



Kirikyali, Narin and Wood, Jonathan and Connerton, Ian F. (2014) Characterisation of a recombinant  $\beta$ -xylosidase (xylA) from *Aspergillus oryzae* expressed in *Pichia pastoris*. *AMB Express*, 4 (68). pp. 1-7. ISSN 2191-0855

**Access from the University of Nottingham repository:**

<http://eprints.nottingham.ac.uk/28794/1/s13568-014-0068-1.pdf>

**Copyright and reuse:**

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the Creative Commons Attribution licence and may be reused according to the conditions of the licence. For more details see: <http://creativecommons.org/licenses/by/2.5/>

**A note on versions:**

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact [eprints@nottingham.ac.uk](mailto:eprints@nottingham.ac.uk)

ORIGINAL ARTICLE

Open Access

# Characterisation of a recombinant $\beta$ -xylosidase (xylA) from *Aspergillus oryzae* expressed in *Pichia pastoris*

Narin Kirikyali, Jonathan Wood and Ian F Connerton\*

## Abstract

$\beta$ -xylosidases catalyse the hydrolysis of short chain xylooligosaccharides from their non-reducing ends into xylose. In this study we report the heterologous expression of *Aspergillus oryzae*  $\beta$ -xylosidase (XylA) in *Pichia pastoris* under the control of the glyceraldehyde-3-phosphate dehydrogenase promoter. The recombinant enzyme was optimally active at 55°C and pH 4.5 with  $K_m$  and  $V_{max}$  values of 1.0 mM and 250  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  respectively against 4-nitrophenyl  $\beta$ -xylopyranoside. Xylose was a competitive inhibitor with a  $K_i$  of 2.72 mM, whereas fructose was an uncompetitive inhibitor reducing substrate binding affinity ( $K_m$ ) and conversion efficiency ( $V_{max}$ ). The enzyme was characterised to be an exo-cutting enzyme releasing xylose from the non-reducing ends of  $\beta$ -1,4 linked xylooligosaccharides ( $X_2$ ,  $X_3$  and  $X_4$ ). Catalytic conversion of  $X_2$ ,  $X_3$  and  $X_4$  decreased ( $V_{max}$  and  $k_{cat}$ ) with increasing chain length.

**Keywords:** *Aspergillus oryzae*; Xylose;  $\beta$ -xylosidase; Enzyme kinetics; Protein expression

## Introduction

Xylanolytic and cellulolytic enzymes encoded by filamentous fungi have been employed in several industrial applications for improving digestibility in animal feed, production of sweeteners, pharmaceuticals, additive chemicals for bio-fuel production and for the replacement of hazardous chemicals in textile and paper manufacture (Michelin et al. 2012). As a consequence it has been a necessity for enzymes involved in cellulose and hemicellulose hydrolysis to be individually identified and characterised in order to utilise them in the process of converting waste agricultural materials into valuable products with greater efficiency.

Hemicellulose is comprised of a linear main chain  $\beta$ -1,4 linked D-xylose backbone with short lateral side chains of different lower molecular weight sugar residues (Dyk and Pletschke 2012). Enzymatic hydrolysis of hemicellulose commences with the removal of side chains that block the sites where xylanases cleave the xylan backbone. Endo-1,4- $\beta$ -xylanase enzymes cleave the glycosidic bonds in a selective manner depending on the chain length, degree of branching of substrate molecules and the presence of

alternative carbohydrate moieties (Polizeli et al. 2005). Cleavage of the xylan backbone yields xylooligosaccharides and the final trimming is carried out by  $\beta$ -xylosidase, whereby short chain oligosaccharides and xylobiose are hydrolysed from the non-reducing termini to release xylose monomers (Polizeli et al. 2005; Teng et al. 2011).

Among xylanolytic enzymes, endo-xylanases and  $\beta$ -xylosidases have attracted attention as they commence and complete the breakdown of hemicellulose fraction respectively (Kulkarni et al. 1999). For comprehensive hydrolysis  $\beta$ -xylosidases play an important role in the removal of xylooligosaccharides from the catalytic environment, which assists by the elimination of the end-product inhibitors of endo-xylanases.

The gene encoding for XylA was previously identified by Kitamoto et al. (1999) and was reported to be responsible for the rapid browning of soy sauce. In addition Kitamoto et al. (1999) were interested in the antisense inhibition of XylA expression in order to hinder the translation in *Aspergillus oryzae* KBN616 to produce a mutant strain that could be used in Japanese food industry. However, XylA is a potentially efficient candidate for the facilitation of hydrolysis of hemicellulose applications in industrial processes. The work presented here reports the expression

\* Correspondence: [ian.connerton@nottingham.ac.uk](mailto:ian.connerton@nottingham.ac.uk)  
Division of Food Sciences, School of Biosciences, University of Nottingham,  
Sutton Bonington Campus, Loughborough LE12 5RD, UK

of a  $\beta$ -xylosidase from *Aspergillus oryzae* in *Pichia pastoris* and the kinetic characterisation of the recombinant enzyme.

