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Thermo-Stable Xylanases from Non Conventional Yeasts

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Abstract

Xylanase is a commercial enzyme that has considerable applications in different types of industries, such as the feed, food, textile and paper industries, amongst others. The main goal of the present work was to select and evaluate the enzyme production from isolated wild yeasts strains obtained from several different Brazilian regions. From a total of 349 strains, two were selected, namely LEB-AAD_5 and LEB-AY_{10}, which produced fairly stable enzymes. The characterization studies showed that the strain LEB-AAD_5 produced an enzyme with the following optimal range: temperature from 57.5 to 67.5 ºC and pH from 4.7 to 5.5, with a half-life of 21.33 hours at 52ºC and pH 5.3, *Vmax* of 1.77 µmol/mL.min and Km of 0.44 g/L. The enzyme from strain LEB-AY_{10} showed the following optimal range: pH from 4.1 to 4.8 and temperature around 80ºC, with a half-life of 11.21 hours at 72ºC and pH 5.3, *Vmax* of 5.47 µmol/mL.min and Km of 1.37 g/L. Xylanase from LEB-AY_{10} strain is more Thermo-stable than that of LEB-AAD_5, both of which belongs to *Cryptococcus* sp. After optimized using two experimental designs, xylanase production increased by 600%, reaching 11.25 IU/mL under the optimal fermentation conditions (30°C, initial pH of 6.0 and 20 g/L of xylan as substrate).

Keywords: Xylanase; Enzyme characterization; Wild yeast selection; Xylan

Introduction

The enzymatic hydrolysis of xylan is one of the most important industrial bioprocesses, xylan being the second most abundant natural polysaccharide [1,2]. It is a heteropolysaccharide consisting of a chain of β-1,4-linked D-xylanopyranose units with substitution by acetyl, arabinosyl and glucopyranosyl residues. The complete hydrolysis of xylan requires the action of several enzymes, including endo-1,4-β-D-xylanase (EC3.2.1.8), which is crucial for xylan depolymerization. Xylanases have applications in animal feed digestion, food industries and as bleaching agents in the pulp and paper industries [3]. However, the main application of xylanases is in the pulp and paper industry, where they are used in pre-treatment prior to bleaching [4].

The main chain of xylan is composed of β-xylopyranose residues [2]. Xylan is the most common hemicellulosic polysaccharide in the cell walls of land plants, representing up to 30%–35% of the total dry weight [5]. Xylan is the major hemicellulose in the hardwood from angiosperms, but is less abundant in the softwood from gymnosperms, accounting for approximately 15%–30% and 7%–12% of their total dry weights, respectively [2]. The xylan from hardwood is an 0-acetyl-4–0-methylglucuronoxylan, consisting of at least 70 β-xylopyranose residues [average degree of polymerization (DP) between 150 and 200], linked by β-1,4-glycosidic bonds. Every tenth xylose residue carries a 4-0-methylglucuronic acid attached to the 2 position of xylose. Hardwood xylans are highly acetylated (e.g. the birchwood xylan contains more than 1 mol of acetic acid per 2 mol of xylose), and acetylation is more frequent at the C-3 than at the C-2 position. The presence of these acetyl groups is responsible for the partial solubility of xylan in water and they are readily removed when the xylan is subjected to alkali extraction [6]. Xylans from softwood are composed of arabino-4-0-methylglucuroxylans and they have a higher 4-0-methylglucuronic acid content than hardwood xylans, the 4-0-methylglucuronic acid residues being attached to the C-2 position. Softwood xylans are not acetylated, and instead of an acetyl group they have α-L- arabinofuranose units linked by α-1,3-glycosidic bonds at the C-3 position of the xylose [2].

Due to the structural heterogeneity of the xylans, xylan-degrading enzyme systems include several hydrolytic enzymes, and xylan derivatives are frequently used to induce the production of xylanase by yeasts and fungi on a laboratory scale [7].

The main goal of this study was to screen wild yeast strain producers of xylanase in a liquid-state culture, using xylan as the main substrate, examine the effects of pH, temperature and substrate concentration on the crude xylanase stabilities, and optimize the production of xylanase from the selected microorganism.

Material and Methods

Screening and growth conditions

The total of 349 yeasts used in the screening process [8] was isolated from the soil and from the stems, fruits and especially from flowers of plants harvested in several Brazilian regions.

