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ORIGINAL PAPER

Production and Characterisation of Xylanase and Endoglucanases Produced by *Penicillium roqueforti* ATCC 10110 Through the Solid-State Fermentation of Rice Husk Residue

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Abstract
Purpose *Penicillium roqueforti* ATCC 10110 was cultivated in rice husk residue, to produce a multienzymatic extract, which was characterised for its potential biotechnological applications.
Methods Optimisation of the fermentation conditions for the xylanase activity production (U/g) was evaluated by using a Doehlert-type experimental design.
Results The optimum xylanase activity (at 32 °C/82 h), was 1.04 U/g, which represented a deviation of 3% from the theoretically optimised value predicted by the quadratic model ($R^2 = 0.92$). The optimum conditions were observed at pH 7.0 and 35 °C. The xylanase activity was favoured, particularly, by the presence (1 M) of Co^{2+} and Cu^{2+} and the kinetic constants were determined ($K_m = 7.22$ mg/ml and $v_{max} = 3.29$ U/g). For the endoglucanase activity, it was not possible to adjust a quadratic model but maximal activities (2.37 ± 0.01 U/g) were obtained at 32 °C for 72 h. For this enzyme, the optimum conditions were pH 4.8 and 50 °C. Also, Co^{2+}, Cu^{2+}, acetone, ethanol and isopropanol increased the endoglucanase activity.
Conclusion The substrate rice husk, without any additives, permitted the acquisition of xylanases and endoglucanases similar to those obtained from synthetic substrates, justifying its application as a substrate for solid-state fermentations.

Keywords Rice husk · Endoglucanases · Solid-state fermentation · Xylanases

Introduction

There is an increasing acknowledgement of socio-environmental awareness in the industrial environment. The ethics involved in the preservation of natural resources, as well as their conscious use, has generated a strong movement toward the reuse of wastes and biomass conversion. In the agro-industry, for example, considerable waste is generated from the harvesting and processing of maize, rice, sugarcane and soybean, among others. Some researchers, have proposed exploiting the wastes in several biotechnological applications, for instance, as substrates for microbial cultivation [1, 2].

During the processing of rice (*Oryza sativa L.*) the machines that select the grains, remove impurities, husk and other parts of the rice [3]. According to Saha and Cota [4], from every 100 kg of processed rice, 20 kg of bark residue are generated. Rice residue is composed mainly of cellulose (36%), hemicellulose (20%), ashes (20%), lignin (19%), proteins (3%) and other compounds (2%) [5]. Considering the possibilities for reuse of agro-industrial residues, bioconversion by solid-state fermentation (SSF) has gained particular attention because a low-value substrate can be converted into a new, high-value commercial product [6–8]. According to Pandey

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[9], SSF has many advantages when compared with chemical reactions, such as low water requirements, low toxic residue generation and simplicity of operation. Fungi cultivation, as the microbiological agent of transformation in SSF, is justified by their mild growth condition requirements [10]. Among the various fungi species, those considered safe are classified as GRAS (Generally Recognised as Safe) by the Food and Drugs Administration (FDA). *Penicillium roqueforti* is termed a GRAS fungi because it is a non-pathogenic microorganism and is commonly associated with cheese production [11].

Another benefit of bioconversions in SSF is the substantial quantity and variety of enzymes produced by the microorganism. Among the fungal enzymes, xylanases (endo-1,4-β-xylanase, EC 3.2.1.8) are responsible for the hydrolysis of xylan—one of the main components of hemicellulose [12]—through the depolymerisation of the main chain, releasing D-xylose [13]. Xylanases have diverse industrial applications, such as cellulose pulp bleaching, juice clarification, baking and many others [14, 15]. Two other enzymes of industrial value, as equally produced by fungi, are endoglucanases (E.C. 3.2.1.4) that are responsible for cleaving inner cellulose bonds and exoglucanases (E.C. 3.2.1.91) that act in reducing and non-reducing regions of cellulose. From a commercial perspective, complex enzymatic extracts (with diverse enzymatic activities acting synergistically) are more interesting than purified enzymes [16] because, for example, purification steps often increase the final product cost.

In this context, the objective of this work was to investigate the cultivation of *P. roqueforti* ATCC 10110 by SSF, using rice husk as the substrate, to obtain a multienzymatic crude extract containing xylanases, and endoglucanases. Also, some fundamental aspects of the xylanases and endoglucanases obtained, were characterised.

