Purification and Characterization of Xylanase SRXL1 from *Sporisorium reilianum* Grown in Submerged and Solid-State Fermentation

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Sporisorium reilianum is a phytopathogenic fungus that produces the maize head smut. Hydrolytic enzymes such as xylanases have not been studied in this basidiomycete, although these enzymes are widely used within the industry. In this study, the characterization of a xylanase produced by *S. reilianum* grown in submerged and solid-state fermentation using different culture media was performed. Submerged fermentation with a medium containing birch xylan and yeast extract showed the highest xylanase activity (12.6 U/mL). The enzyme, purified through ion exchange chromatography, had a molecular weight of 42 kDa, as determined by SDS-PAGE, and a K_m and V_{max} of 1.72 mg/mL and 2.48 µmol/mL/min, respectively. The optimal values of pH and temperature were 5.0 and 70 °C, respectively. The enzyme showed activity over a broad range of temperatures and pHs. Zn^{2+}, Fe^{3+}, and Mn^{2+} ions increased xylanase activity. Sequence protein analysis showed 100% similarity with the theoretical protein encoded by the sr14403 gene of *S. reilianum*, encoding a putative endo-β-1,4-xylanase. This is the first report on the production and purification of a xylanase from this fungus, which has interesting biochemical characteristics for application in biotechnological processes.

Keywords: *Sporisorium reilianum; Xylanase; Maize head smut; Phytopathogenic fungus*

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INTRODUCTION

Xylan is a major component in the cellular wall of plants. This polysaccharide consists of β-1,4-linked xylopyranosyl residues that contain substituent groups of acetyl, 4-O-methyl-D-glucuronosyl, and α-arabinofuranosyl residues linked to the backbone of β-1,4-linked xylopyranose units and is linked by covalent and non-covalent interactions with lignin, cellulose, and other polymers. Due to the heterogeneity and chemical structure of xylan in plants, its degradation requires a complex of hydrolytic enzymes. The endo-β-1,4-xylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37) are the key enzymes for the degradation of xylan by the hydrolysis of glycosidic bonds. Interest in xylanases has increased in the last few years due to its wide application in many industries, particularly in processes such as obtaining xylose and xylooligosaccharides, which are used in the food industry as thickeners and antifreeze additives, pharmaceut-

icals, biofuel production, pulp and paper biobleaching, agro-waste degradation, and their application as elicitors of plant defense mechanisms (Kulkarni *et al.* 1999; Subramaniyian and Prema 2002; Belien *et al.* 2006; Khandeparker and Numan 2008). Furthermore, xylanase activity has been detected in fungi, bacteria, plants, and mollusks (Subramaniyian and Prema 2002; Polizeli *et al.* 2005).

The structural and functional properties of new microbial xylanases have been studied in detail. Xylanolytic enzymes are produced by microorganisms for the degradation of plants' cellular walls to obtain a carbon source from the xylan (Subramaniyian and Prema 2002). In phytopathogenic fungi, this process aids penetration and colonization (Walton 1994). Phytopathogenic fungi have shown high xylanase activity with potential industrial applications (King *et al.* 2011). Ellouze *et al.* (2007) purified and partially characterized a xylanase of *Sclerotinia sclerotioriun* S2. Another study found that *Fusarium oxysporum* produces different xylanases; two of these xylanases were excreted by the fungus when it was grown in tomato vascular tissue as a sole carbon source. The purification and characterization of xylanases from *F. oxysporium* f. sp. *Melonis* and *F. oxysporium* f. sp. *Lycopersici* have also been reported (Alconada and Martínez 1994; Gómez-Gómez *et al.* 2001, 2002). *F. oxysporum* f. sp. *ciceris* presented xylanase activity when chickpea cell walls and xylan was used as a carbon source (Immaculada *et al.* 2005). *Myrothecium verrucaria* produced xylanases by submerged fermentation in the presence of either glucose or xylan as a carbon source (Moreira *et al.* 2005).