## Materials and methods

### Construction of expression vector

The  $\beta$ -xylosidase encoding gene (*xylA*) was kindly provided by Noriyuki Kitamoto (Aichi Industrial Technology Institute, Japan). The gene sequence appears in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession number AB013851. The gene was sub-cloned into pCR<sup>+</sup>2.1 and subsequently into the *Pichia pastoris* expression vector pPpHis4\_GAP\_BglII (TU Graz) under control of the glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter.

### Transformation and expression of recombinant $\beta$ -xylosidase in *Pichia pastoris*

Plasmid DNA (10  $\mu$ g) containing the *xylA* gene sequence was linearized with *Bgl*II for integration at the GAP locus and was transformed into *P. pastoris* GS115 using Invitrogen *Pichia* Easycomp kit (as per manufacturers' guidelines). Positive transformants, displaying the His<sup>+</sup> phenotype on Regeneration Dextrose medium (RD) agar plates (1 M sorbitol, 2% dextrose, 1.34% yeast nitrogen base,  $4 \times 10^{-5}$ % biotin, 0.005% amino acids (L-glutamic acid, L-methionine, L-lysine, L-leucine, L-isoleucine) and 1.5% agar), before transfer onto RD plates containing 50  $\mu$ g ml<sup>-1</sup> bromo-4-chloro-3-indolyl  $\beta$ -D-xylopyranoside (X-xyl) (Sigma-Aldrich, UK) and incubated at 30°C for 2 days. Functional expression of the  $\beta$ -xylosidase under the control of GAP promoter was tested by cleavage of xylopyranoside from the synthetic indicator X-xyl.

A single colony displaying the highest level of blue precipitate was sub-cultured from the RD plate onto a YPD plate (1% yeast extract, 2% peptone, 2% glucose, 2% agar) and incubated at 30°C for 3 days. A seed culture was raised using a single colony inoculated into 50 ml YPD liquid medium, incubated at 28°C overnight. One hundred millilitres of YPD broth was then inoculated to 1 OD<sub>600nm</sub> and incubated for 72 h at 28°C. One ml of culture was removed every 24 h to test expression levels using the synthetic 4-nitrophenyl  $\beta$ -xylopyranoside (PNPX) as described in section 2.4. Following incubation the cells were harvested by centrifugation at 4000  $\times$  g for 10 min.

### Purification of recombinant $\beta$ -xylosidase

Following centrifugation the culture supernatant was concentrated using a Sartorius Sartoclon Slice then diafiltered with 10 volumes of Tris-salt buffer (10 mM Tris, 50 mM NaCl, pH 7.5). The concentrate was then stabilised using 30% (w/v) sucrose based on protein concentration and frozen for long term storage at -20°C. Prior

to enzymatic assays the 30% (w/v) sucrose was removed from the recombinant enzyme concentrates using a Vivaspin concentrator (GE Healthcare, UK) with a 10 kDa molecular weight cut off membrane filter and the filtrate was washed with Tris-salt buffer (10 mM Tris, 50 mM NaCl, pH 7.5).

### Enzyme assays using synthetic substrates

Assays for  $\beta$ -xylosidase activity were performed by measuring the pNP released from p-nitrophenyl glycoside synthetic substrates 4-nitrophenyl- $\beta$ -D-xylopyranoside (PNPX), 4-nitrophenyl- $\beta$ -D-glucopyranoside (PNPG) and 4-nitrophenyl- $\alpha$ -L-arabinofuranoside (PNPAf) in a final volume of 4 ml for 20 min in 50 mM sodium phosphate buffer pH 6.0 at 50°C. Reactions were terminated by the addition of 1 M Na<sub>2</sub>CO<sub>3</sub> and the amount of released pNP was measured at OD<sub>410nm</sub>. One unit (U) of  $\beta$ -xylosidase activity is defined as the amount of enzyme required to release 1  $\mu$ mol of pNP per min under assay conditions. Kinetic parameters ( $K_m$  and  $V_{max}$ ) were determined by the measurement of activity against pNPX using different substrate concentrations (0.5 - 12 mM) using the standard assay procedure. Enzyme assays were performed in triplicate and are presented as mean values with standard error.

### Enzyme assays using xylooligosaccharides

Activities against xylobiose, xylotriose and xylotetraose were determined at varying substrate concentrations (0.25 - 4 mg ml<sup>-1</sup>) in a final volume of 1 ml for 10 min in 50 mM sodium phosphate buffer pH 6.0 at 50°C. All assays were carried out in triplicate and were terminated by the addition of 1 M Na<sub>2</sub>CO<sub>3</sub>. Reaction products were separated according to molecular size by HPLC (Dionex ICS-3000 SP) with CarboPac<sup>TM</sup> PA20 column (3  $\times$  150 mm) and a gradient of 10 - 50 mM sodium hydroxide was applied for 20 min at a flow rate of 1 ml min<sup>-1</sup>. The products were quantified on the basis of standard peak areas from various concentrations of control xylose, xylobiose, xylotriose and xylotetraose solutions. Enzyme assays were performed in triplicate and are presented as mean values with standard deviations.

