Effect of media components on the production of xylanases by *Pseudozyma* spp.

Joana Rita Carreira Cerejo

**Abstract**

**Background:**

There has been much concern on xylanase due to its potential industrial applications in the manufacturing of pulp and paper, baking, food and beverages, textile and animal feeds production. Information about the production of xylanases by yeasts is scarce, however among the genus *Pseudozyma*, some strains are found to be producers of cellulase-free xylanases. There are continuously endeavors to turn xylanase into a profitable market with lower production costs using optimum fermentation medium and growth conditions.

The aim of this study was to evaluate the effect of media components on the production of xylanases by *Pseudozyma* spp.

**Result:**

The xylanolytic potential of this *Pseudozyma* strain was further explored and xylanase production was evaluated when using different organic and inorganic nitrogen sources, as well as simple and complex organic carbon sources.

The screening of nitrogen sources revealed that yeast extract was found to exert a marked influence on xylanase production, reaching a volumetric activity of 529.0 U/ml using D-xylose as carbon source.

Xylanase activity was characterized in terms of temperature and pH profiles. The optimal temperature and pH for xylanase activity is between 45-57 °C, and 4.5-5.0.

**Conclusions**

The present study reveals that *P. aphidis* PYCC 5535 is an efficient xylanase producer and identified the conditions for the production of high-activity xylanolytic extracts with synthetic substrates. The microorganism is promising for industrial application, since it grows in simple and complex substrates and secretes the enzymes, and its set of enzymes displayed properties that potentially match with industrial applications, including those requiring cellulase-free xylanases. The xylanase production by *P. aphidis* is, to the best of our knowledge, superior than any other yeasts described in the literature. Further studies need to be done in order to use low-cost substrates, as lignocellulosic substrates.

**Keywords:**

Xylanase, lignocellulosic substrates, yeast, hemicellulose, xylan, wheat straw, corn straw, eucalyptus residue.
Introduction

Xylanases have played an important role in many industrial biotechnological processes, having a broad range of applications including enzymatic bleaching of paper pulp, juice clarification, extraction of plant oils, texture improvement in bakery, bioconversion of agricultural waste, bioscouring in textiles and improvement of animal feed. Recently, they have received much attention owing to their use in hydrolysis of lignocellulosic biomass to sugars, which can subsequently be converted into liquid fuels, solvents and other chemicals. Overall, xylanases have a worldwide market of around 200 million dollars.

The use of expensive substrates for xylanase production hinders cost-effective industrial processes and wide application in xylan conversion processes. Consequently, there have been attempts to develop xylanase production processes from inexpensive substrates. Xylan is usually available in large amount in lignocellulosic substrates, as by-products of forest, agriculture, agro-industries, wood and pulp and paper industries.

The three main components that constitute lignocellulosic substrates are lignin, cellulose, and hemicellulose, comprising on average 23 %, 40 % and 33 %, respectively. In interaction with a matrix of lignin, these heteropolysaccarides form a highly complex structure. Hemicelluloses include xylans, xyloglucans (heteropolymer of D-xylene and D-glucose), glucomannans (heteropolymer of D-glucose and D-mannose), galactoglucomannans (heteropolymer of D-galactose, D-glucose and D-mannose) and arabinogalactans (heteropolymer of D-galactose and arabinose). Xylan is usually the major constituent of hemicellulose and it is the second most-abundant polysaccharide in nature, next to cellulose, accounting for approximately one-third of the renewable organic carbon on earth with a high potential for degradation to useful end products.

Xylan is a complex, highly branched heteropolysaccharide, comprising a backbone of D-xylopyranose units linked through glycosidic β-1,4 linkages, which may be substituted with side chain branches such as α-arabinofuranosyl, 4-O-methyl-D-glcuronosyl, acetyl, feluric acid and p-coumaric acid. Due to the structural heterogeneity of xylan, its complete hydrolysis requires the action of several enzymes, namely endo-β-xylanases (EC 3.2.1.8), which attacks the main chain of xylans and cleaves the β-1,4 glycosidic linkage between xylose residues in the backbone of xylans, and β-xylosidases (EC 3.2.1.37), which hydrolyze xylooligo-saccharides into D-xylose.

