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Improvement of Highly Thermostable Xylanases Production by *Talaromyces thermophilus* for the Agro-industrials Residue Hydrolysis

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Abstract A newly isolated thermophilic fungal strain from Tunisian soil samples was identified as *Talaromyces thermophilus* and was selected for its ability to produce extracellular hemicellulases when grown on various lignocellulosic substrates. Following the optimization of carbon source, nitrogen source, and initial pH of the growth medium in submerged liquid cultures, yields as high as 10.00±0.15 and 0.21±0.02 U/ml were obtained for xylanase and β-xylosidase, respectively. In fact, wheat bran was found to be a good inducer of hemicellulase enzymes, mainly β-xylosidase. The optimal temperature and pH of the xylanase activity were 75°C and 8.0, respectively. This enzyme exhibited a remarkable stability and retained 100% of its original activity at 50°C for 7 days at pH7.0–8.0. The half-lives of the enzyme were 4 h at 80°C, 2 h at 90°C, and 1 h at 100°C. *T. thermophilus* could therefore be considered as a satisfactory and promising producer of thermostable xylanases. Crude enzyme of *T. thermophilus* rich in xylanase and β-xylosidase was established for the hydrolysis of lignocellulosic materials as wheat bran.

Keywords Xylanase · β-xylosidase · *Talaromyces thermophilus* · Wheat bran · Xylose

Introduction

Xylan is one of the most abundant plant structural polysaccharides. This heterogeneous polymer consists of a backbone of β-1,4-linked xylopyranose residues decorated with acetyl, arabinofuranosyl, and 4-methyl-*O*-glucuronyl side chains [1].

Due to its structural heterogeneity, xylan's complete degradation requires the cooperative action of a variety of xylanolytic enzymes classified according to their action on distinct substrates, endo-1,4-β-xylanase (EC 3.2.1.8) generates oligosaccharides from the cleavage of xylan, and xylan 1,4-β-xylosidase (EC 3.2.1.37) produces xylose from oligosaccharides [2].

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In addition, hemicellulose degradation needs accessory enzymes such as xylan esterases, ferulic and *p*-coumaric esterases, α-1-arabinofuranosidases, and α-4-*O*-methyl glucuronosidases, acting synergistically to efficiently hydrolyze wood xylans and mannans.

Xylanolytic enzymes are generally induced by lignocellulosic residues that contain xylan and are subjected to catabolite repression by easily metabolizable sugars [3]. In fungi, it is well established that extracellular xylanases have been shown to be constitutively expressed at basal levels. These enzymes are crucial for an initial hydrolysis of the polysaccharide in low-molecular-mass degradation products, penetrate into the cells, and induce xylanase expression at a high level [3, 4].

In fact, xylanases from various microorganisms have attracted a great deal of attention in the last few decades because of their biotechnological potential in various industrial processes such as the clarification of wine and juice, the separation of starch, and the production of functional food ingredients and have therefore been involved in the improvement of the quality of bakery products and animal foodstuff supplements [5]. Xylanases have been proven to be particularly efficient in paper and pulp industries where thermostable cellulase-free and alkaline-active xylanase is exceptionally required for the biobleaching of various kinds of pulp [6]. Xylanolytic enzymes have opened new possibilities for the bioconversion of agricultural wastes into easy fermentable products [7]. Recently, xylanases have attracted increasing attention for several new applications: coffee extraction, protoplastation of plants cells, and production of alkyl glycosides for use as surfactants and washing of precision devices and semi-conductors [8].

Tolerance to high pH and temperature is a desirable property of xylanases. In fact, only few fungal strains produce alkali-tolerant, cellulase-free xylanase when grown under alkaline conditions (pH8–10) [9].

As cost of the enzyme is one of the main influential factors in the determination of the product ultimate price, in order to make the enzyme's industrial application more cost-effective, many researchers have recommended the use of production techniques that rely on low-cost substrates [10]. Agro-industrial residues are generally considered efficient and inexpensive substrates for the process of enzyme production [11]. Several uses have been suggested for bioconversion of agro-industrial residues, among them are used as raw material for the production of ethanol after being hydrolyzed into simple fermentable products [12]. A particular concern is the cost effectiveness of the composition of the culture medium. It has recently been estimated that around 30–40% of the production cost was allocated to the growth medium [13].