Materials and Methods

Fermentation Substrate

Tio Mário, a local rice producer (Barreiras, Bahia, Brazil) kindly provided samples of rice husk (*O. sativa* L.) After the hygiene and size reduction, the samples were dried in an oven (TECNAL/São Paulo, Brazil) at 70 °C for 24 h, and milled in a Willey-type knife mill (LABOR/Piracicaba, Brazil) to a granulometry of approximately 2 mm. The substrate was stored in closed polyethylene containers, until the necessary analysis.

Microorganism and Inoculum

The fungus *P. roqueforti* ATCC 10110 (Lot: 041140074, INCQS: 40074) was donated by the *Fundação Oswaldo Cruz* (FIOCRUZ, Rio de Janeiro, RJ, Brazil). The inoculum was prepared in culture medium with agar–agar and potato dextrose agar, in Erlenmeyer flasks (250 mL) and incubated at 27.5 °C for 7 days in a bacteriological incubator (SOLAB/Piracicaba, Brasil). The spores were suspended in aqueous 0.1% Tween 80 and counted in a Neubauer chamber using a binocular microscope (Medilux MDL 150 BAI/BPI) adjusted to 40x magnification.

SSF

10 g of rice husk substrate were autoclaved (121 °C/1 atm/15 min) in Erlenmeyer flasks of 125 mL; after cooling, the sterile substrate was inoculated with 10^7 spores/g substrate and moistened with sterile distilled water until determining the value of water activity (a_w) was standardised as 0.984. The incubation was carried out in a bacteriological incubator (SOLAB/Piracicaba, Brasil). A Doehlert-type experimental design (six different experiments and triplicates of the central point) was conducted to evaluate two independent variables including the incubation time (*t*, which varied in three levels, from 48 to 96 h) and incubation temperature (*T*, which varied in five levels, from 24 to 40 °C). The evaluated responses were the activities of xylanase (U/g) and endoglucanase (U/g). The resulting data were analysed [17, 18] with statistical software (STATISTICA™ v. 10.0, StatSoft), to perform a quadratic model fitting and to obtain the response surfaces.

Multienzymatic Crude Extract

Sodium citrate buffer (50 mM, pH 4.8) was added to the fermented substrate (rice husk + fungi cells) at 5:1 volume (mL): weight (g) ratio and the mixture agitated (200 rpm/35 °C/20 min) in a shaker-type incubator (SOLAB). The biomass was then pressed and finally centrifuged (1250 *g*/10 min). The resulting crude solution was analysed for xylanase and endoglucanase. However, no cellulase activity was determined, so the methods applied for this enzyme were not discussed further in this study.

Determination of the Enzymatic Activities

The enzymatic activity of xylanase was determined, as described by Santos et al. [19], using beechwood xylan as a substrate in sodium citrate buffer (50 mM, pH 4.8). Endoglucanase activity was assessed based on the method reported by Santos et al. [20], using 2 g/L carboxymethylcellulose solution (BIOTEC) as the substrate in sodium

citrate buffer (50 mM, pH 4.8). The reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNS) method [21] and absorbance measured using a spectrophotometer (BEL Photonics MV M51).

Determination of Optimal pH and Temperature of the Xylanases and Endoglucanases

The activities of xylanase and endoglucanases were determined (triplicate) under the fixed condition of pH = 4.8 (50 mM sodium citrate buffer), at various temperatures (from 5 to 85 °C) and, under the fixed condition of 35 °C, using buffers (50 mM) of various pH including glycine–HCl at pH 2, sodium citrate at pH 3–5, sodium phosphate at pH 6–8 and glycine-NaOH at pH 12. The results were expressed as residual activity (%, U/U_o). The reference activities (U_o) were determined as 0.961 U/g for the xylanases and 2.200 U/g for the endoglucanases, at the optimum temperature investigation, and 1.323 U/g for the xylanases and 2.290 U/g for the endoglucanases at the optimum pH investigation.

