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Production, Purification, and Characterization of a Low-Molecular-Mass Xylanase from *Aspergillus* sp. and Its Application in Baking

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Abstract

An extracellular xylanase produced by a Mexican *Aspergillus* strain was purified and characterized. *Aspergillus* sp. FP-470 was able to grow and produce extracellular xylanases on birchwood xylan, oat spelt xylan, wheat straw, and corncob, with higher production observed on corncob. The strain also produced enzymes with cellulase, amylase, and pectinase activities on this substrate. A 22-kDa endoxylanase was purified 30-fold. Optimum temperature and pH were 60°C and 5.5, respectively, and isoelectric point was 9.0. The enzyme has good stability from pH 5.0 to 10.0, retaining >80% of its original activity within this range. Half-lives of 150 min at 50°C and 6.5 min at 60°C were found. K_m and activation energy values were 3.8 mg/mL and 26 kJ/mol, respectively, using birchwood xylan as substrate. The enzyme showed a higher affinity for 4-*O*-methyl-D-glucuronoxylan with a K_m of 1.9 mg/mL. The enzyme displayed no activity toward other polysaccharides, including cellulose. Baking trials were conducted using the crude filtrate and purified enzyme. Addition of both preparations improved bread volume. However, addition of purified endoxylanase caused a 30% increase in volume over the crude extract.

Index Entries: *Aspergillus*; purification; endoxylanase; bread making.

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Introduction

Naturally occurring lignocellulosic plant biomass consists of 20–30% hemicellulosic materials, which are heterogeneous polysaccharides found in association with cellulose and pectin. Xylan is the major constituent of hemicellulose and is the second most abundant renewable resource with a high potential for degradation to useful end products *(1,2)*.

Xylan is a complex heteropolysaccharide consisting of a main chain of highly branched β-1,4-linked xylanopyranosyl residues *(1)*. This linear chain can contain various substituents, including arabinofuranosyl, glucuronyl, and acetyl groups, which have a great influence on its chemical and structural properties and on the enzymatic degradability of xylan *(1,3)*. Consequently, the complete breakdown of xylan requires the action of a consortium of several enzymes. Xylanases are important enzymes for the degradation of plant cell-wall material. Based on sequence similarities, xylan-degrading enzymes were classified into several families of glycosylhydrolases *(4)*. Xylanases have also been divided into two groups: (1) xylanases with alkaline isoelectric points (p*I*s) and low molecular mass and (2) xylanases with acidic p*I*s and high molecular mass *(5)*. These two groups correspond to families F10 and G11 within the glycosylhydrolase classification *(6)*.

Recently, interest in xylanolytic enzymes has increased owing to their potential use in several industrial processes such as biopulping and bleaching; bioconversion of lignocellulose; food processing, including clarification of beer, wine, and juice; increasing digestibility of animal feedstock; and bread making *(1–3,7)*. In the latter, xylanases improved bread volume, which is considered one of the most important parameters with a direct relationship to their organoleptic properties *(2,8)*. These enzymes may contribute to eliminating the use of chemical additives such as bromate.

Commercial enzyme preparations combine several enzyme activities and vary considerably in composition and ratio of these activities depending on the source. Thus, identification and characterization of pure enzymes is needed in order to better understand each enzyme in food processing and to identify the best enzyme for a given process. This knowledge will also be very important to the design of specific routes to produce such enzymes.

In previous work, we isolated a Mexican *Aspergillus* strain from decaying fruit that produced high levels of several xylanolytic enzymes at 37 and 45°C *(9)*. This strain, called *Aspergillus* sp. FP-470, produced several enzymes with xylanolytic activity, some of which showed thermotolerant behavior *(9)*. To evaluate the potential bread-baking application of xylanases produced by different *Aspergillus* strains and to identify the different enzymes in these complex mixtures, we are working on the production and characterization of xylanases obtained from tropical *Aspergillus* strains.