Xylanase producer cultures were selected according to their capability of degrading soluble xylan in Petri plates and s liquid cultures. The selective medium contained the following: 10.0 g/L birchwood xylan (Sigma); 0.6 g/L yeast extract; 7 g/L KH_2PO_4; 2.0 g/L K_2HPO_4; 0.1g/L $MgSO_4.7H_2O$; 1.0 g/L $(NH_4)_2SO_4$ and 15 g/L agar. The pH was adjusted to 5.0 and plates were incubated at 30ºC / 48 hours.

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The first selection was carried out according to the enzymatic ratio (diameter of the non-dyed halo/diameter of the colony) of higher than 2.5 [9]. The next selection step was performed according to the ability of the strain to produce large amounts of the enzyme in liquid cultures.

The selected yeasts were inoculated into a liquid culture medium containing: 10 g/L birchwood xylan; 5 g/L peptone; 3 g/L yeast extract; 7 g/L KH_2PO_4; 2.0 g/L K_2HPO_4; 0.1 g/L $MgSO_4.7H_2O$ and 1.0 g/L $(NH_4)_2SO_4$. The pH was adjusted to 6.0. The cultures were inoculated into 500 mL flasks containing 100 mL of medium and incubated at 30ºC and 150 rpm. Samples were taken at 12 h intervals, centrifuged at 6,000 rpm for 10 min at 4ºC and the supernatants were used to estimate the xylanase activities, as described below. Two strains, LEB-AY_{10} and LEB-AAD_5, were selected and both identified as *Cryptococcus* sp. strains, and their enzymes were characterized.

Enzymatic assay

The xylanase (1,4-β-D-xylanase) activity was assayed by incubating the diluted enzyme extract with a solution of 1% (w/v) birchwood xylan dissolved in 50mM pH 5.3 sodium citrate buffer at 50ºC for 5 min [10]. The amount of reducing sugars liberated was determined using the 3,5-dinitrosalicylic acid method [11]. One unit of xylanase activity (IU) was defined as the amount of enzyme necessary to produce 1 µmol of reducing sugars (D-xylose) from xylan per min at 50ºC.

Optimal activity conditions

First experimental design: A central composite design (CCD) with $k = 2$, was used to generate 11 treatment combinations, with the pH and temperature as the independent variables. Five levels were chosen for each variable, the upper and lower limits being set according to the literature. In the statistical model, Y denotes units of xylanase activity.

Table 1 shows the real levels corresponding to the coded settings, the treatment combinations and the responses of the microorganisms LEB-AY_{10} and LEB-AAD_5. Each design is represented by a second-order polynomial regression model, Eq. (1), to generate the surface plots:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 + \varepsilon \quad (1)$$

The test factors were coded according to the following regression equation: $x_i = (x_i - x_0)/\Delta X_i$. Where x_i is the 'coded' value and X_i is the real value of the i^{th} independent variable, X_0 is the real value at the center point, and ΔX_i is the step change value. The Statistical software 5.0 [12] was used for the regression and for the graphical analysis of the data. The significance of the regression coefficients was determined using the Student t-test, and the second-order model equation was determined by the Fisher test. The variance explained by the model was given by the multiple coefficient of determination, R^2.

Treatment	Coded setting levels		Real levels		Xylanase activity (IU/mL)	
	X_1	X_2	X_1	X_2	LEB-AAD_5	LEB-AY_{10}
1	-1	-1	55	3.8	0.363	0.613
2	1	-1	75	3.8	0.087	0.850
3	-1	1	55	5.8	0.500	0.780
4	1	1	75	5.8	0.207	1.440
5	-1.41	0	50	4.8	0.523	0.677
6	1.41	0	80	4.8	0.223	1.847
7	0	-1.41	65	3.4	0.043	0.683
8	0	1.41	65	6.2	0.573	1.080
9	0	0	65	4.8	0.823	1.257
10	0	0	65	4.8	0.883	1.173
11	0	0	65	4.8	0.743	1.257

Results represent the mean of three experiments.
X_1 = *T(ºC)*, X_2 = *pH*, code settings are X_1 = (temperature – 65)/10, X_2 = (pH – 4.8)/1.0.