A thermotolerant *Talaromyces thermophilus* fungus growing at 50 °C was isolated in our laboratory. In a previous study, our research team reported on the purification of endo-1,4-β xylanase [14] and β-xylosidase [15].

In the present work, we intended to improve xylanase production by *T. thermophilus* by the optimization of submerged culture conditions. The properties of the crude enzyme were also studied to identify its ideal conditions of applications to explore the synergistic action of this xylanolytic complex rich in xylanase, β-xylosidase, and other debranching enzyme activities to break down lignocellulosic materials into simple fermentable sugars, mainly xylose.

Materials and Methods

Source of Fungal Strains

Several samples were collected from Tunisian soil and different thermophilic fungi were screened for their xylanase activity. One of these fungi, identified by CBS (Centraalbureau

voor schimmelcultures, code reference: detail 274-2003) as *Talaromyces thermophilus stolk*, was isolated from El Hamma thermal station in the south of Tunisia and used for the production of the thermoactive xylanase presented in this study.

The *T. thermophilus* strain was also deposited in national strain bank of Tunisia: "Tunisian Collection of Microorganisms" CTM10 103 (Centre of Biotechnology of Sfax, Tunisia).

Screening Medium and Method

The basal medium used for screening the xylanase-producing thermophilic fungi was a slightly modified Mandel's medium [16]. The basal medium contained KH_2PO_4 1 g/l, K_2HPO_4 2.5 g/l, $(NH_4)_2SO_4$ 1.4 g/l, $MgSO_4{\cdot}7H_2O$ 0.3 g/l, $CaCl_2$ 0.3 g/l, yeast extract 1 g/l, urea 0.7 g/l, Tween 80 1 ml/l, and 1 ml/l of an oligoelement solution ($MnSO_4$, 1.6 g/l; $ZnSO_4$, 1.4 g/l; $FeSO_4$, 5 g/l; $CoCl_2$, 2 g/l). One percent of oat splet xylan and 18 g/l Agar-agar (Fluka) were added to this medium. After sterilization, two antibiotics, ampicillin 50 μg/ml and tetracycline 20 μg/ml, were added. The medium was inoculated by the soil samples that were previously suspended in 10 ml of sterile water. Finally, all inoculated media were incubated at 50°C for 5 days.

This *T. thermophilus* strain was identified as a xylanase producer as it formed a clear digestion halos on oat spelt xylan agar plates after being soaked with Congo Red and washed with 1 M NaCl.

Media and Growth Conditions

Cultivation was performed in baffled Erlenmeyer flasks containing 2% of carbon source moistened with 100 ml of slightly modified Mandel's medium [16] KH_2PO_4 1 g/l, K_2HPO_4 2.5 g/l, $(NH_4)_2SO_4$ 1.4 g/l, $MgSO_4{\cdot}7H_2O$ 0.3 g/l, $CaCl_2$ 0.3 g/l, yeast extract 1 g/l, urea 0.7 g/l, and Tween 80 1 ml/l acting as a surfactant agent to promote the enzyme secretion [17] and to facilitate their desorption from the substrate present in the culture medium.

The medium was supplemented with 1 ml/l of oligoelement solution composed by $MnSO_4$ 1.6 g/l, $ZnSO_4$ 1.4 g/l, $FeSO_4$ 5 g/l, and $CoCl_2$ 2 g/l. Oligoelements are necessary for the fungal growth [16]. Following heat sterilization (121 °C) for 20 min, each flask was inoculated with 10^6 spores suspension and incubated for 6 days at 50 °C on a rotary shaker (160 rpm). Experiments were carried out in triplicate.

A step-by-step optimization procedure regarding the effect of important parameters (carbon nitrogen source and initial growth pH) on enzyme production was employed.

Oat spelt xylan, beech wood xylan, birch wood xylan, wheat bran, and rabbit food at 2% each were the carbon sources investigated. Under these conditions, initial Mandel's nitrogen sources (yeast extract 1 g/l, urea 0.7 g/l, $(NH_4)_2SO_4$ 1.4 g/l) and an initial pH medium (pH7.0) were remained fixed.

Ammonium nitrate, yeast extract, ammonium sulfate, pastone, peptone, urea, and their combination were the nitrogen sources examined. Constant C/N ratio was maintained at 12. The initial culture pH ranged between 3.0 and 9.0 and was adjusted using 1 M NaOH or 1 M H_3PO_4 before sterilization.

Enzyme Extraction

After suitable time intervals, aliquots were withdrawn and centrifuged at 6,000×*g* for 15 min to obtain a clear supernatant which was used for all subsequent analyses.

