Xylanase SMXL1 from *Stenocarpella maydis*: Purification and Biochemical Characterization

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This study aimed to develop a method for the purification of a xylanase called SMXL1 produced by *Stenocarpella maydis* and its biochemical characterization. The enzyme was purified using a Rotofor preparative chamber and one chromatographic step in an ion exchange column coupled to equipment FPLC. Posteriorly the protein was characterized, and its effect on the birchwood xylan degradation was determine by HPLC. The purified enzyme showed a molecular weight of 55 kDa calculated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The purification process obtained a yield of 6.5 ± 0.3 %. The activity was stable at a pH range of 4 to 10 and temperatures of 45 to 60 °C. The optimum values of temperature and pH were 55 °C and 4, respectively. The Michaelis constant (K_m) value was 2.61 mg/mL and the V_{max} was 3.02 µmol/mL/min using birchwood xylan as substrate and the Michaelis-Menten equation. The enzyme is inhibited by the cations Mn^{2+} and by Fe^{3+} and degrades the birchwood xylan being the principal products the xylobiose and the xylose. This work is the first report of the purification and biochemical characterization of a xylanase called SMXL1 produced by *S. maydis.*

Keywords: Xylanolytic activity; Phytopathogenic fungus; Enzyme; Ascomycete; Xylan substrate; Xylan degradation

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INTRODUCTION

Xylan, after cellulose, is an abundant polysaccharide in nature. It is found in hemicellulose and constituted by a chain of β-D-xylose molecules linked by bonds β,1-4; it is a polymer present in the amorphous matrix in the vegetal cell wall (Kulkarni *et al*. 1999; Collins *et al*. 2005; Sharma and Kumar 2013). Xylan can be degraded by microorganisms, such as bacteria and fungi, which have enzymatic systems to complete the hydrolysis of xylan. Due to the heterogeneity of xylan as a substrate, different enzymes are required, each with a defined and specific role for the complete degradation of this polymer (Biely 1985; Coughlan and Hazlewood 1993).

Degrading enzymes of xylan are classified into two groups, the endo-xylanases and exo-xylanases; these groups act inside and at the end of the xylan molecule, respectively (Coughlan and Hazlewood 1993). The β-1,4-xylanases (EC 3.2.1.8) are known as endo-xylanases, which hydrolyze the β (1-4) bonds, producing xylooligosaccharides of different sizes (Biely 1985).

There is increasing interest in the study of xylanases due to their applications in different industries, such as pulp and paper, textile, food, and pharmaceutical products, as well as fuel manufacturing, besides in lignocellulosic residues degradation (Subramaniyan and Prema 2002; Techapun *et al*. 2003; Dhiman *et al*. 2008; Deutschmann and Dekker 2012; dos Santos *et al*. 2018; Souza *et al*, 2018; Marques *et al*. 2018, 2019; de Almeida *et al*. 2020). Although xylanase production of microbial origin is well documented, their role in phytopathogenic fungi has received little attention even though it is known that xylanase has potential to degrade polymers in the plant cell wall. For example, the xylanase S2 of *Sclerotinia sclerotiorum* has been partially purified and characterized (Ellouze *et al*. 2007). *Fusarium oxysporum* f. sp. *melonis* produces an extracellular endo-1,4-beta-xylanase in medium with oat spelt xylan and glucose, in which these components are the carbon source (Alconada and Martinez 1994). In *F. oxysporum* f. sp. *licopersici* the gene xyl5 encodes for a xylanase of the family 11. Its transcript is present in tomato roots at the beginning of the infection process, but it has no impact on virulence (Gómez-Gómez *et al*. 2002). *F. oxysporum* f. sp. *cucerus* presented xylanolytic activity using the cell walls of chickpea and xylan as the carbon source (Inmaculada *et al*. 2005). *Myrothecium verrucaria* produced xylanase in submerged fermentation with glucose and xylan (Moreira *et al*. 2005). Álvarez-Cervantes *et al*. (2013) purified and characterized one xylanase (SRXL1) produced by *Sporisorium reilianum* grown in both liquid and solid-state fermentations using xylan as a carbon source. In addition, it is known that the number of genes coding for xylanases in industrially important microorganisms, such as *Trichoderma reesei*, is less than in the phytopathogenic fungi. With this antecedent, the use of the microorganisms able to infect plants to search for new enzymes is very important to develop effective processes used in the enzymatic hydrolysis of polysaccharides present in lignocellulosic materials. This knowledge can then be used to obtain biotechnological products and biofuels (Martinez *et al*. 2008).

Different studies have demonstrated that phytopathogenic fungi produces high xylanolytic activity against xylan and untreated biomass. The substrate preference of these enzymes depends on whether the host plant is monocotyledonous or dicotyledonous, so that these enzymatic characteristics could be used to design a specific process to degrade the lignocellulosic materials (Martinez *et al*. 2008). Phytopathogenic fungi that cause rot of plant tissues have a high potential for the production of extracellular hydrolytic enzymes, such is the case of *Stenocarpella maydis*, the causative agent of white rot of stalk and cob in maize (Bensch and Van Staden 1992; Álvarez-Cervantes *et al*. 2016b). In this ascomycete, an extracellular aspartyl protease was purified and biochemically characterized. Moreover, at least two xylanase isoforms were produced in liquid and solid-state fermentations with different carbon sources, and three xylanase isoforms in solid-state fermentation using natural supports from maize were reported (Hernández-Domínguez *et al*. 2014; Mandujano-González *et al*. 2016).

In this work, the purification method of a xylanase of *S. maydis* that has been called SMXL1 and its biochemical characterization were performed; these results could inform about potential biotechnological applications and its possible association with the life cycle of the fungus.

EXPERIMENTAL

Microorganism and Culture Media

The *S. maydis* strain was donated by Dr. Dan Jeffers (International Maize and Wheat Improvement Center, CIMMYT, Texcoco City, Mexico). The fungus was conserved at 4 °C in a tube with potato dextrose agar (BD Bixon™, Mexico City, Mexico).

The culture media for enzyme production was prepared with the following components (g/L): K_2HPO_4, 0.4; $ZnSO_4$ $7H_2O$, 0.001; KH_2PO_4, 0.6; $FeSO_4$ $7H_2O$, 0.05; $MnSO_4$ H_2O, 0.05; $MgSO_4$ $7H_2O$, 0.5; Birch xylan, 5.0, and yeast extract, 9.0 (Hernández-Domínguez *et al*. 2014).

