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Characterization of β-Xylosidase and α-L-Arabinofuranosidase Activities From *Thermomonospora Fusca* BD25

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Abstract: *Thermomonospora fusca* BD25 produces relatively high levels of activity of β-xylosidase and α-L-arabinofuranosidase. The aim of the work described in this study was to characterize some properties of β-xylosidase and α-L-arabinofuranosidase produced by *T. fusca* BD25 when growing on oat spelt xylan as the main carbon and energy sources. The substrates *p*-NPX and *p*-NPA reacted with β-xylosidase and α-L-arabinofuranosidase with specific activities of 4.01 U mg^{-1} protein and 0.35 U mg^{-1} protein, respectively. The β-xylosidase remained stable at up to 65 °C, but α-L-arabinofuranosidase lost 10 % of its maximum activity at 55 °C. β-xylosidase and α-L-arabinofuranosidase activities remained at 86 % and 83 % of their maximum activities after 9 h of incubation at 50 °C. The maximum relative β-xylosidase activity occurred (0.82 U mg^{-1} protein) at pH 8.0 with a 50 % decrease of maximum relative activity occurring at pH 4.5 and 10. α-L-arabinofuranosidase exhibited maximum relative activity (0.136 U mg^{-1} protein) at pH 9.0 with 54 % and 55 % activities remaining at pH of 4.5 and 11, respectively. The apparent K_m values for the crude β-xylosidase and α-L-arabinofuranosidase preparations were 0.5 mM of *p*-NPX and 0.18 mM of *p*-NPA, while the V_{max} values were 0.83 μmol *p*-nitrophenol ml^{-1} min^{-1} and 0.04 μmol *p*-nitrophenol ml^{-1} min^{-1}, respectively. The addition of D-xylose (10 mM) to the reaction mixture caused a 10 % reduction in β-xylosidase activity as the end-product inhibitor. However, a 15 % reduction in α-L-arabinofuranosidase activity was detected when L-arabinose (10 mM) was added to the reaction mixture.

Key Words: β-xylosidase, α-L-arabinofuranosidase, lignocellulose degradation, *Thermomonospora fusca,* actinomycete.

Thermomonospora fusca BD25'ten Elde Edilen β-Ksilozidaz ve α-L-Arabinofuranozidaz Aktivitelerinin Karakterizasyonları

Özet: Termofilik bir aktinomiset olan *Thermomonospora fusca* BD25, diğer organizmalara oranla daha yüksek seviyede β-ksilozidaz ve α-L-arabinofuranozidaz enzimleri üretmektedir. Bu çalışmada, karbon ve enerji kaynağı olarak yulaf ksileni kullanılan kültür ortamında *T. fusca* tarafından üretilen β-ksilozidaz ve α-L-arabinofuranozidaz enzimlerinin bazı karakteristik özelliklerinin belirlenmesi amaçlanmıştır. β-ksilozidaz ve α-L-arabinofuranozidaz enzimlerinin spesifik aktivitelerini belirlemek amacı ile yapılan çalışmalarda, substrat olarak sırası ile *p*-NPX ve *p*-NPA kullanılmıştır. β-ksilozidaz enziminin spesifik aktivitesi 4,01 U mg^{-1} protein, α-L-arabinofuranozidaz enziminin spesifik aktivitesi ise 0,35 U mg^{-1} protein olarak saptanmıştır. β-ksilozidaz ve α-L-arabinofuranozidaz enzimleri için optimum sıcaklıklar ise sırası ile 65 °C ve 55 °C olarak tesbit edilmiştir. β-ksilozidaz ve α-L-arabinofuranozidaz enzimleri (substrat yokluğunda ve pH 7'de) 50 °C'ta 9 saat süre ile inkübe edildikten sonra sırası ile aktivitelerinin % 86 ve % 83 ünü korudukları saptanmıştır. β-ksilozidaz pH 4,5 ile 10 arasında ise, pH 8'de göstermiş olduğu maksimum aktivitenin % 50 sini göstermiştir. α-L-arabinofuranozidaz ise pH 4,5 ile 11 arasında, pH 9'de göstermiş olduğu maksimum aktivitenin % 54-

55 ini göstermiştir. β-ksilozidaz ve α-L-arabinofuranozidaz için substrat olarak sırası ile *p*-NPX ve *p*-NPA kullanıldığında görünür K_m değerleri 0,5 mM ve 0,18 mM olarak tesbit edilirken, V_{max} değerleri 0,83 μmol ml^{-1} dak^{-1} ve 0,04 μmol ml^{-1} dak^{-1} olarak tesbit edilmiştir. Son ürün inhibitörü olarak D-ksilozun (10 mM) β-ksilozidaz enziminin reaksiyon ortamına ilavesi ise enzimin aktivitesinde yaklaşık olarak % 10 azalmaya neden olduğu saptanırken α-L-arabinofuranozidazın L-arabinoz (10 mM) tarafından %15 oranında inhibe edildiği saptanmıştır.

Anahtar Sözcükler: β-ksilozidaz, α-L-arabinofuranozidaz, lignosellüloz, *Thermomonospora fusca*, ksilen, aktinomiset.

Abbreviations: PAGE, polyacrylamide gel electrophoresis; RBB-xylan, Ramazol Brilliant Blue-Xylan; CMC, Carboxymethylcellulose; P-NPX, p-nitrophenyl-β-D-xylopyranoside; p-NPA, p-nitrophenyl-α-L-arabinofuranoside; p-NPG, p-nitrophenyl-β-D-glucopyranoside; SDS, sodium dodecyl sulphate; EDTA, ethylenediaminetetraacetic acid.

Introduction

Plant cell walls, the major reservoir of fixed carbon in nature, have three major polymeric constituents: cellulose, hemicellulose and lignin (1). The amount of xylan, which is the predominant hemicellulose, varies in different plants, from as much as 35 % of the dry weight of birchwood to as little as 7 % in some gymnosperm (2). Besides terrestrial plants, in which xylans are based on a β-1,4-linked D-xylosyl backbone, marine algae synthesize xylans of different chemical structure, based on a β-1,3-linked D-xylosyl backbone (3). Those containing a mixture of β-1,3 and β-1,4 linkages are found in seaweeds such as *Rhodymenia palmata* (4, 5). In some species of the *Chlorophecae* and the *Rhodophceae,* where cellulose is absent, xylans form a highly crystalline fibrillar material (6).