Table 1: Process variables used in the first CCD, showing the treatment combinations and the mean experimental responses.

Variables	Coded setting levels		
	-1	0	+1
Xylan (g/L)	5	10	15
Yeast Extract (g/L)	1	2	3
$MgSO_4.7H_2O$ (g/L)	0.1	0.3	0.5
$(NH_4)_2SO_4$ (g/L)	0	2.2	4.4
Peptone (g/L)	1	2	3
pH	5	6	7
Temperature (°C)	25	30	35

Table 2: Process variables used in the Plackett & Burman design, showing the treatment combinations.

Treatment	Coded setting levels		Real levels		Xylanase activity (IU/mL)
	X_1	X_2	X_1	X_2	LEB-AY_{10}
1	-1	-1	13	26.5	3.600
2	1	-1	27	26.5	3.233
3	-1	1	13	33.5	1.267
4	1	1	27	33.5	1.367
5	-1.41	0	10	30	1.817
6	1.41	0	30	30	3.067
7	0	-1.41	20	25	2.033
8	0	1.41	20	35	1.400
9	0	0	20	30	10.417
10	0	0	20	30	11.250
11	0	0	20	30	10.833

Results represent the mean of three experiments.
X_1 = *xylan (g/L)*, X_2 = *T(ºC)*, code settings are X_1 = (xylan – 20)/7, X_2 = (temperature – 30)/3.5.

Table 3: Process variables used in the second CCD, showing the treatment combinations and the mean experimental response.

i. **Thermal and pH stability:** The temperature and pH stability of the enzyme extract were determined by incubation at different values. Samples were taken at different intervals, depending on the temperature and pH used, and the residual enzyme activity measured.

ii. **Kinetics determination:** The initial reaction rates were determined at different substrate concentrations ranging from 0.5 to 30 mg of birchwood xylan/ml of 50mM pH 5.3 sodium citrate buffer at 50ºC. The kinetic constants K_m and V_{max} were estimated according to the method of Lineweaver and Burk [13].

iii. **Optimizing the xylanase production:** The plackett and burman design with the aim of optimizing xylanase production by the LEB-AY_{10} strain, under adequate medium and fermentation conditions, a Plackett & Burman (PB) design with 12 assays was applied. The independent variables and levels can be seen in Table 2.

Four central points were added to the 12 PB assays, giving a total of 16 assays. The effect of each variable and error were calculated using Statistica (Statsoft Corporation, USA) software, the enzymatic activity being the main response (dependent variable).

Second experimental design: The PB design indicated the variables that were significant in the enzyme production, and these were assayed using the CCD and the optimal xylanase production conditions obtained from the response surface generated by the mathematical model. The independent variables and levels can be seen in Table 3.

Results and Discussion

Culture selection and growth conditions

The yeast strains were evaluated in Petri plates, however only 9 of the 349 tested showed an enzymatic ratio above 2.5, these strains being shown in Table 4. These strains were assayed for their enzyme production capacity in submerged medium, and the strains with better characteristics in terms of enzyme activity and stability, LEB-AAD_5 and LEB-AY_{10}, were chosen for the following assays.

Figure 1 shows the enzyme production of the microorganism LEB-AY_{10}, also showing its pH behavior. The production started after practically 24 hours of fermentation, with a peak of activity at 36 hours followed by a slight decrease, although the activity can be considered similar due to the standard deviations. The enzymatic activity was about 0.67 IU/mL up to the end of fermentation, and showed good stability with respect to the effects of both pH and temperature.

Strains	Region	Enzymatic Ratio
AAD_5	Cerrado	2.9
AAF_4	Cerrado	6.7
AB_{05}	Atlantic forest1	2.8
AF_4	Amazonian rain forest	4.0
AY_{10}	Atlantic forest 2	3.0
AZ_{15}	Atlantic forest 2	3.0
J_{06}	Pantanal	2.5
Q_{03}	Pantanal	3.8
T_{05}	Atlantic forest 1	3.3

Table 4: Average enzymatic ratios from the Petri plates.