In this article, we report the production, purification, biochemical characterization, and use in bread making of an extracellular low-molecular-mass xylanase produced by the tropical strain *Aspergillus* sp. FP-470.

Materials and Methods

Microorganism

The microorganism used was *Aspergillus* sp. FP-470. This strain was isolated in our laboratory from spoiled fruits. Stock cultures were propagated at 37°C and maintained on potato dextrose agar slants.

Growth and Enzyme Production

Aspergillus was cultivated in basal medium (BM) containing 0.4% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.2% K_2HPO_4, 0.3% yeast extract, and 1% of the appropriate carbon source. Birchwood xylan; oat spelt xylan (both from Sigma, St. Louis, MO); corn hemicellulose, extracted from corncob as reported previously *(9)*; wheat bran; and milled corncobs were used as carbon source.

The medium was sterilized at 121°C, 15 psi for 20 min. Growth and xylanase production were followed in 500-mL Erlenmeyer flasks containing 200 mL of culture medium and shaken at 100 rpm in an incubator shaker (New Brunswick Scientific) kept at 37°C. The initial pH was 5.0. All flasks were inoculated with a spore suspension of *Aspergillus* sp. FP-470 to give a final concentration of 1×10^6 spores/mL of culture medium. For large-scale production, a 14-L fermentor (New Brunswick Scientific) was used. The fermentor was filled with 9 L of BM with 1% corncobs as sole carbon source. One liter of a 24-h-old culture incubated at 37°C in the same medium was used as inoculum. Fermentation was carried out at 37°C, 300 rpm and aeration of 1 vvm at 15 psi.

Enzyme Activity Assays

Xylanases were assayed by measuring the reducing groups released form xylan by the dinitrosalicylic acid method *(10)*. The reaction mixture consisted of 500 µL of 2% xylan; 400 µL of 0.17 *M* acetate buffer, pH 5.0; and 100 µL of enzymatic sample and was incubated at 50°C for 10 min. One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of xylose in 10 min under the assay conditions. Oat spelt xylan and 4-*O*-methyl-D-glucuronoxylan (Sigma) were also used as substrates. Exo- and endopectinases were assayed as reported previously *(11)*. One unit of exopectinase was defined as the amount of enzyme that released 1 µmol of galacturonic acid in 20 min under the assay conditions. One unit of endopectinase was defined as the amount of enzyme that reduced the viscosity of a 1% pectin solution by 50% in 10 min under the assay conditions. Amylase activity was measured as reported previously *(12)*. Briefly, 0.8 mL of 1.2% starch solution was incubated with 100 µL of enzymatic solution for 10 min. Then 100 µL of 1 *N* H_2SO_4 was added to stop the reaction. One hundred microliters of this solution was mixed with 2.4 mL of 0.4% Lugol's solution. The absorbance of the resultant solution was recorded at 620 nm. One unit of amylolytic activity was defined as the amount of enzyme that hydrolyzed 1.0 mg of soluble starch/min under the assay conditions.

Stability

The effect of pH on the stability of xylanase was evaluated by incubating the enzyme at different pH values for 24 h. To test stability at different temperatures, the enzyme was incubated at the desired temperature for 30 min at pH 5.0 in the presence or absence of 1% substrate, 10% sorbitol, or 10% glycerol. Afterward, residual activity was determined in each sample. The optimal temperature and pH were determined by evaluating enzyme activity at different temperatures and pHs, under standard conditions. Half-life of the enzyme was estimated by incubating the enzyme in 20 m*M* acetate buffer, pH 5.0, at 50 and 60°C for different lengths of time. Samples were taken and the residual activity was determined under standard conditions. The effect of different ions was determined by the adding of the corresponding ion at final concentrations of 10 and 20 m*M* to the reaction mixture and assaying the activity under standard conditions.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and Agarose RBB-Xylan Overlays for In Situ Detection of Xylanolytic Activity

Electrophoresis was carried out under denaturing conditions with a resolving gel containing 10% acrylamide and 2.7% bis-acrylamide. Gels were stained with Coomassie blue and destained with a mixture of methanol/acetic acid/water (50:10:40).

