl column chromatography and accounted for approximately 75% of the total protein secreted by *F. oxysporum* (Table 1). A three step purification increased the purity of xylanase only by 1.3 fold with a recovery of 53.3% (Table 1).

*General properties.*—SDS–PAGE, under denatured conditions (Fig. 2) revealed that the purified xylanase is a homogeneous protein of 60.2 kDa. On IEF–PAGE with ampholytes covering the range pH 5.0–8.0, the enzyme focussed as a pure protein corresponding to a pI of 6.60 (data not shown). The enzyme was optimally active at pH

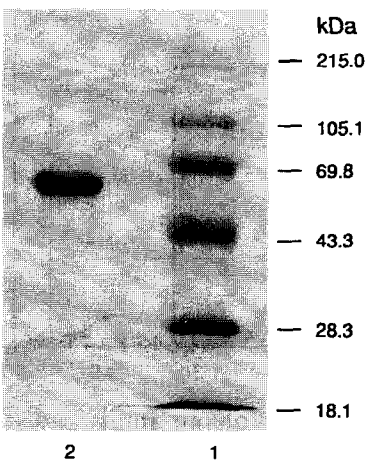


Fig. 2. SDS–PAGE. Lane 1, standard protein markers in the order of increasing molecular mass: β-lactoglobulin, carbonic anhydrase, ovalbumin, albumin, phosphorylase B and myosin (H-chain) (15 μg); lane 2, xylanase from *F. oxysporum* (10 μg).

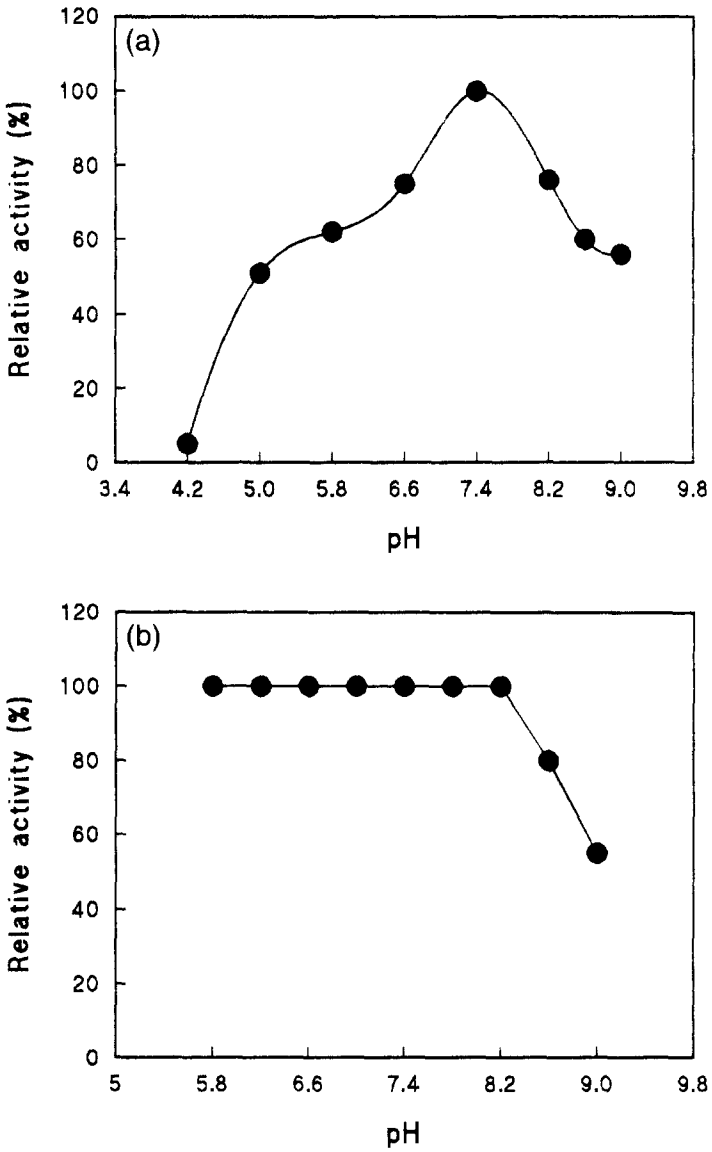


Fig. 3. (a) Effect of pH on the activity of purified xylanase from *F. oxysporum*. (b) Stability of purified xylanase from *F. oxysporum* over the pH region 5.8–9.0.