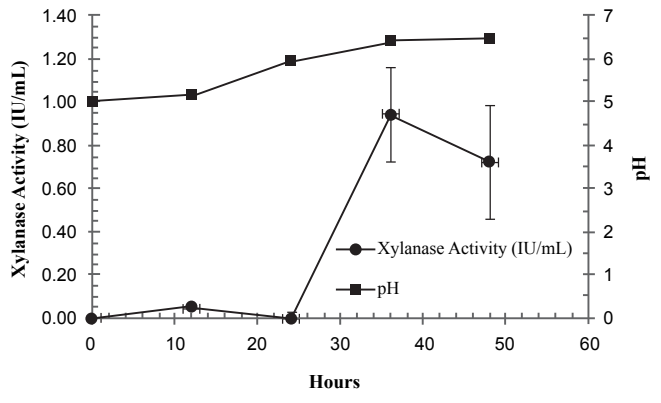

Figure 1: Xylanase activity and pH variation during LEB-AY_{10} fermentation in supplemented medium.

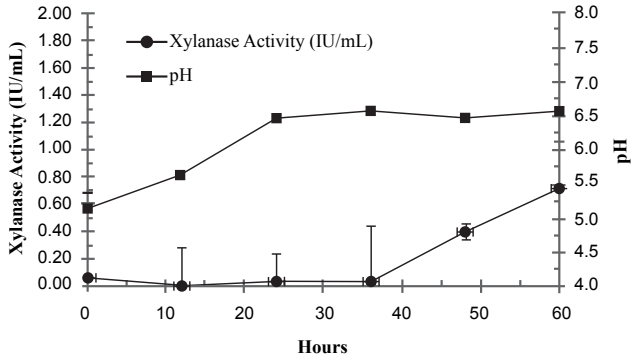

Figure 2: Xylanase activity and pH variation during LEB-AAD_5 fermentation in supplemented medium.

	LEB-AAD_5 strain				LEB-AY_{10} strain			
	SS	DF	MS	F_{value}	SS	DF	MS	F_{value}
Regression	0.803	4	0.077	9.71	1.290	3	0.121	4.90
Residual	0.047	6	0.008	-	0.174	7	0.025	-
Total	0.851	10	-	-	1.465	10	-	-
$F_{4, 6, 0.05}$ = 4.53 , (R^2) = 94.39%. SS, sum of squares, DF, degrees of freedom, MS, mean square. Significance level = 95%.					$F_{3, 7, 0.05}$ = 4.35 , (R^2) = 88.13%. SS, sum of squares, DF, degrees of freedom, MS, mean square. Significance level = 95%.			

Table 5: Analysis of variance (ANOVA) for the model regressions.

Figure 2 shows the pH behavior and enzyme production of the LEB-AAD_5 strain, the highest enzymatic activity of 0.73 IU/mL being produced after 60 hours of fermentation, then decreasing to about 0.50 IU/mL at the end.

Optimization of the conditions for the enzyme activities

The temperature and pH were optimized using factorial design methodology, and the results for the first experimental design are shown in Table 1. The optimal temperature and pH conditions for this enzyme were around 65°C and 4.8, respectively (assays 9, 10 and 11). The data from Table 1 were used to obtain the coded models for the LEB-AAD_5 and LEB-AY_{10} strains, equations (2) and (3), and the ANOVA (Table 5) confirmed that the models described the enzymatic activity well as a function of pH and temperature, since the values for $F_{calculated}$ were higher than those for F_{listed} for both models, with R^2 values higher than 0.88. The response surfaces were built using equations (2) and (3) and are represented in Figure 3 and 4.

$$Y = 0.816 - 0.124 \cdot T - 0.234 \cdot T^2 + 0.126 \cdot pH - 0.266 \cdot pH^2 \quad (2)$$

$$Y = 1.209 + 0.319 \cdot T + 0.164 \cdot pH - 0.206 \cdot pH^2 \quad (3)$$

According to the analysis of the response surfaces and contour plots, it can be seen in Figure 3 that the highest enzymatic activity was achieved for the LEB-AAD_5 strain under the center point conditions, that is at a temperature of 65°C and a pH of 4.8, whereas for the enzyme from LEB-AY_{10}, the highest activity was achieved at a pH around the center point (4.8), but at a temperature as high as 80°C, different from the enzyme produced by the acidophilic fungus *Bispora* sp. MEY-1 and reported by Luo et al. [14], which showed its optimal conditions at 65°C and pH 2.6.