The agarose Remazol-brilliant-blue (RBB)-xylan overlays were prepared by dissolving the agarose and the RBB-xylan by heating in acetate buffer at pH 5.0. This mixture was poured into a plate sandwich with 0.75-mm spacers in a gel caster previously heated at 37°C. Final concentration within the gels was 1% agarose and 0.4% RBB-xylan.

Purification Procedure

One liter of culture filtrate from a 10-L fermentation was concentrated 10-fold in an ultrafiltration device (Amicon, Beverly, MA) with a PM-10 membrane. The concentrated enzyme solution was dialyzed and applied to a Resource Q anionic exchange column (Amersham-Pharmacia) at a flow rate of 3.0 mL/min. The column was equilibrated with 20 m*M* Tris-HCl buffer at pH 8.0. Elution was done with a linear NaCl gradient (0–0.5 *M*) in the same buffer. From this column, five peaks with xylanase activity were obtained. Activity peaks were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the fractions from the greatest activity peak (peak 1) were pooled, dialyzed, and applied to a carboxymethyl-Sepharose column (16 cm long, 1.2 cm in diameter). The column was equilibrated with 40 m*M* acetate buffer, pH 5.0, and eluted with an NaCl gradient (0–1.0 *M*) at a flow rate of 3.0 mL/min. Fractions with the highest activity were pooled, dialyzed, and lyophilized.

General Analytical Techniques

Protein was estimated in dialyzed cell-free samples by the method of Lowry using bovine serum albumin as standard. The dyed xylan (RBB-xylan) was prepared following the method of Biely et al. *(13)*. Hydrolysis products were analyzed by thin-layer chromatography (TLC) on silica gel plates according to Ganju et al. *(14)*.

Isoelectric Focusing

Isoelectric focusing was determined using 5% acrylamide with 6.7%, 3–10 ampholines. Gels were prerun for 20 min. Protein samples were loaded and gels were run for 3.5 h. Staining was done with Coomassie blue. A duplicate gel was used for *in situ* activity with RBB-xylan as substrate, as described earlier.

Baking Trials

Bread was made according to Method AACC 10-09 of the American Association of Cereal Chemistry *(15)*, using 100 g of wheat flour; 4.0 g of dry milk; 3 g of vegetable fat; and 10 mL of a solution containing 5% sugar, 1.5% salt, and 2% dehydrated yeast. All ingredients were placed in a bowl and mixed with approx 10 mL of water. The enzyme sample was added to this mixture and the dough was mixed for 4 min. The dough was then put in a fermentation chamber at 36°C for 2 h. The dough was mixed again and once more put in the fermentation chamber for 55 min. The dough was finally cooked in an oven at 220°C for 25 min in standard bread pans. After cooking time, the volume of each bread was determined by rape seed displacement. Enzyme samples used were the concentrated culture filtrate and purified xylanase. The amount of enzyme used was between 25 and 200 ppm. The baking test was repeated two times.

Results

Xylanase Production

Aspergillus sp. FP-470 was grown on different carbon sources. As can be seen from Table 1, this strain produced xylanases in all carbon sources tested, with the highest production of xylanases obtained when the strain was grown in medium containing corncob. At 48 h of fermentation, xylanases were produced on all other carbon sources. However, under those conditions the production was <50% compared with that obtained on corncob medium. At 72 h only the production on birchwood xylan reached a higher value (about 70% that on corncob).