The enzymatic hydrolysis of hemicellulose is of great interest for biotechnological applications, where most studies focused on enzymes that hydrolyze xylan. Due to the heterogeneity and complex chemical nature of plant driven xylan, its complete breakdown requires the synergistic complex action of several enzymes with diverse specificities and modes of action. Thus, xylan-degrading cells appear to produce an arsenal of polymer-degrading proteins. In addition to the production of a variety of xylanolytic enzymes, many microorganisms produce multiple xylanases, having these diverse physicochemical properties, structures, specific activities and yields, increasing the efficiency and extent of hydrolysis, as well as diversity and complexity of the enzymes. All these enzymes act cooperatively to convert xylan into its constituent sugars.

The xylanolytic systems are extensively studied in filamentous fungi (Aspergillus sp., Trichoderma sp., Penicillium sp., etc.) and bacteria (Bacillus sp., Streptomyces sp.), known as efficient enzyme producers. However, information available on xylanase production and on the nature of xylanase produced from yeasts is very limited, possibly because of the low production of xylanase by such microorganisms. Among yeasts, those belonging to the genera Cryptococcus, Scheffersomyces, Candida, Trichosporon, Dekkera, Hanseniaspora, Metschnikowia, Rhodotorula, Sugiyamaella and Wickerhamomyces were described as cellulose and/or xylanase producers. These xylanolytic microorganisms are mainly found in environments that are rich in degraded plant materials, as well as in the rumen of ruminants.

To reach commercial feasibility, optimizing culture conditions must increase enzyme production by yeasts. The global aim of this study was to improve xylanase production by P. aphidis, studying the influence of the components of the medium.
Material and Methods

Yeast strains, maintenance and standard cultivation conditions

*Pseudozyma aphidis* PYCC 5535\(^T\) was obtained from Portuguese Yeast Culture Collection (PYCC), CREM, FCT/UNL, Caparica, Portugal. Yeasts were plated on Yeast Malt Agar (YM-agar) medium (yeast extract, 3 g/l; malt extract, 3 g/l; peptone, 5 g/l; D-glucose 10 g/l and agar 20 g/l) and incubated for 3 days at 25°C. Stock cultures were prepared by propagation of yeast cells in liquid medium as described below for the inoculum and stored in at -80°C in 20% glycerol for later use. Inoculum was prepared by incubation of stock cultures of *P. aphidis* at 28°C, 140 rpm, for 48 h, in liquid medium containing glucose (40 g/l), NaNO\(_3\) (3 g/l), MgSO\(_4\) (0.3 g/l), KH\(_2\)PO\(_4\) (0.3 g/l) and yeast extract (1 g/l). Xylanase production was performed in 250 ml Erlenmeyer flasks with 50 ml of the culture medium (1/5 working volume). The flasks were inoculated with 10 % v/v inoculum (DO \(_{640}\) nm 0.5) and incubated during 7 days at 28°C (140 rpm). The cultivation media contained a carbon source (40 g/l), and was supplemented with MgSO\(_4\)-7H\(_2\)O (0.3 g/l), KH\(_2\)PO\(_4\) (0.3 g/l) and yeast extract (1 g/l). All experiments were carried out in a biological duplicate. Cultured sampling was performed at 0, 2, 4 and 7 days to follow yeast growth and enzyme activity. One milliliter of culture sample was taken and after centrifugation (6,000 g; 4°C), the supernatants were stored at -20°C for further sugar content and activity assays analyses. Cell growth was followed by measuring optical density (OD) spectrophotometrically at 640 nm. Cell dry weight (CDW) was also used to quantify the biomass concentration, being determined using 1 mL culture broth centrifuged at 6,000 g for 10 min, followed by cell pellet washing with deionized water. The cell pellet was dried in the oven at 100°C for 16h. In order to relate CDW and OD, a culture of *P. aphidis* was grown according to the conditions for inoculum preparation mentioned above, and successive dilutions were performed. Supernatants aliquots and samples were filtered through a 0.45 µm-pore-size filter and analyzed for glucose, xylose, mannose, acetic acid, and furfural by High Performance Liquid Chromatography (HPLC) system High Performance Liquid Chromatography (HPLC) system (Merck Hitachi, Darmstadt, Germany) equipped with a refractive index detector (L-2490, Merck Hitachi, Darmstadt, Germany) and an Rezex ROA-organic acid H+ column (300 mm ~ 7.8 mm, Phenomenex), at 40°C. Sulfuric acid (0.005 M) was used as mobile phase at 0.5 ml/min.