Culture Conditions for Xylanase Production

The production of the xylanase SMXL1 was carried out by culture of *S. maydis* in 1 L Erlenmeyer flasks with 250 mL of production medium. Each flask was inoculated with five mycelium plugs (5 mm diameter), they were incubated at 28 ° C for 7 days in orbital agitation at 160 rpm. The biomass was separated through filtration and the broth obtained with xylanase activity was considered the crude enzyme extract (ECE). The cultivation was done in triplicate. (Hernández-Domínguez *et al*. 2014).

Xylanase Assay and Protein Determination

The xylanase activity was determined by the release of reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). A mixture of 475 µL of birch xylan (0.5%) was prepared in 0.1 M acetate buffer at pH 5.3 and 25 µL of ECE, which was incubated at 50 °C. The amount of enzyme that liberated 1 µmol of xylose per minute was one unit of xylanase. The Bradford method (Bradford 1976) was used to determine the protein content, using bovine serum albumin as a standard.

Purification and Biochemical Characterization

Isoelectric focusing separation was completed with 58 mL of ECE using the Rotofor preparative chamber with 2 mL of ampholytes (pH 3 to 10). The enzymatic activity and protein concentration were determined in all fractions obtained. Those with the higher xylanase activity values were mixed and desalted through a Bio-ScaleTM Mini Bio-Gel® P-6 desalting cartridge equilibrated with 20 mM of phosphate buffer at pH 8.2. This sample was eluted at 2 mL/min and the fractions collected with protein were pooled and introduced in an ion exchange column Bio-Scale Mini UNOsphere Q Cartridge, coupled to an FPLC (Fast Protein Liquid Chromatography, model 2110; BioLogic System, Hercules, CAUSE). The protein separation was done using a linear gradient 0 to 1 M NaCl in phosphate buffer with pH 8.2 at 0.5mL / min. The purification procedure was followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 10% (Laemmli 1970). Gels were stained with the silver technique (Silver Stain kit). To estimate the molecular mass, protein markers ranging from 10 to 250 kDa was used (Precision Plus Protein TM Standards, Hercules, CA, USA) was used.

The activity of the purified xylanase was also detected *in situ* through zymogram (Raghukumar *et al*. 2004), using a native PAGE, with birchwood xylan at 0.5% as substrate in the gel. Once the electrophoretic run was finished, the gel was incubated in agitation at 40 rpm at 50 ºC for 40 min in acetates buffer 0.1 mM at pH 5.3. The enzymatic activity was visualized placing the gel in a Congo red solution at 2% and incubating at room

temperature for 30 min. Posteriorly, washes were made with 1 M NaCl until the white bands were observed.

To determine the optimal pH of the purified enzyme, 100 mM buffers of McIlvaine, Tris-HCl and glycine-NaOH were used for pH 2.0 to 8.0, 8.0 to 9.0, and 9.0 to 11, respectively. Xylan was used as a substrate and the reaction mixtures were incubated at 50 °C. The stability of the purified xylanase to the pH was made by pre-incubating the enzyme for 12 h at 4 °C in each buffer mentioned above, after which the enzyme activity was determined.

The optimum temperature of the enzyme was determined performing the assays at 5, 25, 30, 35, 40, 45, 50, 55, 60, 70, and 80 °C, at its optimal pH. To determine the thermal stability, the purified xylanase was incubated for 35 min at each of the temperatures mentioned above and subsequently its activity was quantified.

The effect of different metal ions at 2 and 10 mM (Zn^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Ca^{2+}, and K^{+}) was evaluated on the purified xylanase and reported as a percentage of activity, considering 100% at the activity value obtained in the absence of any ion or chemical compound. The salts used were $ZnCl_2$, $MgCl_2$, $FeCl_3$, $MnCl_2$, $CuCl_2$, $CaCl_2$ and KCl. The data obtained were compared by means of a two-way ANOVA test. The level of significance was established as $P < 0.05$.

The Michaelis-Menten constant (K_m) and V_{max} were estimated using birch xylan as substrate at 1 to 10 mg/mL, using the Michaelis-Menten equation. The data were analyzed with the non-linear least square-fitting program "Solver" (Excel Microsoft 2016, Redmond, WA, USA). The experiments were conducted in triplicate.

Birch xylan hydrolysis by the purified xylanase

For the enzymatic hydrolysis of xylan, the reaction mixture consisted of 0.5% birch xylan (5 mg/mL in 0.1 M acetate buffer at pH 4.0) with different units of the purified xylanase (0.1- 0.1 U/mL) in 1 mL, and was incubated at 55 °C for 12 h. The tubes were placed in boiling water to stop the reaction. The high-performance liquid chromatography (HPLC; Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the products of hydrolyses, with a 300 × 7.7 mm 8µ HyperREZ XP Carbohydrate H column. Water HPLC grade was applied as eluent at 6 mL/min. The standards xylooligosaccharides of 1, 2, 3, 4, and 5 monomers were used (Pérez-Rodríguez *et al*. 2019).

RESULTS AND DISCUSSION

The enzyme purified to homogeneity in two steps was called SMXL1. The fractions with activity obtained in the isoelectric focusing showed an isoelectric point (pI) around 2.5 (Fig. 1). In the second step of purification (ion exchange chromatography), fractions 17 and 18 had activity and were eluted at around 0.21 and 0.23 mol/L of NaCl (Fig. 2).

The enzyme was purified 4.2-fold with a yield of 6.5% (Table 1). Figure 3 shows the purified protein (SDS-PAGE) and its xylanase activity (zymogram). The molecular weight of the enzyme estimated by SDS-PAGE (denaturing conditions) was 55 kDa.