The strain also produced other hydrolytic enzymes. In fact, when *Aspergillus* sp. FP-470 was grown on oat spelt xylan, hemicellulose, wheat bran, or corncob, cellulolytic (0.88, 1.89, 2.95, and 7.31 U/mL, respectively) and pectinolytic (1.8, 4.76, 5.1, and 9.67 U/mL, respectively) activities were detected. This strain also produced amylases when grown on corncob (data

Table 1
Xylanase Production in Shake Flasks

Carbon source	Culture time[a] 48 h	72 h
Birchwood xylan	15.5 (42)	27.5 (70)
Oat spelt xylan	16.2 (44)	19.0 (48)
Hemicellulose from corncob	13.0 (35)	16.5 (42)
Corncob	37.0 (100)	39.5 (100)
Wheat bran	11.0 (30)	17.0 (43)

[a]Activity is expressed as units/milliliter. Numbers in parentheses denote the activity expressed as percentage of maximum.

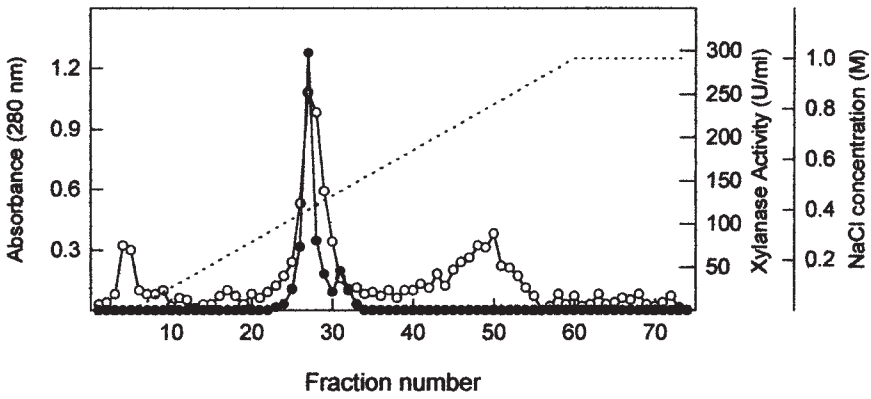

Fig. 1. CM-Sepharose column chromatography of *Aspergillus* sp. FP-470 xylanase. The column was equilibrated with 20 m*M* acetate buffer, pH 5.0. Elution of xylanase activity was done by applying a linear NaCl gradient (0–1.0 *M*) in the same buffer. (○) A 280; (●) xylanase activity; (– – – – –) NaCl gradient.

not shown). On birchwood xylan, only xylanase activity was detected. Since corncob gave the highest yield, it was selected for large-scale xylanase production. At the fermentor level, higher yield was obtained. High xylanase activity was detected at 24 h of fermentation (67 U/mL), with production levels reaching 1.8 times those achieved in shake-flask cultivation. Maximum activity (74 U/mL) was produced at 80 h of culture. However, about 90% of the activity was produced after 24 h (Table 1).

Purification and Characterization

The culture filtrate from the fermentor, used as starting material, contained 142 U/mg of xylanases. It was concentrated by ultrafiltration and dialyzed. The specific activity of this concentrate increased to 169 U/mg. Xylanases were first purified by anion-exchange chromatography on a Resource Q column. The elution profile showed five peaks of xylanase activity. The peaks were analyzed for xylanase activity by SDS-PAGE

Table 2
Summary of Xylanase Purification (XYL22) from *Aspergillus* sp. FP-470

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification factor (-fold)
Culture filtrate[a]	57.81	8200	142	100	1.0
Ultrafiltration	47.18	7953	169	97	1.2
Resource Q	1.66	5280	3181	64	22.4
CM-Sepharose	0.73	3270	4473	40	31.5

[a]The starting volume of culture filtrate was 1.0 L.