Enzyme Assays

The xylanase activity was determined using xylan oat spelt 1% (Sigma) in 0.05 M potassium phosphate buffer, pH7.0. The reaction mixture, whose total volume was 1 ml and that basically contained 0.5 ml of substrate and 0.5 ml of appropriate solution, was incubated at 50°C [18]. After 30 min of incubation at 50°C, the reducing sugar produced in the reaction mixture was assayed by the dinitrosalicylic acid method with D-xylose as the standard [19]. All activity measurements were performed at least in triplicates. One unit of xylanase activity is defined as the amount of enzyme required to release 1 μmol of xylose per minute. The β-xylosidase activity was measured by a spectrophotometric method with *p*-nitrophenyl β-D-xylopyranoside as substrate [20]. The assay mixture contained 900 μl of pNPX 4 mM in 50 mM phosphate buffer, pH7.0, and 100 μl of enzyme solution. After incubation at 50°C for 30 min, the reaction was stopped by the addition of 1 ml of (2 M) Na_2CO_3. The absorbance resulting from the release of *p*-nitrophenol was measured at 410 nm. One unit of enzyme was required to release 1 μmol of *p*-nitrophenol per minute in the reaction mixture. The proteins in the culture supernatant were quantified using the Bradford method [21].

Thin-Layer Chromatography

Products of enzymatic hydrolyses were analyzed by thin-layer chromatography (TLC) on silica gel G-60 using chloroform/acetic acid/water (6:7:1 by volume) as a mobile phase system. Sugars were detected with 0.2% (*w*/*v*) orcinol in sulfuric acid/ethanol (10:90).

Enzyme Characteristics

The optimum temperature of the crude xylanases was determined by performing enzyme assays at temperatures ranging between 40 and 100°C. The enzyme thermostability was determined by measuring the residual activity after incubation of the enzyme for 4 h at temperatures ranging from 50 to 100°C.

The optimum pH for xylanase activity was determined by incubating the supernatant with xylane for 10 min at 50°C in different buffers: 50 mM citrate (pH3, 4, and 5); 50 mM phosphate (pH6, 7, and 8); 50 mM AMPSO buffer (pH9); and 50 mM glycine (pH10 and 11). The stability at 4°C in various pH conditions was assessed by incubating the enzyme at different pH and by measuring the residual activity after 24 h using the standard protocol.

Enzymatic Hydrolysis of Xylan and Agro-industrial Residues

Xylan (2%) was hydrolyzed by 1 ml of crude enzyme (produced on three different carbon sources: xylan, wheat bran, and rabbit food) during 15 h at 70°C and pH7.0.

Furthermore, four types of agro-industrial residues (wheat bran, barley bran, bagasse, and rabbit food) were used for xylose production by enzymatic hydrolysis. Hemicellulose fractions were extracted according to the method of Dien et al. [22]. This method consists of incubated 1 g of each residue 30 min at 180°C in 10 ml phosphate buffer, pH7.0. After the hydrothermal pretreatment, 1 ml of crude enzyme of *T. thermophilus* (containing 10.5 U/ml of endoxylanase, 0.21 U/ml of β-xylosidase, and a total protein of 334 mg/l) was added to each preparation and the samples were incubated at 70°C for 7 h at continuous agitation.

After suitable time intervals, aliquots were withdrawn, centrifuged, and analyzed by HPLC, and the reducing sugars were estimated by dinitrosalicylic acid [19].

High-Performance Liquid Chromatography Analysis

The hydrolysis products were analyzed by HPLC (Aminex HPX-42A, column 7.8×300 mm). In the latter method, the products were separated by elution with water at a flow rate of 0.6 ml/min and detected with a refractive index detector (SHIMADZU, RID-10A). A solution of oligosaccharides (xylose and xylobiose), at 10 g/l each, was used as a standard.

Results and Discussion

Effect of Carbon Sources on Xylanase Production

A variety of complex carbon sources was examined in the present work. The level of xylanase produced by *T. thermophilus* was largely dependent on the carbon source used in the medium (Fig. 1). The highest levels of activity were observed at 5 days of growth for most of the substrates studied. Among xylan as a carbon source, oat spelt xylan was the most effective for xylanase production followed by beech wood or birch wood xylan; it was selected for further studies.