In order to confirm the effects of temperature on the LEB-AY_{10} enzyme, additional assays were carried out with the pH set at 4.8 and the temperature ranging from 50 to 85°C, and the results are expressed in Figure 5.

As can be seen in Figure 5, an increase in temperature also increased the enzyme activity, 80°C being the optimal temperature for this enzyme, equivalent to the conditions described for *Bacillus circulans* BL53 in solid-state cultivation [15]. Thus the optimal conditions for the LEB-AY_{10} xylanase were a temperature of 80°C and a pH value of 4.8.

Thermal and pH stability

The thermal stability of xylanase was studied from the enzyme half-life. Enzymes from both the LEB-AAD_5 and the LEB-AY_{10} strains were incubated at different temperatures and the activities measured after pre-defined time intervals. Based on this data, the half-lives ($t_{1/2}$) were determined as a function of temperature.

For the xylanase from the LEB-AAD_5 strain, the half-lives were determined at 52, 57, 60, 65 and 70°C while for that from the LEB-AY_{10} strain, they were determined at 70, 72, 75, 77, 80 and 85°C. These temperatures were chosen according to the optimal temperature of each enzyme, and thus the ranges were different for each one. The results for both xylanases are shown in Figure 6, where it can be seen that the enzyme produced by LEB-AY_{10} was considerably more stable.

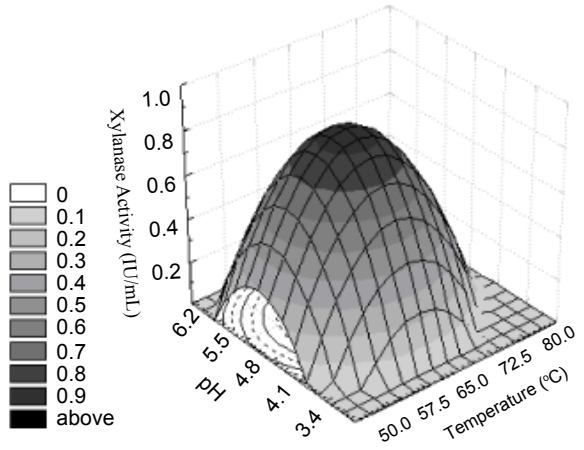

Figure 3: Response surface and contour curves for enzymatic activity as a function of pH and temperature for xylanase from the LEB-AAD_5 strain.

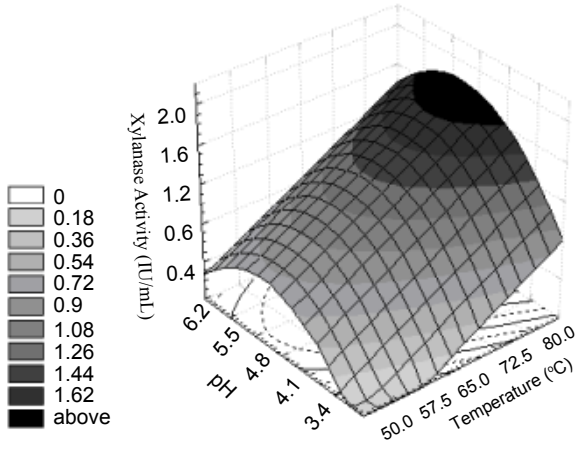

Figure 4: Response surface and contour curves for enzymatic activity as a function of pH and temperature for xylanase from the LEB-AY_{10} strain.

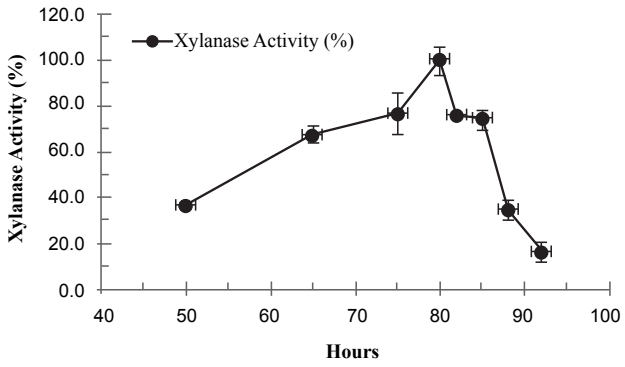

Figure 5: Activities for xylanase produced by the LEB-AY_{10} strain at different temperatures at pH 4.8.

