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Pseudozyma sp. isolation from *Eucalyptus* leaves and its hydrolytic activity over xylan

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ABSTRACT

Eucalyptus leaves were investigated as a source for the isolation of xylanase producing microorganisms. A total of 37 isolates were obtained after a series of enrichment steps. Seven of the isolates were xylanase positive in an agar screening experiment and were further analyzed in liquid media with beechwood xylan as carbon source. A yeast identified as *Pseudozyma* sp. showed the highest xylanase activity in tested conditions. Afterwards, different lignocellulosic residues were studied as substrates for xylanase production by this strain and the best results were obtained with corncob.

Yeast's xylanase with a molecular weight of 19.9 kDa showed the maximum activity at pH 4.8 and 50 °C. Thermostability was observed at 30 °C with a 60% activity retention after 10 days. By the hydrolytic activity of the enzyme was characterized as an endoxylanase, similar as the ones found in family GH 10, from the products obtained by beechwood xylan hydrolysis.

1. Introduction

In order to reduce the use of fossil fuels and develop a bio-based economy, it is necessary to produce biofuels from renewable sources. Lignocellulosic material is the non-edible portion of the plants, is the most abundant and bio-renewable biomass on earth and is the major component of woody and non-woody plants (Isikgor and Becer, 2015; Pennacchio et al., 2018). (). It generally, consists of 11–40% cellulose, 10–36% hemicellulose and 15–30% lignin (Mussatto, 2016). Cellulose and hemicelluloses are polysaccharides, while lignin is an aromatic heteropolymer bounded to xylan to form a matrix that surrounds cellulose (Makhuvele et al., 2017). In nature, lignocellulosic biomass is degraded with the cooperation of several microorganisms, including diverse fungal and bacterial genera producing a variety of cellulolytic, hemi-cellulolytic and ligninolytic enzymes under aerobic and anaerobic conditions (Carro et al., 2016). The main enzymes involved in the general carbohydrate hydrolysis processes are xylanase (endo-1,4-β-D-xylanase, (EC 3.2.1.8)), cellulase (endo-1,4-β-D-glucanase (EC 3.2.1.4), exo-1,4-β-D-glucanase (EC 3.2.1.91)), β-xylosidase (EC 3.2.1.37), and β-glucosidase (EC 3.2.1.21). These enzymes are useful for obtaining C5 and C6 sugars from the lignocellulosic biomass. Xylose and glucose, are used as substrates in fermentation processes to produce biofuels, building blocks and high value-added compounds. In this context, the concept of biorefinery has emerged with the aim of optimizing the processing of biomass to obtain biofuels, biomaterials and bioproducts (International Energy Agency, 2009).

Xylanases have been traditionally used in several industrial processes over the past decades, especially in the food, feed, detergents and pulp industries. However, these enzymes are becoming attractive for the saccharification process of lignocellulosic biomass, once they can hydrolyze hemicellulose and assist in the hydrolysis of cellulose, to obtain fermentable sugars with potential to produce other chemicals with high added value such as xylitol, succinic, acetic, lactic and butyric acids, butanol, 2,3 butanodiol, acetoin, acetone, propanol and furfural (Goldbeck et al., 2016).

The use of xylanases to achieve hemicellulose hydrolysis leads to high sugar yields, high selectivity and requires low energy costs and mild operating conditions (Kumar et al., 2017). The main argument

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against its use is the high costs of enzyme production, representing one of the major bottlenecks for the lignocellulose conversion (Singhania et al., 2015; Wu et al., 2017).

To overcome this problem, research focuses on the discovery of more effective hydrolytic enzymes. The isolation of microorganisms producing hydrolytic enzymes, from a wide variety of sources, such as trees (Pennacchio et al., 2018), decaying wood and bagasse (Lara et al., 2014), the rhizosphere (Rai et al., 2016), as well as different animals such as termites (Ali et al., 2017) and ruminants (Wei et al., 2016) is a strategy that is being used.

Another approach for lowering economic costs is the utilization of cheap substrates, as lignocellulosic residues, for the growth of microorganisms that produce xylanases. These materials can be an environmental problem when discarded, but nonetheless also could be a source of reusable feedstock for biotechnological processes (Menezes da Silva et al., 2017). The production of xylanolytic enzymes using inexpensive and easily available agricultural residues as substrates, could contribute to the reduction of the production costs and help solving environmental pollution problems (Ravindran and Jaiswal, 2016).

Forestry and agricultural industries constitute one of the main economic activities in Uruguay. About half of all industrial production is dedicated to food processing or the refining of agricultural products (MGAP-DIEA, 2017). The transformation of these products (wood pulp, cereals such as barley, corn and rice and citrus fruits) generates vast amounts of residues rich in lignocellulose that could be applied in different processes.

Considering the above, in this study we investigated the ability of the microorganisms inhabiting in *Eucalyptus* leaves, to produce xylanases enzymes. Of the total of the microorganisms isolated we selected *Pseudozyma* sp. EBV97-87 for xylanase production. For this purpose, the effect of different lignocellulosic substrates as carbon source for xylanase production was evaluated. The crude enzymatic extract was then purified and characterized in terms of optimal pH and temperature. Finally, we identified the xylanolytic enzymes present in the enzymatic crude by the products obtained from the hydrolysis of beechwood xylan.