The use of wheat bran, a nutrient-rich intermediate of the wheat processing industry, resulted in satisfactorily appreciable enzymes levels. This actually reflects the significant presence of high proportions of arabinoxylan in this lignocellulosic material, which is the most inexpensive and suitable for hemicellulase induction by filamentous fungi [23]. Other carbon sources used, namely, barley bran and rabbit food, permit obtaining lower xylanase activities.

On the other hand, monosaccharides and disaccharides were poor inducers of xylanase activities by *T. thermophilus*. When 2% of xylose was added as a carbon source, the biosynthesis of xylanase and β-xylosidase decreased drastically to 0.50±0.04 and 0.023± 0.002 U/ml, respectively. This inhibition could be interpreted in terms of catabolite repression as described previously [3].

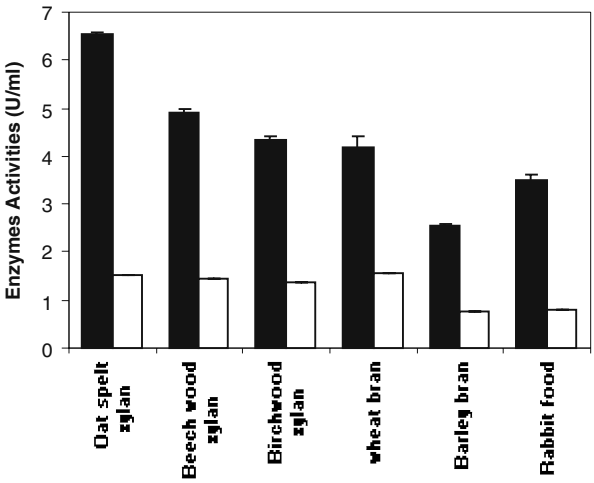

Fig. 1 Effect of carbon sources on xylanase (*filled square*) and β-xylosidase ($\times 10^{-1}$, *empty square*) production by *T. thermophilus*. Enzyme activity was measured after 5 days of culture. The available carbon source was used at concentration of 2% (*w*/*v*). The experiments were conducted three times and standard errors are reported

Conversely, xylose was previously reported as a good inducer of xylanases for certain fungi such as *Aspergillus fischeri* [24] and *Trichoderma longibrachiatum* [25].

Effect of Initial Medium pH on Xylanase Production

The pH value has also been reported to play an important role in controlling the expression of many genes, especially carbohydrolase genes of filamentous fungi [26]. Xylanase production by *T. thermophilus* at different initial pH was examined, maintaining oat spelt xylan as a carbon source previously optimized and initial nitrogen source of Mandel's medium (yeast extract 1 g/l, urea 0.7 g/l, $(NH_4)_2SO_4$ 1.4 g/l).

The effect of initial pH value on enzyme production is depicted in Fig. 2. The highest xylanase and β-xylosidase activities were obtained with initial pH values ranging from 8 to 9. The xylanase and β-xylosidase production was practically lightly affected by the pH value of the growth medium compared to other fungi such as *Aspergillus* [27] and *Penicillium* [28] where it was observed that the enzyme's production depends significantly on the initial pH of fermentation.

Effect of Nitrogen Sources

Keeping the other culture conditions previously optimized (xylan oat spelt as a carbon source and the optimum initial pH (pH8), different nitrogen sources were supplemented to the growth medium. The results show that the levels of enzyme activities were generally lower when complex organic sources such as peptone and yeast extract were employed (Fig. 3). The highly xylanase (9.87±0.10 U/ml) and β-xylosidase (0.20±0.01 U/ml) activities could be obtained with both organic (urea) and inorganic (ammonium nitrate NH_4NO_3) nitrogen; an improvement of 40% of xylanase production was observed. Ammonium sulfate ($(NH_4)_2SO_4$) was by far an effective nitrogen source for hemicellulase production by *T. thermophilus.* These findings are in contrast to previous works where it was reported that fungi produced more xylanases on complex organic nitrogen sources [29]. The sodium nitrate ($NaNO_3$) or potassium nitrate (KNO_3) used as single sources of nitrogen inhibited completely the production of xylanases and restricted the quantity of the biomass produced (data not

