Xylanases, Cellulases, and Acid Protease Produced by *Stenocarpella maydis* Grown in Solid-state and Submerged Fermentation

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Activity levels of extracellular hydrolytic enzymes produced by *Stenocarpella maydis*, a fungal pathogen of maize, have so far not been reported. Production of xylanase, cellulase, and acid protease by this ascomycete using different culture media in solid-state and submerged fermentation was studied. In solid-state fermentation, polyurethane foam was used as an inert support, and corncob, corn leaves, and broken corn were used as biodegradable supports. The highest xylanase activity was produced in the medium with xylan in both fermentation systems, reaching 18,020 U/L and 19,266 U/L for submerged and solid-state fermentation, respectively. Cellulase production was observed only in the culture medium with carboxymethylcellulose, obtaining values of 7,872 U/L in submerged fermentation and 9,439 U/L in solid-state fermentation. The acid protease was produced only in minimal medium with glucose in acidic pH, reaching the highest levels of activity in SSF (806 U/L). The corncob was the best biodegradable support for the production of xylanases and acid protease. Two isoenzymes of xylanase and cellulase were observed in both fermentation systems, and three isoenzymes of xylanase were produced on the biodegradable supports.

Key words: Xylanase; Cellulase; Acid protease; Stenocarpella maydis

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INTRODUCTION

Enzymes are considered of great importance not only in a strict biological sense, but also because of their important applications in scientific and industrial fields such as food manufacturing, production of biofuels, textiles, paper, and pharmaceuticals (Beg *et al.* 2001). Enzymes can be isolated from different organisms including plants, animals, and microorganisms. In the second half of the twentieth century, there was an unprecedented expansion of the use of microorganisms to produce many important metabolites, including enzymes, with industrial applications (Beg *et al.* 2001; Subramaniyan and Prema 2002; Kirk *et al.* 2002).

Xylanase, cellulase, and protease are enzymes widely used in various industrial processes. Xylanases (endo-β-1,4 D-xylanases E.C.3.2.1.8) act synergistically with other

enzymes in the degradation of xylan, which is a heteropolysaccharide, and their total degradation produces xylose (Subramaniyan and Prema 2002). The use of xylanases has increased significantly; in this sense, researchers have focused on the isolation of new microbial strains that produce large amounts of these enzymes. One of the most important applications of these enzymes is in the bleaching of pulp and paper, to increase the brilliance of the product (Polizeli 2005), and they are also used in the food industry in the clarification of juices and wines, coffee mucilage liquefaction, extracting flavors and pigments as well as oils from plants and seeds, conditioning of feed for poultry and pigs, and in the baking industry (Kulkarni *et al.* 1999).

On the other hand, cellulases are a complex of at least three enzymes that degrade cellulose to obtain glucose: endo β-1,4-glucanases (EC 3.2.1.4), exo β-1,4-glucanases (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Bhat 2000). At the industrial level, cellulases play an important role because they have wide applications: in the textile industry, they are used in fading denim, and in detergents, they are added to remove lint. Within the food industry, cellulases are used to facilitate the extraction and filtration of juices and musts of fruit and vegetable; it is also used in extraction of edible oils. Likewise, they are used in the partial hydrolysis of lignocellulosic materials for improving digestion in ruminants (Subramaniyan and Prema 2002). Microbial proteases are widely used for brewing and for obtaining protein hydrolyzates, primarily in the gelatin, meat, leather, and detergent industries. Specifically, acid proteases are endopeptidases that have their maximum catalytic activity at acid pH values. Microbial acid proteases have a preference for aromatic amino acid residues, similar to what occurs with pepsin. Their main application is in replacing rennet in the manufacture of cheese (Rao *et al.* 1998).

Fungi are important producers of xylanase, cellulase, and protease. *Aspergillus* and *Trichoderma* species are most commonly used to produce xylanases; however, xylanases have also been reported in different thermophilic fungi such as *Chaetomium* sp., *Humicola lanuginosa*, *Paecylomyces variotii*, and *Thermoascus auratiacus* (Polizeli *et al.* 2005). Cellulases have been described in the genera *Anaeromyces* and *Caecomyces*, as well as in *Cyllamyces aberencis*, *Trichoderma* sp., and *Aspergillus* sp. (Hou *et al.* 2007; Murray *et al.* 2003). Acid proteases with industrial applications have been isolated from different species of *Mucor* and *Aspergillus* as well as from *Piptoporus soloniensis* (Yegin *et al.* 2011; Siala *et al.* 2009; El-Baky *et al.* 2011).