### Inhibition studies

To investigate the effect of end product xylose on catalytic activity, reactions were carried out in the presence of various xylose concentrations from 1 mM to 80 mM using synthetic substrate concentrations of either 1 mM or 4 mM pNPX. To further confirm the type of inhibition, kinetic constants were determined from experiments carried out using fixed inhibitor concentrations of 5 mM xylose at varying substrate concentrations from 0.25 mM to 8 mM under standard assay conditions.

The effects of monosaccharide sugars (20 mM glucose, mannose, galactose, arabinose, fructose and xylose), metal ions and chemicals (10 – 20 mM LiCl, KCl, ZnCl<sub>2</sub>, SDS, EDTA and DTT) on enzyme activity were tested using 50 mM sodium phosphate buffer pH 6, 1 mM pNPX and 2 µg of enzyme at 50°C for 10 min in a final volume of 4 ml and measured optical density of released p-nitrophenyl at OD<sub>410nm</sub>.

#### Determination of protein concentration

Protein concentrations were determined by the standard assay procedure using Pierce Coomassie® Plus Protein Assay Reagent. Sample diluents were used as the blank and the absorbance measured at OD<sub>595nm</sub>. All assays were performed in triplicate and the OD<sub>595nm</sub> readings of unknown sample were compared against BSA protein standard series which covered the range of concentrations between 50 and 1500 µg ml<sup>-1</sup>.

#### Determination of molecular mass by SDS-PAGE

SDS-PAGE was performed using 8% polyacrylamide gels according to the method described by Laemmli (1970). Protein bands were stained with colloidal Coomassie Blue. Bands from SDS-PAGE were excised and were subjected to trypsin digestion prior to mass spectrometry analysis.

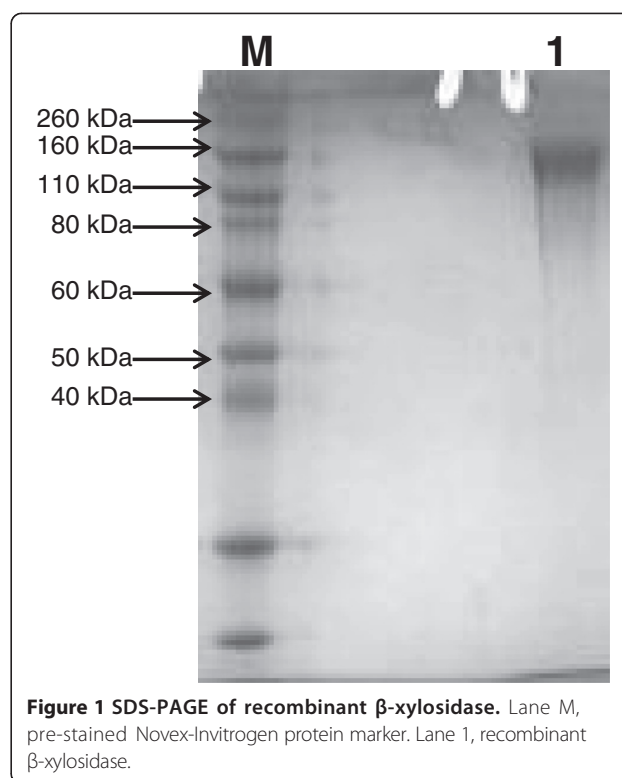
#### Mass spectrometry

Analyses of samples were carried out by LC-ESI-tandem MS on a Q-TOFII mass spectrometer fitted with a nano-flow ESI (electrospray ionization) source (Waters Ltd, UK). Peptides were separated on a PepMap C18 reverse phase, 75 µm i.d., 15-cm column (LC Packings) and delivered on-line to the MS via a CapLC HPLC system. Sequence interpretation for individual peptides was performed using the PepSeq MASCOT tool of the MassLynx™ 4.0 software package (Waters).

## Results

#### Characterisation of recombinant β-xylosidase

The β-xylosidase gene is contained within an open reading frame of 2397 nucleotides with no introns, which encodes a protein of 798 amino acids. A putative signal peptide was identified by SignalP software, thus the mature protein was predicted to be 778 amino acids with a molecular mass of 84.7 kDa. The recombinant enzyme was recovered from *Pichia pastoris* culture supernatant at approximately 100 mg L<sup>-1</sup>. NetNGly 1.0 predicted 12 potential N-glycosylation sites for β-xylosidase, and consistent with this, the recombinant enzyme was heterogeneous with a molecular mass estimated between 153 and 165 kDa on SDS-PAGE (Figure 1). The recombinant protein was excised from SDS-PAGE and the masses and protein sequences of tryptic peptides were determined using mass spectrometry to confirm the protein



**Figure 1** SDS-PAGE of recombinant β-xylosidase. Lane M, pre-stained Novex-Invitrogen protein marker. Lane 1, recombinant β-xylosidase.

product was exo-1,4 β-xylosidase originating from *Aspergillus oryzae*.