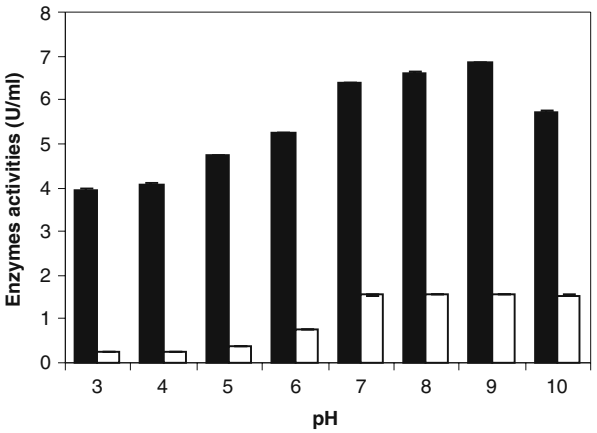

Fig. 2 Effect of initial culture pH on xylanase (*filled square*) and β-xylosidase ($\times 10^{-1}$, *empty square*) production by *T. thermophilus*. The experiments were conducted three times and standard errors are reported

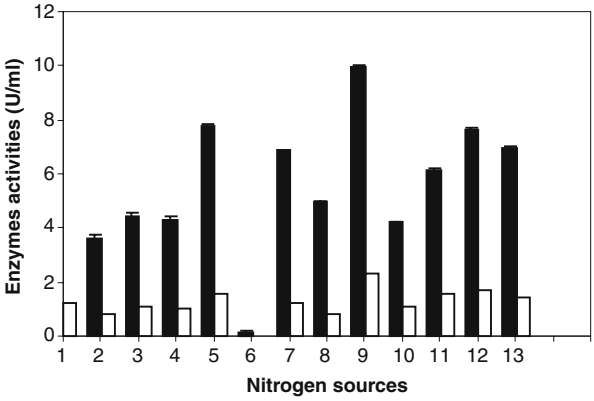

Fig. 3 Effect of organic and inorganic nitrogen sources on xylanase (*filled square*) and β-xylosidase ($\times 10^{-1}$, *empty square*) production by *T. thermophilus*. Enzyme activity was measured after 5 days of culture. *1* urea, *2* yeast extract, *3* peptone, *4* ammonium sulfate, *5* ammonium nitrate, *6* sodium nitrate, *7* urea + ammonium sulfate, *8* urea + sodium nitrate, *9* urea + ammonium nitrate, *10* ammonium sulfate + yeast extract, *11* urea + yeast extract, *12* ammonium nitrate + sodium nitrate + ammonium sulfate, *13* Mandel's medium nitrogen sources (ammonium sulfate + yeast extract + urea). The experiments were conducted three times and standard errors are reported

shown). It seems that nitrate is not assimilated by *T. thermophilus*; this was also observed with *Trichoderma reesei* [30]. Ammonium salts was found to be the most appropriate inorganic nitrogen source for xylanase production. This nitrogen source seems to be necessary for a maximum production of xylanases by several fungi such as *Aspergillus awamori* [27] and *Chaetomuim cellulolyticum* [31].

Application of Optimal Conditions

Each of the optimized parameters was retained in the process and was simultaneously employed in a single fermentation. Therefore, xylanase production was studied for 7 days at 50 °C and at an optimal initial pH of 8 in the optimized medium containing 2% oat spelt xylan as carbon source substrate and urea and ammonium nitrate at a C/N ratio of 12.

The time course of xylanase activities, reducing sugar, and protein concentrations of *T. thermophilus* on optimized medium is presented in Fig. 4. Enzyme biosynthesis began after 24 h of culture; there is a time needed for the spore germination and the mycelium growth. This time is also necessary for the xylan hydrolysis into oligosaccharides acting as xylanase inducers. The xylanase production reached a maximum after 5 days. After optimization, xylanase activity was enhanced from 6.5 to 10 U/ml. We also observed a notable enhancement in the level of β-xylosidase production from 0.15 to 0.21 U/ml.

From the sixth day onwards, a small decrease in xylanase production was observed after a protease activity was detected (data not shown). The production remains stable until the seventh day for β-xylosidase.