Although the production of xylanase, cellulase, and protease by various microorganisms is well documented, secretion of such enzymes by phytopathogenic fungi has been little explored. They have potential for degrading the polymers of plant cell wall, which due to its complexity are a determining factor to efficient degradation of the biomass. However it has been found that the number of genes encoded to xylanases and cellulases in some fungal plant pathogens is larger than that of this type of microorganisms with industrial importance, such as *Trichoderma reesei*. The discovery of novel enzymes is very important to the development of processes for efficient use in depolymerization of the polysaccharides present in the lignocellulosic materials, in order to obtain bioproducts and biofuels. It has been demonstrated that different isolations of phytopathogenic fungus have high activity against xylans and untreated biomass. The preference for biomass type depends the monocot or dicot host preference of the pathogen fungus, in which the enzymes produced are promising for industrial applications. These could be used for more efficient lignocellulosic digestion with specialized activities for the utilization biomass obtained from the preferred hosts

(Subramaniyan and Prema 2002; Martinez *et al.* 2008; King *et al*. 2011). On the other hand, the acid proteases take an important role in the maceration of plant tissues, degrading the structural proteins (Aro *et al*. 2005). Among plant pathogenic fungi with the potential for producing extracellular hydrolytic enzymes, some cause rot in plant tissues; *Stenocarpella maydis*, which is a fungal pathogen of maize, produces white rot of maize stalk and cobs (Bensch and van Staden 1992). Due to the severity of damage in the plant, the hydrolytic enzymatic activities could be interesting to applications in the corn biomass degradation to obtain biotechnological products. In this research, the production of xylanase, cellulase, and acid protease from *Stenocarpella maydis* in different culture conditions was studied.

EXPERIMENTAL

Microorganism and Culture Conditions

The strain of *Stenocarpella maydis* was donated by Dr. Dan Jeffers from the International Center for Maize and Wheat Improvement (CIMMYT), and it was conserved at 4 °C in inclined tubes with potato dextrose agar (Bioxon). Five culture media for submerged (SMF) and solid-state fermentation (SSF) on inert supports were used. Basal medium was used for three of them, and its composition was (in g/L): yeast extract, 9; 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 (Téllez-Téllez *et al.* 2008), and either xylan, 5 (YEX), carboxymethylcellulose, 10 (YEC), or glucose, 10 (YEG). Minimal medium with glucose (MMG), (glucose 2%, NH_4 $2SO_4$ 0.5%, and YNB 0.17%; Mercado-Flores *et al.* 2003) and YPD medium (yeast extract 0.5%, peptone 1%, and glucose 1%) were also used.

SMF and SSF on inert supports were carried out in 250-mL Erlenmeyer flasks with 15 mL of either media. Each flask was inoculated with one mycelial plug (5 mm in diameter) and incubated at 28 °C for 14 days. SMFs were shaken at 160 rpm on a rotary shaker. Each SSF flask contained 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 an inert support (Díaz-Godínez *et al.* 2001). SSF was also carried out using biodegradable supports. To each flask was added 15 mL of water and 10 g of either corncob, corn leaves, or broken corn previously milled to a particle size of 0.841 µm and washed twice with sterile distilled water and dried at 60 °C for 24 h. Each flask was also inoculated with one mycelial plug (5 mm in diameter) and incubated at 28 °C for 14 days. All cultures were sterilized before inoculation. Three flasks were taken as samples every 24 h for 13 days. Samples were also taken at zero and 12 h of fermentation.

Enzymatic Extract and Biomass Evaluation

The enzymatic extract (EE) from SMF was obtained by filtration of the cultures using filter paper (Whatman No. 4). The biomass (X) was determined as the difference of dry weight (g/L). EE from SSF was obtained by compression of the PUF in a Büchner funnel and was also filtered. The solid retentate with PUF was dried to constant weight. The calculated X was the difference between the initial and final PUF weight (Díaz-Godínez *et al.* 2001). The pH of each EE obtained from SMF and SSF on PUF was measured by potentiometry. To each SSF flask on biodegradable supports, 30 mL of 50

mM acetate buffer, pH 5.3 was added, and the flask was shaken for 15 min in an ice bath. After that, the liquid obtained by filtration was considered to be EE.

An assay of the biomass $X = X(t)$ produced by SMF and SSF on PUF was done using the Velhurst-Pearl or logistic equation,

$$\frac{dX}{dt} = \mu\left[1 - \frac{X}{X_{\max}}\right]X \quad (1)$$

where μ is the maximal specific growth rate and $X_{\max}$ is the maximal (or equilibrium) biomass level achieved when $dX/dt = 0$ for $X > 0$. The solution of Eq. 1 is as follows,

$$X = \frac{X_{\max}}{1 + Ce^{-\mu t}} \quad (2)$$

where $C = (X_{max} - X_0)/X_0$ and $X = X_0$, the initial biomass value.