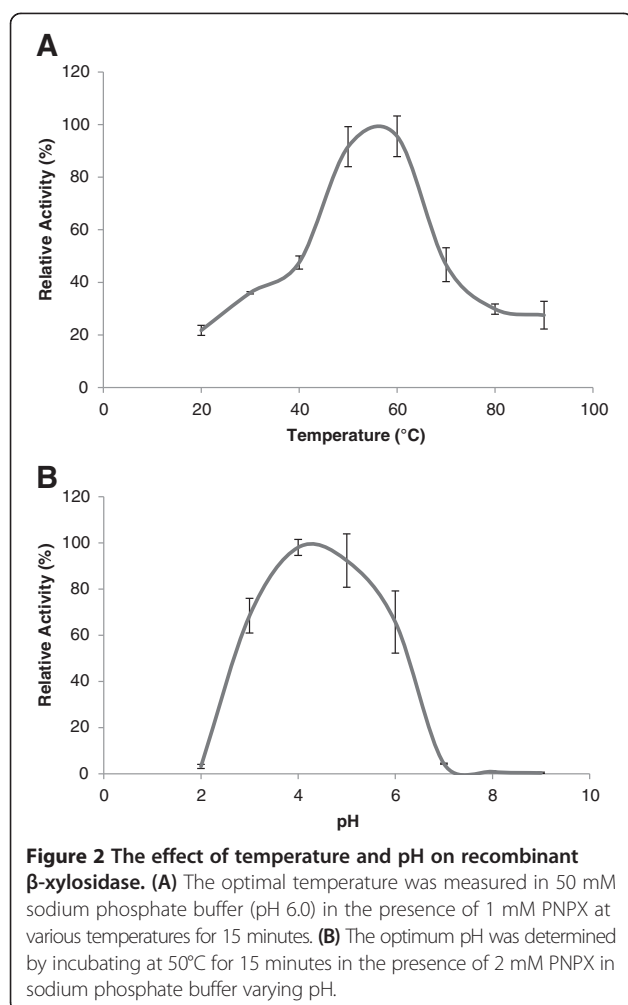
#### Determination of optimal conditions

The optimum temperature was determined by incubation of the recombinant enzyme in the presence of 50 mM sodium phosphate buffer pH 6.0 and 2 mM 4-nitrophenyl β-xylopyranoside (PNPX) for 15 min at varying temperatures between 20°C and 90°C. The enzyme displayed optimum activity at 55°C and retained more than 90% catalytic activity between the ranges of 50 – 60°C (Figure 2A).

The optimum pH of enzymatic activity was assayed in phosphate buffer system of varying pH values from 2–9 in the presence of 2 mM PNPX. The enzyme displayed activity within a narrow pH range, with an optimum of pH 4.5 and at least 65% activity from pH 3 – 6; less than 5% activity was observed at pH 7 – 9 (Figure 2B).

#### Substrate specificity and kinetic analysis

The substrate specificity of the recombinant enzyme was determined using various 4-nitrophenyl glycoside synthetic substrates and xylooligosaccharides. Recombinant XylA hydrolysed 4-nitrophenyl β-xylopyranoside efficiently but had trace hydrolytic activities against 4-nitrophenyl-β-D-glucopyranoside or 4-nitrophenyl-α-L-arabinofuranoside. Specific activities were determined as 150, 2 and 0.9 U mg<sup>-1</sup> for PNPX, PNPAf, PNPG respectively.



Kinetic constants for synthetic and natural substrates were determined using Hanes plots (Table 1). The enzyme exhibited  $K_m$  and  $V_{max}$  values of 1.0 mM and 250  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  respectively for the hydrolysis of 4-nitrophenyl  $\beta$ -xylopyranoside. Addition of the reaction product xylose resulted in enzyme inhibition. Kinetic constants were determined using Hanes plots from experiments carried out using a fixed inhibitor concentration of 20 mM xylose at varying PNPX concentrations (0.5 -

4 mM). The  $K_m$  was altered whilst the  $V_{max}$  remained unchanged, suggesting that xylose is a competitive inhibitor. A  $K_i$  of 2.7 mM for xylose was determined from Dixon plots. The addition of 20 mM fructose also had an inhibitory effect. Hanes plots indicated that the catalytic conversion of substrate into product was altered in terms of a reduction in the  $V_{max}$  and substrate affinity ( $K_m$ ), exhibiting the characteristics of uncompetitive inhibition.