Xylanases production using synthetic substrate and agro-industrial residues

The study of xylanase production using synthetic substrates was carried out based on stepwise modification of the standard cultivation media. To study the effect of nitrogen source on xylanase production, organic and inorganic sources were used, added in different concentration to the standard cultivation media. As organic sources, peptone, urea and yeast extract were used and the inorganic sources used were NaNO\(_3\), NH\(_4\)NO\(_3\), NH\(_3\)Cl and NH\(_4\)SO\(_4\). In combination with the study of nitrogen source, the effect of carbon source was also investigated, using a simple source (xylose) and a complex source (xylan). Cultivation was carried out at 28°C for 7 days and aliquots were taken at day 0, 2, 4 and 7 days for yeast growth and enzyme activity. Xylanase activity was determined colorimetrically by measuring the release of reducing sugars from xylan using 3,5-dinisalicylic acid (DNS) method as described by Miller\(^{16}\) using D-xylene as standard. The reaction mixture consisting of 300 µL 1% beechwood xylan solution in 5.5 pH phthalate and 100 µL of suitably diluted enzyme solution was incubated at 50°C for 30 min. The reaction was stopped by adding 600 µL of DNS (dinitrosalicylic acid). The mixture was then boiled for 6 min and cooled to room temperature. Color changes were observed when reducing sugars were liberated from the reaction occurred between xylan (and subsequent xylooligosaccharides) and xylanases. The reducing sugars were determined using spectrophotometer at 540 nm, against a standart curve with different D-xylene concentrations processed with the same procedures as the samples. One unit of enzymatic activity was defined as the amount of enzyme required to liberate 1 µmole of reducing sugars per minute under the assay condition. For determination of the enzyme optimal pH, the enzymatic reaction was carried out at different pH using 50 mM buffer solutions ranging from pH 3.0-10.0; citrate buffer for pH 3.0-6.0 and potassium phosphate buffer for pH 6-10 (at 50°C). To determine the optimum temperature, the enzymatic reaction was carried out at different temperature (20-72°C) using 50 mM potassium phthalate at pH 5.5.
Protein quantification was performed with BCA Protein Assay Kit (Thermo Scientific – Pierce). Samples were prepared on a 96 wells microplate, in a final volume of 200 μL (175 μL reagent, 25 μL sample) and measured spectrophotometrically at 562 nm in Multiskan GO Microplate Spectrophotometer (Thermo Scientific) against a BSA standard curve between 0 and 2 g/L.

Results and discussion

The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates. The nitrogen source has profound influence on xylanase production as it is essential element for protein biosynthesis, and so, can be an important limiting factor in enzymes production. Besides, the nitrogen source can affect the pH of the medium, which in turn, may influence the enzyme activity and stability.

Thus, the effect of nitrogen source supplementation on xylanase production was studied. In the present study, the effect of nitrogen sources was tested using organic (yeast extract, peptone and urea) and inorganic (sodium nitrate, ammonium nitrate, ammonium chloride and ammonium sulfate) compounds.

Xylan with nitrogen sources - nitrate

The use of a nitrate source, more specifically NaNO₃ in cultivation media for xylanase production is referred in the literature as a good nitrogen source for xylanase production in other organisms, for instance filamentous fungus. Xylanase production was tested using xylan medium without nitrate and with NaNO₃ (3g/l) (Figure 1-A and Table 1).

Comparing the production of xylanase in the absence of NaNO₃ and using 3 g/l of NaNO₃, volumetric endoxylanase activity increased 300 % for the last condition. In the presence of NaNO₃, maximum OD₆₄₀nm is lower than without nitrate (12.28 against 17.15, respectively), while total protein is higher (8.21 mg/ml compared with 2.32 mg/ml in the absence of NaNO₃). This might indicate that the presence of NaNO₃ promotes the production of endoxylanase (i.e. growth of the target protein) but also other extracellular enzymes, reducing the purity of xylanases in the extracellular crude extract), and consequently specific activity.

In order to see the effect of nitrate concentration on xylanase production, concentrations of 3 and 5 g/l were used using sodium nitrate as nitrogen source (Figure 1-B and Table 1).

An increase of the concentration of nitrate led to 60% higher volumetric activity (313.73 ± 11.05 U/ml), while maintaining similar biomass and protein content, which means that this condition promoted higher specific activity (42.3 U/mg protein).