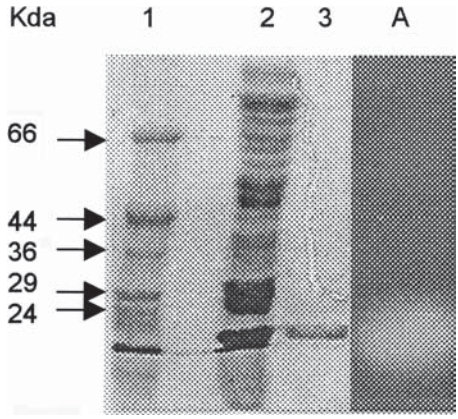

Fig. 2. SDS-PAGE (lanes 1–3) and zymogram (lane A) of xylanases from *Aspergillus* sp. FP-470. Lane 1, molecular weight markers; lane 2, concentrated culture filtrate; lanes 3 and A, xylanase and activity stain, respectively, obtained after CM-Sepharose column.

coupled with *in situ* detection of activity. The fractions from the peak with the highest activity were pooled, dialyzed, and applied to a CM-Sepharose column. From this column a single peak with xylanase activity was obtained (Fig. 1).

Fractions of this peak were pooled and dialyzed to be used for characterization. This purification procedure yields a xylanase with specific activity of 4473 U/mg, exhibiting a purification factor of 31.5 and a yield of 40% (Table 2). The apparent molecular mass of the xylanase purified with the preceding protocol was found to be 22 kDa, as estimated by SDS-PAGE (Fig. 2). The purity of the 22-kDa xylanase (XYL22) attained here was more than 30-fold, and it was considered enough to be used for biochemical characterization.

Effect of pH and Temperature

The effect of pH and temperature on XYL22 is shown in Fig. 3. XYL22 showed higher activity at pH 5.5. Between pH 5.0 and 7.0 more than 80% of

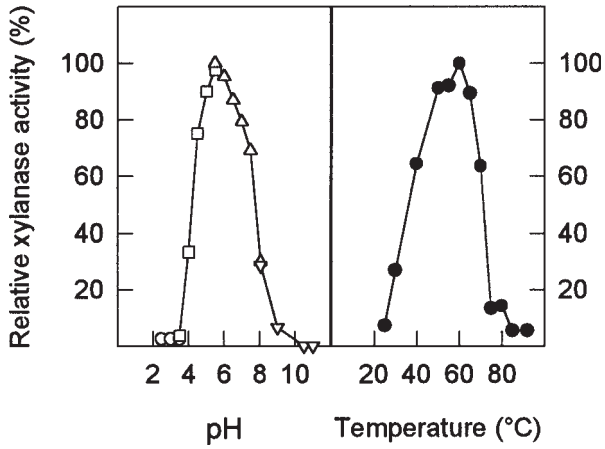

Fig. 3. Effect of pH (at 50°C) and temperature (at pH 5.0) on enzyme activity. Buffers used to test different pH values were (○) 50 m*M* glycine-HCl (pH 2.5–3.4), (□, ●) 50 m*M* sodium acetate (pH 3.5–5.5), (△) 50 m*M* Tris-maleate (pH 5.5–8.5), and (▽) 50 m*M* glycine-NaOH (pH 8.5–11.0).

activity was displayed. More than 50% of activity was lost below pH 4.0 and above 8.0 (Fig. 3). After incubating for 10 min, optimum temperature for XYL22 was found to be 60°C. However, >90% of activity was seen between 50 and 65°C. The Arrhenius law was followed from 20 to 60°C and an activation energy of 26 kJ/mol was calculated. A sharp decrease in the activity was observed above 60°C (Fig. 3).

Stability

The enzyme showed good stability at a pH range from 3.5 to 10.5 (Fig. 4A). At this range, >80% of the activity was retained. A maximum was observed at pH 5.5.

Thermal stability of XYL22 was monitored by measuring the residual enzymatic activity on incubation for 30 min with or without substrate, sorbitol, or glycerol at the prescribed temperatures. The results showed that XYL22 was sensitive to temperature inactivation above 40°C, losing about 30% of its activity at 50°C in the absence of substrate. Under these conditions, activity was completely lost at 65°C (Fig. 4B). High stability was observed when the incubation was carried out in the presence of 1% birchwood xylan. With either glycerol or sorbitol, the enzyme maintained its activity until 55°C. The half-life of XYL22 was determined to be 6 min at 60°C and 150 min at 50°C.