Activation/Inactivation of Xylanases and Endoglucanases

The effects of Cu^{2+}, Co^{2+}, Mg^{2+}, Na^{+}, Al^{3+}, K^{+} and NH_4^{+} at 1.0 mol, EDTA (ethylenediamine tetraacetic acid) at 1% v/v, H_2O_2 (hydrogen peroxide, oxidising agent) at 5% v/v, and the solvents acetone, ethanol, isopropanol, dichloromethane and formaldehyde at 10% v/v, on the enzymatic activities of xylanase and endoglucanases were evaluated (triplicate). In all instances, the activities were determined initially (U_o) and after incubation in the presence of the compound evaluated (U). The results were expressed as residual activity (%, U/U_o) and the analyses were performed in triplicate. Incubations were performed in sodium citrate buffer (50 mM, pH 4.8) for both the xylanases and endoglucanases. The xylanase U_o values were 1.344 U/g for the solvents and 1.262 U/g for the other compounds, whereas the endoglucanase U_o was 2.40 U/g for all compounds.

Estimation of the Xylanase Kinetic Parameters

For the xylanase produced under the optimised conditions, the kinetic parameters (based on the Michaelis–Menten model), v_{max} (U/g) and K_m (mg/mL), were estimated [22] by linearisation of the kinetic data obtained (triplicate) under various concentrations of beechwood xylan (3–10 mg/mL) in sodium citrate buffer (50 mM, pH 4.8). The turnover number was determined as $k_{cat} = v_{max}/U_o$, where $U_o = 1.110$ U/g.

Xylanase Stability under Freezing Conditions

For the xylanase produced under the optimised conditions, the stability, expressed as residual activity (%, U/U_o), in citrate buffer (50 mM, pH 4.8) under freezing (−20 °C), was also evaluated (triplicate) over 45 days. The initial xylanase activity (U_o) was 1.262 U/g.

Results and Discussion

Production of a Multienzymatic Crude Extract

The culture obtained under SSF by *P. roqueforti* ATCC 10110 on rice husk meal, was conducted in accordance with the matrix presented in Table 1, for the factors of temperature (T, °C) and incubation time (t, h), as well as the responses of xylanase (U/g) and endoglucanases (U/g) activities.

For the xylanase activity, the analysis of the effects of each factor on the responses (data not presented) indicated, at the 85% confidence level that three terms were statistically significant ($p < 0.15$) to the quadratic model including the linear terms of temperature and time and the quadratic term of time. After the removal of non-significant terms, analysis of variance (ANOVA) was performed (Table 2) and the model obtained (Eq. 1) presented a good fit to the data (R^2 and $R_{adj}{}^2 > 0.88$). Biological/biochemical systems, due to their variable characteristics, allow the selection of less rigorous confidence levels (up to 85%) to maintain a greater number of significant terms in the model.

$$Xylanase\ activity\ (U/g) = 1.04 + 0.08(T) + 0.017(t) - 0.023(t^2) \quad (1)$$

Equation 1 also allowed estimation of the maximum theoretical xylanase activity at 32 °C for about 82 h

Table 1 Doehlert matrix with coded values (real values are presented in parenthesis) for the factors: temperature (T, °C) and time (t, h) and the responses: xylanases (*XYL*, U/g) and endoglucanases (*CMC*, U/g) activities obtained from the cultivation of *Penicillium roqueforti* in 10 g of rice husk and $a_w = 0.984$

Trial	T (°C)	t (h)	*XYL* (U/g)	*CMC* (U/g)
1	+1 (40)	0 (72)	0.921	2.343
2	+0.5 (36)	+0.866 (96)	1.040	2.315
3	−1 (24)	0 (72)	1.113	2.287
4	−0.5 (28)	−0.866 (48)	0.781	2.333
5	+0.5 (36)	−0.866 (48)	0.662	2.269
6	−0.5 (28)	+0.866 (96)	1.009	2.333
7	0 (32)	0 (72)	1.006	2.361
8	0 (32)	0 (72)	1.100	2.370
9	0 (32)	0 (72)	1.070	2.390

Table 2 Analysis of variance (ANOVA) for xylanase production (U/g) (by *Penicillium roqueforti* in rice husk) for the factors: temperature (T, °C) and time (t, h). The regression (R^2) and the adjusted regression (R_{adj}^2) coefficients are also depicted

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value
Regression	0.174	3	0.058	20.517	0.0031*
Residue	0.014	5	0.003		
Lack of fit	0.010	3	0.003	1.375	0.4473
Pure error	0.005	2	0.002		
Total	0.188	8			
R^2	0.9249				
R_{adj}^2	0.8839				

*statistically significant, $p < 0.05$

fermentation, as 1.07 U/g, with an equivalent theoretical productivity of 0.013 U/g.h. These conditions were applied experimentally (triplicate) and resulted in a xylanase activity of 1.04 ± 0.02 U/g, with an equivalent yield of 0.013 U/g.h. This result represented a deviation of 3% from the predicted value of the model and was considered adequate because it was smaller than the 5% standard value. Once the model was validated (ANOVA and experimental repetition), the response surface and the contour curve (Fig. 1) were obtained. From Fig. 1, it is possible to observe a strong effect exerted by the time of cultivation (t) on the production of xylanases (a reflected by the coefficients of the model).