2. Materials and methods

2.1. Plant material

Leaves from an individual *Eucalyptus globulus* tree located at the Faculty of Agronomy, UdelaR, (34°50′12.9″S 56°13′17.8″W), were collected in March 2017 and transported to the laboratory in sterile sealed bags. The tree was identified by MSc. Fernando Irisity of Forestry Department of Faculty of Agronomy, UdelaR.

Corncobs were obtained from corn brought at a local market. Rice straw was provided from a local producer from Melo, Cerro Largo, Uruguay (32°28′51″S 54°34′37″W). Orange peel was provided by Azucitrus (NovaCore S.A.) from Paysandú, Uruguay (32°17′05.0″S 58°04′38.7″W). Brewers spent grain was obtained from a local organic beer factory.

Eucalyptus dunnii bark was provided by UPM Forestal Oriental, aforestry and wood supplying company located in Río Negro, Uruguay (32°50′53.3″S 57°57′24.3″W). All plant material was dried at 45 °C and milled to pass a 5 mm screen in a Retsch® SK 100 Cross Beater Mill.

2.1.1. Eucalyptus dunnii bark delignification with peracetic acid

Extractive-free milled *Eucalyptus dunnii* bark (Sluiter et al., 2008) was delignified with peracetic acid (Evtuguin et al., 2003). Samples were stored at room temperature in a sealed bag.

2.1.2. Bark chemical composition analysis

Cellulose, hemicellulose and lignin content were determined by the method developed by NREL (Sluiter et al., 2012), with minor modifications. Modifications introduced to the NREL/TP-510-42618 method were reported previously (Reina et al., 2016).

2.2. Reagents

Beechwood xylan, carboxymethyl cellulose (CMC), xylose, 3,5-dinitrosalicylic acid (DNS), orcinol, Bovine serum albumin (BSA) and protein standards were obtained from Sigma-Aldrich (St Louis, USA). Xylooligosaccharides with degree of polymerization (DP) 2–5 were obtained from Megazyme (Bray, Ireland). Culture media were purchased from Difco ™ (Detroit, USA). All other chemicals were of analytical grade unless otherwise stated.

2.3. Microorganism isolation

Fragments of 1 cm of *Eucalyptus globulus* leaves were transferred to minimal medium yeast nitrogen base (YNB) and M9 supplemented with xylan (0.5%) as carbon and energy source. Three rounds of serial culturing (1:10 dilutions) were followed, all incubated at 28 °C and 150 rpm (orbital shaker IKA KS4000 ic). In each step, an aliquot of cell culture was spread onto potato dextrose agar plates (PDA) and tryptic soy agar plates (TSA), and the plates were inspected daily. All colonies found were isolated and maintained for later experiments. Bacterial and yeast strains were maintained as frozen cultures in 17% glycerol at −20 and at −70 °C. In the case of filamentous fungi, pieces of agar with grown colonies were transferred to sterile vials with distilled water and maintained at 5 °C.

2.4. Screening for xylanase and cellulase activity on solid media

For xylanase/cellulase activity screening, pure cultures of isolates were grown in YNB agar with 0.5% xylan/CMC. Cultures were incubated at 28 °C and when visual growth was observed, plates were flooded with an aqueous solution of Congo red (0.1% Congo red in distilled water). After 15 min, the Congo red solution was poured off and plates were further flooded with 1 M NaCl for 15 min (Teather and Wood, 1982). Xylanase/Cellulase activity was detected by observation of clear zones around colonies. Strains with a hydrolysis halo radius > 0.5 cm were selected for further analysis.

2.5. Screening for xylanase activity on liquid media

Pure cultures of isolates selected by the screening described above were grown in two different media with addition of 0.5% beechwood xylan. Potato dextrose broth (PDB) and yeast nitrogen base (YNB) were used as nutrient and minimal medium for fungi strains and tryptic soy broth (TSB) and M9 for bacteria strains. A single colony from fresh culture of yeast or bacteria was inoculated in 100 mL Erlenmeyer flasks containing 20 mL of the corresponding medium. For fungal strains, a piece of 1 cm diameter of mycelial growth in solid media was used as inoculum for the experiments. Cultures were incubated for 24, 48 and 120 h (for bacteria, yeast and filamentous fungi respectively) at 28 °C and 150 rpm (orbital shaker). At the end of the incubation period, culture filtrates were collected in clean and sterile tubes for xylanase quantification.

A cellulase assay was performed on selected strain. Inoculum was performed as described above and culture was grown in YNB with addition of 0.5% CMC. After incubation at 28 °C and 150 rpm (orbital shaker) for 48 h, culture filtrates were collected in clean and sterile tubes for cellulase quantification. All experiments were performed in duplicates.