Effect of Metal Ions

XYL22 activity was measured in the presence of different metal ions at final concentrations of 10 and 20 m*M*. As can be seen from Table 3, none of the metal ions tested were required for catalytic activity. All of the ions reduced xylanase activity in a concentration-dependent manner. Zn^{2+}, Co^{2+},

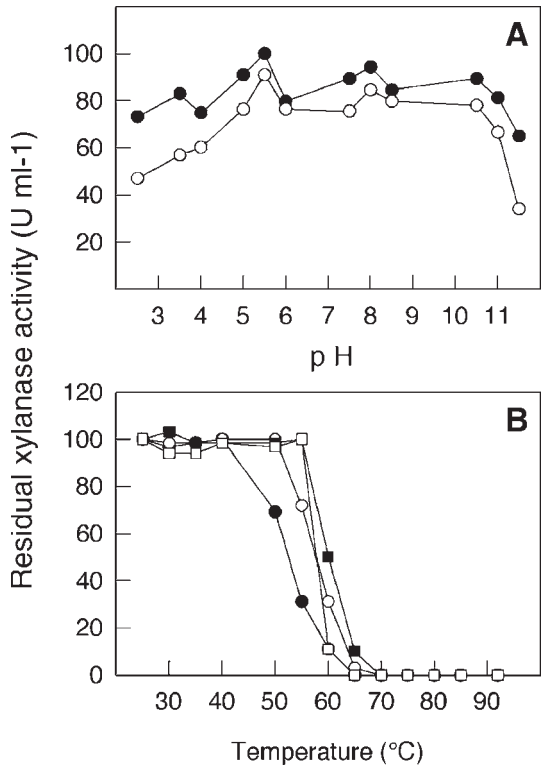

Fig. 4. Effect of pH **(A)** and temperature **(B)** on stability of purified xylanase. (A) incubation was carried out for 24 h at 4 (●) and 25°C (○) in glycine-HCl buffer (pH 2.5–3.4), sodium acetate (pH 3.5–5.5), Tris-maleate (pH 5.5–8.5), and glycine-NaOH (8.5–11.0) all at 50 m*M*. (B) Incubation was carried out for 30 min in 0.17 *M* acetate buffer, pH 5.0, with the enzyme alone (●) or in the presence of 1% birchwood xylan (○), 10% glycerol (□), or 10% sorbitol (■).

Table 3
Effect of Metal Ions
on Purified Xylanase Activity

	Relative activity (%)	
	Ion concentration	
Ion	10 m*M*	20 m*M*
None	100	100
Ba^{2+}	82	60
Mg^{2+}	78	60
Ca^{2+}	64	35
Zn^{2+}	22	0
Co^{2+}	27	0
Cu^{2+}	0	0
Fe^{3+}	35	0

Table 4
Relative Activity and Kinetic Parameters of XYL22 on Different Substrates

Substrate	Relative activity (%)	K_m (mg/mL)	V_{max} (%) (μmol/[min·mg])
Birchwood xylan	100	3.80	62
Oat spelt xylan	95.5	10	44
4-*O*-methyl-D-glucuronoxylan	74.3	1.9	44

Cu^{2+}, and Fe^{3+} produced the strongest effect, inhibiting activity completely at the 20 m*M* concentration. Furthermore, the effect of Na^+ and K^+ was also tested. Between 0 and 50 m*M*, Na^+ had a slight inhibitory effect and a maximum reduction of 15% was observed at 50 m*M*. The addition of K^+ had no negative effect, and, in fact, a slight stimulatory effect of about 5% was observed at 50 m*M*.