The xylanase activity of *Sporisorium reilianum* has not been studied. This basidiomycete fungus is the causative agent of corn smut (Ghareeb *et al.* 2011). Recently, the genome sequence of *S. reilianum* was reported, and it was found that there are at least three possible genes that encode xylanases (the sr14403 gene, sr15309 gene, and sr15773 gene relate to endo-1,4-beta-xylanase) (Schirawski *et al.* 2010). However, the proteins encoded by these genes have not been studied in this fungus. This is the first report on the production and purification of a xylanase called SRXL1 from *S. reilianum*, which has interesting biochemical characteristics for application in biotechnological processes.

EXPERIMENTAL

Organism and Growth Conditions

A strain of *S. reilianum* donated by Dr. Santos Gerardo Leyva Myr, from Universidad Autónoma Chapingo, México, was used. The strain was grown at 28 °C on Petri dishes with YPD medium.

The fungus was grown in submerged fermentation (SmF) and solid-state fermentation (SSF). A mineral medium (MM) modified from Téllez-Téllez *et al.* (2008) was prepared containing (in g/L): KH_2PO_4, 0.6; $MgSO_4–7H_2O$, 0.5; K_2HPO_4, 0.4; $FeSO_4–7H_2O$, 0.05; $MnSO_4–H_2O$, 0.05; and $ZnSO_4–7H_2O$, 0.001. Four fermentations using MM were developed in triplicate. Fermentation media were supplemented with glucose (10.0 g/L) and yeast extract (5.0 g/L) (GYM); birch xylan (2.5 g/L) and yeast extract (1.25 g/L) (XYM); only birch xylan (0.5 g/L) (XM); and birch xylan (0.5 g/L), ammonium sulfate (0.5 g/L) (XAM), and Potato Dextrose Broth (PDB; Difco). SmFs were carried out in 50-mL Erlenmeyer flasks containing 10 mL of either media described above. SSFs were performed in 250-mL Erlenmeyer flasks with 0.5 g of polyurethane foam (PUF) cubes (0.5 x 0.5 x 0.5 mm) of low density (17 kg m^{-3}) as inert support impregnated with 15 mL of either media (Díaz-Godínez *et al.* 2001). Each flask was

inoculated with a culture of *S. reilianum* grown for 24 h at 28 ºC on a rotary shaker (150 rpm) and adjusted to a final absorbance of 0.2 at 600 nm. All cultures were incubated at 28 °C for 15 days on a rotary shaker at 150 rpm. Three flasks were taken as samples at 0, 6, 12, 24, 48, 72, 96, 120, 144, 168, and 192 h of fermentation. The enzymatic extract (EE) was obtained by filtration of the cultures using filter paper (Whatman No. 4) and stored at -20 °C until analysis. The pH of each EE was measured by potentiometry.

Enzyme Assay

Xylanase activity in EE was assayed by quantifying reducing sugars using the DNS (3,5-dinitrosalicylic acid) method (Miller 1959). The assay mixture contained 475 µL of substrate (0.5% birch xylan in 0.1 M acetate buffer at pH 5.3) and 25 µL of EE and was incubated at 50 °C. One unit of xylanase activity was defined as the amount of enzyme that liberated 1 µmol of xylose per minute under assay conditions.

Zymogram Analysis

Xylanase activity obtained in both SSF and SmF using XYM was detected *in situ* through zymograms (Raghukumar *et al.* 2004).

Purification and Characterization of Xylanase

For the purification process, EE obtained from the XYM at 72 h was used. EE previously centrifuged (8,000 x g for 8 min at 2 °C) was eluted from a cationic exchange column (Bio-Scale Mini Unosphere S, Bio-Rad) with a NaCl gradient (0 to 1.0 M) for 35 min at a flow rate of 1.0 mL/min in an acetate (25 mM, pH 5.3) buffer. Fractions were dialyzed (12 400 Da, Sigma D9527), and both xylanolytic and protein activities (Bradford 1976) were evaluated. The molecular mass of the xylanase SRXL1 was estimated under denaturing conditions using SDS-PAGE (Laemmli 1970). A molecular weight marker (10 to 250 kDa) (Precision Plus Protein^{TM} Standards, BioRad) was used.