In the literature, diverse productivities have been obtained for xylanases activities using the same rice residue substrate, such as Masutti et al. [23], which cultivated (25 °C/672 h) *Pleurotus ostreatus* and obtained 0.0040 U/g.h and also Arulanandham and Palaniswamy [24], which cultivated *Penicillium frequentans* (30 °C/96 h) and obtained 3.062 U/g.h. For other substrates, such as corn residues, Hedge and Ramesh [25] cultivated *Aspergillus furmigatus* (25 °C/168 h) and obtained, for example, 0.00744 U/g.h.

The endoglucanase production by *P. roqueforti* ATCC 10110 (Table 1) was analysed in the same multienzymatic extract as described above for xylanase. However, only the quadratic terms of time and temperature were statistically significant at a lower (83 vs. 85% for the xylanases) confidence level ($p < 0.17$). Under these conditions, the ANOVA (data not shown) showed that the regression analysis presented satisfactory values for p (0.06) and F (4.6) but the R^2 and R_{adj}^2 coefficients obtained were lower than 0.60. Consequently, the model was not considered adequate and will not be presented in this discussion. However, the production of endoglucanases (Table 1) presented the best results (2.37 ± 0.01 U/g) at the central points, with an equivalent

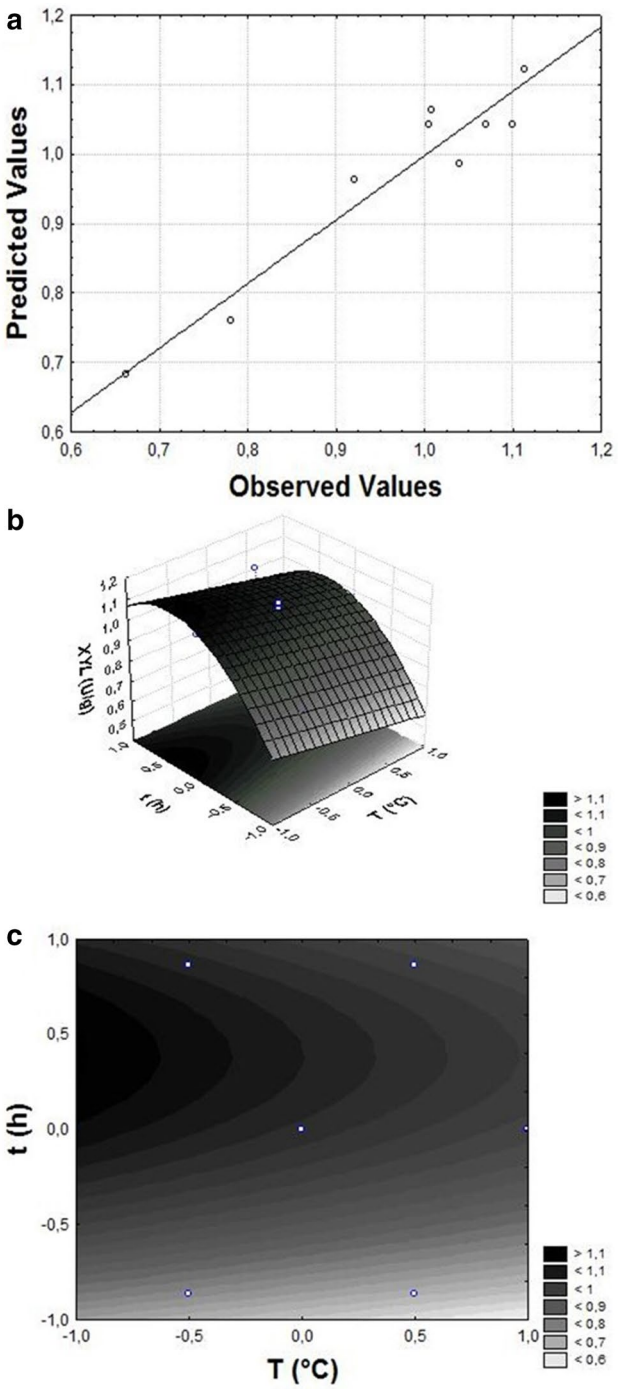

Fig. 1 **a** Observed and predicted values, **b** response surface, and **c** contour curve for xylanase production (*XYL*, U/g) (from *Penicillium roqueforti* ATCC 10110 in rice husk) for the factors temperature (T, °C) and time of cultivation (t, min)