7.4 and at 50 °C, and retained 55–60% original activity over the pH 8.0–9.0 (Fig. 3A). The enzyme was stable between pH 5.8–8.2 at 40 °C for 24 h, but under identical conditions, the stability over pH 8.6–9.0 was only 50–55% (Fig. 3B). At pH 7.0, the enzyme was stable up to 50 °C for at least 1 h, but above 65 °C, the enzyme lost > 50% of its original activity in 30 min.

Table 2
Relative activity of a major xylanase from *F. oxysporum* towards aryl β-D-glycosides and polysaccharides

| Substrate | Relative activity (%) |
|---|---|
| Oat spelt xylan (Sigma) | 100 |
| Birchwood xylan (Sigma) | 55 |
| Birchwood xylan (Roth 7500) | 55 |
| CMC (low viscosity) | 3.1 |
| Filter paper | 0.8 |
| Avicel | 0.3 |
| *p*-Nitrophenyl β-D-xyloside | 0.0 |
| *p*-Nitrophenyl β-D-glucoside | 0.0 |
| Laminarin | 0.0 |
| Lichenan | 0.0 |

Two mL reaction mixture containing either aryl β-D-glycoside (2 mM) or polysaccharide (1%; w/v) in 50 mM sodium acetate buffer, pH 6.0 was incubated at 50 °C for 5 min–1 h. Activities were determined by measuring either *p*NP at 410 nm or reducing sugar by DNS method [16].

*Substrate specificity.*—Xylanase from *F. oxysporum* hydrolysed both oat spelts (husks) and birchwood xylans, CMC, Avicel and filter paper, but not *p*NPG, *p*NPX, laminarin, and lichenan (Table 2). The relative activity on oat spelts xylan (Sigma) was 1.8 fold higher than that on birchwood xylans obtained from Rotec (Roth 7500) and Sigma. Also, the relative activities on CMC, filter paper, and Avicel were 32, 125, and 333 fold lower than those on oat spelts xylan.

*Kinetics.*—The $K_m$ values for oat spelts xylan, insoluble birchwood xylan (Sigma) and CMC were comparable, while the $K_m$ was 2–3 fold lower towards the soluble birchwood xylan (Roth 7500) than the other substrates (Table 3). However, the comparison of $k_{cat}$ values shows that the enzyme hydrolysed oat spelts xylan 1.5–2.0 and 16 times faster than birchwood xylans and CMC, respectively. Comparison of $k_{cat}/K_m$ ratio shows that this xylanase hydrolysed the soluble birchwood xylan (Roth 7500) 1.8, 2.2, and 35 times more efficiently than oat spelt xylan (Sigma), insoluble birchwood xylan (Sigma), and CMC, respectively (Table 3).

*Binding to avicel and insoluble xylan.*—Two xylanases, one arabinofuranosidase and one esterase from *Pseudomonas fluorescens* subsp. *cellulosa* contain cellulose binding domain and are capable of binding to crystalline cellulose [22,23]. However, the xylanase from *F. oxysporum* did not bind to Avicel or to xylan.