The optimal temperature for xylanase SRXL1 production was assayed at different incubation temperatures (4 to 80 °C). Thermal stability was determined by evaluation of xylanase activity at 50 and 70 °C after incubation of the mixture reaction at 4, 25, 30, 35, 40, 45, 50, 55, 60, 70, and 80 °C for 60 min.

The optimum pH for xylanase SRXL1 production was evaluated at different pH values using various buffer systems (0.1 M citrate buffer for pH 3; 0.1 M acetate buffer for pH 4, 5, and 6; 0.1 M Tris buffer for pH 7, 8, and 9, 0.1 M glycine-NaOH buffer for pH 10 and 11). All assays were incubated at 50 and 70 °C.

The pH stability was assayed by evaluation of xylanase activity after incubation of the EE at 4 °C overnight in each buffer described above.

The effect of different metal ions (Zn^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Ca^{2+}, and K^{+}) at concentrations of 2 and 10 mM in the enzymatic reaction mixture on the xylanase activity was evaluated.

K_m and V_{max} were estimated by the Michaelis-Menten equation using the non-linear least square-fitting program ‘‘Solver’’ (Excel, Microsoft); birch xylan (1 to 10 mg/mL) was used as the substrate.

The proteomic analysis “Protein identification by LC-MS/MS (Ion Trap)” was performed using the ‘CBMSO PROTEIN CHEMISTRY FACILITY’ service, a member of the ProteoRed network.

RESULTS

Growth and Xylanase Production

The fungus did not grow in XAM and XM media. Figure 1a shows the xylanase activity produced by SmF. In GYM, there was no activity during the first 48 h of fermentation, but values of about 2.7 U/mL were observed thereafter. The activity profile obtained in PDB showed that the highest activity value was 4.07 U/mL at the beginning of fermentation and 2 U/mL during the rest of the fermentation time. The XYM produced 12.6 U/mL of xylanase activity between 72 and 96 h of fermentation. After that, the values decreased to close to 9 U/mL. Furthermore, Figure 1b shows xylanase activity obtained in SSF. In both GYM and PDB, the xylanase activity was minimal during the first 100 h of fermentation. After this time, the activity values were close to 1.1 U/mL. The activity values obtained in XYM were approximately 3 to 4.3 U/mL throughout the fermentation. In general, pH values increased from the beginning of fermentation to reach values near to 8.0.

Purification and Characterization of Xylanase

The purification factor of xylanase SRXL1 was 36-fold (Table 1). Figure 2 shows the SDS-PAGE and zymogram results for purified xylanase with a molecular mass of 42 kDa. K_m and V_{max} were 1.72 mg/mL and 2.48 μmol/mL/min, respectively.

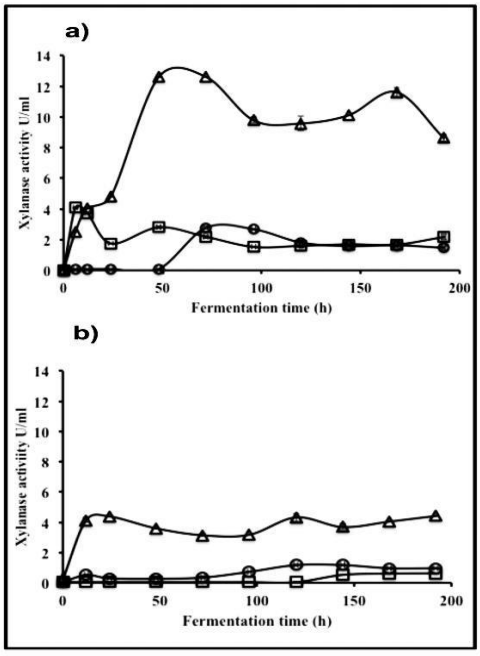

Fig. 1. Xylanase activity produced by *Sporisorium reilianum* grown in SmF (a), SSF (b), GYM (○), PDB (□), and XYM (△).