Enzyme Characteristics

The thermoactivity and thermostability of the crude xylanase from *T. thermophilus* are shown in Fig. 5a. The optimum temperature of xylanase was 75 °C at pH7.0. It exhibited remarkable stability and retained 100% of its original activity at 50 °C for 3 days at pH7.0–8.0 (Fig. 5b),

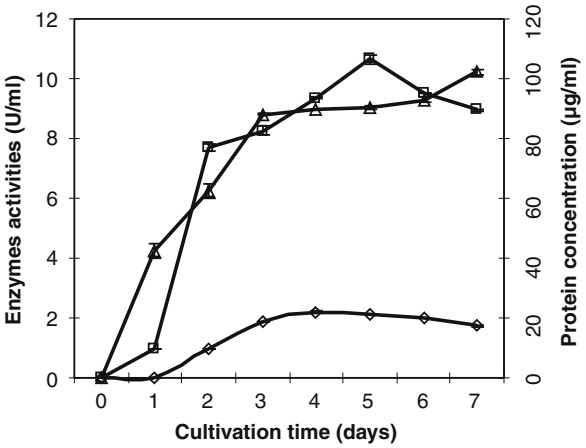

Fig. 4 Xylanase (*square*) and β-xylosidase ($\times 10^{-1}$, *circle*) activities and proteins concentrations (*triangle*) produced by *T. thermophilus* grown under optimal (carbon source: xylan oat spelt; nitrogen sources: urea and ammonium nitrate and initial culture, pH 8.0). The experiments were conducted three times and standard errors are reported

and the values of the half-life times were 4, 2, and 1 h at temperatures of 80, 90, and 100 °C, respectively. The xylanase of *T. thermophilus* turns out to be among the most thermostable enzymes secreted by thermophilic fungi.

The optimum pH of the crude xylanase ranged between 7.0 and 8.0, and the enzyme was very stable over a considerable pH range from 6 to 9 (70% of residual activity after 24 h).

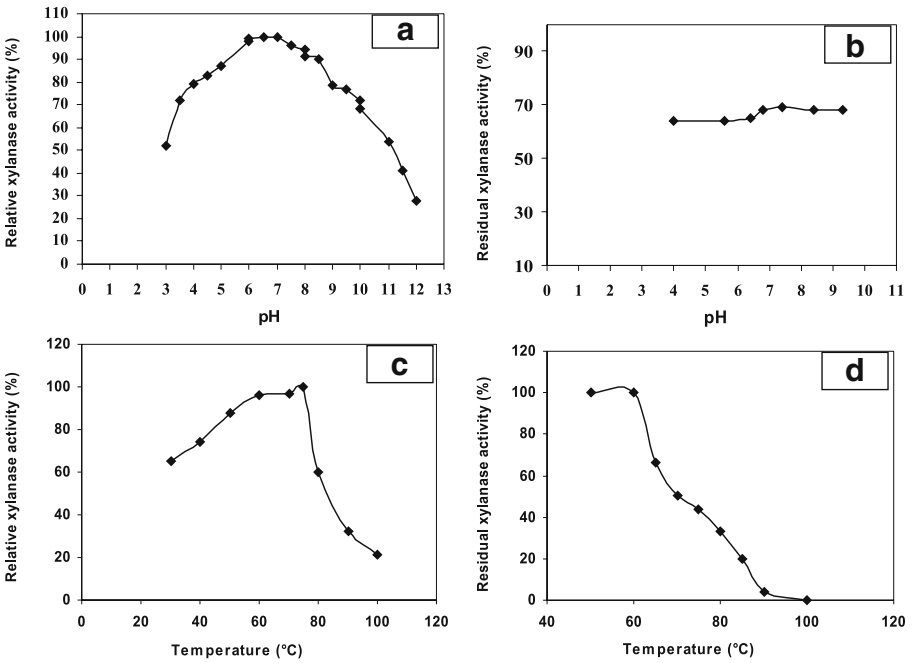

Fig. 5 Optimal pH (**a**), pH stability (**b**), optimal temperature (**c**), and thermal stability (**d**) of the crude extracellular xylanase produced by of *T. thermophilus*

Most of the xylanase reported work efficiently in pH optima ranging from 4.5 to 6.5 and the temperature optima ranging from 55 to 65 °C [32]. However, stability in an alkaline environment and high-temperature condition is rarely observed. Only *Melanocarpus albomyces* has a half-life of 2 h at 70 °C at pH 10 [33]. Alkaline-tolerant and thermotolerant xylanase from *Thermomyces* sp. with a half-life of 4 h at 70 °C, pH 6.5, [34] and from *Thermoactinomyces thalophilus* with a half-life of 2 h at 65 °C, pH 8.5 [35], were also reported.

Crude enzyme exhibits a number of highly appealing and promising features that make it a strong candidate for future industrial applications mainly in the pulp bleaching technology since it is cellulase-free xylanase produced (data not shown).