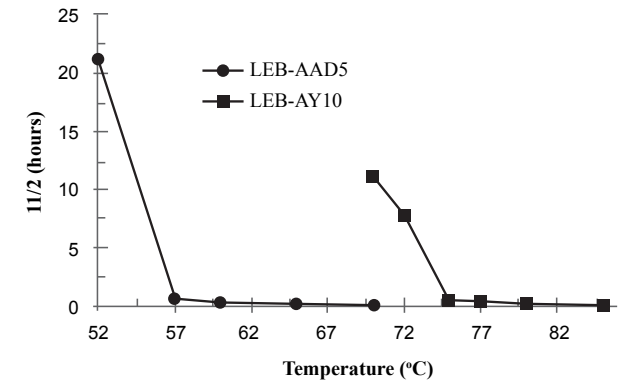

Figure 6: Thermal stability in terms of the half-lives for the xylanases from the LEB-AAD_5 and LEB-AY_{10} strains.

The enzyme produced by the LEB-AY_{10} strain can be considered as significantly more thermostable as compared to others found in the literature, if the values of $t_{1/2}$ are compared. Moreover, this enzyme maintained 75% of its initial enzyme activity after 5 minutes of incubation at 80°C. On the other hand, the enzyme from *Penicillium capsulatum*, reported by Ryan et al. [5], lost its activity in less than 3 min at 75°C and the enzyme from *Aspergillus niveus,* reported by Sudan and Bajaj [16], lost 90% of its activity after 5minutes incubation at 70°C.

However, the thermostability close to the optimal activity temperature was quite poor, a common behavior for enzymes, so they should be used at temperatures below the optimal ones, as stated by Santos et al. [17].

With respect to pH stability, a set of experiments was carried out with this proposal, incubating the enzymes in citrate buffer solution (50mM) at pH values from 3.5 to 6.0. The temperature was maintained at 52°C for the LEB-AAD_5 strain enzyme, and at 72°C for the LEB-AY_{10} strain one. Samples were collected and their half-lives evaluated, and the data are shown in Figure 7.

As can be seen in Figure 7, the variations in the half-lives of the two enzymes with pH, are significantly different. On the other hand, it can be seen that both enzymes show good stability at an equivalent range of pH values between 4.5 and 5.3, this range being close to that of optimal activity for these enzymes. Therefore, different from the effect of temperature, they can be used close to the pH for optimal activity, with practically no effect on their stability.

Kinetic parameters

Enzyme extracts for each strain were used to determine the kinetic parameters (K_m and V_{max}), with the substrate concentration ranging from 0.5 to 30 g/L of xylan. The plots for reaction rate *versus* substrate concentration show that the enzymes followed the Michaelis-Menten kinetic model, with K_m and V_{max} being obtained using the linearization procedure of Lineweaver and Burk [13] (Figures 8 and 9).

The enzyme produced by the LEB-AAD$_5$ strain showed a K_m of 0.379 g/L and a V_{max} of 1.73 µmol/mL.min, whereas the values for the enzyme from the LEB-AY$_{10}$ strain were, respectively, 0.970 g/L and 5.0 µmol/mL.min. Sudan and Bajaj [16] observed a K_m of 2.5 g/L and V_{max} of 26 µmol/mg.min for the xylanase produced by *Aspergillus niveus*, while Bakir et al. [18] reported K_m and V_{max} values of 18.5 g/L and 90 µmol/mg.min, respectively, for the xylanase from *Rhizopus oryzae*. Both papers indicated much higher values for the K_m and V_{max} than that found in the present work.

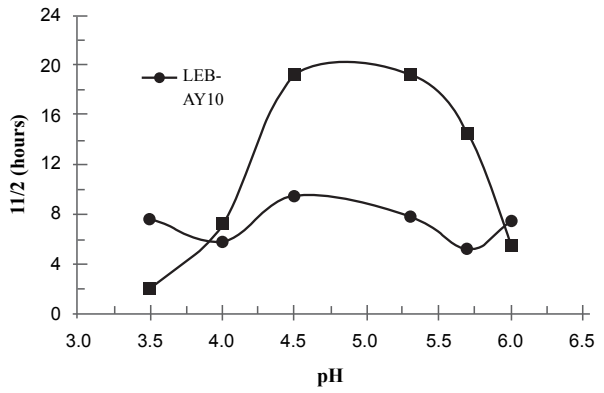

Figure 7: Half-lives of enzymes produced by the LEB-AY$_{10}$ and LEB-AAD$_5$ strains as a function of pH.