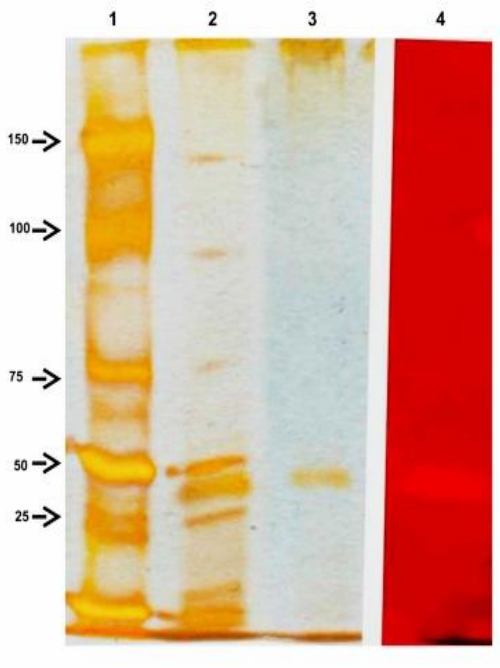

Fig. 2. SDS-PAGE and zymogram of xylanase SRXL1 from *S. reilianum*. Lane 1, molecular mass markers (kDa); lane 2, EE; lane 3, xylanase SRXL1; lane 4, zymogram.

Table 1. Purification of Xylanase SRXL1

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Purification fold	Recovery (%)
Crude enzyme	143	124.4	0.87	100	1
Econo-Pac High S Cartridge	2.4	45	18.7	36	21.5

Commonly, xylanase activity is determined at 50 °C; however, SRXL1 showed the greatest activity at 70 °C, although between 45 and 60 °C, the activity observed was approximately 90% in relation to maximal activity (Fig. 3a), implying that the activity reported in Fig. 1 could present the same profile if it is evaluated at 70 °C, but with an increase of 10% approximately in each of the reported values. Since the optimum temperature was 70 °C and the activity was evaluated at 50 °C, thermal stability was assayed at these two temperatures, corroborating the minimum difference between the activity values (Fig. 3b). The optimum pH for the xylanase SRXL1 was 5.0 (Fig. 4a). The enzyme showed stability over a broad range of pH values (Fig. 4b). The presence of 2 mM Mn^{2+} ions enhanced the xylanase activity to 18%; however, at 10 mM, the activity decreased to 12%. Fe^{2+} ions (10 mM) had little positive effect, increasing the activity to 8%. Mg^{2+}, Ca^{2+}, and K^{+} ions decreased the xylanase activity in both concentrations evaluated. Cu^{2+} at 10 mM showed an inhibitory effect, and Zn^{2+} showed no effect (Table 2). The sequencing analysis of eight fragments of protein showed 100% similarity with the theoretical protein encoded by the sr14403 gene of *S. reilianum*, encoding a putative endo-β-1,4-xylanase (Schirawski *et al.* 2010). The predicted values of molecular weight and pI of the gene product are 37.2 kDa and 9.2, respectively.

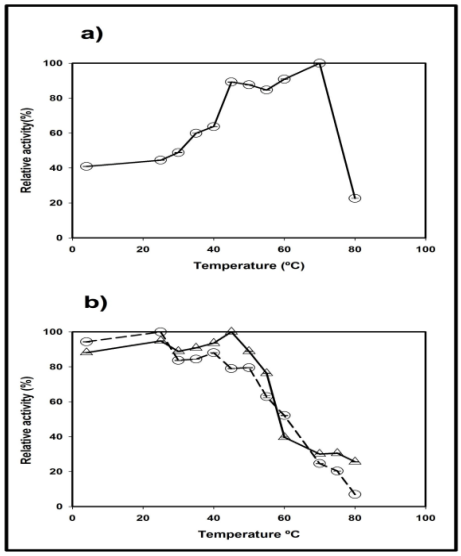

Fig. 3. Effect of temperature on the activity (a) and on the stability of xylanase SRXL1 (b). Activity assayed at 50 °C (○) and 70 °C (△).