2.5.1. Xylanase and cellulase activity and total protein concentration

Xylanase assay was performed according to Bailey et al. with few modifications (Bailey et al., 1992). The reaction mixture of 1.5 mL of a 2.0% (w/v) suspension of beechwood xylan in 50 mM sodium citrate buffer at pH 4.8 and 0.5 mL of the enzyme diluted in the same buffer,

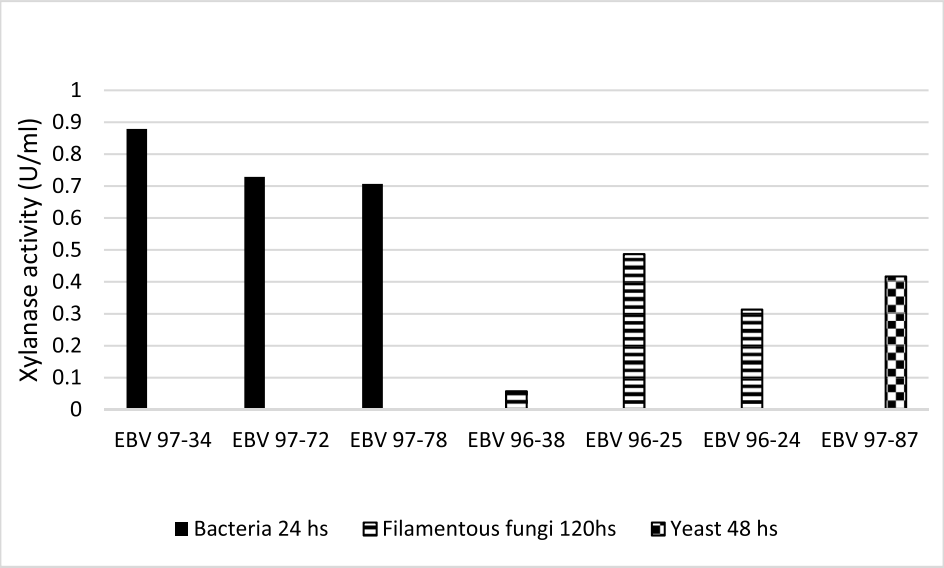

Fig. 1. Xylanase activity by isolated strains in rich culture media.

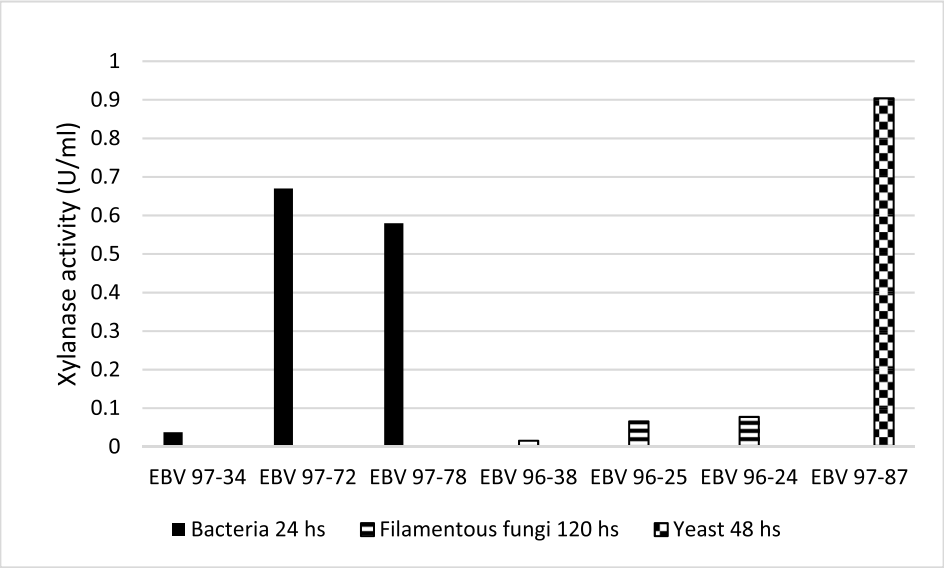

Fig. 2. Xylanase activity by isolated strains in minimal culture media.

was incubated at 30 °C for 15 min. Reducing sugars were determined by adding 3.0 mL of 3,5-dinitrosalicylic acid solution (Adney and Baker, 2008) and incubating the mixture at 95 °C for 5 min. Absorbance was measured at 540 nm on a UV-spectrophotometer (Thermo Scientific). One unit of enzyme was defined as the amount of enzyme catalyzing the release of 1 μmol of reducing sugars as xylose per min. under the specified conditions.

Filter paper cellulase activity (FPase) was assayed by incubating 0.5 mL of enzyme solution and 1.0 mL of sodium citrate buffer (50 mM, pH 4.8) with 50 mg filter paper (Whatman no. 1, 0.25 mm pore size); at 50 °C for 60 min. Reducing sugars were determined as above. One international unit of FPase activity is the amount of enzyme that forms 1 μmol of reducing sugars as glucose per minute during the hydrolysis reaction.

Protein concentrations in crude extract and in the partially purified extract were determined according the Bradford method (Bradford, 1976) using Biorad reactive (München, Germany) and BSA for standard curve preparation.