Table 3
Kinetic constants of a major xylanase from *F. oxysporum*

| Substrates | $K_m$ (mg mL$^{-1}$) | $k_{cat}$ (min$^{-1}$) | $k_{cat}/K_m$ (mL min$^{-1}$ mg$^{-1}$) |
|---|---|---|---|
| 1. Oat spelt xylan | 11.1 | 66.9 | 6.0 |
| 2. Birchwood xylan (Roth 7500) | 3.8 | 40.4 | 10.6 |
| 3. Birchwood xylan (Sigma) | 9.5 | 46.0 | 4.8 |
| 4. CMC (low viscosity) | 13.1 | 4.2 | 0.3 |

Table 4
Hydrolysis products released from xylo-oligosaccharides by a major xylanase from *F. oxysporum*

| Xylo-oligosaccharides | Molar ratio (%) | | | |
|---|---|---|---|---|
| | xylose | xylobiose | xylotriose | xylotetraose |
| Xylotriose | 47 | 53 | – | – |
| Xylotetraose | 22 | 48 | 30 | – |
| Xylopentaose | 14 | 43 | 34 | 9 |

*Mode of action on xylo-oligosaccharides and MeUmb(Glc)$_n$.*—Xylanase from *F. oxysporum* was not active on xylobiose, but hydrolysed 20 mM xylotriose, xylotetraose, and xylopentaose completely, in 1 h at 40 °C (Table 4). As expected, the enzyme released equal amounts of xylose and xylobiose from xylotriose, whereas the major hydrolysis products released from xylotetraose and xylopentaose were xylobiose and xylotriose with a molar ratio of 5:3 and 4:3, respectively (Table 4). The accumulation of 30–34% of the total product as xylotriose from xylotetraose and xylopentaose together with complete hydrolysis of xylotetraose and xylopentaose during 1 h incubation, suggested that the purified enzyme is an endoxylanase.

When MeUmbGlc$_2$ was used, the xylanase preferentially attacked the glycosidic bond adjacent to MeUmb, whereas the enzyme hydrolysed the second and third glycosidic bonds of MeUmbGlc$_3$ from the non-reducing end with equal efficiency (Fig. 4). Also, when MeUmbGlc$_4$ and MeUmbGlc$_5$ were used, the xylanase preferentially attacked the second glycosidic bond from the non-reducing end, whereas the preferential attack on MeUmbGlc$_6$ was on the glycosidic bond adjacent to the non-reducing end (Fig. 4). Thus, the xylanase from *F. oxysporum* preferentially attacked either the internal glycosidic bonds or the glycosidic bonds adjacent to the non-reducing end of higher cello-oligosaccharides.

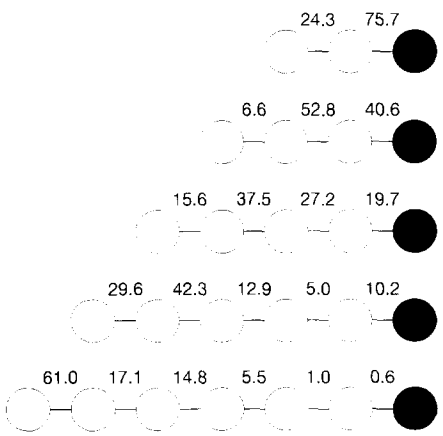


Fig. 4. Bond cleavage frequency for the hydrolysis of MeUmb(Glc)$_n$ by a major xylanase from *F. oxysporum*. This was determined based on the initial rate of the reaction and calculated as described [21].