The use of xylanases for delignification in the paper industry has been slowed down by the lack of large-scale availability of enzymes active at pH above 8 and temperature of around 60 °C, which are the prevalent conditions in many bleaching processes [6]. Xylanase of *T. thermophilus*, which has the required properties, was compared with some commercial xylanases. As seen in Table 1, this xylanase seems to be promising for use in the pulp bleaching processes.

Enzymatic Hydrolysis of Xylan

Xylan was hydrolyzed by three types of enzymatic extracts (produced on three different carbon sources: xylan, wheat bran, and rabbit food).

Figure 6a shows that the enzymatic extract obtained on wheat bran as a carbon source was the most effective for xylan hydrolysis. At the beginning of the reaction, the higher quantity of reducing sugars was released by the crude preparation obtained on xylan as a carbon source. After 8 h of incubation, the quantity of reducing sugars released by the crude preparation obtained on wheat bran as a carbon source increased and exceeded that of the first one extract. This could be explained by the presence of other debranching enzymes better induced by wheat bran that contributed by their synergetic action to the degradation of the oligosaccharides.

Table 1 Characteristics comparison of some commercial xylanases with *T. thermophilus* xylanase.

Trademark name	Distributor	Source	Operational condition	Optimum condition	Reference
This xylanase	–	*Talaromyces Thermophilus*	–	50–75 °C pH 6–9 Half-life (1 h 100 °C, pH 7)	This study
Cartazme	Clariant, UK, Basel, Switzerland Sandoz, UK	*Thermomonospor fusca*	35–55 °C, pH 3–5, 2–10 h	45–55 °C, pH 5	[6]
Ecopulp X200	ICI Forest Products Primalco Biotec	*Trichoderma reesei*	50–55 °C, pH 5–6, 6 h	50–55 °C, pH 5–6	[6]
Irgazyme 40	Nalco-Genecor, Finland Ciba Giegy, Switzerland	*Trichoderma longibrachiatum*	50–55 °C, pH 6.6–7.8, 1 h	50–60 °C, pH 7–8	[6]
Pulpzyme	Novozymes, Denmark	*Bacillus* sp. VI-4	50–70 °C, pH 7–8, 3 h	50 °C, pH 9.5	[6]

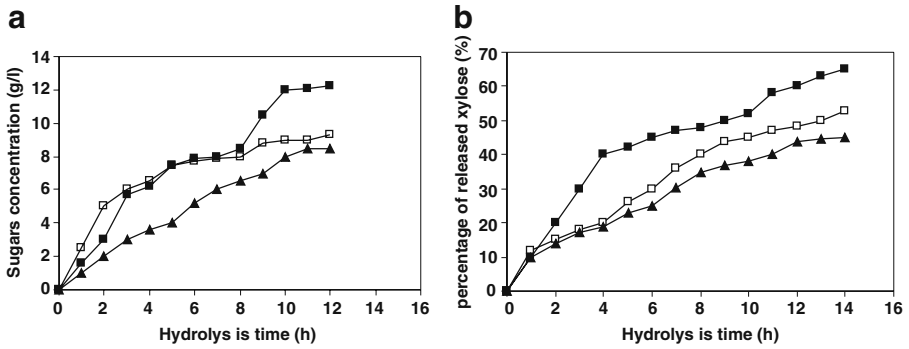

Fig. 6 Hydrolysis of xylan at 70 °C by three types of enzymatic extracts: enzymatic extract produced on xylan (*empty square*), enzymatic extract produced on wheat bran (*filled square*), and enzymatic extract produced on rabbit food (*triangle*). **a** Measuring of liberated reducing sugar. **b** HPLC analysis of hydrolysis products

The released sugars were analyzed by HPLC using mainly xylose, xylobiose, and xylotriose as standards. During the hydrolysis reaction, crude enzymes released mainly xylose (Fig. 6b), which is the major product of xylan degradation. The presence of a small quantity of xylobiose detected after the hydrolysis was explained by the presence of β-xylosidase activity which degrades xylobiose to xylose.

The hydrolysis products of xylan by the crude enzyme were also analyzed by TLC. The results (Fig. 6c) obtained show that oligosaccharides with comparatively high polymerization formed in the initial stage of reaction are progressively degraded to oligosaccharides with lower DP. In the late reaction stages, the xylo-oligosaccharides with low DP tend to be hydrolyzed into xylose (Fig. 7).