2.6. Identification of selected yeast

Selected yeast from screening assays was identified by analysis of the sequences of the D1/D2 variable domains of the large subunit rRNA gene. Extraction of genomic DNA was carried out with ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research). Amplification of D1/D2 region was performed with primers NL-1 and NL-4 (Kurtzman and Robnett, 1997). The thermal profile was 94 °C 5 min, followed by 35 cycles of 94 °C 30 s, 55 °C 1 min, 72 °C 2 min, and a final extension step at 72 °C for 7 min. Sequencing of the purified PCR product was performed at Macrogen Inc. (Seoul, Korea). Sequences were aligned with Vector NTI (Life Technologies) and sequence similarity searches were performed with BLAST network service of the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The yeast was deposited in the Colección Española de Cultivos Tipo (CECT) as *Pseudozyma* sp. EBV 97–87 (CECT 13162).

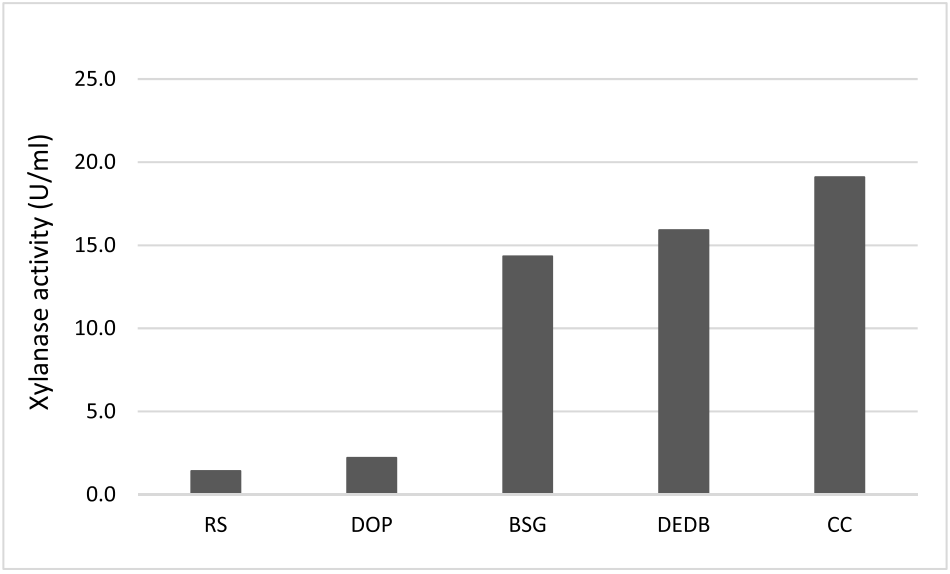

Fig. 3. Xylanase activity of extracellular crude extract from *Pseudozyma* sp. EBV 97–87 at 30 °C and 96 h using a different carbon source (rice straw (RS), dried orange peel (DOP), brewers spent grain (BSG), delignified *E. dunnii* bark (DEDB) and corn cob (CC).

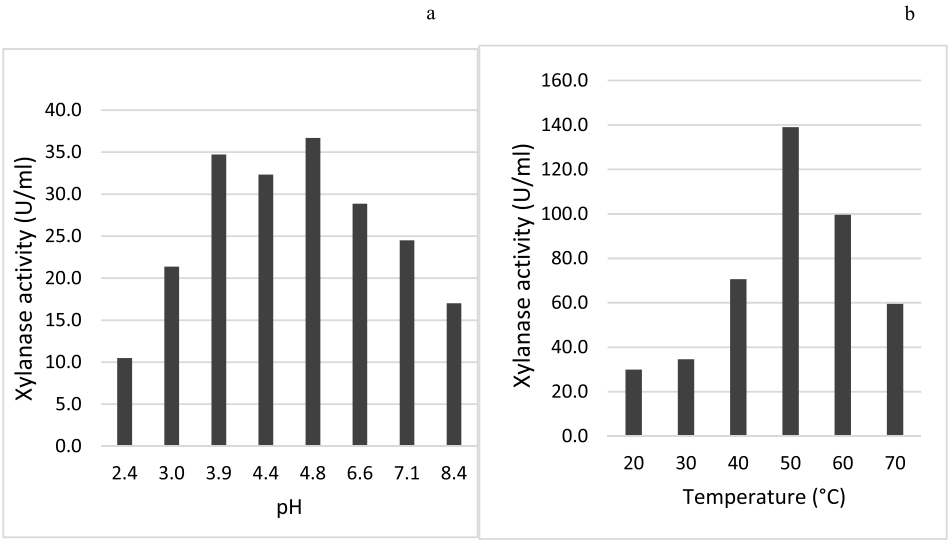

Fig. 4. Effect of pH and temperature on xylanase activity in the extracellular purified extract from *Pseudozyma* sp. EBV 97–87.

2.7. Xylanase production with different agricultural residues

A pure culture of *Pseudozyma* sp. EBV 97–87 was grown in 50 mL of modified Czapek-Dox medium (CDm) prepared in a 250 mL flask under submerged fermentation conditions. CDm medium composition was (g/L): 7.65 $NaNO_3$, 3.04 KH_2PO_4, 1.52 $MgSO_4{\cdot}7H_2O$, 1.52 KCl; supplemented with 0.1% w/v yeast extract and 0.5% peptone. CDm medium was supplemented with 1% agricultural residues sterilized. Residues used were orange peel, rice straw, delignified *E. dunnii* bark, corncob and brewers spent grain.