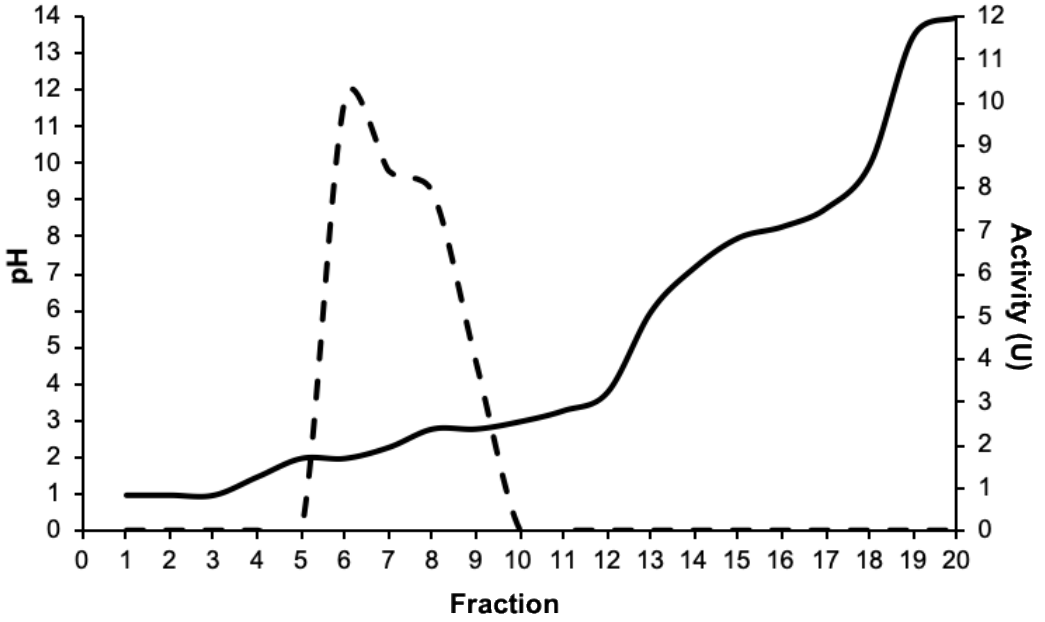

Fig. 1. Isoelectric focusing profile of ECE with xylanase activity of *S. maydis* grow in from liquid culture: pH (solid line) and xylanase activity (dashed line)

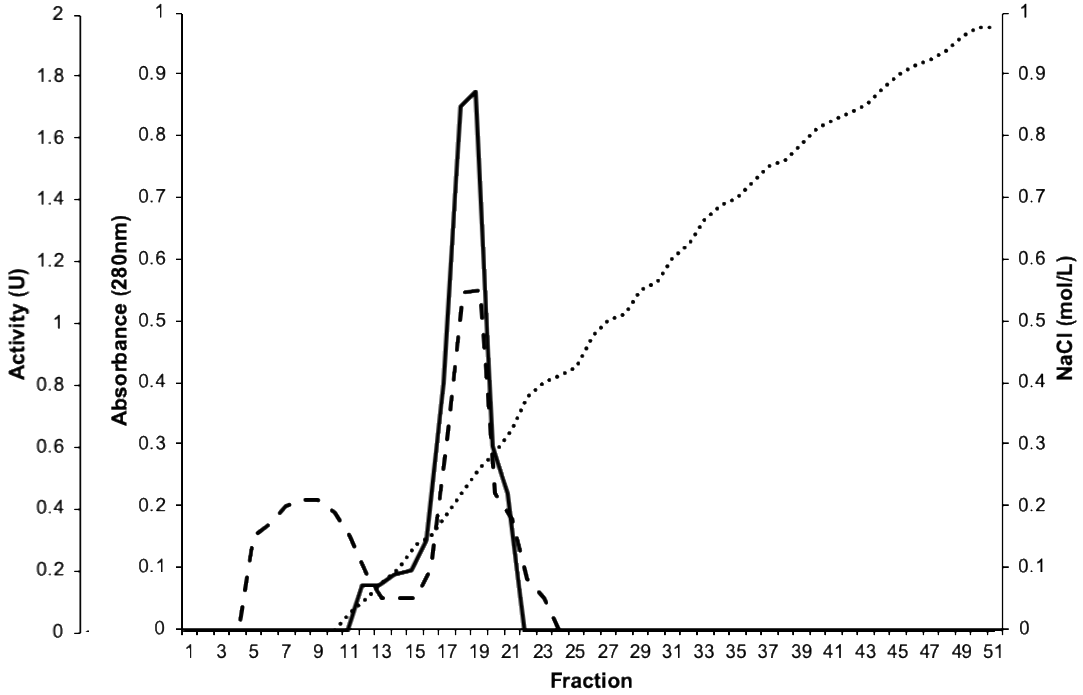

Fig. 2. Ion exchange chromatography of xylanase SMXL1 using an FPLC equipment; Proteins at 280 nm (dashed line), xylanase activity (solid line), and NaCl gradient (dotted line)

Table 1. Purification Steps of Xylanase SMXL1 from *S. maydis*

Purification Step	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg protein)	Yield (%)	Purification (Fold)
ECE	235 ±10.8	130.8 ± 7.0	0.6 ± 0.04	100 ± 7.0	1 ± 0.04
Isoelectric focusing	17 ± 1.4	21.0 ± 1.8	1.2 ± 0.2	16.1 ± 2.1	2.2 ± 0.5
Anion exchange	3.8 ± 0.2	8.6 ± 0.4	2.3 ± 0.1	6.5 ± 0.3	4.2 ± 0.1

The enzyme was stable for 12 h at 4 °C across a wide range of pH values. At pH 3 there was 40% residual activity, while at pH values of 4 to 6 and 7 to 10, the activity values were 70 and 80 to 100%, respectively. The optimum pH for activity of SMXL1 was 4.0 (Fig. 4A), and was stable at temperature values between 45 and 60 °C for 35 min, where it maintained 70 to 100% activity. The optimum temperature for this xylanase was 55 °C (Fig. 4B).