Xylan is the most common backbone structure found in hemicellulose. However, the hemicellulose structure also has branched hetero-polysaccharides, which require a more complex battery of enzymes to achieve hydrolysis. A concerted action of enzymes which randomly cleave inter-monomeric bonds (endo-enzymes), enzymes which remove monomers from the end of the chain (exo-enzymes), and enzymes which hydrolyse dimers is needed. These enzymes include endo-1,4-β-xylanase (1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8), β-xylosidase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37), α-glucuronidase, α-L-arabinofuranosidase (EC 3.2.1.55) and acetyl xylan esterase. A concerted action of hemicellulolytic enzymes is needed in order to achieve a complete degradation of branched acetyl xylan to carbohydrate monomers (7-9).

The ability to degrade lignocellulose is widespread among different types of microorganisms, including actinomycetes. Actinomycetes generally grow as branching hyphae, well adapted to the penetration and degradation of insoluble substrates such as lignocellulose. Within this group of bacteria the thermomonosporas are of particular interest as they produce multiple thermostable enzymes involved in the degradation of lignocellulose (10-14).

Xylan-degrading enzymes are produced by a wide range of microorganisms. However, there is only limited information available on the production of these, except for endoxylanase enzymes, which contribute to the complete saccharification of hemicellulose. The aim of the work described in this study was to characterize some properties of β-xylosidase and α-L-arabinofuranosidase activities produced by *T. fusca* BD25 when growing on oat spelt xylan as the main carbon and energy sources.

Materials and Methods

Growth and maintenance of *Thermomonospora fusca* BD25

The growth and maintenance conditions of the bacterial strain have been described in previous studies (13, 15).

Biomass measurement and protein estimation

Bacterial growth and protein estimation have been described in a previous study (13).

Enzyme assays

β-xylosidase and α-L-arabinofuranosidase activities were assayed as described by Bachman & McCarthy (16) and MacKenzie *et al.*(17) respectively. The substrates used were *p*-nitrophenyl-β-D-xylopyranoside (*p*-NPX, 5 mM) and *p*-nitrophenyl-α-L-arabinofuranoside (*p*-NPA, 2 mM) in 100 mM phosphate buffer, pH 6.5, at 50 °C, respectively. One unit of enzymatic activity was defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per minute.

Purification of β-xylosidase and α-L-arabinofuranosidase

Gel filtration was carried out using a 2.5 id x 70 cm column, which was packed with Sepharose 6B column material according to the manufacturer's instructions. The crude enzyme preparations were loaded onto the column. The samples were eluted using potassium phosphate buffer (pH 7.0) at a flow rate of 20 ml h^{-1}. Positive fractions for enzyme activity were pooled and used for anion exchange chromatography.

Anion exchange chromatography was carried out using Diethylaminoethyl-Sepharose (DEAE-Sepharose) fast flow column material. The material was packed into a Pharmacia-XK50 column (5.0 id x 5.0 cm) according to the manufacturer's instructions. The pooled active fractions of gel filtration were loaded onto an anion exchange column and eluted with a NaCl gradient (0 to 1 M) in Tris-HCl buffer (50 mM, pH 8.5) (at a flow rate of 0.5 ml min^{-1}). Fractions showing enzyme activity were concentrated by disposable concentration units and the salt content of the sample was removed by washing with sample buffer. Enzyme activities were assayed at each step by the standard assay method.

Temperature studies of β-xylosidase and α-L-arabinofuranosidase

The effect of temperature on the stability of the β-xylosidase and α-L-arabinofuranosidase produced by *T. fusca* BD25 was studied by incubating the enzyme for 15 min in the absence of the substrates over a range of temperatures (50 °C to 90 °C). Activities were measured at 50 °C using the standard enzyme assay methods. Similarly, the effect of time and temperature on the stability of the enzymes was determined by incubating the enzyme in the absence of substrate over a period of 27 h at various temperatures in the range of 50 °C to 80 °C at 7.0. The pH was adjusted with 1 M NaOH or HCl at specified reaction temperatures. Again the activity was measured at 50 °C using the standard assay methods. Finally the effect of temperature on the activity was monitored by measuring the activity using the appropriate substrates at specific temperatures ranging from 50 °C to 90 °C.

pH studies of β-xylosidase and α-L-arabinofuranosidase

The activities of β-xylosidase and α-L-arabinofuranosidase over a pH range of 3.0-12.0 were investigated using the standard assay methods. However, the potassium phosphate buffer was replaced with Britton-Robinson Universal Buffer (18), which contained 6.008 g l^{-1} citric acid; 1.769 g l^{-1} boric acid; 3.839 g l^{-1} KH_2PO_4 and 5.266 g l^{-1} diethylbarbituric acid. The pH was adjusted with 1 M NaOH or HCl. Aliquots (50 µl) of enzyme preparations were mixed with Universal Buffer (950 µl) of each pH value. These samples were then left to equilibrate for 30 minutes, then the pH was checked. Finally, enzyme activities were assayed by standard assay methods. The activities were recorded as a percentage of the maximum activity.

Kinetic and inhibition studies of β-xylosidase and α-L-arabinofuranosidase

i- Determination of K_m and V_{max}

The kinetics of crude β-xylosidase and α-L-arabinofuranosidase preparations have been determined by previously reported methods (19, 20). β-xylosidase and α-L-arabinofuranosidase assays were carried out at 50 °C, at pH 6.5. K_m values for β-xylosidase and α-L-arabinofuranosidase were calculated by using the substrates *p*-NPX and *p*-NPA at concentrations of 0-5.0 and 0-2.5 mM, respectively.

ii- Inhibitions of β-xylosidase and α-L-arabinofuranosidase

The effect of end-product inhibition on β-xylosidase and α-L-arabinofuranosidase by xylan-hydrolysis products was determined by addition of D-xylose, D-glucose, L-arabinose, xylobiose and cellobiose (0-30 mM) to the reaction mixture.

Inhibition by metal ions and reagents such as boric acid, citric acid, mercury, nickel and ethylenediaminetetraacetic acid (EDTA) was investigated by incorporating appropriate salts (1 mM) in the assay mixture.