The degradation of various xylooligosaccharides ( $X_2$ ,  $X_3$  and  $X_4$ ) by recombinant XylA was analysed by HPLC. Xylose was released from all substrates and the rate of xylose released decreased with increasing chain length of the xylooligosaccharide. Table 1 shows reductions in the catalytic conversion parameters  $V_{max}$  and  $k_{cat}$  with respect to the increasing chain length of the xylooligosaccharides. The relative affinity of XylA towards the natural substrate xylotetraose ( $X_4$ ) was significantly greater than xylobiose ( $X_2$ ) or xylotriose ( $X_3$ ) with respect to the  $K_m$  values.

#### Effect of carbohydrates on catalytic activity

The catalytic activity of recombinant  $\beta$ -xylosidase was measured using 1 mM pNPX as substrate in the presence of 20 mM sugar solutions to test whether the enzyme was inhibited or enhanced (Figure 3A). In the presence of 20 mM xylose and fructose the catalytic activity was reduced to 18% and 26% respectively, with none of the others (arabinose, mannose, galactose, glucose and sucrose) showing any change in catalytic activity.

#### Effect of metal ions and chemical compounds on enzyme activity

The effects of various metal ions and reagents on  $\beta$ -xylosidase activity were assayed at 10 mM and 20 mM concentrations (Figure 3B). Most notably the addition of  $\text{Zn}^{2+}$  (10 mM) enhanced enzyme activity by 80%. The detergent SDS at 20 mM reduced the catalytic activity by 40%.

#### Discussion

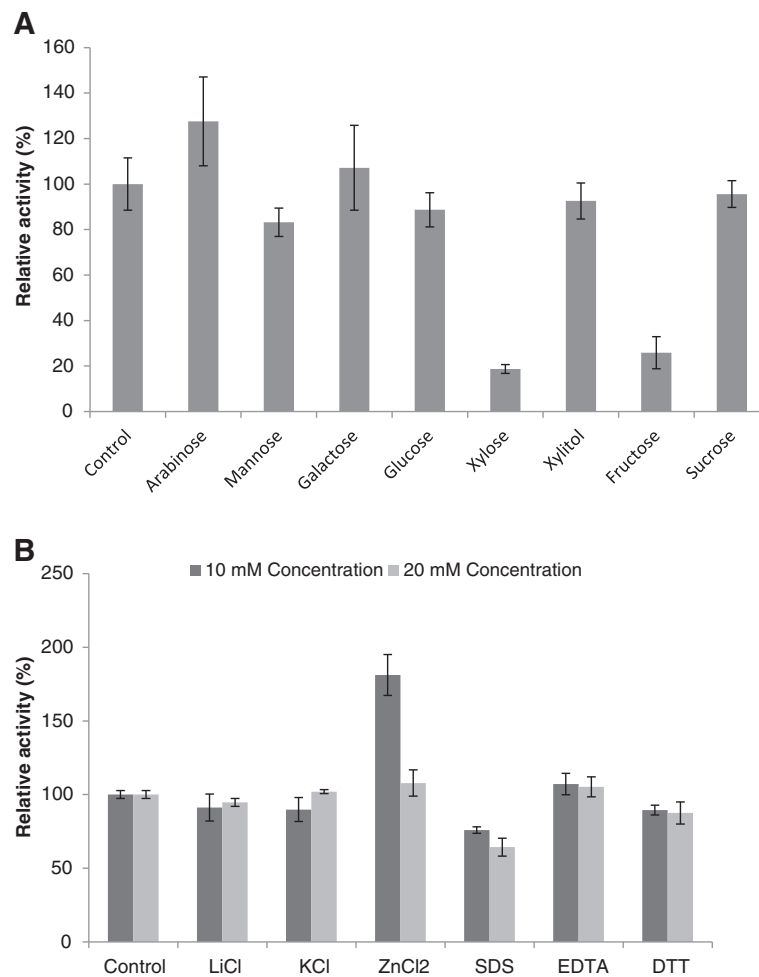
A  $\beta$ -xylosidase encoding gene (*xylA*) from *Aspergillus oryzae* KBN616 was expressed in a soluble, active form under control of the constitutive GAP promoter in

**Table 1** Kinetic analysis of synthetic and natural substrates determined in 50 mM sodium phosphate buffer (pH 6.0) at 50°C

Substrate	$K_m$ (mM)	$V_{max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	$k_{cat}$ ( $\text{sec}^{-1}$ )	$k_{cat}/K_m$ ( $\text{mM}^{-1} \text{s}^{-1}$ )
pNPX	1 $\pm$ 0.3	250 $\pm$ 0.001	352.7	352.7
pNPX + 20 mM Xylose	2.9 $\pm$ 0.5	250.5 $\pm$ 23	353.4	121.9
pNPX + 20 mM Fructose	0.1 $\pm$ 0.06	14.5 $\pm$ 3	20.6	206
Xylobiose ( $X_2$ )	2.6 $\pm$ 0.3	25.5 $\pm$ 0.1	36.0	13.8
Xylotriose ( $X_3$ )	3.07 $\pm$ 0.3	21.3 $\pm$ 0.3	30.1	9.7
Xylo-tetraose ( $X_4$ )	0.62 $\pm$ 0.4	14.5 $\pm$ 0.003	20.5	33.1

$\pm$  standard deviation of the means of triplicate data.