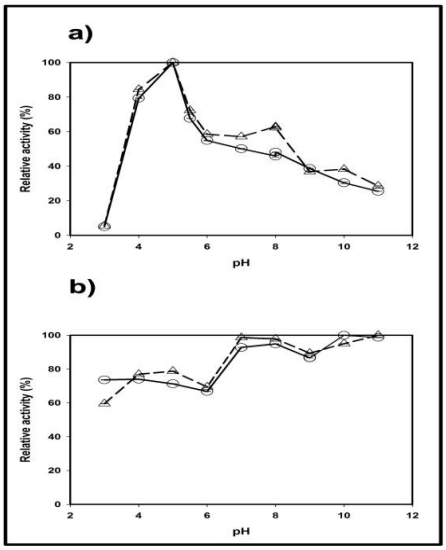

Fig. 4. Effect of pH on the activity (a) and on the stability of xylanase SRXL1 (b). Activity assayed at 50 °C (○) and 70 °C (△).

Table 2. Effect of Metal Ions on the Activity of Xylanase SRXL1

	Residual activity (%)	
Metal ions	**2 mM**	**10 mM**
Control	100	100
Zn^{2+}	99	103
Mg^{2+}	91	79
Fe^{3+}	82	108
Mn^{2+}	118	88
Cu^{2+}	74	0
Ca^{2+}	102	55
K^{+}	88	58

DISCUSSION

Growing Conditions and Production of Xylanase

In the present work the xylanase SRXL1 from *S. reilianum* was studied, which could be related with the degradation of hemicellulose of the cell wall plants, such as in other phytopathogenic fungi (Riou *et al.* 1991). The medium with xylan was the best for the production of the enzyme; however, the activity was detected in presence of glucose. In this study, the xylanase activity produced in SmF was higher than those previously reported. Ellouze *et al*. (2008) reported that the fungus *Sclerotinia sclerotiorum* S2 grown on wheat straw, presented a maximum xylanase activity of 2.5 U/mL. Furthermore, *Fusarium verticilliodes* grown on corn produced activity of 8 U/mL (Saha 2001). The fungus *Chaetomium thermophilum* showed different xylanase activities depending on the carbon source used in the liquid medium (0.5, 2.7, and 3.9 U/mL on glucose, oat spelt xylan, and birch wood xylan, respectively) and in SSF on wheat straw found an activity of 1.6 U/mL (Ahmed *et al*. 2012). Nair *et al*. (2008) reported the xylanase activities produced in SmF by *Aspergillus fumigatus*, *Penicillium* sp. and *Trichoderma* sp. (20, 15, and 7 U / mL). *S. reilianum* showed a strong dependence on organic nitrogen, as this fungus did not grow in the absence of yeast extract and even in the presence of ammonium sulfate. There have been some reports about the increase of xylanase production in *Aspergillus* and *Penicillium* genus in the presence of yeast extract in the culture medium (Bakri *et al.* 2003; Sudan and Bajaj 2007). Zakariashvili and Elisashvili (1993) reported that the production regulation of lignocellulolytic enzymes from basidiomycetes fungus, in which the xylanase is found, depends on the nature and concentration of nitrogen sources. On the other hand, xylan acted as the xylanase activity inducer. However, the fungus has a basal expression level because the activity was observed in that medium without the inducer. Polizeli *et al.* (2005) observed the presence of constitutive xylanases at low levels. Generally, the production of xylanases by filamentous fungi is usually higher in SSF than in SmF, using lignocellulosic residues (Haltrich *et al.* 1996; Polizeli *et al.* 2005). In this study, the SmF produced higher xylanase activity than SSF, perhaps because this organism is yeast, unlike filamentous fungi, which can cause the mycelium to be disrupted by the agitation forces when grown in SmF, decreasing the xylanase production (Subramaniyan and Prema 2002). Téllez-Téllez *et al*. (2008) reported for first time an atypical behavior in the production of enzymes by fungi, since *Pleurotus ostreatus* produced higher laccases activities in SmF than in SSF. Xylanase activity was produced at an alkaline pH, and this has been reported previously in *Aspergillus niveus* RS2 (Sudan and Bajaj 2007).