Polyacrylamide gel electrophoresis (PAGE)

Concentrated crude supernatant and cell extract of *T. fusca* BD25, grown on oat spelt xylan for 72 h, were separated by non-denaturing discontinuous polyacrylamide gel electrophoresis. Gels were constructed according to the method of Laemmli (21) using 10 % (w/v) acrylamide. Electrophoresis was carried out at 80 V in double strength running buffer (pH 8.8) which contained 0.303 % (w/v) Tris and 1.44 % (w/v) glycine. Gels were run in an Atto model AE-6450 vertical electrophoresis tank (Atto, Japan). Electrophoresis was stopped when the dye-front marker had run just off the bottom of the gel. Bands of β-xylosidase and α-L-arabinofuranosidase activities were detected by the replica gel technique with *p*-NPX and *p*-NPA respectively, as described by Bachmann and McCarthy (16).

Results

Substrate specificity of β-xylosidase and α-L-arabinofuranosidase

The substrate specificity of partially purified β-xylosidase (23.78 fold, 26.85 U mg^{-1}) and α-L-arabinofuranosidase (15.94 fold, 20.95 U mg^{-1}) enzymes and crude enzyme preparations

were studied using colorimetric assays. Compounds known to be hydrolysed by xylan-degrading enzymes from different sources, which are related to lignocellulose, such as oat spelt xylan, birchwood xylan, arabinogalactan, cellulose, carboxymethylcellulose (CMC) and synthetic substrate compounds of *p*-nitrophenol- attached to sugar units were used as substrates. Negative reactions were obtained against some of the substrates such as cellulose, arabinagalactan and Ramazol Brilliant Blue-xylan (RBB-xylan) by β-xylosidase and α-L-arabinofuranosidase of *T. fusca* BD25. Only *p*-NPX and *p*-NPA reacted with these crude enzymes, with specific activities of 4.01 U mg^{-1} protein and 0.35 U mg^{-1} protein, respectively (see Table 1). β-xylosidase and α-L-arabinofuranosidase activities of *T. fusca* BD25, which were separated by non-denaturing PAGE, also reacted when *p*-NPX and *p*-NPA were used as substrates respectively, but not RBB-xylan, CMC and other *p*-nitrophenol compounds (see Figure 1, lanes 1, 2 and 3, 4, respectively).

Partially purified β-xylosidase and α-L-arabinofuranosidase did not exhibit detectable endoxylanase, endoglucanase and β-glucosidase activities. However, very low levels of cross-

Table 1. The activities of the partly purified β-xylosidase and α-L-arabinofuranosidase from *T. fusca* BD25 against various substrates.

Substrate	β-Xylosidase Activities	α-L-Arabinofuranosidase Activities
Oat spelt xylan[1]	–	–
Birchwood xylan[1]	–	–
Arabinogalactan[1]	–	–
RBB-Xylan[1]	–	–
CMC[1]	–	–
Cellulose[1]	–	–
p-NP-β-D-glucopyranoside[2]	–	–
p-NP-α-D-glucopyranoside[2]	–	–
p-NP-β-D-mannopyranoside[2]	–	–
p-NP-α-D-mannopyranoside[2]	–	–
p-NP-β-D-galactopyranoside[2]	–	–
p-NP-α-D-galactopyranoside[2]	–	–
p-NP-α-L-fucopyranoside[2]	–	–
p-NP-β-D-fucopyranoside[2]	–	–
p-NP-β-L-fucopyranoside[2]	–	–
p-NP-α-L-arabinopyranoside[2]	–	–
p-NP-α-L-arabinofuranoside[2]	–	+
p-NP-β-D-xylopyranoside[2]	+	–

[1]10 mg ml^{-1}

[2]3 mM.

activities were detected after prolonged incubation (2 h) by β-xylosidase and α-L-arabinofuranosidase enzyme preparations.

Thermostability and the effect of temperature on the activity of the β-xylosidase and α-L-arabinofuranosidase

In this study, crude β-xylosidase and α-L-arabinofuranosidase activities of *T. fusca* BD25 were used to assess temperature and pH effects on the enzymes. Thermal inactivation of β-xylosidase and α-L-arabinofuranosidase was studied by pre-incubating the enzyme preparations for a fixed period of time in the absence of substrates at a number of temperatures, then by cooling and carrying out the standard assay procedures described in materials and methods. The β-xylosidase remained stable at up to 65 °C, but α-L-arabinofuranosidase lost 10 % of its maximum activity at 55 °C (see Figure 2.a).

The effect of temperature on the β-xylosidase and α-L-arabinofuranosidase activities were monitored by measuring the activities using the standard assay methods at specific temperatures ranging from 50 °C to 90°C. The optimum activities occurred at 70 °C for β-xylosidase and at 65 °C for α-L-arabinofuranosidase (see Figure 2.b). The relative β-xylosidase activities in the temperature range of 50 °C to 70 °C remained between 90 and 100 % of 100 % activity at 70 °C, but the relative β-xylosidase activities decreased to 46 % at 90 °C. In the temperature range of 50-65 °C, α-L-arabinofuranosidase exhibited 45-100 % of its maximum activity, and exhibited 33 % of maximum activity at 75 °C.

Finally, the effect of temperature on the stability of the β-xylosidase and α-L-arabinofuranosidase were determined by incubating the enzyme in the absence of substrates over a period of 27 hours within a temperature range of 50 °C to 80 °C.

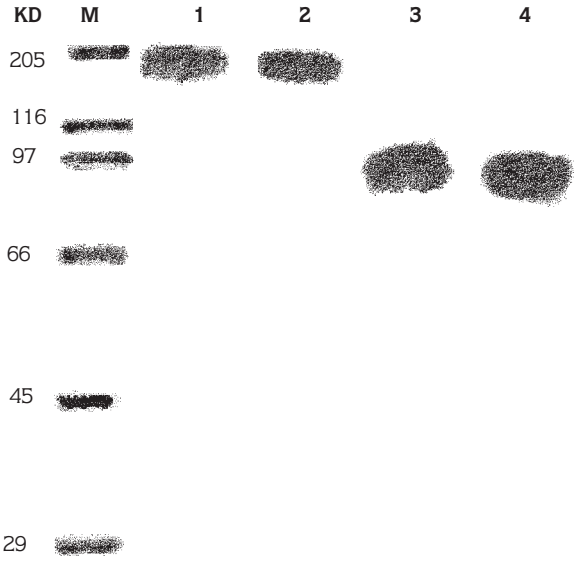

Figure 1. Zymograms of *T. fusca* BD25 β-xylosidase and α-L-arabinofuranosidase separated by PAGE and developed as zymograms. Lines: Crude cell extract (lane 1) and purified (lane 2) β-xylosidase and crude (lane 3) and purified (lane 4) α-L-arabinofuranosidase separated by PAGE. M: molecular mass markers.