productivity of 0.033 U/g.h. This average value for endoglucanase activity is similar (less than 2% difference) to the average value of all the experiments (Table 1). This suggests that the levels defined for the factors were insufficient to cause statistically significant differences for the

endoglucanase activities. In this instance, the best condition could be selected as the central point condition or any other combination under the conditions applied in the experiments (Table 1). Pericin et al. [26], also using *P. roqueforti* but on a markedly different substrate (pumpkin oil cake), produced substantially more endoglucanases, with a maximum productivity of almost 0.5 U/g.h.

Optimum pH and Temperature of Xylanases and Endoglucanases

Figure 2a, b present the various pH and temperature profiles of xylanase activity. For pH, it was possible to observe (Fig. 2a) residual activities greater than 100% between pH 5.0 and 8.0, suggesting different levels of enzymatic activation, possibly by charge equilibrium. Even at pH 3, the residual activity obtained was around 80% but at the extreme pHs analysed (2.0 and 12.0), there was a considerable loss of activity. Among the various temperatures evaluated, the xylanases presented (Fig. 2b) the highest residual activity (100%) at the reference condition of 35 °C and demonstrated a reduction of 10% at 45 °C. For the other temperatures investigated, the reduction in activity was greater than 20%, reaching 46% at 85 °C. Khandeparkar and Bhosle [27], when evaluating a xylanase from *Enterobacter* sp. MTCC 5112, observed optimal activities under more drastic conditions (glycine-NaOH buffer, pH 9.0 at 100 °C) than those observed in this work. Knob and Carmona [28] and Terrasan et al. [29] observed a more similar optimum pH (6.0), with xylanases from *Penicillium sclerotiorum* and *Penicillium janczewskii*, respectively. Also, similar temperatures were defined by Querido et al. [30], for a *Penicillium expansum* xylanase (40 °C), and Saha and Ghosh [31], for a *Penicillium citrinum* xym2 xylanase (45 °C). In contrast, the xylanase produced by *Myceliophthora thermophila*, had an optimum pH of 12 [32].

Figure 2c, d illustrate the effect of pH and temperature for the endoglucanases activities contained in the multienzymatic extract by SSF of *P. roqueforti* ATCC 10110 on rice husk. In Fig. 2c, it is observed that the endoglucanases were inhibited at all pH values tested, concluding that for the endoglucanases produced by *P. roqueforti* ATCC 10110 the optimum activity occurs at pH 4.8. However, the loss of activity was not greater than 15% at any pH tested, revealing a good pH range of enzyme activity. Silva et al. [33] determined the action of endoglucanases was optimal at pH