Isoforms of Xylanase and Molecular Weight

S. reilianum showed only one xylanase isoform in both SmF and SSF. Polizeli *et al.* (2005) showed that the production of xylanase isoforms is differentially regulated and can show post-translational modifications such as glycosylation and self-aggregation, as well as proteolytic digestion. Endoxylanases can also be expressed by different alleles of a gene or even by separate genes. Inmaculada *et al*. (2005) found two xylanases of 24 and 20 kDa in *F. oxysporum* in a medium containing xylan. However, cultures of chickpea cell walls showed two additional enzymes with molecular weights of approximately 60 and 40 kDa. The xylanase SRXL1 from *S. reilianum* had a molecular weight of 42 kDa. Bakir *et al.* (2001) observed a xylanase from *R. oryzae* with a molecular weight of 22 kDa; Carmona *et al.* (2005) purified xylanase II with a molecular weight of 32 kDa from *A. versicolor*. Inmaculada *et al.* (2005) purified a xylanase from *Fusarium oxysporum* f. sp. *Ciceris* with a molecular weight of 21.6 kDa. Alconada and Martinez (1994) reported the purification of a xylanase from *F. oxysporum* f. sp. *melonis* with a molecular mass of 80 kDa. These results suggest the ability to produce diverse xylanase types whose expressions may be conditioned by different environmental factors as well as by the producer microorganisms (Polizeli *et al.* 2005; Inmaculada *et al.* 2005).

Characterization of Xylanase

Optimal values of pH and temperature, as well as the stability of these factors in xylanase SRXL1, suggest that this enzyme can be used in different biotechnological processes. In general, endoxylanases show activity between 40 and 80 °C and from pH 4.0 to 6.5, but optimal conditions have been found outside these ranges (Polizeli *et al.* 2005). The presence of different ions modified the xylanase activity level; the presence of Mn^{2+} ions enhanced the activity, while Cu^{2+} inhibited the enzyme. Recent studies have shown that Cu^{2+}, Ca^{2+}, Co^{2+}, Ag^{+}, and Fe^{3+} ions can act as xylanase activity inhibitors (Carmona *et al.* 2005; Drobrev and Zhekova 2012) and that Mn^{2+}, Ca^{2+}, and Fe^{3+} can increase the activity (Carmona *et al.* 2005; Sudan and Bajaj 2007; Sharma and Bajaj 2005; Dobrev and Zhekova 2012). K_m values of xylanases for *Aspergillus niveus* RS2, *Fusarium oxysporum*, and *Rhizopus oryzae* (2.5, 2.24, and 18.5 mg/mL, respectively) were 0.45-, 0.30-, and 10.0-fold higher than that observed for xylanase SRXL1 (Sudan and Bajaj 2007; Inmaculada *et al.* 2005; Bakir *et al.* 2001). This is the first report on purification and characterization of a xylanase from *S. reilianum*, encoded by the sr14403 gene. Although other xylanases from phytopathogenic fungal species have been reported, xylanase SRXL1 has potential biotechnological applications such as obtainment of xylose and xylooligosaccharides, applications in the food industry as thickeners and antifreeze additives, in pharmaceuticals, biofuels, paper pulp biobleaching, agro-waste degradation, and as elicitors for inducing plant defense mechanisms (Kulkarni *et al.* 1999; Subramaniyian and Prema 2002; Khandeparker and Numan 2008; Belien *et al.* 2006).

CONCLUSIONS

1. *S. reilianum* showed xylanase activity in SmF and SSF, using different culture media. The liquid medium with xylan showed the highest activity in both fermentation systems.
2. Basal levels of xylanase were observed in media without xylan.