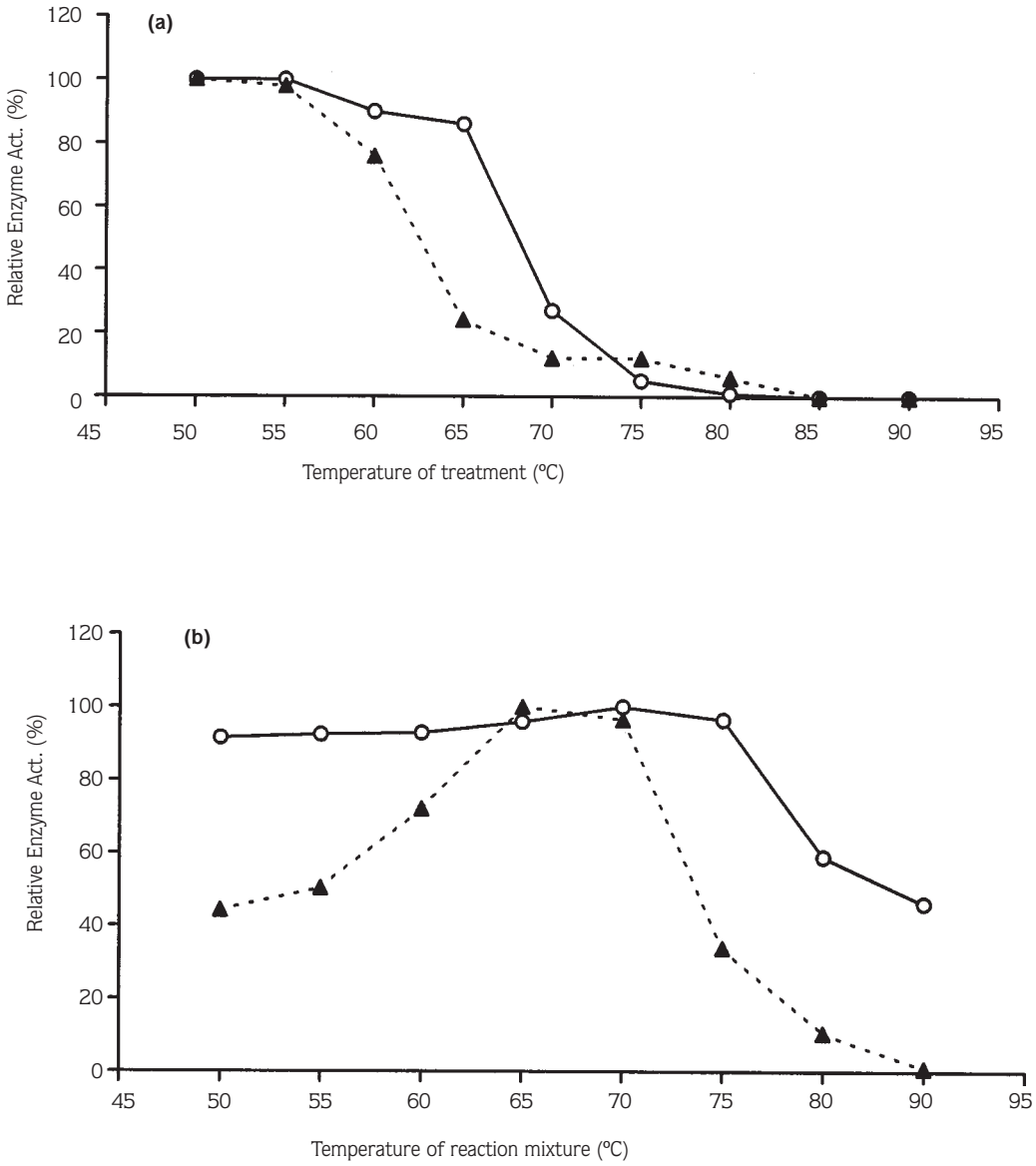

Figure 2. (a) The effect of temperature on the stability of β-xylosidase (-o-) and α-L-arabinofuranosidase (-▲-). The enzyme supernatants were incubated for 15 min at each temperature prior to the enzyme assays using the standard assay methods at 50 °C.
(b) The effect of temperature on the activity of β-xylosidase and α-L-arabinofuranosidase. Data are presented as means of three replicates. Standard deviations were ≤ 5 % in all cases. The enzyme activities are expressed relative to the maximal values (0.307 U mg^{-1} and 0.035 U mg^{-1} protein, respectively).

From the results (see Figure 3), it may be seen that the β-xylosidase and α-L-arabinofuranosidase activities remained at 86 % and 83 % of their maximum activities after 9 h of incubation at 50 °C. However, β-xylosidase and α-L-arabinofuranosidase showed a half life of 1.5 h and 1 h at 75 °C at pH 7.0, respectively (see Figure 3).

pH studies of β-xylosidase and α-L-arabinofuranosidase

The activity of the β-xylosidase and α-L-arabinofuranosidase over a pH range of 3-12 was investigated using the standard assay methods. The effect of pH on β-xylosidase and α-L-arabinofuranosidase activity is shown in Figure 4. The pH profile shows the maximum relative β-xylosidase activity occurring (0.82 U mg^{-1} protein) at pH 8.0 with a 50 % decrease of maximum relative activity occurring at pH 4.5 and 10.0. α-L-arabinofuranosidase exhibited maximum relative activity (0.136 U mg^{-1} protein) at a pH 9.0 with 54 % and 55 % activities remaining at pH of 4.5 and 11.0 respectively (Figure 4).

Kinetic studies of β-xylosidase and α-L-arabinofuranosidase

The affinities of *T. fusca* BD25 β-xylosidase and α-L-arabinofuranosidase for the substrates *p*-NPX and *p*-NPA respectively were examined using Lineweaver-Burk plots. The apparent *Km* values for the crude β-xylosidase and α-L-arabinofuranosidase preparations were 0.5 mM of *p*-NPX and 0.18 mM of *p*-NPA, while the V_{max} values were 0.83 μmol *p*-nitrophenol ml^{-1} min^{-1} and 0.04 μmol *p*-nitrophenol ml^{-1} min^{-1}, respectively (see Figure 5).