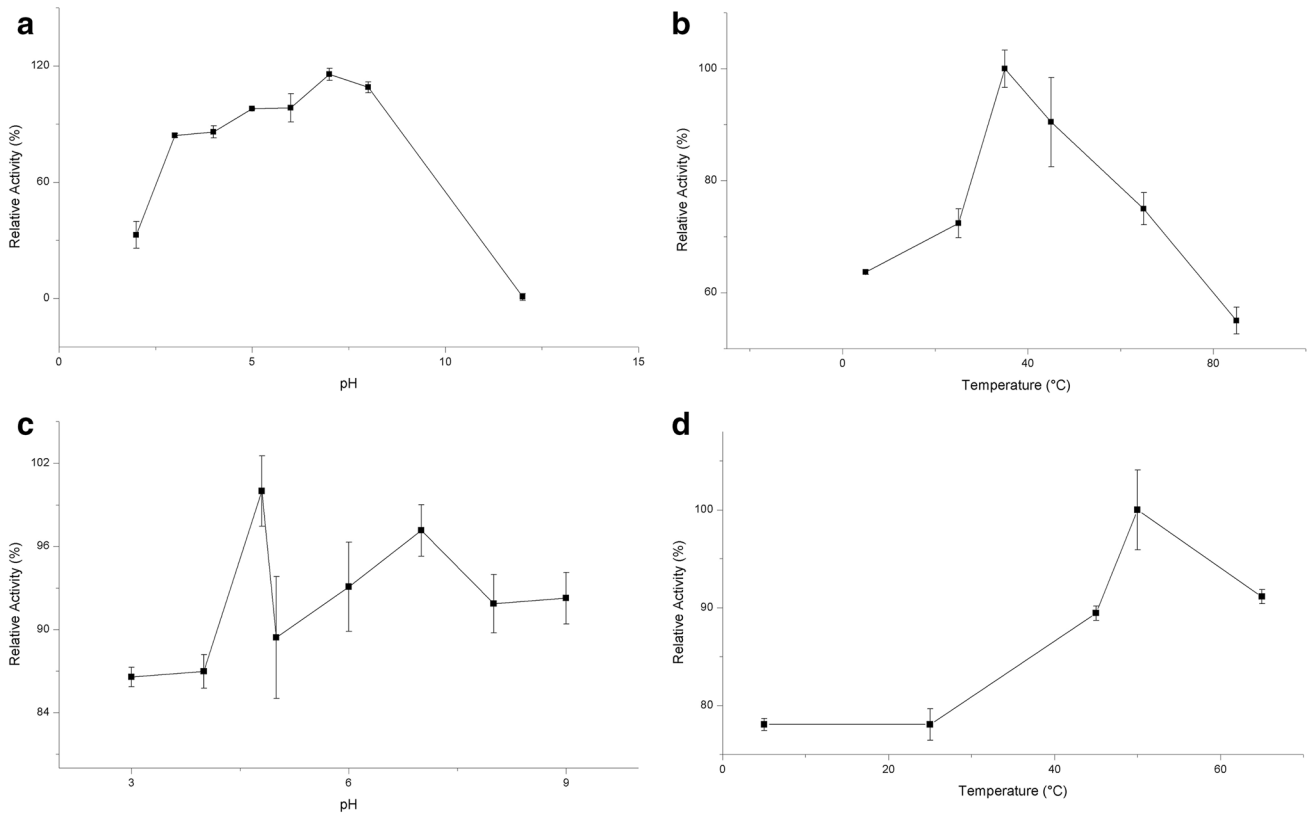

Fig. 2 Residual activities (%, U/U_o) of **a** and **b** xylanase and **c** and **d** endoglucanase produced (from *Penicillium roqueforti* ATCC 10110 in rice husk) at different values of **a** pH (at 35 °C) and **b** temperature (at pH = 4.8). The values of U_o (pH 4.8/35 °C) employed in **a** and **c** were: 0.961 and 2.20 U/g respectively, and for **b** and **d** were 1.323 U/g and 2.20 U/g, respectively

5.5 and for endoglucanases from *Streptomyces* sp., Azzeddine et al. [34] determined an optimum at pH 6. For the temperature (Fig. 2d), the endoglucanases presented the highest residual activity (100%) at the reference condition (50 °C), whereas at 5 and 25 °C, the largest reduction in activity (~22%) was observed. At the other temperatures tested, the reduction in activity was not greater than 11%. Similar results were found by Lin et al. [35], with *Jonesia quinghaiensis*, and by Das et al. [36], with *Penicillium notatum* NCIM NO-923, and Huang et al. [37], with *Arthrobacter* sp. HPG166. All these studies also demonstrate low endoglucanases activities at temperatures lower than 40 °C.

Activation/Inactivation of Xylanases and Endoglucanases

The activity of enzymes can be increased or decreased by certain compounds in solution (such as metal ions and solvents) by interfering with their three-dimensional structure or blocking the active site [38, 39]. Therefore, in this study, some compounds were analysed for their influence, during incubation of the multienzymatic crude extract, on the residual activities of the xylanases and endoglucanases (Table 3). Considering the xylanase activities, activation (residual activity > 100%) by salts was observed in the descending order $Co^{2+} > Cu^{2+} > NH_4^+ > Al^{3-} > K^+$. The presence of Mg^{2+} had a minor effect (residual activity ~97%), whereas, the presence of Na^+ was inhibitory (residual activity <90%). Khandeparkar and Bhosle [27] also determined, an enzymatic activation (202%) with Cu^{2+} and Co^{2+} ions, which are frequently identified as enzymatic co-factors, for an *Enterobacter* sp. MTCC 5112 xylanase. Terrasan et al. [29] determined an increase in activity in the presence of NH_4^+ (~131%), for xylanases from *P. janczewskii* but an inhibition was observed with Co^{2+} (~84%), while Mg^{2+} presented a minor influence (~104%). Guan et al. [40] determined an increase in activity of around 52% with Mg^{2+}, for xylanase from *Cladosporium oxysporum*.