Estimation of kinetic parameters in the above equations was performed using a non-linear least square-fitting program "Solver" (Excel, Microsoft) (Téllez-Téllez *et al.* 2008). The coefficient $Y_{E/X}$ was calculated as the relation between the maximal enzymatic activity and the biomass observed at the same time (U/g X). The productivity (P, U/L h) was obtained as the relation between the maximal enzymatic activity and its time of production.

Agar Plate Assays for Enzymatic Activities of Xylanase, Cellulase, and Acid Protease

Cellulase and xylanase activities were observed in agar plates, using bacteriological agar (1.8 %) and carboxymethylcellulose or xylan (0.2 %), respectively. *S. maydis* was inoculated and incubated at 28 °C for 5 to 7 days. After that, Congo red (0.2%) was added for 30 min at room temperature, and each plate was finally washed with 1 M NaCl.

For protease activity in agar plates, a medium with 1.2 g of albumin in 60 mL of 0.5 M citrate buffer, pH 4.0, previously sterilized by filtration and mixed with 480 mL of sterile YNB medium (1.7 g/L YNB and 20 g/L bacteriological agar) and 60 mL of sterile 0.5 M citrate buffer, pH 4.0, was prepared. The plates were inoculated with the fungus and incubated at 28 °C for 7 to 10 d. In all cases, a clear halo around the colony was taken as a positive test for the enzymatic activities assayed.

Enzyme Assays

Xylanase and cellulase activities in EEs were 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 1.0% carboxymethylcellulose in 0.1 M acetate buffer at pH 5.0, for xylanase and cellulase activities, respectively) and 25 µL of EE and was incubated at 50 °C. The acid protease activity was measured by the method previously reported by Saheki and Holzer (1975), using denatured hemoglobin at pH 3. One unit of xylanase, cellulase, or acid protease activity was defined as the amount of enzyme that liberated 1 µmol of xylose, glucose, or tyrosine, respectively, per minute under assay conditions. Protein content was determined by the Lowry method (Lowry *et al.* 1951).

Zymogram Analysis

Hydrolytic activities were detected *in situ* through zymograms, using 0.2 U of activity of either xylanase or cellulase activity of both SMF and SSF and 0.1 U of xylanase activity of the EE obtained from biodegradable (Raghukumar *et al.* 2004).

Effect of pH on Acid Protease Production

To evaluate the effect of pH on acid protease production, minimal media at pH 7 and pH 3 were prepared, as described previously by Ruiz-Herrera *et al.* (1995). Both media were inoculated with PDA discs (Difco) containing 3 days' growth of *S. maydis* and incubated at 28 ºC and 160 rpm for 120 h. The extracellular fraction was obtained by centrifugation (supernatant). The growth was washed with sterile distilled water and transferred as follows: the mycelium from the medium at pH 3 was inoculated into the pH 7 medium; the mycelium from the medium at pH 7 was inoculated into the pH 3 medium; and the cultures were incubated at 28 ºC and 160 rpm for 120 h. The extracellular fractions were obtained, and acid protease activity was determined in the supernatants.

RESULTS

Plate Assays for Enzymatic Activities

Figure 1 shows the activities of xylanase, cellulase, and acid protease of *Stenocarpella maydis* determined in plates. Acid protease showed a large halo of hydrolysis beginning at day 5, and the other enzymes had the highest halo of hydrolysis on day 7.