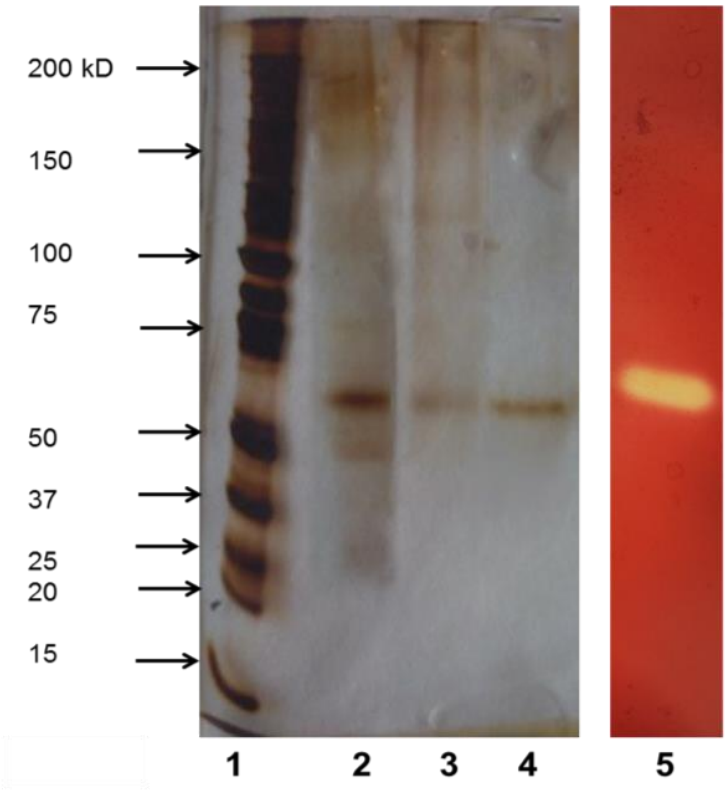

Fig. 3. Electrophoretic pattern (SDS-PAGE) of xylanase SMXL1: Lane 1- molecular weight markers (kDa); lane 2- ECE; lane 3- fraction of Isoelectric focusing; lane 4- fraction obtained of Ion exchange chromatography; and lane 5- zymogram

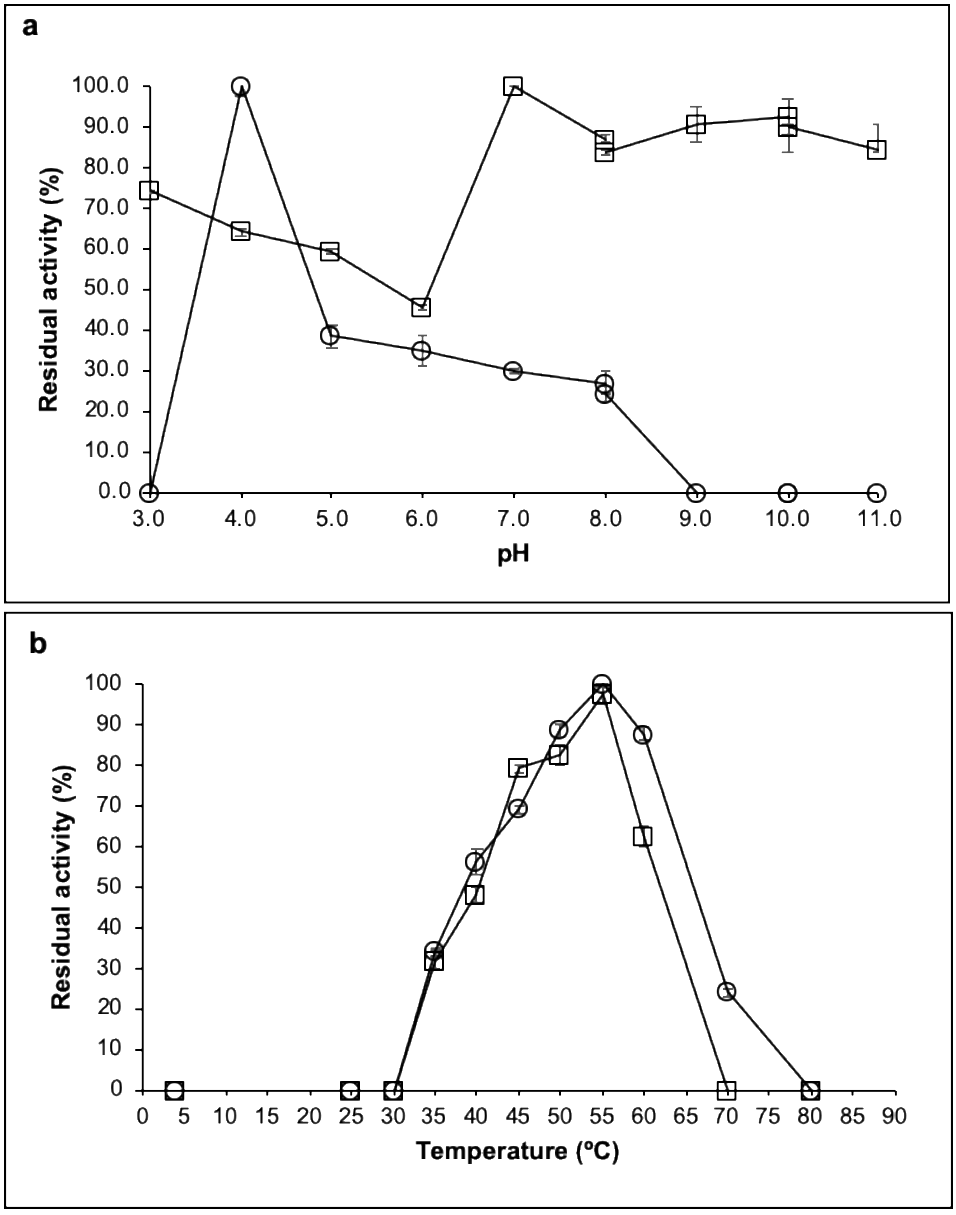

Fig. 4. The effect of pH and temperature on the activity of the xylanase SMXL1: (a) Optimal pH (○), pH stability (□); (b) Optimal temperature (○), temperature stability (□)

Such as is observed in the Fig. 5, the activity was inhibited predominantly by Mn^{2+} at 2 and 10 mM. Inhibition was likewise observed for Cu^{2+} but only at 10 mM; at 2 mM this cation increased the enzymatic activity ($P < 0.001$). The Fe^{3+} showed a slight inhibition effect at the two concentrations assayed, while for the K^{+} and the Zn^{2+} only at 10 mM this same effect was observed ($P < 0.05$). The Mg^{2+} and the Ca^{2+} did not have effect.

The purified enzyme degraded the birch xylan, in which the principal product was xylobiose; following this was xylose and after xylotriose when 1 to 7 U/mL were used and from 8 to 10 U/mL the amount of xylose was smaller than that of xylotriose (Fig. 6). Xylopentose and xylotetrose were not found in the hydrolysis products.

The K_m value was 2.61 mg/mL and the V_{max} was 3.02 µmol/mL/min (Fig. 7).