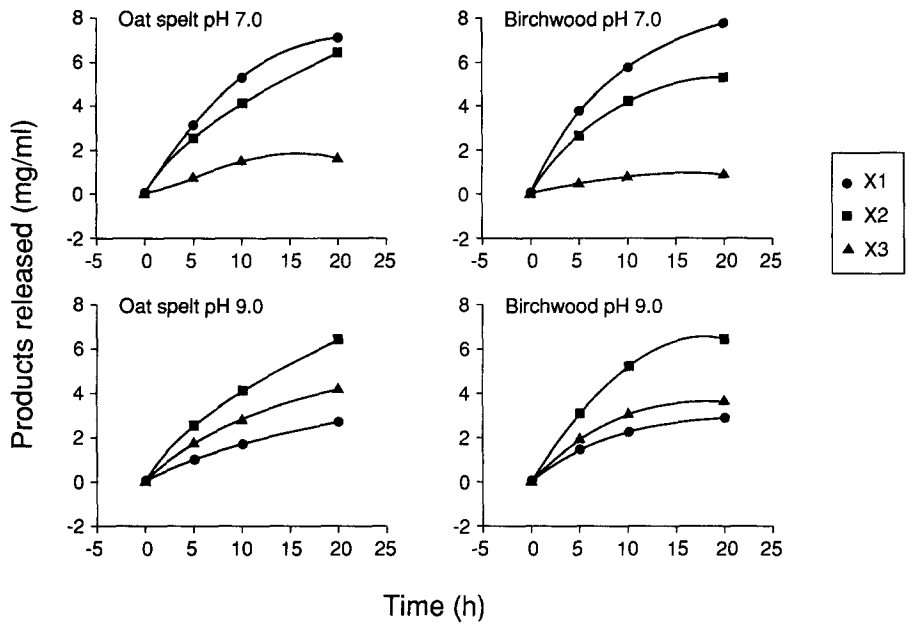


Fig. 5. HPLC analysis of products released from oat spelt and birchwood xylans by the xylanase from *F. oxysporum*.

*Analysis of extent of hydrolysis and the products released from oat spelts and birchwood xylans.*—The hydrolysis oat spelts and birchwood xylans by xylanase from *F. oxysporum* was studied at pH 7.0 and 9.0. The enzyme hydrolysed both xylans up to 75% at pH 7.0 and up to 65% at pH 9.0 in 24 h. HPLC analysis of the hydrolysis products released from oat spelts and birchwood xylans at different time intervals and at pH 7.0 showed that the reaction mixtures contained mainly xylose and xylobiose (Fig. 5). However, analysis of the samples taken from reaction mixtures maintained at pH 9.0 revealed that both oat spelts and birchwood xylans reaction mixtures contained twice as much xylobiose as xylose and xylotriose at all time points (Fig. 5).

*Transglycosylation activity.*—The xylanase from *F. oxysporum* catalysed transglycosylation reaction in the presence of either xylotriose, xylotetraose or xylopentaose as a donor and MeUmbGlc as an acceptor. HPLC analysis of samples taken at different time intervals revealed the formation of at least 6–7 transfer products in the presence of above xylo-oligosaccharides (data not shown). However, the retention time (approx. 10 min) of the main transfer product formed in the presence of all three xylo-oligosaccharides coincided with that of $MeUmbGlc_3$.

## 4. Discussion

An extracellular xylanase from *F. oxysporum* was purified to homogeneity by gel filtration, affinity and ion-exchange chromatographies with a recovery of 53.3%. Inter-

estingly, this xylanase was the major protein accounting for around 75% of the total protein secreted by *F. oxysporum*, strain F3.

The molecular mass (60.2 kDa) of xylanase from *F. oxysporum* was relatively high compared to xylanases reported from fungi and bacteria [24–27] and similar to that recorded for xylanases from *Sclerotium rolfsii* UV-8 (56 kDa) [13], *Aeromonas caviae* W-61 (41 and 58 kDa's) [28], and *Clostridium acetobutylicum* ATCC 842 (65 kDa) [29]. The pI of this xylanase (6.6) was similar to that reported for xylanases from *Bacillus* sp. C-59-2 (6.3) [30], *Trichoderma reesei* QM9414 (6.6) [31], and *Aspergillus niger* (6.7) [32].

Unlike the xylanases from other fungi [8,13,17,33–36], this xylanase showed much higher pH optimum (7.4) and greater stability under alkaline conditions. In this respect, the purified xylanase was similar to xylanases from *Nocardiopsis dassonvillei* [37], *Bacillus* sp. 41M-1 [38], *Bacillus* sp. TAR-1 [39], and *Bacillus* sp. NCIM 59 [40]. Thus, xylanase from *F. oxysporum* could be useful for biotechnological applications, especially in the treatment of paper pulp. The temperature optimum for the above xylanase was also in the range of those reported for xylanases from other mesophilic fungi and bacteria [8].