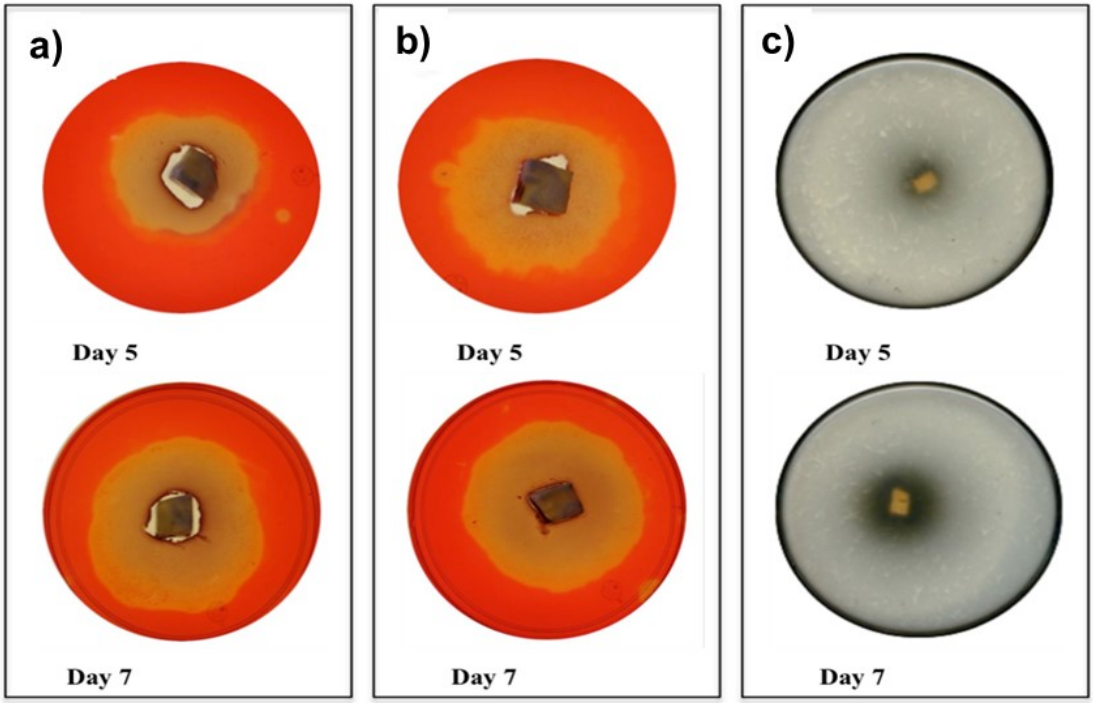

Fig. 1. Enzymatic activity in plates: a) xylanase, b) cellulase, and c) acid protease

Enzymatic Production

Xylanase activity was higher with YEX medium in SMF and SSF on PUF, reaching values of 18,020 U/L at 144 h and 19,266 U/L at 72 h, respectively. The maximal values of xylanase activity in SMF with media YEC, YEG, and YPD were 16,857 U/L at 96 h, 8,458 U/L at 120 h, and 6,742 U/L at 96 h, respectively. The maximal values of xylanase activity for SSF on PUF were 9,439 U/L at 216 h, 8,593 U/L at 72 h, and 14,162 U/L at 96 h, respectively (Fig. 2).

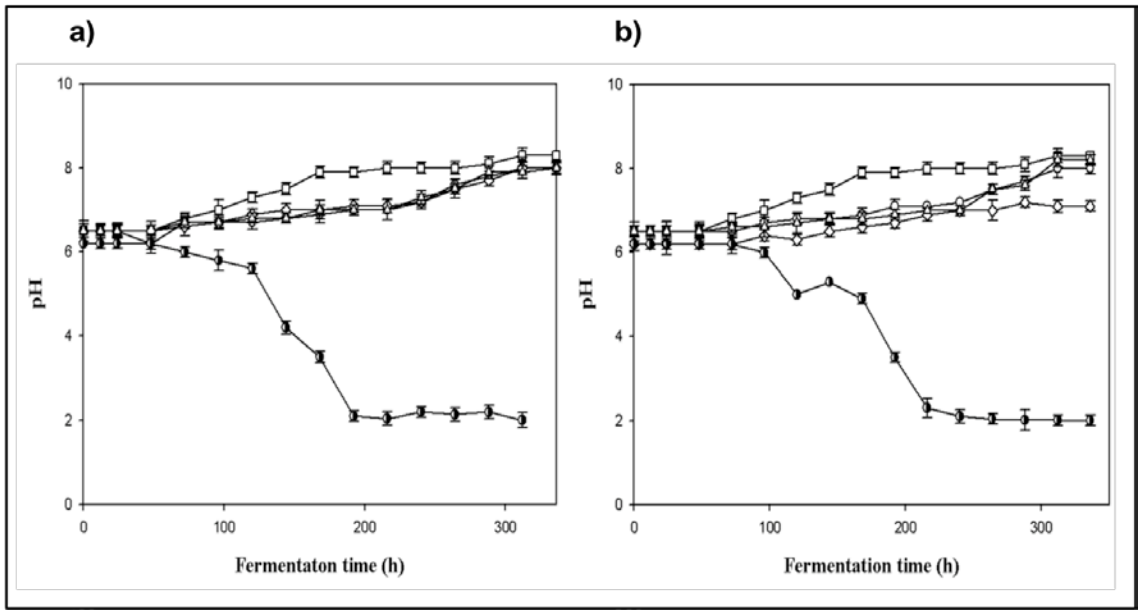

Fig. 2. Profile of pH of the culture medium when *Stenocarpella maydis* grown in: a) SMF and b) SSF on PUF. YEC (○), YEX (□), YEG (△), YPD (◇), MMG (◐)