As inoculum a cell suspension in sterile saline solution (0.9% NaCl) was used, reaching a final concentration of 10^5 cell/mL in the culture media. Flasks were incubated at 150 rpm (orbital shaker) at 28 °C for 96 h and xylanase activity was determined. Experiments were performed in duplicates.

2.8. Xylanase extract characterization

The extract from CDm with 1% beechwood xylan was subjected to salting out for concentration and characterization. Crude extract (50 mL) was centrifuged at 4 °C and 5000 rpm and the supernatant was subjected to 70% ammonium sulphate precipitation, and left overnight at 4 °C. The resulting precipitate was collected by centrifugation at 10,000 rpm for 15 min. Precipitates were then resuspended in minimum volume of 50 mM citrate buffer (pH 4.5) at 30 °C and 150 rpm for 1 h. Xylanase activity and protein concentration were determined before and after concentration. These samples were used to characterize the xylanolytic extract and hydrolyze beechwood xylan.

The effect of pH in the activity was determined by incubating the reaction mixtures at different pH for 15 min at 30 °C. The buffers used were 0.1 M citrate (pH 2.4–4.8), 0.1 M phosphate (pH 6.6–8.4). Optimum temperature was determined by incubating the reaction mixture at various temperatures ranging from 20 to 70 °C in increments of 10 °C, and keeping constant pH 4.8 for 15 min. Experiments were performed in duplicates.

Thermal stability was measured by incubating the purified extract in a 20 mM citrate buffer pH 4.8, at different temperatures (4, 30 and 50 °C) for 444 min. Residual activity was calculated at different time

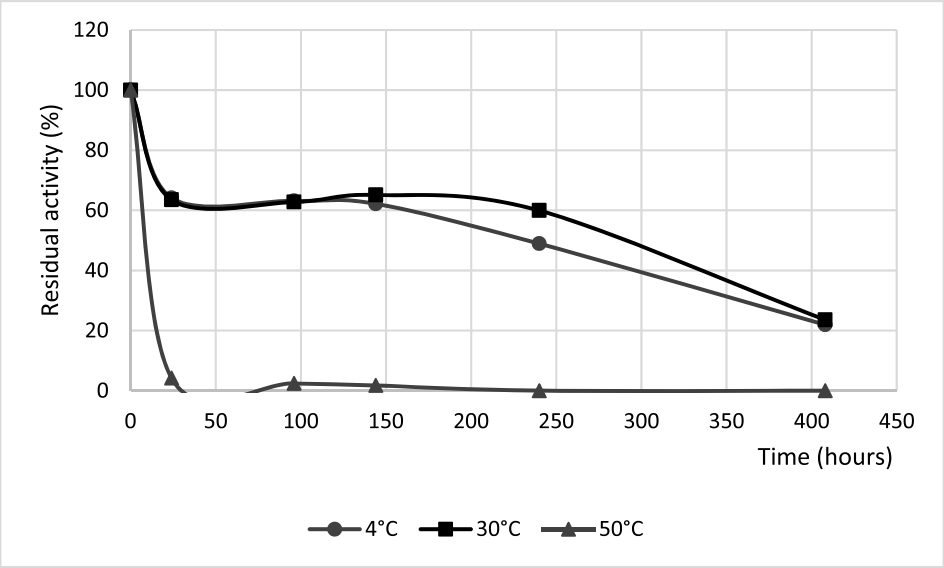

Fig. 5. Thermal stability of xylanase in extracellular purified extract from *Pseudozyma* sp. EBV 97–87. Residual activity is expressed as percentage of the initial activity.

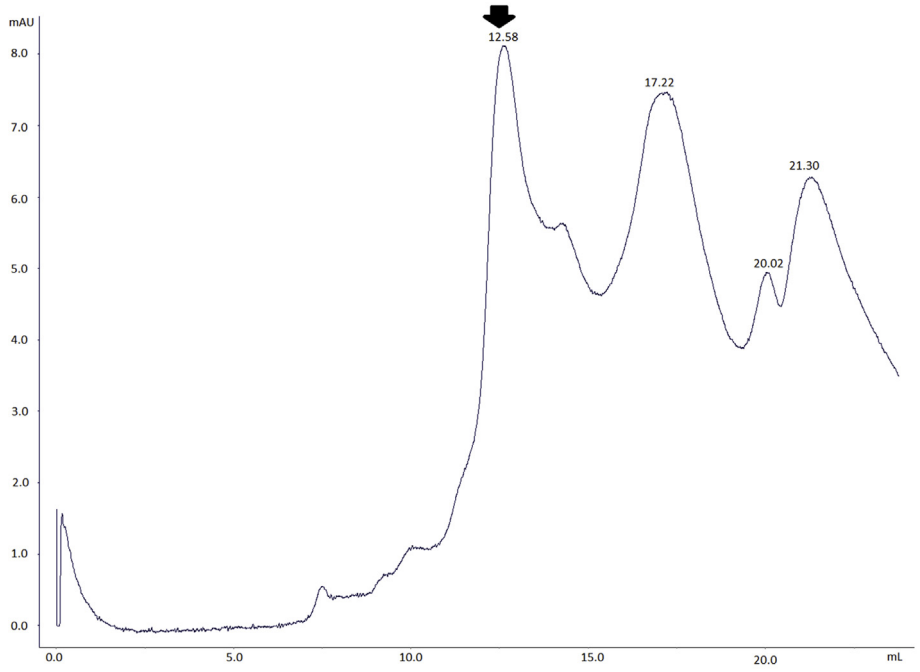

Fig. 6. Protein elution peak profile of *Pseudozyma* sp. EBV 97–87 after gel filtration. Peak with xylanase activity is indicated with an arrow in the figure. Absorbance was measured at 280 nm.

intervals as a percentage of the initial activity measured under standard conditions.