Figure 1. Relative xylanase volumetric activity of extracellular extracts of P. aphidis PYCC 5535ᵀ cultivated in xylan 40 (g/l). The conditions used were without nitrate, with sodium nitrate (3 g/l) (A) and 3 g/l and 5 g/l of NaNO₃ (B).
Xylan with nitrogen sources: other inorganic and organic sources

Other inorganic nitrogen source used was ammonia (Figure 2 and Table 1), tested with different compounds (NH₄NO₃, NH₄Cl and (NH₄)₂SO₄) at 3 g/l. These sources were chose based on several studies done in fungi. The influences of these nitrogen sources are compared in.

Comparing the effects of nitrogen source supplementation in the form of nitrate and in the form of ammonia, lower volumetric activities (2 times lower for the condition with highest volumetric activity) were obtained for the last condition, as well as specific activity.

Among them, NH₄Cl led to the highest xylanase specific activity, 3-fold higher compared with those obtained with NH₄NO₃ and (NH₄)₂SO₄. No relevant differences in the protein content were observed between the three conditions but lower biomass was obtained in the case of NH₄Cl, comparing with the other two. Two possibilities can explain these results: either the xylanase that are produced in the case of NH₄Cl have more activity than those produced in NH₄NO₃ and (NH₄)₂SO₄ and P. aphidis PYCC 5535 T need to produce less biomass in order to have the same amount of enzyme or the enzyme produced in NH₄NO₃ and (NH₄)₂SO₄ are others enzymes besides xylanases. In this situation, we can clearly see that rather to be dependent of the nitrogen content present in the media, xylanase production is affected by the source of nitrogen, having the presence of chloride or nitrate different consequences in the xylanase production and activity.

Three organic nitrogen sources were used at 3 g/l: yeast extract (additional 2 g/l to the standard medium) peptone and urea.

The best condition for xylanase production was using peptone (combined with yeast extract present in the standard cultivation media). Those kind of nutrients have been reported as most balanced and conditioned nitrogen sources in terms of accessibility and composition for getting high yield of xylanase from different bacteria.

Peptone has been reported as the best nitrogen source for xylanase production by Bacillus, Streptomyces and Trichoderma viride D3 spp. This intermediate product of protein hydrolysis is composed by peptides and amino acids, furnishing an available source of nitrogen. On the other hand, yeast extract contain approximately 37-44% of protein content, carbohydrates, amino acids and nucleic acids, elements that are necessary for the metabolism of the yeast. The combination of peptone (3 g/l) and yeast extract (1 g/l) results in the highest levels of enzyme production, reflected in the highest xylanase volumetric activity, 371.35 U/ml. In the absence of yeast extract, peptone only generated the 173.27 U/ml xylanase volumetric activity (data not shown). The specific activity was higher for the condition with yeast extract and maximum biomass was similar, indicating that with the same biomass growth, the condition using peptone and yeast extract led to a higher xylanase production. Another important aspect is the formation of ammonium ions due to the use of yeast extract with peptone, which is a known protease inhibitor that minimizes the proteolytic degradation of xylanase under inducing conditions, also a presumable motive for the difference that were seen.

Using 3 g/l yeast extract as sole nitrogen source, an increase of 6% was obtained (71.31 U/ml) compared to the condition without nitrogen source, except 1 g/l yeast extract. The xylanase volumetric activity was 5-fold lower than obtained with the condition with peptone in the medium, however biomass growth was similar, indicating that in the absence of peptone, the production was less specific and other protein than xylanases were produced. Although both yeast extract and peptone are equally nutrient-rich in terms of amino acids, peptides, vitamins and other nutrients, preferential utilization and ability to support enzyme production is registered using peptone.

Using urea, the lowest xylanase volumetric activity (10.8 U/ml) and the lowest biomass (OD₆₄₀nm of 3.1) were obtained. When urea is dissolved in aqueous medium, it formed ammonium bicarbonate and hydroxide ions. As the fermentation prolonged, it is normal that the pH medium increases due to metabolic activity of yeast. Adding with a medium that is already in alkaline condition, the enormous degree of alkalinity might hamper P. aphidis PYCC 5535 T grow, thus producing the lowest biomass and xylanase.
D-Xylose and nitrogen sources

The carbon source plays a major role on xylanase activity and in the economics of enzyme production. The final objective of this study is to generate maximal xylanase production from inexpensive substrates, preferentially from lignocellulosic residues. After pretreatment of lignocellulosic residues, a significant fraction of the hemicellulosic hydrolyzates is often composed by D-xylose. Therefore, D-xylose was also tested as carbon source, using several of the nitrogen sources previously supplemented to xylan media.