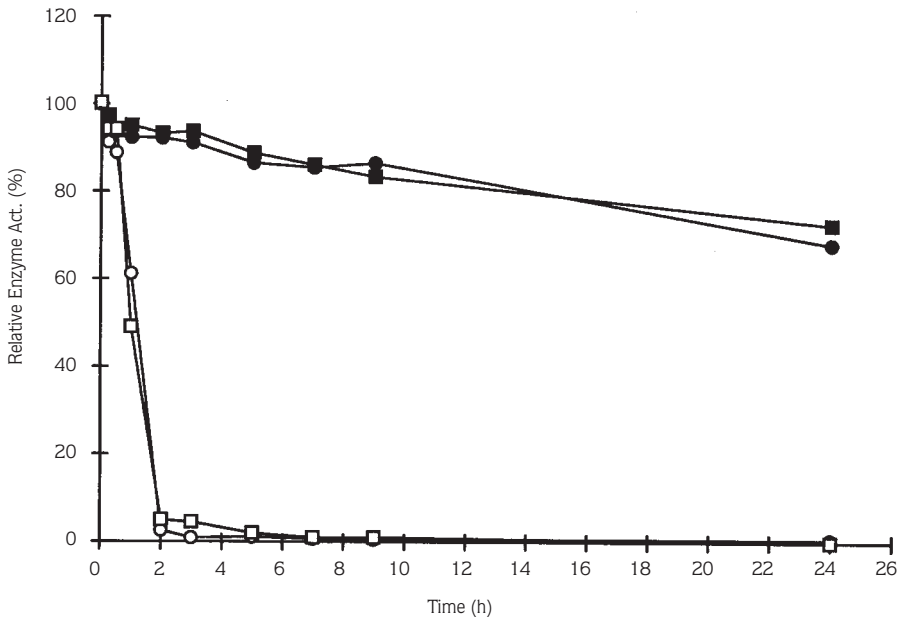

Figure 3. The effect of temperature on the stabilities of β-xylosidase [at 50°C, (-●-); 75 °C, (-o-)] and α-L-arabinofuranosidase [at 50 °C (-■-); 75 °C (-❑-)] at pH 7.0.

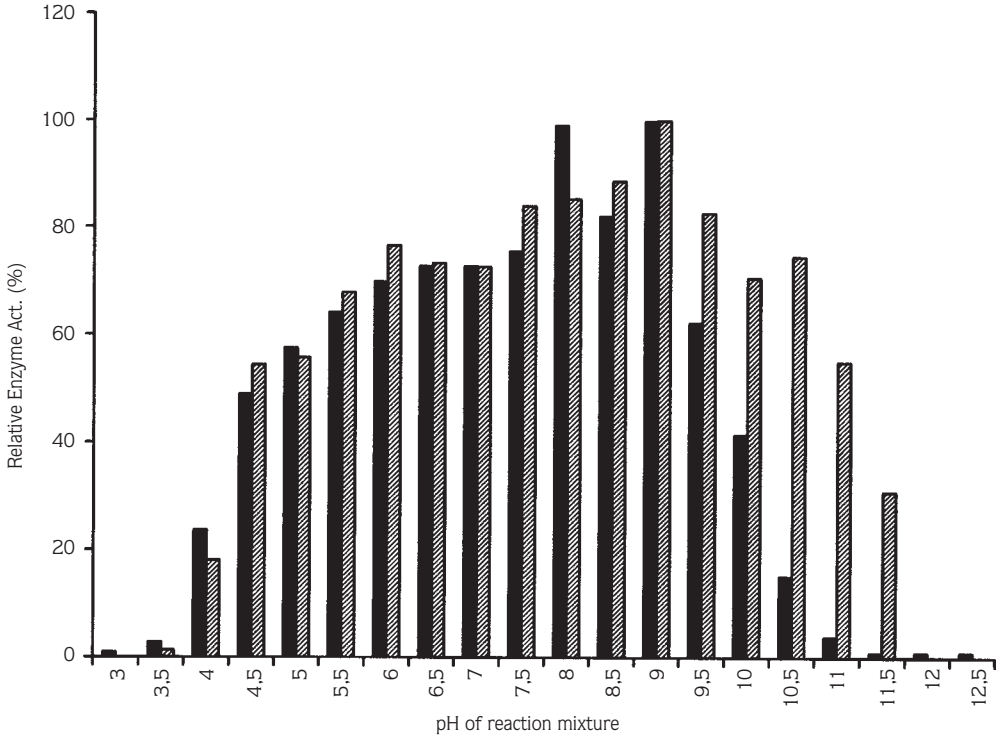

Figure 4. The effect of pH on the activities of β-xylosidase (■) and α-L-arabinofuranosidase (▨). The reactions were monitored at each of the specified pH values using the Universal buffer ranging from 3.0 to 12.0. The enzyme activities are expressed relative to the maximal value (0.82 U mg^{-1} and 0.136 U mg^{-1}, respectively).

The inhibition effects of D-xylose, D-glucose, L-arabinose, xylobiose and cellobiose on the activity of β-xylosidase and α-L-arabinofuranosidase as hydrolysis end-products of lignocellulose were also determined by adding the appropriate concentration (0-30 mM) of sugars to the reaction mixture. Both of the enzyme activities were affected by the different concentrations of sugars. β-xylosidase activity was not inhibited by the addition of D-glucose or L-arabinose (0-30 mM), but a 10 % reduction in β-xylosidase activity was detected with the addition of D-xylose (10 mM). However, a 15 % reduction in α-L-arabinofuranosidase activity was detected when L-arabinose (10 mM) was added to the reaction mixture.

The effect of different metal ions and chemical agents on the activity of β-xylosidase and α-L-arabinofuranosidase was determined by adding the appropriate salts (1 mM in the final reaction mixture) to the reaction mixture. The enzymes retained full activity in the presence of boric acid, citric acid, urea, sodium dodecyl sulphate (SDS), EDTA, Zn^{2+} and Mg^{2+}. β-xylosidase and α-L-arabinofuranosidase activities were completely inhibited by $HgCl_2$ (1 mM).