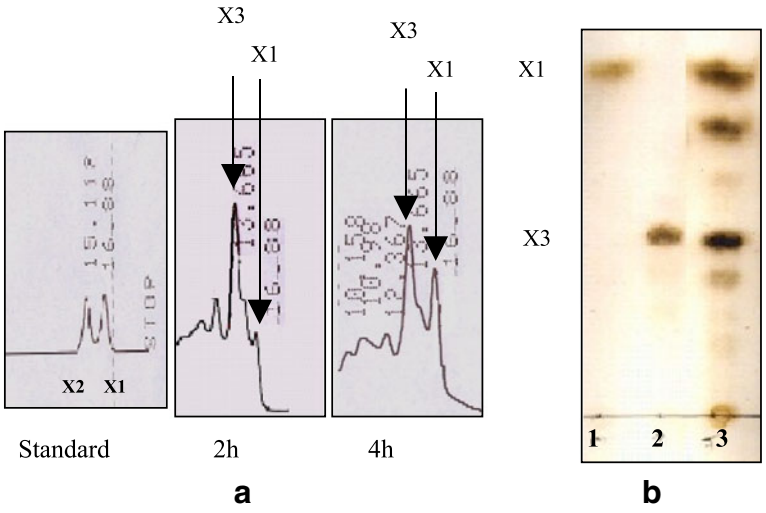

Fig. 7 **a** Chromatographic response analysis of xylo-oligosaccharide after oat spelt xylan hydrolysis by the crude xylanase after different times (2 and 4 h). *X1* xylose, *X2* xylobiose, *X3* xylotriose. **b** TLC analysis of the mixture derived from oat spelt xylan hydrolysis by the crude xylanase after 6 h. *X1* xylose, *X3* xylotriose

Hydrolysis of Agro-industrial Residues

The potential of hemicellulotic enzymes produced by *T. thermophilus* was examined during the saccharification of some agro-industrial residues.

The results summarized in Table 2 show that enzymatic hydrolysis of the different substrates liberated various concentrations of reducing sugars. These concentrations were largely dependent on incubation time and lignocellulosic material composition. The incubation time was prolonged to 10 h for all substrates, but we did not observe any increase in reducing sugar concentration (data no shown).

The HPLC analysis proves the liberation of xylose from all lignocellulosic substrate and shows the ability of the xylanase of *T. thermophilus* to act synergistically with other xylanolytic enzyme to produce xylose. Wheat bran can be considered as a potential substrate for xylose production with 4.6 mg/g of substrate.

The crude enzyme of *T. thermophilus* rich in xylanase, β-xylosidase, and other debranching enzyme activities was advantageously applied for the production of xylo-oligosaccharides, mainly xylose from xylan, and other substrates. The increasing interest in biotechnological processes employing lignocellulosic residues is quite justifiable because these materials are cheap, renewable, and readily available sources of sugars [36–38].

Conclusion

The present work has established the potential of the newly isolated thermophilic *T. thermophilus* fungus for hemicellulase production. *T. thermophilus* is an interesting source of new thermostable xylanase and can therefore be considered a strong candidate with excellent potential for future industrial and commercial applications. At 50°C, the xylanase provides the kind of enzymatic stability that present-day industrialists yearn for. The main hydrolysis products yielded from xylan by this xylanolytic complex were xylose and other xylo-oligosaccharides such as xylotriose. This complex of *T. thermophilus* can be considered as a good alternative for lignocellulosic material waste saccharification and mainly for xylose production. This certainly prompts further investigations on the continuous production of xylo-oligosaccharides on a large scale. Considering the important implications of the results obtained in the present study, further studies are currently under way in our laboratory to improve xylanase production from *T. thermophilus* fungus to make it suitable for future industrial application. Large-scale batch fermentation giving 25 U/ml of xylanase activity (data not shown) was investigated. Alternatively, genetic manipulations by classical mutation techniques and more recently by the use of recombinant DNA technology (disruption of CREA gene and heterologous expression of xylanolytic gene) were in the course of realization to improve xylanase production.

Table 2 Hydrolysis of agro-industrial residues by the crude xylanase and xylose production.

Sample	Amount of liberated sugar (mg/g of substrate)[a]	Percentage of released xylose (mg/g)
Wheat bran	183	4.6
Barley bran	160	1.3
Bagasse	161	3.9
Rabbit food	54	1

[a] Sugar concentration expressed in milligrams per gram of agro-industrial residues

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