Table 3 Residual activities (triplicate) for xylanases and endoglucanases (from *Penicillium roqueforti*) incubated with different additives

Additives	Xylanases (%, U/U_o)	Endoglucanases (%, U/U_o)
Cu^{2+}	154.28 ± 2.21	136.31 ± 3.54
Co^{2+}	211.71 ± 3.15	244.84 ± 1.16
NH_4^+	127.26 ± 3.42	92.66 ± 4.39
Mg^{2+}	97.10 ± 3.99	ND
Na^+	88.90 ± 1.50	102.70 ± 2.92
Al^{3+}	107.72 ± 0.41	NT
Pb^+	NT	93.43 ± 1.34
K^+	114.23 ± 1.10	74.12 ± 1.34
EDTA	91.07 ± 1.10	79.14 ± 8.11
H_2O_2	106.76 ± 3.32	NT
Ethanol	167.27 ± 0.39	180.34 ± 0.67
Acetone	176.56 ± 3.74	176.09 ± 2.41
Dichloromethane	102.04 ± 3.98	NT
Ethyl Ether	90.26 ± 1.04	NT
Isopropanol	82.10 ± 3.06	110.82 ± 1.77
Formaldehyde	98.64 ± 4.92	169.91 ± 3.72

ND not detected, *NT* not tested

Among the solvents analysed, acetone and ethanol resulted in activation of the xylanases from *P. roqueforti* ATCC 10110 (Table 3). Polar solvents, such as these, are capable of interfering with the three-dimensional configuration of proteins (being good precipitating agents at higher concentrations [41]) but do not suggest that the enzymatic activity is preserved under long incubation periods. The presence of formaldehyde had no strong influence on xylanase, while isopropanol resulted in inhibition (Table 3). The activities of xylanases by Bacillus have been reported practically unchanged in the presence of 5% (v/v) ethanol [42], activated by 50% (v/v) acetone and inhibited by 50% (v/v) formaldehyde [43]. The use of EDTA, resulted in an inhibitory effect on the xylanase from *P. roqueforti* ATCC 10110 (Table 3). Similar results were observed by Boonchuay et al. [38] and Sorgatto et al. [44], in which the xylanases from *Streptomyces thermovulgaris* TISTR1948 and *Aspergillus terreus*, respectively, were reduced by up to 40% of their initial activity in the presence of EDTA. Conversely, H_2O_2 showed a minor activation (Table 3), probably due to its oxidative character but this phenomenon, as well as that observed with ethanol and acetone, is unlikely to occur during relatively longer incubations. Sanghvi et al. [45] determined a residual activity of 15% in the presence of only 5% (v/v) acetone, for a *Bacillus* sp. BHO502 xylanase.

Regarding the residual endoglucanase activities (Table 3), activations were observed with Cu^{2+} and Co^{2+}, whereas NH_4^+, K^+ and Pb^+ caused inhibition. Na^+ did not result in any significant influence. Pol et al. [46] also determined Co^{2+} as an activator and K^+, Pb^+ and Cu^{2+} as inhibitors of *Penicillium pinophilum* MS 20 endoglucanase activity. In accordance with our results, Sadhu et al. [47] also observed an inhibition by EDTA. Similarly, Azzeddine et al. [34] found Co^{2+}, Cu^{2+} and NH_4^+, as activators of *Streptomyces* sp. B-PNG23 endoglucanase action. In the current endoglucanase study of *P. roqueforti* ATCC 10110, all the solvents tested had a positive effect on the endoglucanase activity, increasing up to 80% the catalytic activity of the enzyme. In contrast, Silva et al. [33] determined isopropanol as an activator of endoglucanase activity, whereas ethanol and acetone did not affect or improved the

endoglucanases produced by *Myceliophthora heterothallica* F.2.1.4. Differences between the same enzymes from different sources or medium are expected because enzymes are highly specific and complex biochemical molecules and their differences can be manipulated in favour of different processes.