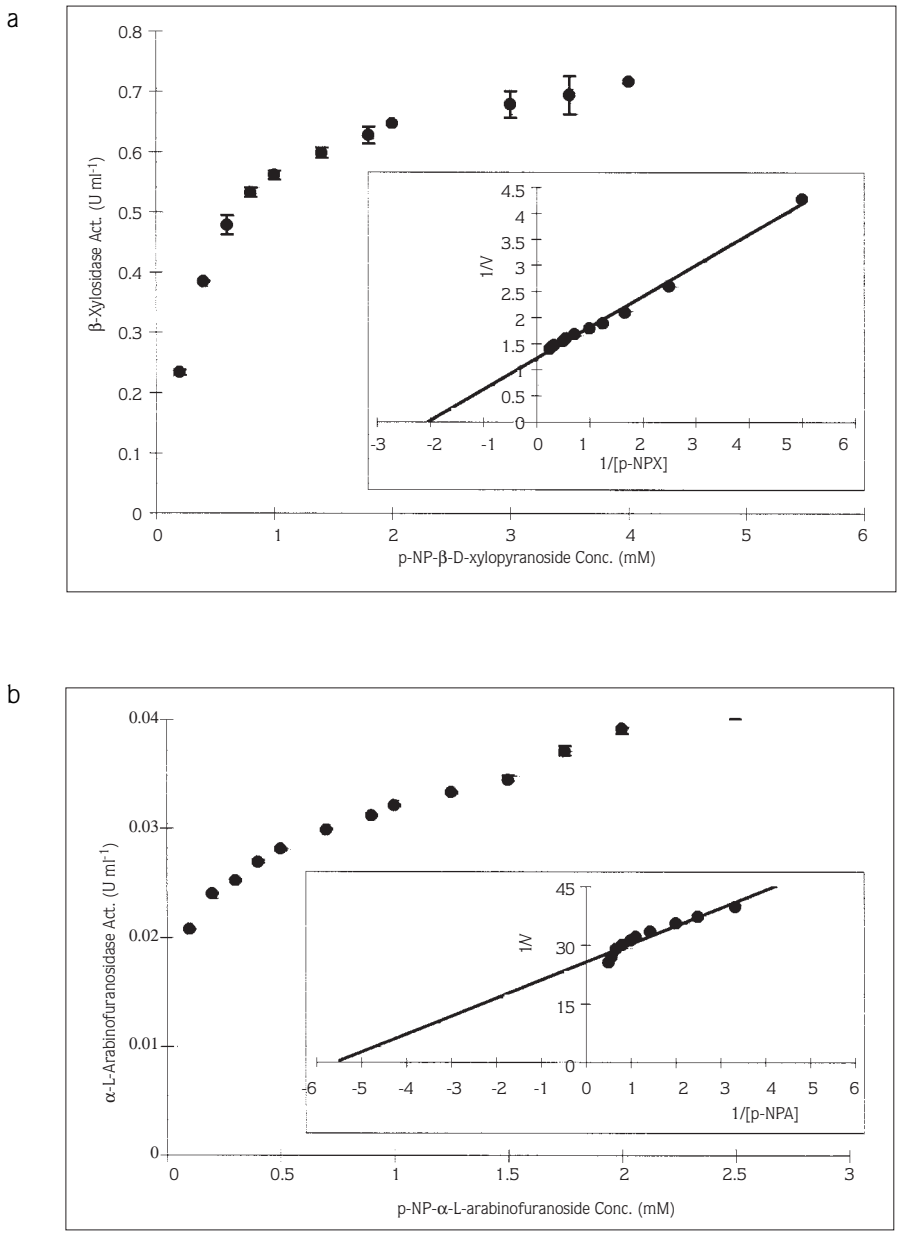

Figure 5. The effect of substrate concentrations on the activities of β-xylosidase (a) and α-L-arabinofuranosidase (b). The reactions were monitored at 50 °C and at pH 7.0 (100 mM phosphate buffer). Also shown are the Lineweaver-Burk plots. The data are presented as means ± SEM for triplicate measurements.

Discussion

Substrate specificity of β-xylosidase and α-L-arabinofuranosidase

The purpose of this work was to investigate the β-xylosidase and α-L-arabinofuranosidase activities produced by *T. fusca* BD25. A crude extracellular supernatant, such as would be used industrially was used for the determination of substrate specificity, activity conditions, reaction rates, stability and inhibitory studies.

The activities of crude and partially purified β-xylosidase and α-L-arabinofuranosidase enzymes were tested on different substrates. The partially purified β-xylosidase and α-L-arabinofuranosidase of *T. fusca* BD25 did not exhibit detectable endoxylanase, endoglucanase and β-glucosidase activities against oat spelt xylan, CMC and *p*-nitrophenyl-β-D-glucopyranoside (*p*-NPG), respectively. However, very low levels of cross activity were detected after prolonged incubation (2 h) by β-xylosidase and α-L-arabinofuranosidase enzyme preparations. These were probably due to the presence of small amounts of cross-contaminating activities of responsible enzymes, since these activities eluted from the anion-exchange column very closely (14). Relatively broad substrate specificities are common amongst enzymes which are involved in the hydrolysis of cellulosic substrates, and fungal β-xylosidases with additional α-L-arabinofuranosidase activity (22) or β-glucosidase activity (23) have been reported.

Thermostability and the effects of temperature and pH on the activity of the β-xylosidase and α-L-arabinofuranosidase from *T. fusca* BD25

The optimum pH and temperature for β-xylosidases from *Thermomonospora curvata* (24), *Thermomonospora sp.*(20), *T. fusca* BD21 (16) and *Streptomyces*. sp. CH-M-1035 (25) have been reported in the range 6-9, and 50-70 °C, respectively. The β-xylosidase from *T. fusca* BD25 appears in this study to be more thermostable ($t_{1/2}$ 1.5 h at 75 °C) than the β-xylosidases of fungi and other bacteria, such as *Bacillus pumilus* (26), *Penicillium votmanni* (22) and *Neurospora crassa* (27), and also exhibits activity across a relatively broad pH range of 5 to 9.

In comparison with the other bacterial and fungal α-L-arabinofuranosidases, including those from *Aspergillus awamori* (28), *Streptomyces* sp. 17-1 (29), and *Clostridium stercorarium* (30), which have been reported to have maximum activities at pH 4.6-6.0 and at 50-60 °C, the α-L-arabinofuranosidase from *T. fusca* BD25 is among the most active and thermostable enzymes ($t_{1/2}$ 1 h at 75 °C). The temperature and pH dependencies of xylan-degrading enzymes with extracellular lignocellulose-degrading enzymes from *T. fusca* are summarized in Table 2.