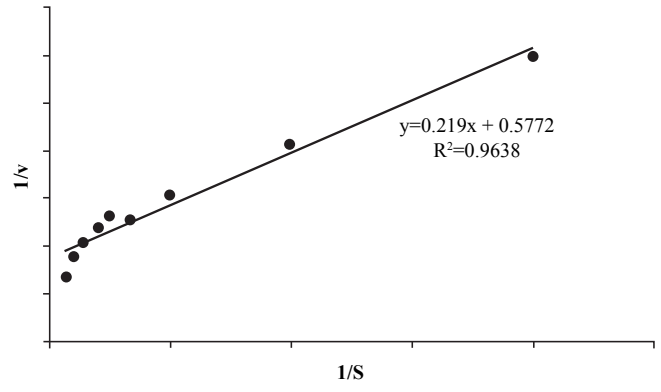

Figure 8: Lineweaver-Burk linearization for the enzyme produced by the AAD$_5$ strain.

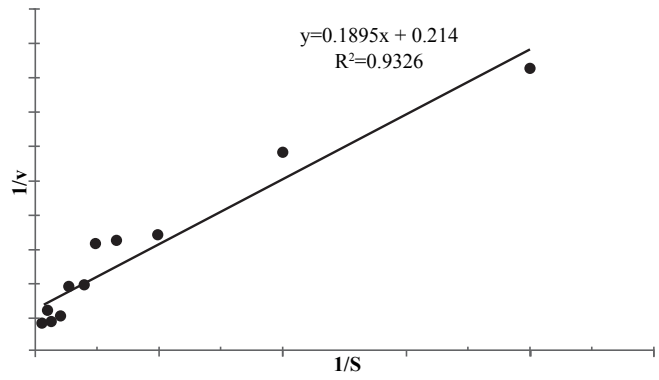

Figure 9: Lineweaver-Burk linearization for the enzyme produced by the AY$_{10}$ strain.

Assays	Xylan (g/L)	Yeast Extract (g/L)	$MgSO_4.7H_2O$ (g/L)	$(NH_4)_2SO_4$ (g/L)	Peptone (g/L)	pH	Temp. (°C)	Xylanase activity (IU/mL)
1	1	-1	1	-1	-1	-1	1	2.018
2	1	1	-1	1	-1	-1	-1	0.516
3	-1	1	1	-1	1	-1	-1	0.726
4	1	-1	1	1	-1	1	-1	0.256
5	1	1	-1	1	1	-1	1	1.247
6	1	1	1	-1	1	1	-1	0.598
7	-1	1	1	1	-1	1	1	0.644
8	-1	-1	1	1	1	-1	1	1.118
9	-1	-1	-1	1	1	1	-1	0.193
10	1	-1	-1	-1	1	1	1	3.211
11	-1	1	-1	-1	-1	1	1	1.448
12	-1	-1	-1	-1	-1	-1	-1	0.177
13	0	0	0	0	0	0	0	1.252
14	0	0	0	0	0	0	0	1.466
15	0	0	0	0	0	0	0	1.395
16	0	0	0	0	0	0	0	1.418

Table 6: Process variables used in the Plackett & Burman design showing the treatment combinations and the mean experimental response.

Optimization of enzyme production

Plackett and burman design: Since the enzyme from the LEB-AY$_{10}$ strain was shown to be more stable and its production was also higher than that from the LEB-AAD$_5$ strain, two experimental designs were carried out in order to optimize its production, using the pre-defined medium. Table 6 shows the codified values for the PB design and the enzyme activity after 24h of fermentation.

The PB design indicated that the variables of temperature, xylan concentration and $(NH_4)_2SO_4$ were statistically significant at 90% of confidence, so these were considered in the next CCD, except for $(NH_4)_2SO_4$, which showed negative effects and was therefore maintained at 1 g/L.