Estimation of the Kinetic Constants of the Xylanases

From the kinetic data obtained (Fig. 3), it was possible to estimate the kinetic parameters for xylanase by *P. roqueforti* ATCC 10110 as $K_m = 7.22$ mg/mL, $v_{max} = 3.29$ U/g and $k_{cat} = 2.99$ 1/s. The catalytic performance of an enzyme is generally expressed by its k_{cat}, and for a purified xylanase of *P. janczewskii* acting on the beechwood substrate [31], its performance was better (300 times higher) than that observed in this present work (a crude extract). The xylanases from *Penicillium oxalicum* GZ-2, also presented a comparatively higher k_{cat} value (86.9 1/s) [48] than that found in the current study but for the partially purified xylanase from *Aspergillus oryzae* [49], a more similar value was estimated (1.34 1/s). Considering, however, that the multienzymatic extract obtained from *P. roqueforti* ATCC 10110 with rice husk, did not undergo any separation/purification step in this work, the process presented in this study is more economically advantageous, despite the lower kinetics values, compared to purified xylanases.

Freezing Stability of Xylanases

The profile of xylanase residual activity during incubation at −20 °C is presented in Fig. 4. From these data, it is possible to observe a low stability under the analysed conditions.

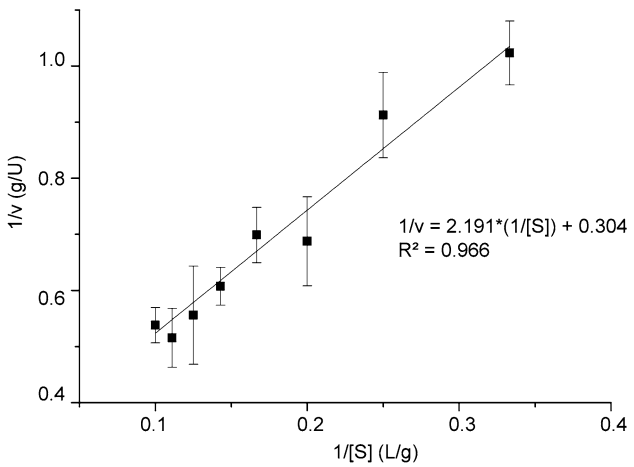

Fig. 3 Linearization (by Linewave-Burk) of kinetic data for estimation of the kinetic parameters (K_m and v_{max}) of xylanase (from *Penicillium roqueforti* ATCC 10110 in rice husk) beechwood xylan as the substrate

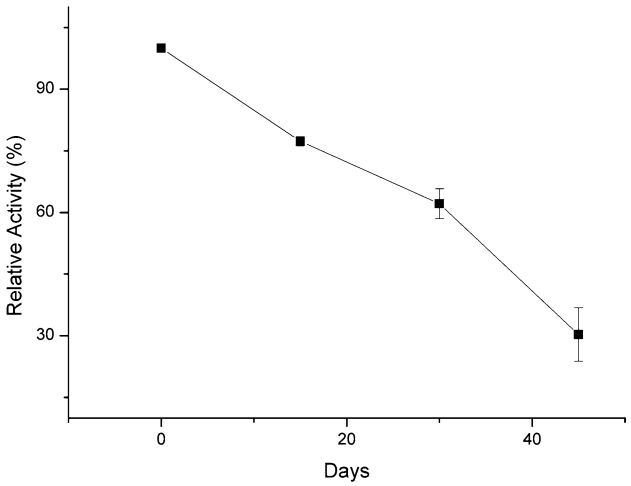

Fig. 4 Residual activities (%, U/U_o) of xylanase (from *Penicillium roqueforti* ATCC 10110 in rice husk) throughout the incubation time under −20 °C in citrate buffer (pH = 4.8, 50 mM). The initial activity (U_o) was 1.262 U/g

At about 15 days, the residual activity was around 75% and this decreased further to 50% at the end of the analysis (45 days). This suggests a necessity for further investigation about increasing the stabilisation of this crude extract for longer periods under freezing conditions.

Conclusions

The application of rice husk, as a substrate in SSF by *P. roqueforti* ATCC 10110, is a promising idea because, as shown in this study, it was possible to produce a multienzymatic crude extract, with good xylanase activity and similar characteristics to other xylanases obtained from various sources and substrates described in the literature, most of which, were purified enzymes. The use of this residue as a biotechnological substrate (not only for enzyme production but other bioproducts), can make a valuable contribution to ecological and economic aspects of local economies. In addition, a process with an innocuous (GRAS classification) fungus, such as *P. roqueforti* ATCC 10110, is advantageous because the microbiological risks are minimised. Besides xylanase, the crude extract obtained was confirmed for endoglucanases, two enzymes important for the bioconversion of lignocellulosic material.

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