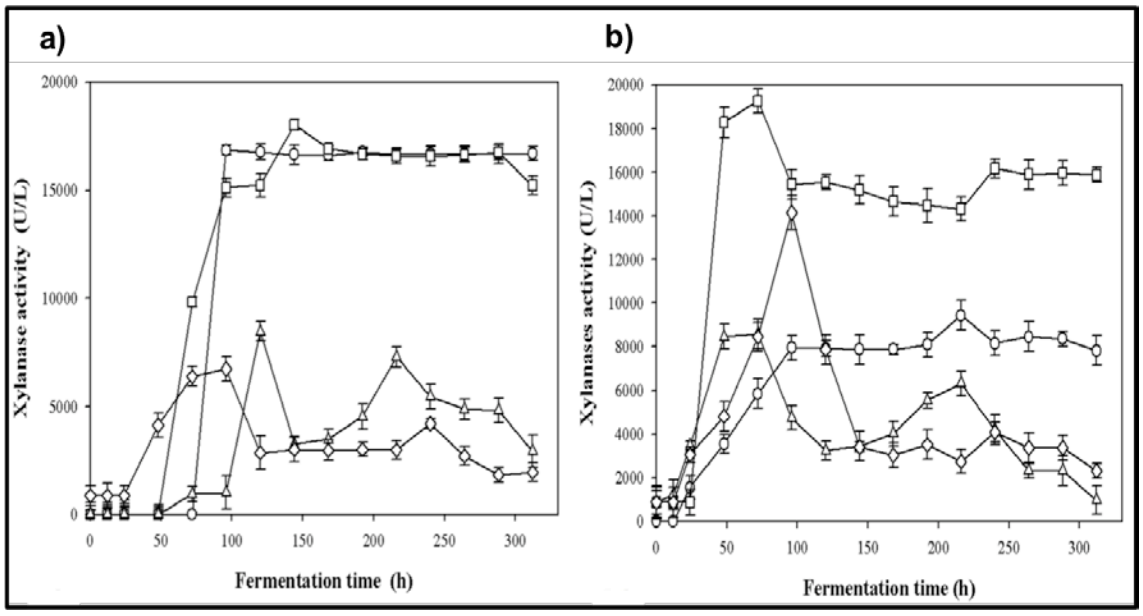

Fig. 3. Xylanase production by *Stenocarpella maydis* in: a) SMF and b) SSF on PUF. YEC (○), YEX (□), YEG (△), YPD (◇)

MMG medium did not show any xylanase activity. In all cases, the enzyme production was observed when the pH of the culture medium was between 6.5 and 8.0 (Fig. 3). Figure 4 shows the xylanase activity obtained in SSF on biodegradable supports, reaching values of 24, 17, and 13 U/g dry support for cobs, broken corn, and corn leaves, respectively. Zymograms showed two xylanase isoenzymes in EEs with the highest activity in each media for both SMF and SSF on PUF and three isoenzymes in SSF on natural supports (Fig. 5 a, b, and c).

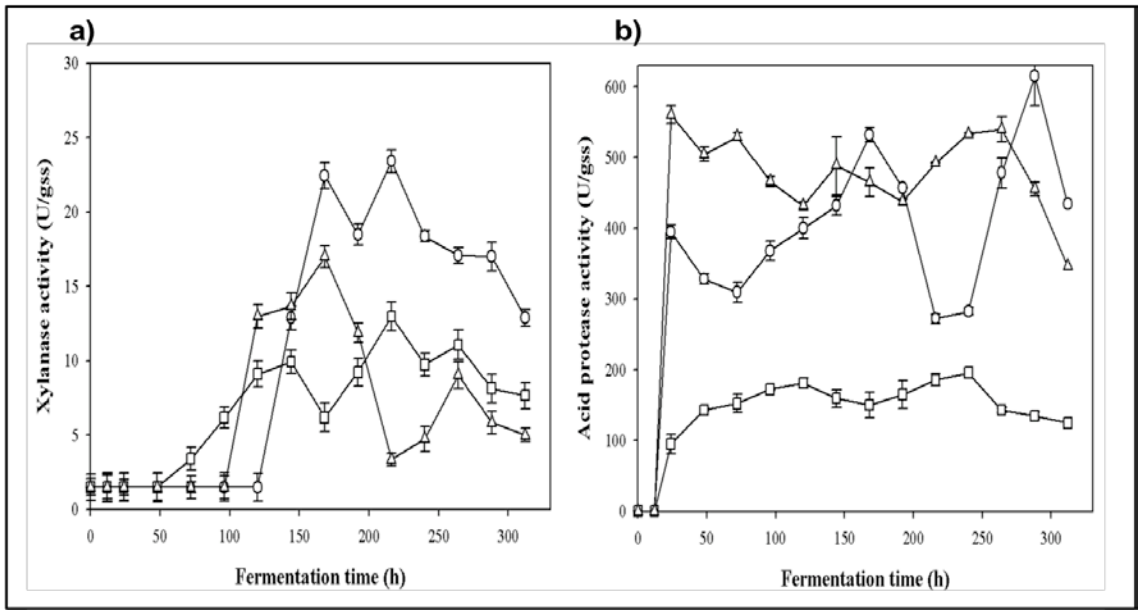

Fig. 4. Enzymes production on natural supports by *Stenocarpella maydis,* a) Xylanases and b) Acid Protease. Cob (○), corn leaves (□) and broken corn (△)