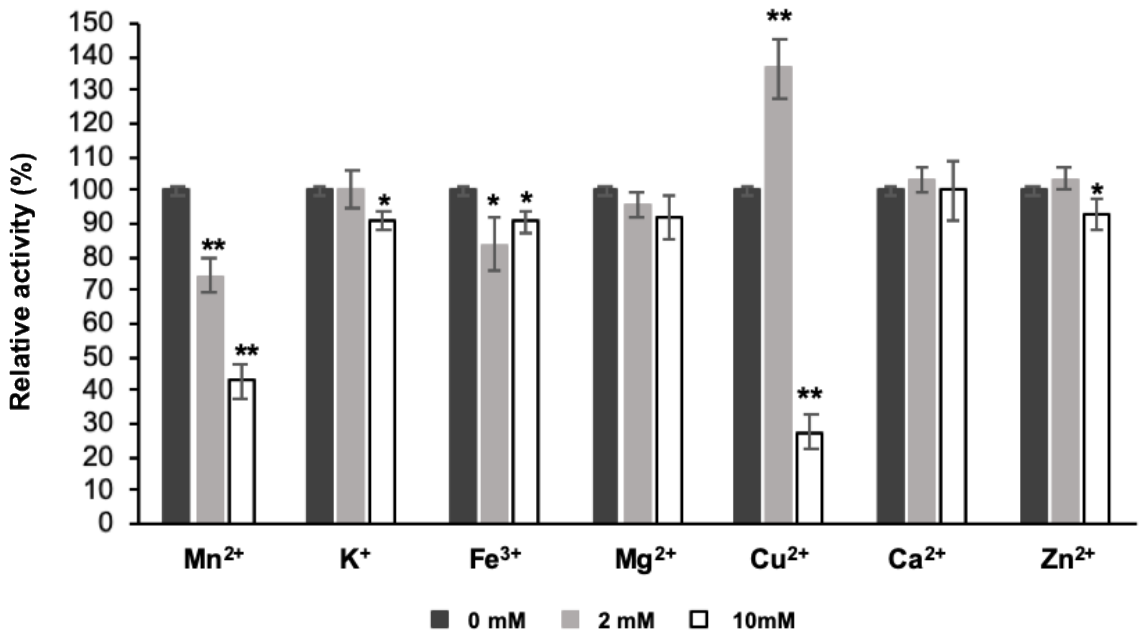

Fig. 5. Metal ions effect on the activity of xylanase SMXL1 of *S. maydis*. * P < 0.05, ** P < 0.001

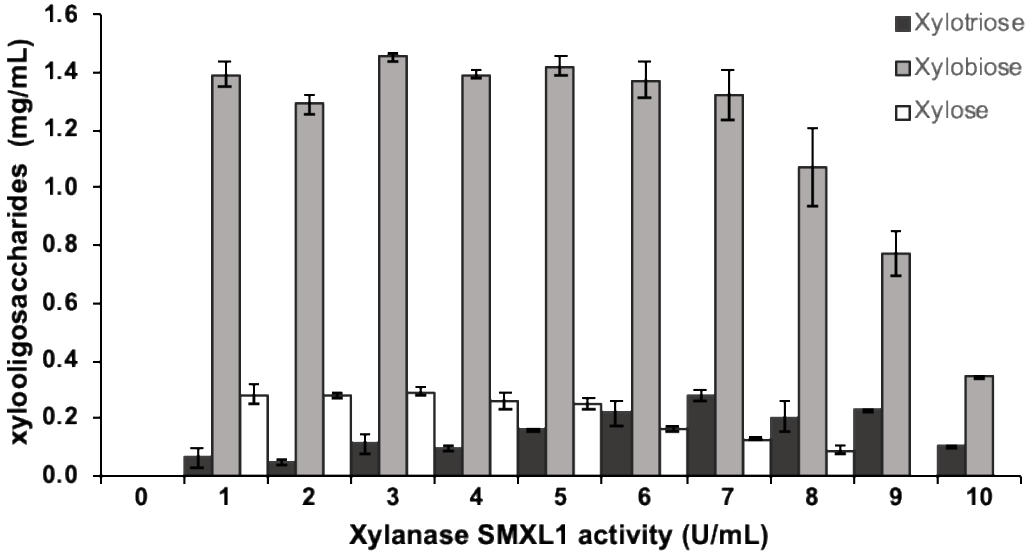

Fig. 6. Hydrolysis products of birch xylan by the xylanase SMXL1 xylanase from *S. maydis*

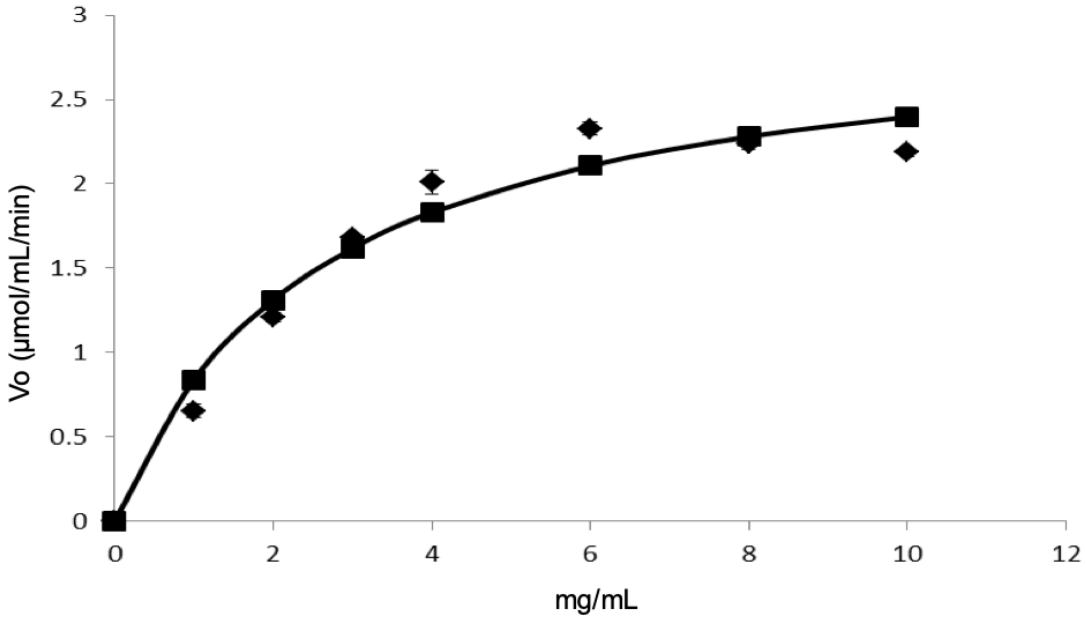

Fig. 7. Effect of xylan concentration on the initial velocity of the xylanase SMXL1 from *S. maydis*

DISCUSSION

In this study, a xylanase called SMXL1 was produced by *S. maydis* grown in liquid culture using a medium previously reported (Hernández-Domínguez *et al.* 2014). The purification process described was simple and involved only two steps. However, yields were low. This can be attributed to the presence of more isoforms in the ECE, because in recent work, using the same culture conditions and the same fungus, two xylanase isoforms by zymograms were observed (Hernández-Domínguez *et al*. 2014). The purified xylanase showed a pI of 2.5. However, other fractions of isoelectric focusing had a reported activity at pH of 2.1, which is probably due to the presence of another isoform.