3. The xylanase SRXL1 from *S. reilianum* was purified through ion exchange chromatography and had a molecular weight of 42 kDa and a K_m and V_{max} of 1.72 mg/mL and 2.48 μmol/mL/min, respectively.
4. The optimal values of pH and temperature of SRXL1 were 5.0 and 70 °C, respectively, and the fungus showed activity over a broad range of temperatures and pH values.
5. Zn^{2+}, Fe^{3+}, and Mn^{2+} ions increased the activity of the purified enzyme.
6. Sequence protein analysis showed 100% similarity with the theoretical protein encoded by the sr14403 gene of *S. reilianum*, encoding a putative endo-β-1,4-xylanase.

ACKNOWLEDGMENTS

We wish to thank the Mexican Council of Science and Technology (CONACyT) for supporting this research (Project No. 167459). J. Álvarez-Cervantes was supported by a FOMIX-HIDALGO scholarship.

REFERENCES CITED

Ahmed, S., Imdad, S. S., and Jamil, A. (2012). "Comparative study for the kinetics of extracellular xylanases from *Trichoderma harzianum* and *Chaetomium thermophilum*," *Electron. J. Biotechnol*. 15(3), 3-3.

Alconada, T., and Martínez, M. (1994). "Purification and characterization of an extracellular endo-1,4-β-xylanase from *Fusarium oxysporum* f. sp. Melonis," *FEMS Microbiol. Lett.* 118(3), 305-310.

Bakir, U., Yavascoglu, S., Guvence, F., and Erroyin, A. (2001). "An endo-b-1,4-xylanase from *Rhizopus oryzae*: Production, partial purification and biochemical characterization," *Enzyme Microbial Technol.* 29(6-7), 328-334.

Bakri, Y., Jacques, P., and Thonart, P. (2003). "Xylanase production by *Penicillium canescens* 10–10c in solid state fermentation," *Appl. Biochem. Biotechnol.* 108(1-3), 737-748.

Belien, T., Van Campenhout, S., Robben, J., and Volckaert, G. (2006). "Microbial endoxylanases: Effective weapons to breach the plant cell-wall barrier or, rather, triggers of plant defense systems?," *Mol. Plant Microbe Interact.* 19(10), 1072-1081.

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Anal. Biochem.* 72(1), 248-254.

Carmona, E. C., Fialho, M. B., Buchgnani, E. B., Coelho, G. D., Brocheto-Braga, M. R., and Jorge, J. A. (2005). "Production, purification and characterization of a minor form of xylanase from *Aspergillus versicolor*," *Process Biochem* 40(1), 359-364.

Díaz-Godínez, G., Soriano, J., Augur, C., and Viniegra-González, G. (2001). "Exopectinases produced by *Aspergillus niger* in solid-state and submerged fermentation: A comparative study," *J. Ind. Microbiol. Biotechnol.* 26(5), 271-275.

Dobrev, G., and Zhekova, B. (2012). "Purification and characterization of endoxylanase Xln-2 from *Aspergillus niger* B03," *Turk. J. Biol.* 36(1), 7-13.

Ellouze, O., Mondher, M., Issam, S., Ferid, L., and Marzouki, M. N. (2007). "Induction, properties and application of xylanase activity from *Sclerotinia sclerotiorum* S2 fungus," *J. Food Biochem.* 31(1), 96-107.

Ghareeb, H., Becker, A., Iven, T., Feussner, I., and Schirawski, J. (2011). "*Sporisorium reilianum* infection changes inflorescence and branching architectures of maize," *Plant Physiol.* 156(4), 2037-2052.

Gómez-Gómez, E., Roncero, M. I. G., Di Pietro, A., and Hera, C. (2001). "Molecular characterization of novel endo-β-1,4-xylanase gene from the vascular wilt fungus *Fusarium oxysporum*," *Curr. Genet.* 40(4), 268-275.

Gómez-Gómez, E., Ruiz-Roldan, A., Di Pietro, A., and Hera, C. (2002). "Role in pathogenesis of two endo-beta-1,4-xylanase genes from the vascular wilt fungus *Fusarium oxysporum*," *Fungal Genet. Biol.* 35(3), 213-222.

Haltrich, D., Nidetzky, B., Kulbe, K., Steiner, W., and Zupancic, S. (1996). "Production of fungal xylanases," *Bioresource Technol.* 58(2), 137-161.