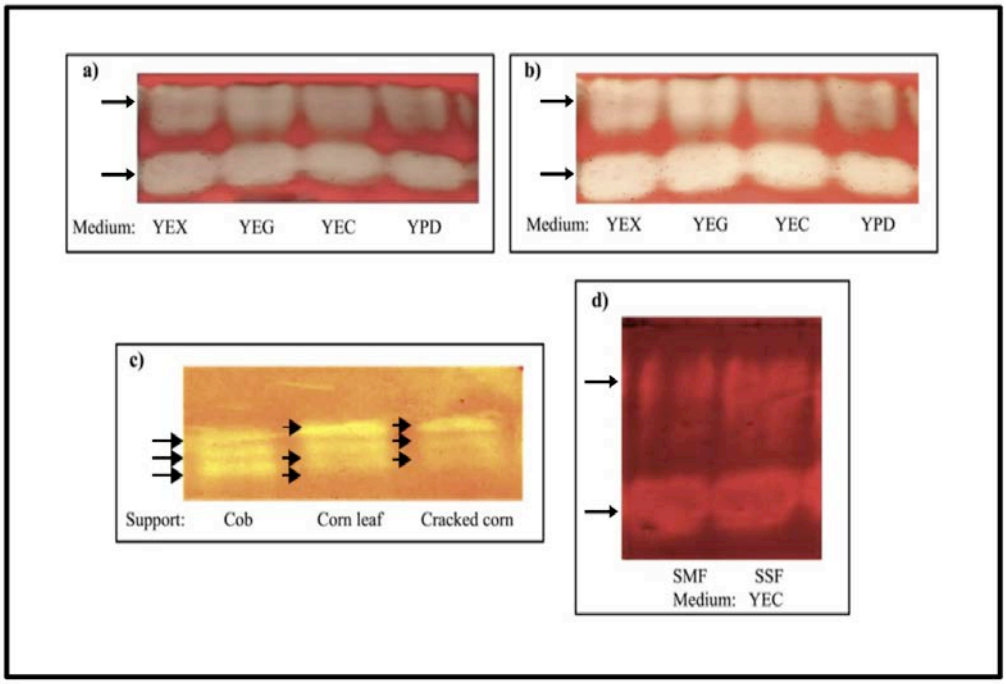

Fig. 5. Xylanase zymogram patterns: a) SMF, b) SSF on PUF, c) natural supports, and d) cellulase xymogram pattern

Cellulase activity was observed only in SMF and SSF on PUF with YEC medium, showing 7,872 U/L at 240 h and 9,439 U/L at 216 h, respectively (Fig. 6a). No activity of this enzyme was found in natural supports. Enzyme production was observed when the pH of the culture medium was between 7.2 and 7.6 (Fig. 3). Two cellulase isoenzymes were observed in the EEs with the highest cellulase activity (Fig. 5d). The acid protease activity was observed in MMG, and the highest levels of activity in SSF on PUF was observed at pH 2 with 806 U/L at 120 h and in SMF at pH 5 with 219 U/L at 312 h (Fig. 6b). The highest acid protease activities obtained in SSF on cobs, broken corn, and corn leaves were 614 at 288 h, 560 at 24 h, and 195 U/g dry support at 240 h, respectively (Fig. 4b). Acid pH is very important for acid protease production, as culture at pH 7 did not show activity, while activity was observed in culture at pH 3 and culture transferred at pH 3 from culture at pH 7 (Fig. 7).