It is reported that *F. oxysporum* f. sp. *ciceris* produced extracellular xylanases that were classified into basic, neutral, and acidic depending on their pI values (10, 8, and 4, respectively). Production of the first two is favored by the cell walls of chickpea, while acidic occurred in the presence of xylan as a carbon source (Inmaculada *et al*. 2005). The presence of xylanase isoforms could favor the infection of the plants by this phytopathogenic fungus, although the environmental conditions change (Álvarez-Cervantes *et al.* 2016a). The xylanase SMXL1 showed a pI value lower than those reported for fungal xylanases, which are in the range 3.4 to 10.2 (Álvarez-Cervantes *et al.* 2016a).

In general, xylanases show molecular weights ranging from 15 to 145 kDa; basic xylanases present a maximum weight of 30 kDa; and acidic xylanases show higher weight than 30 kDa (Álvarez-Cervantes *et al*. 2016a). The xylanase SMXL1 is acidic with a molecular weight of 55 kDa. It has been suggested that the ability to produce different xylanases is conditioned by both environmental factors and producer microorganisms (Inmaculada *et al*. 2005; Hernández-Domínguez *et al*. 2014; Álvarez-Cervantes *et al*. 2016a).

Xylanases show activity at pH of 4 to 6.5, temperatures from 40 to 80 °C, and K_m values between 1.3 and 348 mg/mL (Polizeli *et al*. 2005; Belien *et al*. 2006; Pollet *et al*. 2009; Álvarez-Cervantes *et al*. 2016a), and the xylanase SMXL1 had these characteristics.

The xylanase SMXL1 was not stable at low temperatures, however, the xylanases of *Aspergillus niger* and *Rhizopus* sp. showed stability at those same conditions (dos Santos et al. 2018).

It is considered that xylanases have an important role in the pathogenic process, allowing them to attack the plant through the rupture of the cell wall. An example of this has been previously reported by Diebold *et al*. (2004) and Kubicek *et al*. (2014), in which the endo-xylanases have the function of promoting the virulence of phytopathogenic microorganisms, thereby inducing a defense response of plants. This could be a function of xylanase SMXL1 in *S. maydis*.

Microbial enzyme systems currently have a major biotechnological potential applicable in numerous industries in the field of so-called clean technologies or sustainable technologies (Collins *et al*. 2005; Belien *et al*. 2006; St. John *et al*. 2006; Kubicek *et al*. 2014; Álvarez-Cervantes *et al*. 2016a; Kaushik and Malik 2016; Kumar *et al*. 2016). In this sense, the xylanase SMXL1 presented biochemical characteristics for possible biotechnological use. It showed activity and stability in acidic pH ranges and temperatures between 45 and 60 °C. The xylanolytic activity is affected by the presence of metal ions. Specifically, Ca^{2+} and Mg^{2+} are activators, while Co^{+2}, Hg^{+2}, Fe^{+2}, Mn^{+2}, and Cu^{+2} are inhibitors of these enzymes (Álvarez-Cervantes *et al*. 2016a). Regarding the xylanase SMXL1, the Cu^{2+} at 2 mM was an activator, and at 10 mM a strong inhibitory effect was observed, while to the xylanase produced by *Penicillium roqueforti* ATCC 10110 strain in where this cation had an activator effect on the enzyme at 1M (Marques *et al*. 2018). In other hand, the Mn^{2+} was an inhibitor of the enzyme purified, and the Ca^{2+} and Mg^{2+} did not show an activator effect.

The endo-xylanases catalyze the hydrolysis of xylan or xylo-oligomers with different degrees of polymerization to xylooligosaccharides but they cannot hydrolyze the xylobiose (Wong *et al*. 1988; Álvarez-Cervantes *et al*. 2016a). The results showed that the xylanase SMXL1 was an enzyme that degraded the birch xylan producing xylotriose and xylobiose. The presence of xylose could have been due to the xylostriose hydrolysis of the enzyme.

This work is the first report where the purification and biochemical characterization was made of a xylanase of S. *maydis* called SMXL1.

CONCLUSIONS

1. The xylanase SMXL1 from *S. maydis* was purified using the Rotofor preparative chamber and through ion exchange chromatography.
2. The purified enzyme had a molecular weight of 55 kDa and a *Km* and V_{max} of 2.61 mg/mL and 3.02 µmol/mL/min, respectively.
3. The optimal values of pH and temperature of SMXL1 were 4.0 and 55 °C, respectively, and was stable at pH of 4 to 10 and temperatures of 45 to 60 °C.
4. The xylanase activity was inhibited by Mn^{2+} and by Fe^{3+} at 2 and 10 mM, while that for the cations K^{+} and Zn^{2+} only a slightly effect was observed at 10 mM.
5. The Mg^{2+} and the Ca^{2+}, do not have effect on the purified enzyme.
6. This enzyme hydrolyzed birch xylan at 55 °C and a pH value of 4, where the principal product was the xylobiose.