2.8.1. Size exclusion chromatography

To study the molecular weight (MW) and determine the xylanase enzymes present in the extract, size exclusion chromatography (SEC) analysis was performed in an AKTA Purifier System (AKTA Purifier10, General Electrics), using the Superdex™ 75 10/300 GL column (GE Healthcare) following the manufacturer's instructions.

The column was calibrated using the following set of standards: blue dextran (MW > 2000 kDa), thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa) and ribonuclease (13.7 kDa). Each eluted peak was pooled, and xylanase activity was measured.

2.8.2. Beechwood xylan hydrolysis and product analysis

Reactions were conducted in citrate buffer (pH 4.8), with 2% beechwood xylan and SEC purified xylanase (2 U/mL) at 30 °C. After 30 min, reaction was stopped by boiling. A sample of reaction mixture was applied to a Thin Layer Chromatogram sheet (TLC, Silica gel 60 F254, Merck, Darmstadt, Germany) with Xylo-oligosaccharides standard mixture (2 mg/mL) containing xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5) and xylohexaose (X6), and was placed in a chamber containing 3:6:1 solvent mixture of chloroform: acetic acid: H_2O. The compounds were visualized with 0.5% orcinol solution (w/v) in 5% H_2SO_4 in ethanol (v/v), followed by heating.

The hydrolysis products were also analyzed by HPLC using a Shimadzu LC – 20AT equipped with a refractive index detector

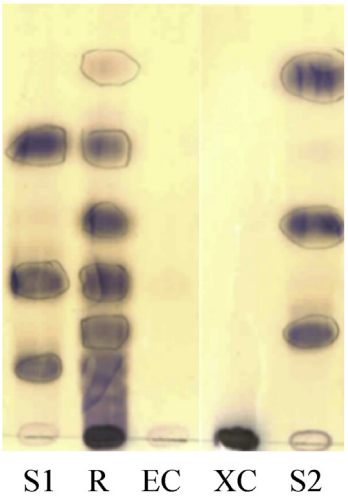

Fig. 7. Thin Layer Chromatogram (TLC) of beechwood xylan hydrolysis products with purified xylanase from *Pseudozyma* sp. EBV 97–87 incubated at 30 °C for 30 min. Standards used S1: xylobiose, xylotetraose and xylohexaose. R: hydrolysis reaction. EC: Enzyme control. XC: xylan control. Standards S2: xylose, xylotriose, xylopentaose.

(Shimadzu RID10-AT) using a Supelcogel C610H (30 cm × 4.6 mm) column at 55 °C, eluted at 0.5 ml/min, 0.005 N sulfuric acid for monomeric and oligomeric sugars (up to xylotetraose).

3. Results and discussion

3.1. Microorganism isolation and xylanase production screening

A total of 37 microorganisms were isolated from *E. globulus* leaves, 15 bacteria, 22 filamentous fungi and 2 yeasts. All strains were tested for xylanase activity in solid media and 7 were selected, 3 filamentous fungi (EBV96_24, EBV96_25, EBV96_38), 3 bacteria (EBV97_34, EBV97_72, EBV97_78) and 1 yeast (EBV97_87). The microorganisms selected were characterized for their extracellular xylanase activity in two liquid media (minimum and rich) using xylan as inducer in both cases (Figs. 1 and 2).

Even though all strains showed xylanase activity in both media, the yeast was the only one capable of producing more activity in minimal medium with xylan. Considering results obtained in both screenings (halo radius = 2.5 cm and xylanase 0.9 U/mL), the yeast was selected to complete the identification and characterization in terms of xylanase production.

3.2. Yeast identification

The selected yeast strain with good xylanase activity titers in a minimal medium was identified by molecular analyses based on the D1/D2 domain of the 26S rDNA as *Pseudozyma* sp. Genus *Pseudozyma* encompasses a small group of basidiomycetous yeasts, of which at least 20 species have been described (Joo et al., 2016). The yeasts of this genus were frequently isolated from plant material (Oliveira et al., 2014; Watanabe et al., 2015). It is noteworthy that members of this genus are able to produce a range of biotechnologically relevant enzymes such as cutinase (Seo et al., 2007), lipase (Bussamara et al., 2010) and xylanase (Adsul et al., 2009; Borges et al., 2014), and biomolecules such as biosurfactants (Faria et al., 2014). It was interesting to notice that this basidiomycetous yeast, like others reported *Pseudozyma*, produced extracellular cellulase-free xylanase (Faria et al., 2015). This fact was found out when a hydrolysis halo was observed on beechwood xylan but did not show cellulase activity in CMC agar nor in a minimal liquid media with CMC after 48 h of growth. The use of crude cellulase-free xylanase in biotechnological industries is cost effective since purification of xylanase is not necessary (Anthony et al., 2003).