Pseudozyma aphidis was cultivated during 7 days at 28°C and 140 rpm in D-xylose medium, supplemented with 3 g/l NaNO₃, 10 g/l yeast extract, 10 g/l yeast extract combined with 20 g/l peptone. Biomass growth was monitored by OD measurements at 640 nm during 7 days. Xylanase volumetric activities were determined (Figure 3) and total protein was quantified in the extracellular extracts. The values are depicted in Table 1.

Figure 2. Relative xylanase volumetric activity of extracellular extracts of *P. aphidis* PYCC 5535ᵀ cultivated in xylan 40 (g/l) and supplemented with inorganic (A) and organic nitrogen sources (B).

Figure 3. Relative xylanase volumetric activity of extracellular extracts of *P. aphidis* PYCC 5535ᵀ cultivated in xylose (40 g/l), supplemented with sodium nitrate (3 g/l), yeast extract (10 g/l) and yeast extract and peptone (10 g/l and 20 g/l, respectively) (A); Volumetric xylanase activity of extracellular extracts of *P. aphidis* PYCC 5535ᵀ cultivated in xylose (40 g/l) (blue) and xylan (4% (w/v)) (red) (B).
Surprisingly, the presence of higher concentrations of yeast extract led to xylanase volumetric activities 4.6-fold higher (529.0 U/ml) than with NaNO₃. Biomass growth was also promoted, reaching a maximum OD₆₄₀nm of 26.1 in the 4th day of cultivation. The concentration of extracellular protein produced in this condition was 8.36 mg/ml, quite similar to the protein content when 20 g/l peptone is present together with yeast extract (8.14 mg/ml). Biomass growth was maximal with peptone, reaching a maximum OD₆₄₀nm of 33.7 in the 7th day of cultivation, but xylanase volumetric activity obtained in this condition was 2.6-fold lower compared with the condition without peptone. The concentration of nitrogen present in those complex components is difficult to be exactly known since composition is not regular and may even change between different batches.

Analyzing the variation of pH during time, the conditions with peptone in its composition reached a final pH over 8, far above from the condition with yeast extract, which final pH was 6.7. This can be due to the formation of ammonium ions due to metabolism of yeast extract with peptone, a usual phenomenon seen in other studies²³.

The behaviour of yeast culture (and production of xylanase) can be different in the presence of different inducers. Thus, it is important to be sure about the effect of yeast extract when xylan and xylose are used as carbon source. Pseudozyma aphidis was cultured in 10 g/L yeast extract using xylan or xylose as carbon source during 7 days. Growth was monitored during 7 days and xylanase volumetric activities were determined (Figure 3).