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REFERENCES CITED

Alconada, T., and Martinez, M. (1994). "Purification and characterization of an extracellular endo-1,4-ß-xylanase from *Fusarium oxysporum* f. sp. *melonis*," *FEMS Microbiology Letters* 118(3), 305-310. DOI: 10.1111/j.1574-6968.1994.tb06845.x

Álvarez-Cervantes, J., Hernández-Domínguez, E. M., Arana-Cuenca, A., Díaz-Godínez, G., and Mercado-Flores, Y. (2013). "Purification and characterization of xylanase SRXL1 from *Sporisorium reilianum* grown in submerged and solid-state fermentation," *BioResources* 8(4), 5309-5318. DOI: 10.15376/biores.8.4.5309-5318

Álvarez-Cervantes, J., Domínguez-Hernández, E. M., Mercado-Flores, Y., and Díaz-Godínez, G. (2016a). "Mycosphere essay 10: Properties and characteristics of microbial xylanases," *Mycosphere* 7(10), 1600-1619. DOI: 10.5943/mycosphere/si/3b/2

Álvarez-Cervantes, J., Hernández-Domínguez, E. M., Tellez-Tellez, M., Mandujano-González, V., Mercado-Flores, Y., and Diaz-Godinez, G. (2016b). "*Stenocarpella maydis* and *Sporisorium reilianum*: Two pathogenic fungi of maize," in: *Fungal Pathogenicity*, S. Sultan (ed.), IntechOpen, Rijeka, Croatia, pp. 45-60. DOI: 10.5772/62662

Belien, T., Van-Campemhout, S., Robben, J., and Volckaert, G. (2006). "Microbial endoxylanases: Effective weapons to breach the plant cell-wall warrier or, rather, triggers of plant defense systems?," *Molecular Plant Microbe Interactions* 19(10), 1072-1081. DOI: 10.1094/MPMI-19-1072

Bensch, M. J., and Van Staden, J. (1992). "Ultrastructural histopathology of infection and colonization of maize by *Stenocarpella maydis* (= *Diplodia maydis*)," *Journal Phytopathology* 136(4), 312-318. DOI: 10.1111/j.1439-0434.1992.tb01313.x

Biely, P. (1985). "Microbial xylanolytic systems," *Trends in Biotechnology* 3(11), 286-290. DOI: 10.1016/0167-7799(85)90004-6

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-dye binding," *Analytical Biochemestry* 72(1–2), 248-254. DOI: 10.1016/0003-2697(76)90527-3

Collins, T., Gerday, C., and Feller, G. (2005). "Xylanase families and extremophilic, xylanases," *FEMS Microbiology Reviews* 29(1), 3-23. DOI: 10.1016/j.femsre.2004.06.005

Coughlan, M. P., and Hazlewood, G. P. (1993). "Beta-1,4-D-xylan-degrading enzyme systems: Biochemistry, molecular biology and applications," *Biotechnology and Applied Biochemistry* 17(3), 259-289. DOI: 10.1111/j.1470-8744.1993.tb00244.x

de Almeida, A. F. J. L, Oliveira, S. L., de Araújo, F. A. G., Ferreira, O. M. L., Rangel de O. J., and Franco, M. (2020). "Optimization of the solid-state fermentation conditions and characterization of xylanase produced by *Penicillium roqueforti* ATCC 10110 using yellow mombin residue (*Spondias mombin* L.)," *Chemical Engineering Communications* 207(1), 31-42. DOI: 10.1080/00986445.2019.1572000

Deutschmann, R., and Dekker, R. F. H. (2012). "From plant biomass to bio-based chemicals: Latest developments in xylan research," *Biotechnology Advances* 30(6), 1627-1640. DOI: 10.1016/j.biotechadv.2012.07.001

Dhiman, S. S., Sharma, J., and Buttan, B. (2008). "Industrial application and future prospects of microbial xylanases: A review," *BioResources* 3(4), 388-402. DOI: 10.15376/biores.3.4.388-402

Diebold, G., Mosenthin, R., Piepho, H. P., and Sauer, W. C. (2004). "Effect of supplementation of xylanase and phospholipase to a wheat-based diet for weanling pigs on nutrient digestibility and concentrations of microbial metabolites in ileal digesta and feces," *Journal of Animal Science* 82(9), 2647C2656. DOI: 10.2527/2004.8292647x

dos Santos, T. C., dos Santos R. N., Silva, T. P., Ferreira, B. R. C., Aguiar-Oliveira, E., Rangel de O. J., and Franco, M. (2018). "Production, optimisation and partial characterisation of enzymes from filamentous fungi using dried forage cactus pear as substrate," *Waste and Biomass Valorization* 9(4), 571-579. DOI: 10.1007/s12649-016-9810-z

Ellouze, O., Mondher, M., Ussam, S., Ferid, L., and Marzouki, M. N. (2007). "Induction, properties, and application of xylanase activity from *Sclerotinia sclerotiorum* S2 fungus," *Journal of Food Biochemistry* 31(1), 96-107. DOI: 10.1111/j.1745-4514.2007.00101.x

Gómez-Gómez, E., Ruiz-Roldan, A., Di Pietro, A., and Hera, C. (2002). "Molecular characterization of novel endo-ß-1,4-xylanase genes from the vascular wilt fungus *Fusarium oxysporum*," *Current Genetics* 40(4), 268-275. DOI: 10.1007/s00294-001-0260-0

Hernández-Domínguez, E. M., Rios-Latorre, R. A., Álvarez-Cervantes, J., Loera-Corral, O., Román-Gutiérrez, A. D., Díaz-Godínez, G., and Mercado-Flores, Y. (2014). "Xylanases, cellulases, and acid protease produced by *Stenocarpella maydis* grown in solid-state and submerged fermentation," *BioResources* 9(2), 2341-2348. DOI: 10.15376/biores.9.2.2341-2348

Inmaculada, J., De la Rosa, O., Navas, J. A., Jiménez, R. M., and Tena, M. (2005). "Extracellular xylanases from two pathogenic races of *Fusarium oxysporum* f. sp. *ciceris*: Enzyme production in culture and purification and characterization of major isoform as an alkaline endo-beta-(1,4)-xylanase of low molecular weight," *Antonie Van Leeuwenhoek* 88(1), 48-59. DOI: 10.1007/s10482.004-7584-y

Kaushik, P., and Malik, A. (2016). "Production and characterization of xylanases from fungal isolate *Aspergillus terreus* and development of low cost fermentation media," *Journal of Medical and Biological Eng*ineering 5(1), 15-18. DOI: 10.12720/jomb.5.1.15-18

Kubicek, C. P., Starr, T. L., and Glass, N. L. (2014). "Plant cell wall-degrading enzymes and their secretion plant pathogenic fungi," *Annual Review of Phytopathology* 52, 427-451. DOI: 10.1146/annurev-phyto-102313-045831

Kulkarni, N., Shendye, A., and Rao, M. (1999). "Molecular and biotechnological aspects of xylanases," *FEMS Microbiology Reviews* 23(4), 411-456. DOI: 10.1111/j.1574-6976.1999.tb00407.x