**Figure 3** Enzyme activity profiles in the presence of (A) 20 mM carbohydrates and (B) metal ions and chemical compounds within reaction mixtures. All reactions were performed in 50 mM sodium phosphate buffer (pH 6.0) and 2 mM PNPX at 50°C. Control reaction performed in the absence of carbohydrates and chemicals under identical conditions.

*Pichia pastoris*. The predicted presence of a native signal sequence (SignalP) was confirmed through secretion of the mature protein by the expression host. XylA was predicted to have a molecular mass of 86.4 kDa and 12 potential N-linked glycosylation sites. Kitamoto *et al.* (1999) previously identified purified the native enzyme from *A. oryzae* culture supernatant, in which the enzyme produced a single protein band with an apparent molecular mass of 110 kDa on SDS-PAGE. However the molecular mass range of XylA determined by SDS-PAGE from *P. pastoris* was 153 to 165 kDa indicating differences in post-translational modification consistent with the predicted glycosylation sites. Heterologous proteins expressed in *P. pastoris* are subject to glycosylation and several plant cell wall degrading enzymes expressed in *P. pastoris* are reported to be hyper-glycosylated, including  $\beta$ -xylosidase from *Paecilomyces thermophila* (Juturu and Wu 2013), cellobiose dehydrogenase from *Neurospora crassa* (Zhang

*et al.* 2011) and endo-xylanase from *Actinomadura* sp. S14 (Sriyapai *et al.* 2011).

The biochemical properties of the recombinant  $\beta$ -xylosidase closely match the native enzyme with respect to the observed optimal pH range (pH 4.5 – 5) and temperature (55°C) for enzyme activity. These are comparable to other fungal xylosidases (Saha 2003; La Grange *et al.* 2001; Wakiyama *et al.* 2008; Zanoelo *et al.* 2004; Rasmussen *et al.* 2006), which exhibit optimal activities between pH 3–5 at 60°C.

The recombinant enzyme was most active against p-nitrophenyl- $\beta$ -D-xylopyranoside (PNPX), with minimal activities towards 4-nitrophenyl- $\beta$ -D-glucopyranoside (PNPG) and 4-nitrophenyl- $\alpha$ -L-arabinofuranoside (PNPAf). Activity towards a broad range of synthetic substrates by other fungal  $\beta$ -xylosidases has been reported, although maximum activity is generally towards PNPX (Margolles-Clark *et al.* 1997; Ohta *et al.* 2010; Wakiyama *et al.* 2008;

Katapodis et al. 2006). The exception to this is *Aspergillus awamori* X-100  $\beta$ -xylosidase, which is reported to exhibit a greater  $k_{cat}$  against PNPAf (Eneyskaya et al. 2007). The recombinant enzyme exhibited kinetic constants for the hydrolysis of PNPX of 1.0 mM and 353  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  for  $K_m$  and  $V_{max}$  respectively. A range of kinetic constants have been reported for the hydrolysis of PNPX by fungal  $\beta$ -xylosidases but these values are similar to those reported for purified  $\beta$ -xylosidases from *Sporotrichum thermophile* (Katapodis et al. 2006) and *Fusarium proliferatum* (Saha 2003). The hydrolysis of various xylooligosaccharides ( $X_2$ ,  $X_3$  and  $X_4$ ) was monitored by HPLC. In the presence of individual xylooligosaccharides, xylose was detected as an initial product of catalysis indicating that the recombinant  $\beta$ -xylosidase is an exo-cutting enzyme. The  $k_{cat}$  values for the xylooligosaccharides decrease with increasing chain length in the order of  $X_2$  ( $36.0 \text{ sec}^{-1}$ ) >  $X_3$  ( $30.1 \text{ sec}^{-1}$ ) >  $X_4$  ( $20.5 \text{ sec}^{-1}$ ). However, the reduction in the  $K_m$  value for xylotetraose ( $X_4$ ) results in a greater catalytic efficiency towards this substrate. The observed changes in the kinetic constants ( $K_m$  and  $k_{cat}$ ) with respect to xylooligosaccharide chain length show similar patterns to those reported for the  $\beta$ -xylosidases originating from *Talaromyces emersonii*, *Trichoderma reesei* and *Aspergillus nidulans* (Rasmussen et al. 2006; Dilokpimol et al. 2011). In contrast *Neurospora crassa*  $\beta$ -xylosidase is reported to show a reduction in catalytic efficiency towards xylotetraose compared to shorter chain xylooligosaccharides (Kirikyali and Connerton, 2014). This is largely due to a relative reduction in the affinity of the *N. crassa* enzyme towards the xylotetraose substrate ( $>K_m$  value).