Kinetic studies of β-xylosidase and α-L-arabinofuranosidase from *T. fusca* BD25

Crude enzyme preparations were used throughout, and this can be justified since any process applications are unlikely to be based on purified enzymes. The apparent K_m and V_{max} values for the crude preparation of β-xylosidase were 0.5 mM of *p*-NPX and 0.83 μmol *p*-nitrophenol ml^{-1} min^{-1}, respectively. These values are in accordance with the published values for the crude β-xylosidase activity of different *Thermomonospora* strains (16, 20). β-xylosidases from *Neurospora crassa* (27) and *Aspergillus niger* (31) showed a greater affinity for this substrate than the enzyme from this study, with K_m values of 0.05 mM and 0.22 mM, respectively.

Table 2. Temperature and pH dependencies of extracellular lignocellulose-degrading enzymes from *T. fusca*. Half-life ($t_{1/2}$) is defined as the time required to reduce the enzyme activity to 50 % of the initial activity at the defined temperature.

Enzyme	Optimum temp. (°C)	Optimum pH	$t_{1/2}$	References
Endoxylanase	70	4.5-8.0	30 min at 80 °C	(14, 33)
Endoglucanase	70	6.0	24 h at 65 °C	(34)
Peroxidase	55-65	6.0-8.0	15 h at 60 °C	(35)
β-xylosidase	40-60	5.0-9.0	8 h at 65 °C	(22, this work)
α-L-arabinofuranosidase	40-60	6.0	3 h at 60 °C	(30, this work)

However, other bacterial and fungal enzymes studied, such as those from *T. curvata* (24) showed less affinity for this substrate than the enzyme from *T. fusca* BD25.

The K_m and V_{max} values for the crude preparation of α-L-arabinofuranosidase were 0.18 mM of *p*-NPA and 0.04 μmol *p*-nitrophenol ml^{-1} min^{-1}, respectively. From the results (see Figure 5b), it may be seen that the reaction mixture of α-L-arabinofuranosidase preparation was contaminated with β-xylosidase activity, since the activities of these enzymes eluted from the anion-exchange column very closely. Relatively few α-L-arabinofuranosidases have been examined in detail, but these enzymes have been studied in another strain of *T. fusca* (32) and in *Streptomyces* sp. 17-1 (29), with similar properties described to the properties of the enzyme from *T. fusca* BD25. Comparison with the published information on other bacterial and fungal α-L-arabinofuranosidases reveals that the enzyme studied here is among the most active, thermostable and end-product inhibition-resistant α-L-arabinofuranosidase.

β-xylosidase and α-L-arabinofuranosidase activities were completely inhibited by 1 mM Hg^{2+}. The activity of β-xylosidase and α-L-arabinofuranosidase were unaffected by the presence of urea, SDS, EDTA, Zn^{2+} and Mg^{2+} (1 mM). Hg^{2+} is known to react with protein sulphydryl groups as well as histidine and tryptophan residues (33). *T. fusca* has been extensively studied as a source of cellulase activity, and comparison of the results presented here with those of other workers illustrates similarities and differences between endoglucanases and β-xylosidase and α-L-arabinofuranosidase activities. All these enzymes contain sulphydryl groups in their active sites (34). The addition of EDTA did not affect the activities of β-xylosidase and α-L-arabinofuranosidase, suggesting that no metals are needed for their enzymatic reactions. The enzymes β-xylosidase and α-L-arabinofuranosidase from *T. fusca* BD25 were not stimulated by the addition of metal ions. However, it has been reported that β-xylosidase from *T. curvata* (24) is stimulated by Co^{2+} and Pb^{2+}.

From this work, the optimum conditions for the activity of β-xylosidase and α-L-arabinofuranosidase from *T. fusca* BD25 can be summarized as:

The extrecellular α-L-arabinofuranosidase and mainly intracellular β-xylosidase activities from *T. fusca* BD25 remained stable up to 65 °C.

The β-xylosidase and α-L-arabinofuranosidase activities from *T. fusca* BD25 showed a half life of 1.5 h and 1 h at 75 °C, at pH 7.0, respectively.

The optimum pH and temperature for the activities of β-xylosidase and α-L-arabinofuranosidase by *T. fusca* BD25 were at pH 8.0 and at 70 °C; and at pH 9.0 and at 65 °C, respectively.

The apparent K_m values for the crude β-xylosidase and α-L-arabinofuranosidase preparations were 0.5 mM of *p*-NPX and 0.18 mM of *p*-NPA, while the V_{max} values were 0.83 μmol *p*-nitrophenol ml^{-1} min^{-1} and 0.04 μmol *p*-nitrophenol ml^{-1} min^{-1}, respectively.

The addition of xylose and arabinose (10 mM) to the reaction mixture of β-xylosidase and α-L-arabinofuranosidase preparation resulted in approximately 10 % and 15 % reductions in activities, respectively.

The enzymes retained full activity in the presence of urea, SDS, EDTA, Zn^{2+} and Mg^{2+}. β-xylosidase and α-L-arabinofuranosidase activities were completely inhibited by $HgCl_2$ (1 mM).

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References

1. Betts, W.B., Dart, R.K., Ball, A.S. & Pedlar, S.L. Biosynthesis and structure of lignocellulose. In: *Biodegradation, Natural and Synthetic Materials*. (Betts, W.B. ed.). London; 1992, Springer-Verlag, pp. 139-156.
2. Whistler, R.L. & Richards, E.L. Hemicellulose. In: *The Carbohydrates. Chemistry and Biochemistry. 2nd edition. Vol. 2A* (Eds. Pigman, W. and Horton, D.). New York. 1970, Academic Press. pp. 447-469.
3. Dekker, R.F.H. & Richards, G.N. Hemicellulases: Their occurrence, purification, properties and mode of action. *Adv. Carbohydr. Chem. Biochem.* 32; 277-352. 1976.
4. Barry, V.C. & Dillon, T. The occurrence of xylan in marine algae. *Nature,* 146; 620. London. 1940.
5. Kato, Y. & Nevins, D. Enzymic dissociation of zea shoot cell wall polysaccharides: (III)purification and partial characterization of an endo-(1,4)β-D-xylanase from a *Bacillus subtilis* enzyme preparation. *Plant Physiol.* 75; 753-758. 1984.
6. Joseleau, J.P., Comtat, J. & Ruel, K. Chemical structure of xylans and their interaction in the plant cell walls. In: *Xylans and Xylanases*. (Eds. Visser, J., Beldman,G., Kusters-van-Someren, M.A. and Varagen, A.G.J.) Amsterdam, London, New York.,Tokyo 1992. *Elsevier*. pp. 1-15.
7. Eriksson, K.-E.L., Blanchette, R.A. & Ander, P. Biodegradation of cellulose. In: *Microbial and Enzymatic Degradation of Wood and Wood Components*. Heidelberg. 1990. Springer-Verlag.