Inmaculada, J., De la Rosa, O., Navas-Cortes, J. A., Jiménez-Diaz, 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 isoforma as an alkaline endo-β-(1,4)-xylanase of low molecular weight," *A van Leeuw. J. Microb.* 88(1), 48-59.

Khandeparker, R., and Numan, M. T. (2008). "Bifunctional xylanases and their potential use in biotechnology," *J. Ind. Microbiol. Biotechnol.* 35(7), 635-644.

King, B. C., Waxman, D., Nenni, N. V., Walker, L. P., Bergstrom, G. C., and Gibson, D. M. (2011). "Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi," *Biotechnol. for Biof.* 4(4), 1-14.

Kulkarni, N., Shendye, A., and Rao, M. (1999). "Molecular and biotechnological aspects of xylanases," *FEMS Microbiol Rev*. 23(4), 411-456.

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

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem*. 31(3), 426-428.

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 J. of Microbiol*. 36(1), 7-11.

Nair, S.G., Sindhu, R., Shashidhar, S. (2008). "Fungal xylanase production under solid state and submerged fermentation conditions," *Afr. J. Microbiol. Res.* 2(4), 82-86.

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," *Appl. Microbiol. Biotechnol.* 67(5), 577-591.

Raghukumar, C., Muraleedharan, U., Gaud, V. R., and Mishara, R. (2004). "Xylanases of marine fungi of potential use for biobleaching of paper pulp," *J. Ind. Microbiol. Biotechnol.* 31(9), 433-441.

Riou, C, Freyssinet G, Fevre M. (1991). "Production of cell Wall-degrading enzymes by the phytopathogenic fungus *Sclerotinia sclerotiorum*," 57 (5), 1478-1484.

Saha, B. (2001). "Xylanase from a newly isolated *Fusarium verticillioides* capable of utilizing corn fiber xylan," *App. Microbiol. Biotechnol*. 56(5-6), 762-766.

Schirawski, J., Mannhaupt, G., Munch, K., Brefort, T., Schipper, K., Doehlemann, G., Di Stasio, M., Rossel, N., Mendoza, M. A., Pester, D., Muller, O., Winterberg, B., Meyer, E., Ghareeb, H., Wollenberg, T., Munsterkotter, M., Wong, P., Walter, M., Stukenbrock, E., Guldener, U., and Kahmann, R. (2010). "Pathogenicity determinants in smut fungi revealed by genome comparison," *Science* 330(6010), 1546-1548.

Sharma, P., and Bajaj, B. K. (2005). "Production and partial characterization of alkali tolerant xylanase from an alkalophilic *Streptomyces sp*. CD3," *J. Sci. Industrial Res.* 64(9), 688-698.

Subramaniyan, S., and Prema, P. (2002). "Biotechnology of microbial xylanases: Enzymology, molecular biology and application," *Crit. Rev. Biotechnol.* 22(1), 33-46.

Sudan, R., and Bajaj, B. K. (2007). "Production and biochemical characterization of xylanase from an alkali tolerant novel species *Aspergillus niveus* RS2," *World J. Microb. Biot.* 23(4), 491-500.

Téllez-Téllez, M., Férnadez, F. J., Montiel-González, A. M., Sanchez, C., and Díaz-Godínez, G. (2008). "Growth and laccase production by *Pleurotus ostreatus* in submerged and solid-state fermentation," *Appl. Microbiol. Biotechnol.* 81(4), 675-679.

Walton, J. D. (1994). "Deconstructing the cell wall," *Plant Physiol*. 104(4), 1113-1118.

Zakariashvili, N. G., and Elisashvili, V. I. (1993). "Regulation of *Cerrena unicolor* lignocellulolytic activity by a nitrogen source in culture-medium," *Microbiol.* 62(5), 525-528.

Article submitted: July 11, 2013; Peer review completed: Aug. 21, 2013; Revised version received and accepted: Aug. 28, 2013; Published: August 30, 2013.