Similar to the data reported for the  $\beta$ -xylosidases from *Penicillium sclerotiorum* (Knob and Carmona 2009), *Talaromyces thermophilus* (Guerfali et al. 2009) and *Paecilomyces thermophila* (Yan et al. 2008) the presence of 20 mM concentrations of the metal ions  $\text{Li}^+$ ,  $\text{K}^+$  or  $\text{Zn}^{2+}$  had no effect on enzyme activity. The  $\beta$ -xylosidase of *Talaromyces thermophilus* has been reported to retain 44% activity in the presence of 10 mM of the detergent SDS (Guerfali et al. 2009), and similarly XylA retained catalytic activities of 75% and 60% respectively at 10 and 20 mM SDS.

Xylose has been determined to be a competitive inhibitor of recombinant *A. oryzae*  $\beta$ -xylosidase. In the presence of 20 mM xylose with varying substrate concentrations the  $K_m$  was altered with no corresponding effect on  $V_{max}$ . This is consistent with competitive inhibition in which the inhibitor interferes with the catalytic properties of enzyme by affecting substrate binding affinity by conferring a  $K_i$  of 2.7 mM. In this respect the recombinant enzyme displays similar characteristic to the  $\beta$ -xylosidases from *A. niger* ( $K_i$  2.9 mM) (Gomez et al. 2001) and *T. Reesei* ( $K_i$  2.4 mM) (Rasmussen et al. 2006). However, xylose tolerant  $\beta$ -xylosidases have been reported

to exist with  $K_i$  values up to 200 mM (Yan et al. 2008; Zanoelo et al. 2004). Fructose has the novel characteristics of an uncompetitive inhibitor in which the inhibitor interacts with the enzyme-substrate complex to prevent product formation. In this case the binding of xylooligosaccharide to the active site of  $\beta$ -xylosidase creates a binding site for fructose. High substrate concentrations of substrate will increase the occupancy of the active site and the binding sites for fructose, and therefore the effective inhibition. The functional and physiological consequences of this finding should be considered when the enzyme has to function in the presence of mixed substrates.

#### Competing interests

JW has become an employee of Biocatalysts Ltd since completion of the project. The authors declare no other competing interests.

#### Authors' contributions

NK carried out the biochemical studies and drafted the manuscript. JW carried out the protein expression studies and helped to draft the manuscript. IFC conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

#### Acknowledgements

This work has been supported by project funds from BBSRC and Biocatalysts Ltd. The authors would like to thank Noriyuki Kitamoto (Aichi Industrial Technology Institute, Japan) for kindly providing the XylA gene. We also would like to thank our technicians, Lorraine Gillet, Nicola Cummings for their advice and David Coles for his assistance with HPLC and Dr Susan Liddell for her assistance with Mass Spectrometry.

Received: 5 July 2014 Accepted: 7 August 2014

#### References

- Dilokpimol A, Nakai H, Gottfredsen CH, Appeldoorn M, Baumann MJ, Nakai N, Schols HA, Hachem MA, Svensson B (2011) Enzymatic synthesis of  $\beta$ -xylosyl-oligosaccharides by transxylosylation using two  $\beta$ -xylosidases of glycoside hydrolase family 3 from *Aspergillus nidulans* FGSC A4. *Carbohydr Res* 346:421–429
- Dyk JSV, Pletschke BI (2012) A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes – Factors affecting enzymes, conversion and synergy. *Biotechnol Adv* 30:1458–1480
- Eneyskaya EV, Ivanov DR, Bobrov KS, Isaeva-Ivanova LS, Shabalin KA, Savel'ev AN, Golubev AM, Kulminkaya AA (2007) Biochemical and kinetic analysis of the GH 3 family  $\beta$ -xylosidase from *Aspergillus awamori* X-100. *Arch Biochem Biophys* 457:225–234
- Gomez M, Isorna P, Rojo M, Estrada P (2001) Chemical mechanism of beta-xylosidase from *Trichoderma reesei* QM 9414: pH dependence of kinetic parameters. *Biochimie* 83:961–967
- Guerfali M, Maalej I, Gargouri A, Belghith H (2009) Catalytic properties of the immobilized *Talaromyces thermophilus*  $\beta$ -xylosidase and its use for xylooligosaccharides production. *J Mol Cat B* 57:242–249
- Juturu V, Wu JC (2013) Heterologous expression of  $\beta$ -xylosidase gene from *Paecilomyces thermophila* in *Pichia pastoris*. *World J Microbiol Biotechnol* 29:249–255
- Katapodis P, Nerinckx W, Claeysens M, Christakopoulos P (2006) Purification and characterization of a thermostable intracellular beta-xylosidase from the thermophilic fungus *Sporotrichum thermophile*. *Pro Biochem* 41:2402–2409
- Kirikyali N, Connerton IF (2014) Heterologous expression and kinetic characterisation of *Neurospora crassa*  $\beta$ -xylosidase in *Pichia pastoris*. *Enzyme Microb Technol* 57:63–68
- Kitamoto N, Yoshino S, Ohmiya K, Tsukagoshi N (1999) Sequence analysis, overexpression and antisense inhibition of a beta-xylosidase gene, *xylA*, from *Aspergillus oryzae* KBN616. *Appl Environ Microbiol* 65:20–24