8. Coughlan, M.P. & Hazlewood, G.P. β-1,4-D-xylan degrading enzyme systems: Biochemistry, molecular biology and applications. *Biotechnol. Appl. Biochem.* 17; 259-289. 1993.

9. Thomson, J.A. Molecular biology of xylan degradation. *FEMS Microbiol. Rev.* 104; 65-82. 1993.

10. McCarthy, A.J. Lignocellulose degrading actinomycetes. *FEMS Microbiol. Rev.* 46; 145-163. 1987.

11. Wong, K.K., Tan, L.U.L. & Saddler, J.N. Multiplicity of β-1,4-xylanase in microorganisms: Function and Applications. *Microbiol. Rev.* 52; 305-317. 1988.

12. Ball, A.S. & McCarthy, A.J. Production and properties of xylanases from actinomycetes. *J. Appl. Bacteriol.* 66; 439-444. 1989.

13. Tuncer, M., Rob, A., Ball, A.S., & Wilson, M.T. Optimisation of extracellular lignocellulolytic enzyme production by a thermophilic actinomycete *Thermomonospora fusca* BD25. *Enzyme & Microbial Technol.* 22; - , - . (in press) 2000.

14. Tuncer, M. A study of the enzymology of lignocellulose degradation by *Thermomonospora fusca* BD25. Ph.D. Thesis. University of Essex. UK. 1999.

15. Tuncer, M. Rob, A., Ball, A.S., Eady, R.R., Henderson, N. & Wilson, M.T. Optimization of production of extracellular non-haem peroxidases by *Thermomonospora fusca* BD25 in aerobic bio-reactor conditions. *Biochem. Soc. Transac.* 25; S 65. 1997.

16. Bachmann, S.L. & McCarthy A.J. Purification and characterisation of a thermostable β-Xylosidase from *Thermomonospora fusca. J. Gen. Microbiol.* 135; 293-299. 1989.

17. MacKenzie, C.R., Bilous, D., Schnieder, H. and Johnson, K. G. Induction of cellulolytic and xylanolytic enzyme systems in *Streptomyces* spp. *Appl. Environ. Microbiol.* 53;2835-2839. 1987.

18. Johnson, W.C. & Lindsey, A.J. An improved universal buffer. *Analyst.* 64; 958-993. 1939.

19. Ristroph, D.L. & Humphrey, A.E. Kinetic characterisation of the extracellular xylanases of *Thermomonospora sp. Biotechnol. & Bioengin.* 27; 832-836. 1985.

20. Ristroph, D.L. & Humphrey, A.E. The β-xylosidase of *Thermomonospora. Biotechnol. Bioengin.* 27; 909-913. 1985.

21. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* London. 227; 680-685. 1970.

22. Deleyn, F., Claeyssens, M., Van Beelmen, J. & de Bruynde, K. Purification and properties of β-xylosidase from *Penicillium wortmanni. Can. J. Biocehem.* 56; 43-50. 1978.

23. Uziie, M., Matsuo, M. & Yasui, T. Possible identify of β-xylosidase and β-glucosidase of *Chaetomium trilaterale. Agri. Biol. Chem.* 49; 1167-1173. 1985.

24. Stutzenberger, F. J. & Bodine, A.B. Thermostable β-xylosidase from *Thermomonospora curvata. J. Indust. Microbiol. & Biotechnol.* 20; 55-60. 1998.

25. Flores, M.E., Pérez, R. & Huitrón, C. β-xylosidase and xylanase characterization and production by *Streptomyces* sp. CH-M-1035. *Lett. Appl. Microbiol.* 24; 410-416. 1997.

26. Claeyssens, M., Saman, M., Kersters-Hilderson, H. & de Bruyne, C.K. β-D-xylosidase from *Bacillus pumilus*: molecular properties and oligomeric structure. *Biochemica et Biophysica Acta*. 405; 475-481. 1975.

27. Deshpande, V. Lachke, A., Misra, C., Keskar, S. & Rao, M. Mode of action and properties of xylanase and β-xylosidase from *Neurospora crassa*. *Biotechnol. Bioeng*. 28; 1832-1837. 1986.

28. Wood, T.M. & McCrae, S.I. Arabinoxylan-degrading enzyme-system of the fungus *Aspergillus awamori*: purification and properties of an α-L-arabinofuranosidase. *Appl. Microbiol. Biotechnol*. 45; 538-545. 1996.

29. Kaji, A., Sato, M. & Tsutsui, Y. An a-L-arabinofuranosidase produced by wild-type *Strptomyces* sp. 17-1. *Agric. Biol. Chem*. 45; 925-931. 1981.

30. Schwarz, W.H., Bronnenmeier, K., Krause, B., Lottspeich, F. & Staudenbauer, W. I. Debranching of arabinoxylan: properties of the thermoactive recombinant α-L-arabinofuranosidase from *Clostridium stercorarium* (*arfb*). *Appl. Microbiol. Biotechnol*. 43; 856-860. 1995.

31. John, M., Schmidt, B. & Schmidt, J. Purification and some properties of five endo-1,4-β-xylanases and β-D-xylosidase produced by a strain of *Aspergillus niger*. *Can. J. Biochem*. 57; 124-134. 1979.

32. Bachmann, S.L. & McCarthy, A.J. Purification and co-operative activity of enzymes constituting the xylan-degrading system of *Thermomonospora fusca*. *Appl. Environ. Microbiol*. 57; 2121-2130. 1991.

33. Volkin, D.B. & Klibanov, A.M. Minimizing protein inactivation. *In: Protein function, a practical approach*. (Ed. Creighton, T.E.), Oxford. 1989. IRL Press. pp. 1-24.

34. Ghangas, G.S. & Wilson, D.B. Cloning of the *Thermomonospora fusca* endoglucanase E2 gene in *Streptomyces lividans*:Affinity purification and functional domains of the cloned gene product. *Appl. Environ. Microbiol*. 54; 2521-2526. 1988.