Second experimental design: For the CCD, all the concentrations of the non-statistically significant components were fixed according to level 0 of the previous PB design, while the temperature and xylan concentration varied according to Table 3.

The data from the second experimental design were used to obtain

the coded model for enzymatic activity as a function of the xylan concentration (g/L) and temperature (°C), as expressed by equation (4). The ANOVA (Table 7) confirmed that the models described the enzymatic activity well, since $F_{calculated}$ was higher than F_{listed}, with a value for R^2 higher than 0.98. Thus a response surface was built according to equation (4) as represented in Figure 10.

$$Y = 10.833 - 4.137 \cdot Xylan^2 - 0.637 \cdot T - 4.498 \cdot T^2 \quad (4)$$

It can be seen from the surface and the contour plots that higher values of enzymatic activity can be achieved under the center point conditions. Therefore the results from the second experiment determined the optimal conditions for maximum the enzyme activity, namely 20g/L xylan and a temperature of 30°C (assays 9, 10 and 11). Considering the results of the previous PB, the final optimal conditions for all the variables are expressed in Table 8.

	SS	DF	MS	F_{value}
Regression	165.540	3	16.437	42.33
Residual	2.718	7	0.388	-
Total	168.258	10	16.826	-

$F_{3, 7, 0.05}$ = 4.35 , (R^2) = 98.35%.
SS, sum of squares, DF, degrees of freedom, MS, mean square.
Significance level = 95%.

Table 7: Analysis of variance (ANOVA) for the model regression of enzyme produced by LEB-AY_{10}.

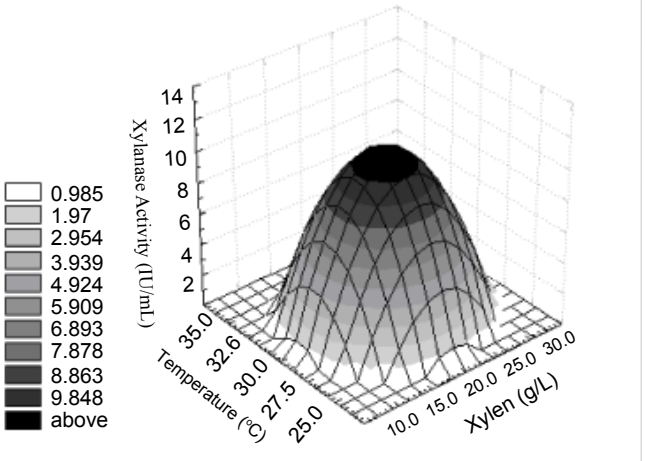

Figure 10: Response surface for enzymatic activity as a function of temperature and xylan concentration for the LEB-AY_{10} strain.

Variables	Optimized values
Xylan (g/L)	20
Yeast Extract (g/L)	1
$MgSO_4.7H_2O$ (g/L)	0.1
$(NH_4)_2SO_4$ (g/L)	0
Peptone (g/L)	1
pH	5
Temperature (°C)	30

Table 8: Optimized process variables for the production of xylanase by the LEB-AY_{10} strain.

Conclusions

In the first part of this work, the strains LEB-AAD_5 and LEB-AY_{10}, both identified as *Cryptococcus* sp. were selected from a total of 349 strains as producers of fairly stable enzymes. The enzymes were characterized and that produced by the LEB-AY_{10} strain showed optimal temperature and pH values of around 80°C and 4.5, respectively, a half-life of 11.21 hours at 72°C and pH 5.3, a V_{max} of 5.47 μmol/mL.min and a K_m of 1.37 g/L. Since this enzyme was the more stable of the two assayed, its production was optimized in flask fermentations using two experimental designs, resulting in an increase in enzyme activity of 600%, reaching 11.25 IU/mL under the optimized fermentation conditions.

The results of this work showed the potential application of the new yeast strains in the production of industrial xylanase due to its thermostability behavior. In addition, the enzymatic activity obtained in this work is relevant when compared to others' works reported in the literature where xylanases were produced by *Cryptococcus* genus. Gomes [19] and Iefuji [20] found an enzymatic activity of 24.00 IU/mL and 0.88 IU/mL respectively; however, their thermostability was not studied.

Therefore, the enzyme produced by the *Cryptococcus* LEB-AY_{10} strain is a potential candidate for large-scale production.

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