Catalytic Properties

The substrate specificity of XYL22 was evaluated on birchwood xylan, oat spelt xylan, 4-*O*-methyl-D-glucuronoxylan, hemicellulose, corncob, carboxymethylcellulose, avicel, soluble starch, filter paper, polygalacturonic acid, and pectin. Birchwood xylan was found to be the best substrate for purified xylanase, followed by oat spelt xylan (Table 4). Relative activity on 4-*O*-methyl-D-glucuronoxylan was about 74% in relation to birchwood xylan, while activity on hemicellulose and corncob was about 23 and 5%, respectively. XYL22 was not able to degrade carboxymethylcellulose, avicel, soluble starch, filter paper, polygalacturonic acid, or pectin.

As calculated from Lineweaver-Burk plots, the K_m and V_{max} for birchwood xylan were 3.8 mg/mL and 62 μmol/(min·mg of protein), respectively, and 1.9 mg/mL and 44 μmol/(min·mg of protein), respectively, for 4-*O*-methyl-D-glucuronoxylan. The apparent K_m and V_{max} values for oat spelt xylan were also determined; values are given in Table 4.

Reaction products of xylan hydrolysis detected by TLC were found to be low-molecular-weight xylooligosaccharides (X2–X4); no xylose could be detected in any case. Therefore, this enzyme exhibits a typical endo fashion mode of action *(5)*.

The purified xylanase was tested in bread making using concentrations from 25 to 200 ppm. As can be seen in Fig. 5, crude enzyme preparation increased the loaf volume about 12% at a concentration of 50 ppm, and at higher concentrations (100, 150, and 200 ppm), the loaf volume was nearly the same as the control (Fig. 5). The same effect was observed with the purified enzyme. However, the increase in loaf volume was nearly 2.5 times higher than that obtained with the crude preparation (Fig. 5). A maximum increase of 30% was obtained using 25 ppm of the purified enzyme. From 25 to 100 ppm, loaf volume was almost the same, but with 150 and 200 ppm, loaf volume was similar to the control. The maximum increase shown here,

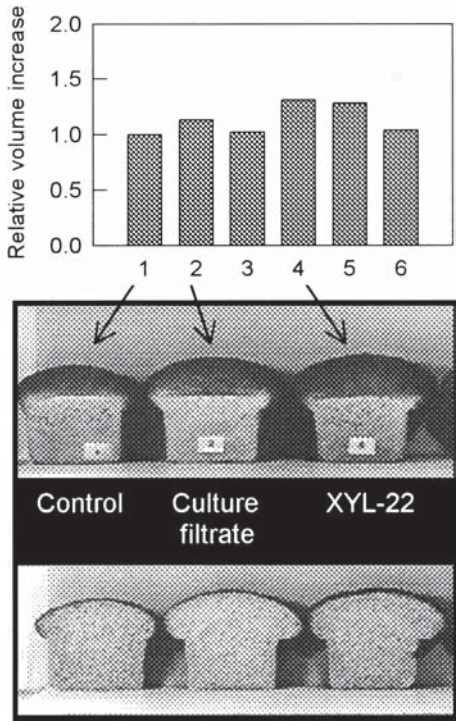

Fig. 5. **(Top)** Volume increase of breads prepared (1) without xylanase; with different amounts of crude culture filtrate at 50 (2) and 150 ppm (3); and with purified xylanase at 25 (4), 150 (5), and 200 ppm (6). **(Bottom)** Representative breads are shown: (1) control without xylanases and breads with (2) 50 ppm of concentrated crude culture filtrate or (4) 25 ppm of XYL22.

using XYL22, was comparable with that attained with commercial preparations commonly used for bread making (data not shown).