Kumar, V., Marin, N., and Shukla, P. (2016). "Thermostable microbial xylanases for pulp and paper industries: Trends, applications and further perspectives," *World Journal of Microbiology and Biotechnology* 2(2), 34-38. DOI: 10.1007/s11274-015-2005-0

Laemmli, U. K. (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature* 227(5259), 680-685. DOI: 10.1038/227680a0

Mandujano-González, V., Tellez-Jurado, A., Anducho-Reyes, M. A., Arana-Cuenca, A., and Mercado-Flores, Y. (2016). "Purification and characterization of the extracellular aspartyl protease APSm1 from the phytopathogen fungus *Stenocarpella maydis*," *Protein Expression and Purification* 117, 1-5. DOI: 10.1016/j.pep.2015.09.017

Marques, G. L., dos Santos R. N., Silva, T. P., Oliveira, F. M. L., Aguiar-Oliveira, E., Rangel de O. J., and Franco, M. (2018). "Production and characterisation of xylanase and endoglucanases produced by *Penicillium roqueforti* ATCC 10110 through the solid-state fermentation of rice husk residue," *Waste and Biomass Valorization* 9(11), 2061-2069. DOI: 10.1007/s12649-017-9994-x

Marques, G. L., Silva, T. P., Lessa, O. A., de Brito, A. R., Reis, N. S., Fernandes, A. G. de A., Ferreira, M. L. O., Oliveira, J. R., and Franco, M. (2019). "Production of xylanase and endoglucanase by solid-state fermentation of jackfruit residue," *Revista Mexicana de Ingeniería Química* 18(2), 673-680. DOI: 10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Marques

Martinez, D., Berka, R. M., Henrissat, B., Saloheimo, M., Arvas, M., Baker, S. E., Chapman, J., Chertkov, O., Coutinho, P. M., Cullen, D., Danchin, E. G., Grigoriev, I. V., Harris, P., Jackson, M., Kubicek, C. P., Han, C. S., Ho, I., Larrondo, L. F., de Leon, A. L., Magnuson, J. K., Merino, S., Misra, M., Nelson, B., Putnam, N., Robbertse, B., Salamov, A. A., Schmoll, M., Terry, A., Thayer, N., Westerholm-Parvinen, A., Schoch, C. L., Yao, J., Barabote, R., Nelson, M. A., Detter, C., Bruce, D., Kuske, C. R., Xie, G., Richardson, P., Rokhsar, D. S., Lucas, S. M., Rubin, E. M., Dunn-Coleman, N., Ward, M., and Brettin, T. S. (2008). "Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*)," *Nature Biotechnology* 26(5), 553-560. DOI: 10.1038/nbt1403

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry* 31(3), 426-428. DOI: 10.1021/ac60147a030

Moreira, F. G., dos Reis, S., Ferreira, C. M. A., Souza, M. C. G., and Peralta, R. M. (2005). "Production of hydrolytic enzymes by the plant pathogenic fungus *Myrothecium verrucaria* in submerged cultures," *Brazilian Journal of Microbiology* 36(1),7-11. DOI: 10.1590/S1517-83822005000100002.

Pérez-Rodríguez, J., Téllez-Jurado, A., Álvarez-Cervantes, J., Ibarra, J. A., Jaramillo-Loranca, B. E., Anducho-Reyes, M. A., and Mercado-Flores, Y. 2019. "Study of the intracellular xylanolytic activity of the phytopathogenic fungus *Sporisorium reilianum*," *Mycoscience* In Press, Corrected Proof. DOI: 10.1016/j.myc.2019.10.005

Polizeli, M. L. T. M., Rizzatti, A. C. S., Monti, R., Terenzi, H. F., Jorge, J. A., and Amorim, D. S. (2005). "Xylanases from fungi: Properties and industrial applications," *Applied Microbiology and Biotechnology* 67(5), 577-591. DOI: 10.1007/s00253-005-1904-7

Pollet, A., Balien, T., Fierens, K., Delcour, J., and Courtin, C. (2009). "*Fusarium graminearum* xylanases show different functional stabilities, substrate specifies and inhibition sensitivities," *Enzyme and Microbial Technology* 44(4), 189-195. DOI: 10.1016/j.enzmictec.2008.12.005

Raghukumar, C., Muraleedharan, U., Gaund, V. R., and Mishara, R. (2004). "Xylanases of marine fungi of potential use for biobleaching of paper pulp," *Journal of Industrial Microbiology and Biotechnology* 31(9), 433-441.

Sharma, M., and Kumar, A. (2013). "Xylanases: An overview," *British Biotechnology Journal* 3(1), 1-28. DOI: 10.9734/BBJ/2013/1784

Souza, L.O., de Brito, A. R., Bonomo, R. C. F., Santana, N. B., de Almeida, A. F. J. L., Aguiar-Oliveira, E., de Araújo F. A. G., Oliveira F. M. L. Rangel de O. J., and Franco, M. (2018). "Comparison of the biochemical properties between the xylanases of *Thermomyces lanuginosus* (Sigma®) and excreted by *Penicillium roqueforti* ATCC 10110 during the solid state fermentation of sugarcane bagasse," *Biocatalysis and Agricultural Biotechnology* 16, 277-284. DOI: 10.1016/j.bcab.2018.08.016

St. John, F. J., Rice, J. D., and Preston, J. F. (2006). "Characterization of XynC from *Bacillus subtilis* subsp. *subtilis* strain 168 and analysis of its role in depolymerization of glucuronoxylan," *Journal of Bacteriology* 188(24), 8617-8626. DOI: 10.1128/JB.01283-06

Subramaniyan, S., and Prema, P. (2002). "Biotechnology of microbial xylanases: Enzymology, molecular biology, and application," *Critical Reviews in Biotechnol*ogy 22(1), 33-46. DOI: 10.1080/07388550290789450

Techapun, C., Poosaran, N., Watanabe, M., and Sasaki, K. (2003). "Thermostable and alkaline-tolerant microbial cellulose-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocess: Advances review," *Process Biochemistry* 38(9), 1327-1340. DOI: 10.1016/S0032-9592(02)00331-X

Wong, K. K. Y., Tan, L. U. L., and Saddler, J. N. (1988). "Multiplicity of beta-1,4-xylanase in microorganism: Functions and applications," *Microbiological Reviews* 52(3), 305-317.

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