Determination of the substrate specificity, kinetic constants, and mode of action, using xylo- and MeUmb cello-oligosaccharides revealed that the xylanase from *F. oxysporum* has low levels of cellulase activity. Although, xylanases from fungi and bacteria do not hydrolyse either natural or derivatised cellulose [7,8,17], there are some xylanases which show low levels of cellulase activity [41–43] and appear to be similar to the xylanase purified from *F. oxysporum*. However, the binding studies using either Avicel or insoluble xylan indicated that xylanase from *F. oxysporum* does not possess either a xylan or a cellulose binding domain. Although, the presence of cellulose binding domain in cellulase [44], xylanase [22], arabinofuranosidase [22], and esterase [23] has been reported, its role in cellulolysis has not been well understood. In most fungi, the cellulose binding domain appears to be essential for the hydrolysis of crystalline cellulose [2]. However, the removal of cellulose binding domain from *Cellulomonas* cellulase reduced its catalytic activity, but only to a small extent [45]. A truncated xylanase from *Pseudomonas fluorescens* subsp. *cellulosa*, lacking its cellulose binding domain, exhibited identical catalytic properties as a full-length enzyme [46]. Also, the *Pseudomonas fluorescens* subsp. *cellulosa* xylanase B, arabinofuranosidase, and esterase which possess cellulose binding domain do not hydrolyse the crystalline cellulose [22,23]. These results strongly suggest that the ability of $\beta$-1,4-glycanases to hydrolyse crystalline cellulose does not infer the presence of cellulose binding domain or vice versa. Thus, the ability of purified xylanase from *F. oxysporum* to solubilize filter paper and Avicel appears to be an intrinsic property of this enzyme.

Like many xylanases [7,8,17,33], the purified xylanase hydrolysed xylotriose, xylotetraose, and xylopentaose. The presence of relatively high concentrations of xylobiose and xylotriose in xylotetraose, xylopentaose, and xylan reaction mixtures suggested that the enzyme preferentially cleaved the internal glycosidic bonds of these oligosaccharides. Also, the determination of mode of action using MeUmb cello-oligosaccharides, indicated that this xylanase is specific for the internal glycosidic bonds of higher

cello-oligosaccharides. Thus, the data presented in this paper strongly suggest that the major xylanase from *F. oxysporum* is an endoxylanase.

One of the interesting properties of this enzyme is that it hydrolysed oat spelts and birchwood xylans almost to the same extent (65–75%) at pH 7.0 and 9.0 in 24 h at 40 °C. This again suggests that the xylanase from *F. oxysporum* could be of commercial interest for the removal of residual xylan from paper pulp under alkaline conditions. However, it is not clearly understood why the reaction mixtures contained xylose:xylobiose:xylotriose at a ratio of 1:2:1 at pH 9.0, whereas at pH 7.0, the main products were xylose and xylobiose from oat spelts and birchwood xylans, respectively.

Another interesting observation was that the xylanase from *F. oxysporum*, in addition to hydrolysis, catalysed the transferase reaction with xylotriose, xylotetraose, and xylopentaose as donors and MeUmbGlc as an acceptor. Evidence for transferase reaction during the hydrolysis of xylo-oligosaccharides has been obtained with endoxylanase from *Aspergillus oryzae* [47], *Aspergillus niger* [48], *Cryptococcus albidus* [49], *Trichoderma longibrachiatum* [50], and *Trichoderma harzianum* [51]. Furthermore, β-glucosidase [52] and two endoglucanases [53,54] purified from *F. oxysporum* showed the ability to synthesize higher oligosaccharides by transglycosylation reaction. As transglycosylation is known to catalyse the synthesis of desired sugars on an industrial scale, the cellulases and xylanase characterised from *F. oxysporum* could be of commercial importance.

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