Discussion

Aspergillus sp. FP-470 produces a complex mixture of xylanases. This is especially true when the strain grows on complex substrates such as corncob or wheat bran (Fig. 2). Corncob and wheat bran were also used as carbon sources for the production of xylanase and b-xylosidase by *A. tamarii* with high yield *(16)*. With the purification protocol used herein, it was possible to obtain a 30-fold purification of a low-molecular-mass xylanase with a yield of 40%. The apparent purity of the enzyme was demonstrated by SDS-PAGE (Fig. 2), and its molecular mass was estimated to be 22 kDa. The purified 22-kDa xylanase exhibited a p*I* of 9.0. Thus, the enzyme can be grouped with the alkaline and low-molecular-mass endoxylanases according to Wong and Saddler *(5)*. The p*I* is higher compared with other xylanases produced by *Aspergilli*. Some xylanases from *Aspergillus* have acidic p*I*, as is the case of *Aspergillus* sp., with p*I*s of 4.7 *(17)*,

3.65, and 4.5 *(18,19)*. In addition, neutral p*I* has been reported for *A. nidulans*, with a p*I* of 6.4 *(20)*, and *A. niger*, with a p*I* of 6.7 *(19)*. However, it has been reported that *A. niger* produced two endoxylanases with p*I*s of 8.6 and 9.0 for xylanase I and II, respectively *(21)*. Xylanase II has a molecular mass of 13 kDa, which is almost half that of the xylanase reported here.

The optimal pH (5.5) and temperature (60°C) were in the range of many other fungal strains and are closely related to *A. sydowii* MG 49 except for the molecular mass (30 kDa) *(22)*. Activation energy of 26 kJ/mol was slightly lower with respect to the value found for the *A. niger* xylanases I and II, which is 30 and 38 kJ/mol, respectively *(21)*. The great majority of xylanases from mesophilic organisms have their maximum activity between 40 and 65°C *(23)*. In our case, the highest activity was found to be 60°C. Other xylanases from *A. niger*, *A. nidulans*, and *Trichoderma harzianum* E58 have the same optimal temperature *(20,23,24)*. The half-life of XYL22 (6 min at 60°C) was similar to that of *A. fisherii* Fxn1, 8 min at 60°C *(25)* and that of *Aspergillus* sp. 15 and 7 min for xylanases I and II, respectively *(17)*. The stability was greatly enhanced in the presence of substrate *(9,25)*. Although the thermal stability of the enzyme is limited, it could be improved by the addition of xylan.

It is difficult to compare the kinetic values of xylanases obtained by other researchers in view of the different xylan substrates and assay conditions. The K_m values of XYL22 from *Aspergillus* sp. FP-470 with birchwood xylan (3.8 mg/mL) and 4-*O*-methyl-D-glucuronoxylan (1.9 mg/mL) were within the range of many xylanases (0.24–20 mg/mL), showing high affinity for these less branched xylans.

Aspergillus sp. FP-470 grew on corncob-produced xylanases, pectinases, amylases, as well as cellulases. Some xylanases have been reported to possess cellulase activity *(21)*. However, on the basis of our results it is apparent that purified XYL22 is free of cellulase activity. The enzyme had no detectable activity on carboxymethylcellulose, filter paper, avicel, pectin, polygalacturonic acid, or starch.

Addition of XYL22 to bread dough increased loaf volume and improved crumb structure of the baked product. However, in cases with more than 150 or 50 ppm for crude culture filtrate and purified enzyme, respectively, dough became sticky. This effect was also shown for *T. viridae* and *A. niger* xylanases *(8,26)*.

Xylanases produced by *Aspergillus* sp. FP-470 showed higher activity on soluble pectosans extracted from wheat than on the insoluble fractions *(27)*. Furthermore, it was demonstrated that these enzymes modify the water retention ability of wheat flour in a first-order kinetics *(27)*, supporting the idea that the main effect of the enzymes is to improve the redistribution of water from hemicellulose to gluten and starch, which will give a more extensible gluten network.

The apparent low thermal stability of XYL22 could be an advantage in bread making since the enzyme will lose its activity during baking and no deleterious effect during storage would be expected.

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