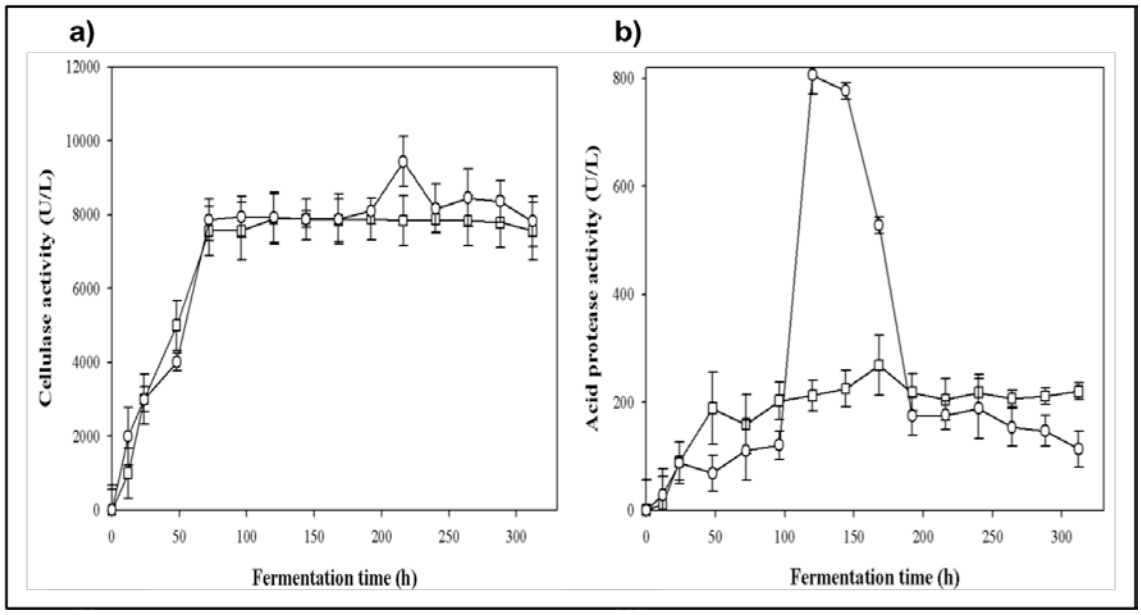

Fig. 6. Enzyme production by *Stenocarpella maydis* of a) cellulase in YEC medium and b) acid protease in MMG medium. SMF (□) and SSF (○).

Growing Conditions of *Stenocarpella maydis*

The growth of *S. maydis* in both SMF and SSF on PUF using different culture media is shown in Fig. 7. In general, cultures in SSF on PUF showed higher values of X_{max} and μ than did SMF. The X_{max} values were 8.52, 5.65, 7.04, 18.49, and 12.56 g/L, and the μ values were 0.045, 0.047, 0.056, 0.047, and 0.050 h^{-1} in cultures grown in SSF on PUF with YEX, YEC, YEG, YPD, and MMG media, respectively. In SMF, the X_{max} values were 5.59, 3.10, 6.35, 15.72, and 4.54 g/L, and the μ values were 0.044, 0.042, 0.028, 0.021, and 0.040 h^{-1} in YEX, YEC, YEG, YPD, and MMG media, respectively. In general, values of $Y_{E/X}$ for xylanase and cellulase activities were higher in SMF than in SSF on PUF. However, P values showed an inverse behavior, and values of $Y_{E/X}$ and P for acid protease were higher in SSF on PUF (Table 1, Fig. 8).

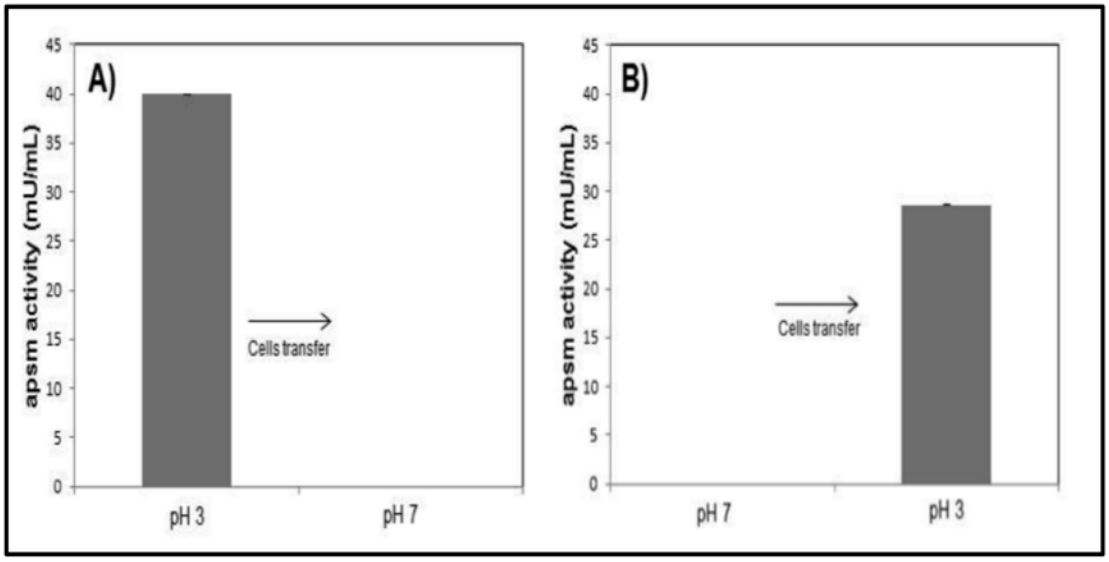

Fig. 7. Effect of pH on the production of acid protease. *Stenocarpella maydis* was grown for 24 h in minimal medium at pH 3 and pH 7. The pH values were determined in the EE. (A) The mycelium was transferred from the media at pH 3 to the media at pH 7; (B) The mycelium was transferred from the media at pH 7 to the media at pH 3, as described in experimental section.