Analyzing Figure 3, xylanase activity is 4.6-fold higher in xylose than in xylan, when supplemented with yeast extract. Similar extracellular protein content was obtained, but differences in yeast growth were observed in xylose or xylan cultures (Table 1). Using D-xylose as carbon source, higher biomass (OD₆₄₀nm) is obtained, compared with the condition using xylan. Thus, the use of D-xylose as carbon source not only promotes specificity, being xylanase production favoured over other enzymes, but also yeast growth, with OD₆₄₀nm values above 30.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Moles of N (x 10⁻³)</th>
<th>Vol. activity (U/ml)</th>
<th>Protein (mg/ml)</th>
<th>Spec. activity (U/mg protein)</th>
<th>Max OD</th>
<th>Carbon source</th>
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<tr>
<td>without nitrate</td>
<td>0.4</td>
<td>67.2 ± 14.4</td>
<td>2.32</td>
<td>29.0</td>
<td>17.15</td>
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</tr>
<tr>
<td>NaNO₃</td>
<td>2.1</td>
<td>193.9 ± 29.4</td>
<td>8.21</td>
<td>23.6</td>
<td>12.28</td>
<td>Xylan</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>3.3</td>
<td>313.73 ± 11.05</td>
<td>7.42</td>
<td>42.282</td>
<td>12.35</td>
<td>Xylan</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1.3</td>
<td>91.8 ± 21.1</td>
<td>6.10</td>
<td>15.1</td>
<td>13.10</td>
<td>Xylan</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>4.1</td>
<td>116.6 ± 12.5</td>
<td>9.14</td>
<td>12.8</td>
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</tr>
<tr>
<td>(NH₄)₂SO₄</td>
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<td>97.2 ± 16.5</td>
<td>7.46</td>
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<td>71.3 ± 24.0</td>
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<td>40.3</td>
<td>18.85</td>
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<td>5.21</td>
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<td>14.9</td>
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<td>114.8 ± 15.8</td>
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<tr>
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<td>529.0 ± 19.5</td>
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<td>26.1</td>
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<tr>
<td>10 g/l yeast extract + 20 g/l peptone</td>
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<td>205.9 ± 26.7</td>
<td>8.14</td>
<td>34.2</td>
<td>33.7</td>
<td>Xylose</td>
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Table 1. Xylanase volumetric activity, specific activity, total protein content in extracellular extracts and maximum biomass (OD₆₄₀nm) in xylan and xylose cultures.
Overall, *P. aphidis* responded differently to nitrogen sources. The highest xylanase activities obtained are about 2-fold higher than those from reported for *P. brasiliensis*. Comparing to other yeasts genera, *P. aphidis* produces xylanolytic enzymes with volumetric activities far superior (50-fold) to those reported (0.1–10 U/ml). For instance, *Cryptococcus albidus*, *Cryptococcus laurenti*, and *Trichosporon* are reported to produce xylanases under xylan induction, reaching specific activities of 0.44 U/mg, 11.05 U/mg, and 4.00 U/μg, respectively. These values are far below those obtained in this study. The values obtained in this study are in the level of xylanase activity obtained for filamentous fungi, industrially important as xylanase producers. Xylan extracts from some filamentous fungi exhibits cellulase activity, while *P. aphidis* is a producer of cellulase-free xylanases, which might be useful for some industrial applications.

**Xylanase profile in relation to temperature and pH**

Xylanase volumetric activity of the extracellular crude extract reported in the previous section was quantified in the range of pH 3 to 8 using two buffers (citrate and potassium phosphate buffer) to find out the optimal pH for the enzyme (Figure 4-A). The extract exhibited xylanase activities higher than 80% of maximum between 4.0 and 5.5 and higher than 50% between 4.0 and 6.5. The optimum pH for the xylanase activity is around 4.5. Xylanolytic activity decreased remarkably after pH 6.5, to values below 40%.

A temperature range between 20 and 70°C was used to study the effect of temperature on xylanase activity (Figure 4-B). The optimum temperature was found to be approximately 45°C, with more than 60% activity for a temperature interval between 35 and 70°C. Xylanase volumetric activity decreases at lower temperature, reaching 20% at 20°C, probably due to the lower kinetic energy of the system.

These results are slightly different from those obtained in previous work with *P. aphidis*, where a temperature of 50°C was obtained and the optimum pH was found to be 5.5. In studies carried out with *Pseudozyma brasiliensis*, the optimum pH and temperature were found to be 4.0 and 55°C, respectively. These data were similar to other studies, where most of the yeast and fungal species showed maximal xylanase activity at pH ranging from pH 4.0 and 6.5 and temperatures between 40 and 80°C. In the literature, other microorganisms are cited with similar optimum temperatures for xylanolytic activity, such as 45°C for *Aspergillus terreus* and 50°C for *Aspergillus aculeatus*. 
Conclusion

The present study reveals that *P. aphidis* PYCC 5535\textsuperscript{T} is a highly potential and promising microorganism as it produced high levels of xylanase when cultured on xylan and xylose. The microorganism is promising for industrial application, since it grows in simple and complex substrates and secretes the enzymes, and its set of enzymes displayed properties that potentially match with industrial applications, including those requiring cellulase-free xylanases. The xylanase production by *P. aphidis* is, to the best of our knowledge, superior than any other yeasts described in the literature. Further studies need to be done in order to use low-cost substrates, as lignocellulosic substrates.

![Figure 3. Effect of pH (A) and temperature (B) on xylanase activity of extracellular extracts of *P. aphidis* PYCC 5535\textsuperscript{T} cultivated in xylose (40 g/l) for 7 days, at 28°C and 140 rpm.](image-url)