3.3. Xylanase production with different agricultural residues

The use of residues for enzyme production is an attractive strategy as it contributes to valorize them and reduce the formation of wastes

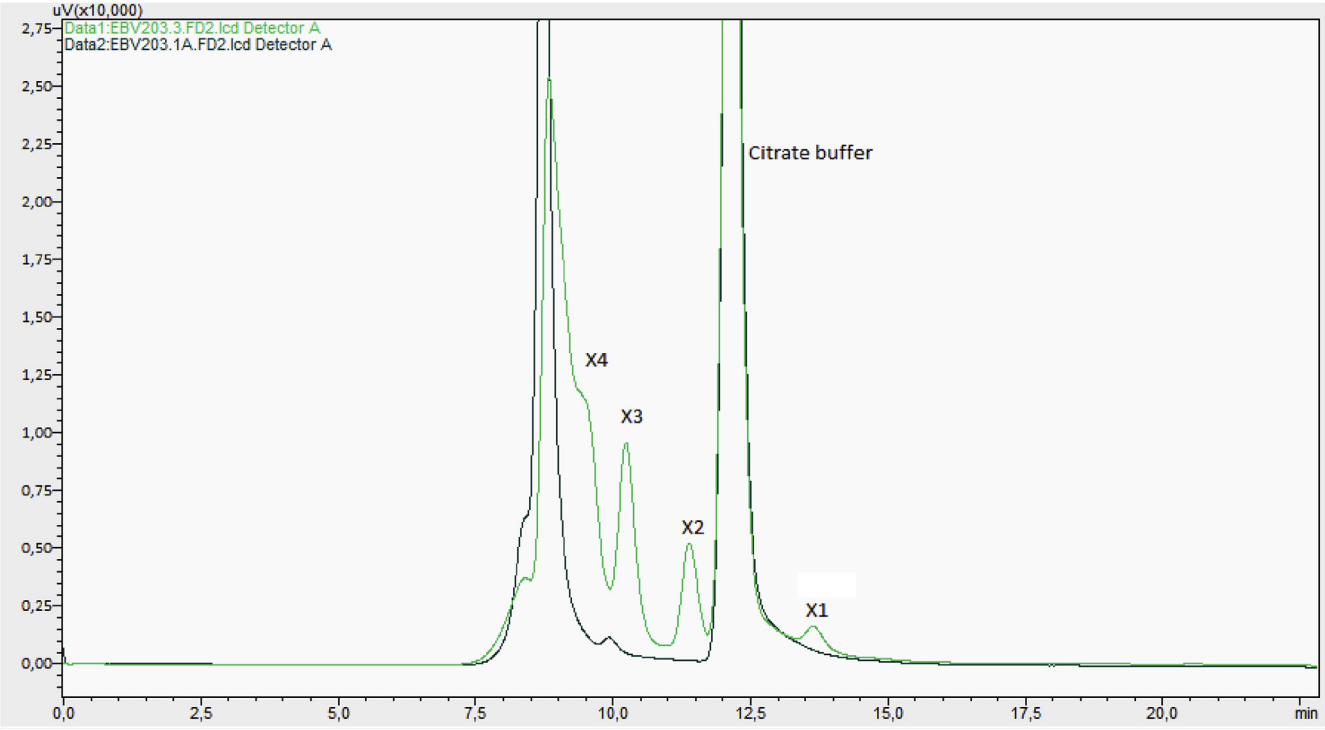

Fig. 8. HPLC chromatograms of beechwood xylan hydrolysis products with purified xylanase from *Pseudozyma* sp. EBV 97–87 incubated at 30 °C for 30 min (green). Xylan control (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

with high content of organic matter and easily available and inexpensive (Rocha dos Santos et al., 2015) (Lee et al., 2018). With this aim our group used different agroindustrial and forest residues as carbon source in a mineral medium, to reduce the costs of xylanase production. Rice straw (RS), delignified *E. dunnii* bark (DEDB), dried orange peel (DOP), corn cob (CC) and brewers spent grain (BSG) were used. Fig. 3 shows the xylanase activity obtained for *Pseudozyma* sp. EBV 97-87 growing with these substrates.

The highest activities were observed in medium supplemented with brewers spent grain, delignified *E. dunnii* bark and corn cob.

The results obtained in this work agree with a previous report, Watanabe et al. used bioethanol distillery wastewater from the hydrolysis of rice straw (LBDW) as carbon source for cultivation of *P. antarctica* GB-4 (0) and obtained a xylanase activity of 17.3 U/mL after 72 h (Watanabe et al., 2015). A recent study about xylanase activity in *Moesziomyces antarcticus* and *M. aphidis* (formerly *Pseudozyma*) reported a better xylanase activity (518.2 U/mL) in a medium with 4% brewers spent grain as carbon source after 10 days (Torres Faria et al., 2019).