- Knob A, Carmona EC (2009) Cell-associated acid  $\beta$ -xylosidase production by *Penicillium sclerotiorum*. *N Biotechnol* 26:60–67
- Kulkarni N, Shendye A, Rao M (1999) Molecular and biotechnological aspects of xylanases. *FEMS Microbiol Rev* 23:411–456
- La Grange DC, Pretorius IS, Claeysens M, Zyl WHV (2001) Degradation of xylan to D-xylose by recombinant *Saccharomyces cerevisiae* coexpressing the *Aspergillus niger*  $\beta$ -xylosidase (*xlnD*) and the *Trichoderma reesei* xylanase II (*xyn2*) genes. *Appl Environ Microbiol* 67:5512–5519
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
- Margolles-Clark E, Ihnen M, Penttila M (1997) Expression patterns of ten hemicellulase genes of the filamentous fungus *Trichoderma reesei* on various carbon sources. *J Biotechnol* 57:167–179
- Michelin M, Polizeli MLTM, Ruzene DS, Silva DP, Vicente AA, Jorge JA, Terenzi HF, Teixeira JA (2012) Xylanase and beta-xylosidase production by *Aspergillus ochraceus*: New perspectives for the application of wheat straw autohydrolysis liquor. *Appl Biochem Biotechnol* 166:336–347
- Ohta K, Fujimoto H, Fujii S, Wakiyama M (2010) Cell-associated beta-xylosidase from *Aureobasidium pullulans* ATCC 20524: Purification, properties and characterization of the encoding gene. *J Biosci Bioeng* 110:152–157
- Polizeli MLTM, Rizzatti ACS, Monti R, Terenzi HF, Jorge JA, Amorim DS (2005) Xylanases from fungi: properties and industrial applications. *Appl Microbiol Biotechnol* 67:577–591
- Rasmussen LE, Sorensen HR, Vind J, Vikso-Nielsen A (2006) Mode of action and properties of the  $\beta$ -xylosidase from *Talaromyces emersonii* and *Trichoderma reesei*. *Biotechnol Bioeng* 94:869–876
- Saha BC (2003) Purification and properties of an extracellular beta-xylosidase from a newly isolated *Fusarium proliferatum*. *Bioresour Technol* 90:33–38
- Sriyapai T, Somyoonsap P, Matsui K, Kawai F, Chansiri K (2011) Cloning of a thermostable xylanase from *Actinomyces* sp. S14 and its expression in *Escherichia coli* and *Pichia pastoris*. *J Biosci Bioeng* 111:528–536
- Teng C, Jia H, Yan Q, Zhou P, Jiang Z (2011) High-level expression of extracellular secretion of a  $\beta$ -xylosidase gene from *Paecilomyces thermophila* in *Escherichia coli*. *Bioresour Technol* 102:1822–1830
- Wakiyama M, Yoshihara K, Hayashi S, Ohta K (2008) Purification and properties of an extracellular  $\beta$ -xylosidase from *Aspergillus japonicus* and sequence analysis of the encoding gene. *J Biosci Bioeng* 106:398–404
- Yan QJ, Wang L, Jiang ZQ, Yang SQ, Zhu HF, Li LT (2008) A xylose tolerant  $\beta$ -xylosidase from *Paecilomyces thermophila*: Characterisation and its co-action with the endogenous xylanase. *Bioresour Technol* 99:5402–5410
- Zanoelo FF, Polizeli MLTM, Terenzi HF, Jorge JA (2004) Purification and biochemical properties of a thermostable xylose-tolerant  $\beta$ -xylosidase from *Scytalidium thermophilum*. *J Ind Microbiol Biotechnol* 31:170–176
- Zhang R, Fan Z, Kasuga T (2011) Expression of cellobiose dehydrogenase from *Neurospora crassa* in *Pichia pastoris* and its purification and characterisation. *Protein Expr Purif* 75:63–69

doi:10.1186/s13568-014-0068-1

**Cite this article as:** Kirikyali et al.: Characterisation of a recombinant  $\beta$ -xylosidase (*xylA*) from *Aspergillus oryzae* expressed in *Pichia pastoris*. *AMB Express* 2014 **4**:68.

Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Immediate publication on acceptance
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](http://springeropen.com)