3.4. Characterization of xylanase from Pseudozyma sp. EBV 97-87

A xylanolytic extract was obtained by *Pseudozyma* sp. EBV 97–87 when was grown on Czapek-Dox mineral medium with 1% beechwood xylan. A partial purification with ammonium sulfate (70%) was carried out. A purification factor of 1.7 was obtained and this partial purified extract was used for characterization.

3.4.1. Effect of pH and temperature on the activity

As shown in Fig. 4 the partially purified extract of *Pseudozyma* sp. EBV 97–87 exhibited activity in the pH range of 3.0–8.0 with a maximum in the range of 3.9–4.8. The optimum temperature was found in the range between 40 and 60 °C, with the maximum at 50 °C (Fig. 4 b). These results agree with previous reports where the maximum xylanase activity from *Pseudozyma* sp. was between pH 4.0 and 6.5 and optimum temperatures between 40 °C and 65 °C (Adsul et al., 2009; Borges et al., 2014; Torres Faria et al., 2019; Watanabe et al., 2015). It is interesting to note that the relatively high activity at low temperatures (21% of maximum activity at 30 °C) may allow the combination of enzymatic hydrolysis and microbial conversion processes to produce chemicals such as xylitol (Torres Faria et al., 2019).

3.4.2. Thermal stability

Thermal stability of the xylanolytic extract was studied at three temperatures, 4, 30 and 50 °C at different times (Fig. 5). It was established that at 4 and 30 °C the extract showed similar profiles of activity loss, but at 50 °C the extract had only 10% of residual activity after 24 h. Although optimum temperature is around 50 °C, the extract was not stable at this temperature. Other reports on thermal stability of *Pseudozyma* xylanases showed the same result (Adsul et al., 2009; Watanabe et al., 2015). Specifically, a work from Faria et al. reported 83% of activity loss after 24 h at 50 °C for a *Moesziomyces* spp. xylanase (Torres Faria et al., 2019). It is noteworthy that at 4 and 30 °C between 50 and 60% of activity was retained after 10 days and 20% after 17 days at these temperatures. These results are of great importance since they indicate that the extract can be conserved for long periods of time.

3.4.3. Molecular weight

A size exclusion chromatography was carried out to determine the number of xylanases in the extract and their molecular weight. Only one peak of the chromatogram was positive for xylanase activity (Fig. 6) and its molecular weight was determined to be 19.9 kDa. Xylanases reported for *Pseudozyma* to date have similar or higher molecular weights 33 kDa in *P. antarctica* GB-4 (0) (Watanabe et al., 2015), 33.5 kDa and 20.1 kDa in *P. hubeiensis* NCIM 3574 (Adsul et al., 2009) and 24 kDa in *P. brasiliensis* GHG001 (Borges et al., 2014).

3.4.4. Beechwood xylan hydrolysis

To deepen into the characterization of the xylanase produced by *Pseudozyma* sp. EBV 97–87 the products obtained from the beechwood xylan hydrolysis were studied. The sugars released by hydrolysis of the xylan were identified as xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), and other xylooligosaccharides (Figs. 7 and 8).

These results were also observed by Watanabe et al., after using a purified a xylanase from *Pseudozyma antarctica* GB-4 (0) for beechwood xylan hydrolysis. They studied whether this xylanase had β-xylosidase activity and found a negative result. They were able to classify the enzyme according to the catalytic module sequence in the GH10 family, which exhibits a higher affinity for shorter and linear β-1,4-xyloligosaccharides than xylanases from GH11 family (Arumugam et al., 2019; Biely et al., 1997; Kolenová et al., 2006; Rahmani et al., 2019; Watanabe et al., 2015).

On the other hand, PbXynA xylanase, purified from *Pseudozyma brasiliensis* GHG001 by Borges et al. showed as hydrolysis products of beechwood xylan a set of XOS (X2 to X6), and only after 20 h small amounts of free xylose (Borges et al., 2014).

Other reports indicate differences in the xylooligosaccharides formed. When the xylanase from *Pseudozyma hubeiensis* NCIM 3574 was used for hydrolysis of a different xylan structure, such as cereal arabinoxylan, XOS of 3–7 xylose units were obtained, but neither X1 nor X2 was produced from the hydrolysis of wheat xylan (Adsul et al., 2009). This difference could be due to the structure of the xylan. In cereals an arabinoxylans structure with ferulic acid as a substituent is present and could interfere in the active site of the enzyme and so in the products formed (Biely et al., 2016). In that sense a further characterization of the xylanolytic extract of *Pseudozyma* sp. EBV 97–87 isolated by our group could be tried by hydrolysis studies of other hemicellulosic materials.

4. Conclusion

Our results show that natural habitats such as *Eucalyptus* tree leaves are a useful source of microorganisms with lignocellulose degradation enzymes. In particular, an isolated *Pseudozyma* strain was able to produce a good titer of xylanase. This enzyme hydrolyzed beechwood xylan to xylose, xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose, indicating endoxylanase activity. The xylanase was active in a wide pH and temperature range and was stable at low and moderate temperatures for prolonged periods of time. These characteristics together with the possibility of producing it using cheap agricultural wastes such as corncobs, opens an opportunity for possible biorefinery applications.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101282.

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