DISCUSSION

Enzymatic production of Xylanase, Cellulase, and Acid Protease from *S. maydis*

In this research, the ability to produce xylanase, cellulase, and acid protease activities in *S. maydis* was demonstrated using plate assay for enzymatic activities. The production of these enzymes has been studied in a few plant pathogenic fungi, such as xylanases of *Sporisorium reilianum*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Myrothecium verrucaria*, and *Ustilago maydis* (Álvarez-Cervantes *et al*. 2013; Ellouze *et al.* 2007; Alconada and Martinez 1994; Gómez-Gómez *et al*. 2001, 2002; Inmaculada *et al*. 2005; Moreira *et al*. 2005; Cano-Canchola *et al*. 2000) and cellulases of *Magnaporthe oryzae* and *U. maydis* (Van Vu *et al.* 2012; Schauwecker *et al.* 1995; Cano-Canchola *et al*. 2000); however, the study by King *et al*. (2011), made it possible to determine that some phytopathogenic species of *Fusarium*, *Rhizoctonia*, *Sclerotinia*, *Cylindrocarpon*, *Colletotrichum*, *Sclerotium*, *Botrytis*, *Bipolaris*, *Alternaria*, *Curvularia*, *Drechslera*, *Cladosporium*, and *Phoma*, showed moderate or high hydrolytic activities in degradation of at least one of the following substrates; xyloglucan from tamarind, filter paper, alfalfa, soybean stems, switchgrass, corns stalks, arabinoxylan from wheat, and xylan from birch. Moreover, the extracellular acid proteases of phytopathogenic fungi have been reported only in *S. reilianum*, *Botrytis cinerea*, *Fusarium culmorum*, *Monilinia frutigena*, *S. sclerotiorum*, *U. maydis*, *Glomerella cingulate*, and *Cryphonectria parasitica*. In these studies, the biochemical properties of acid proteases were reported, trying to relate their activity with pathogenesis (Mandujano-Gonzalez *et al.* 2013; Movahedi and Heale 1990; ten Have *et al.* 2004; Rolland *et al.* 2009; ten Have *et al.* 2010; Urbanek and Yirdaw 1984; Hislop *et al.* 1982; Choi *et al.* 1993; Jara *et al.* 1995; Poussereau *et al.* 2001; Plummer *et al.* 2004).

The enzymes production in this study was found to be related to the growth conditions in both solid or liquid fermentation, and the culture medium used. It has been described that the filamentous fungi are the best source of cellulolytic and hemicellulolytic enzymes that act in the degradation of polymers of the plant to be used as a source of carbon and energy. Its production is coordinately regulated and is induced by the corresponding substrate (Amore *et al*. 2013), as observed in the case of xylanase and cellulase activities of *S. maydis*, where the xylan and cellulose acted as inducers, respectively. It has been reported that the carbon source is important for increasing the enzymatic activity levels. The rumen fungi *Neocallimastix patriciarum* and *Piromonas communis* increased their α-L-arabinofuranosidase and β-D-xylosidase activities in the presence of xylan, and the α/β-glucosidase activities were higher with maltose or starch (Williams and Orpin 1987). Sumair *et al.* (2010) reported that *Pleurotus eryngii* had high xylanase activity using xylan as a carbon and energy source.

Catabolite repression is a mechanism that suppresses inducible enzyme production by the presence of carbon sources of easier assimilation such as glucose (Aro *et al*. 2005). In this sense it was noted that in minimal medium with glucose (MMG), detectable hydrolytic activities were not found; however, significant levels of xylanase were observed in media containing glucose and yeast extract (YEG and YPD). A similar effect was found for *S. reilianum*, where in medium containing glucose and yeast extract, the production of xylanase was observed (Álvarez-Cervantes *et al*. 2013). There are reports that show increased production of xylanolytic enzymes in *Aspergillus* and *Penicillium* in the presence of yeast extract (Bakri *et al.* 2003; Sudan and Bajaj 2007). In basidiomycetes fungi, the regulation of ligninocellulolytic enzymes production depends not only on the presence of xylan, but also on the nature and concentration of the carbon source (Zakariashvili and Elisashvili 1993). On the other hand, Polizeli *et al.* (2005) observed the constitutive presence of xylanase at lower levels, which may be happening in *S. maydis*.

The production of the acid protease was found in the minimal medium with glucose and ammonium sulfate, being favored by acidic pH values similar to that found in the phytopathogenic fungi *S. reilianum* and *U. maydis*, where proteolytic activities Eap1 and pumAe are produced in the presence of ammonium sulfate when the fungus acidified the culture medium (Mandujano-Gonzalez *et al.* 2013; Mercado-Flores *et al.* 2003). This effect in *U. maydis* is attributed to the activity of a proton pump (Ruiz-Herrera *et al*. 1995). In other fungi, acidification is due to production of organic acids, as in the case of oxalic acid in *S. sclerotiorum*, which is required during pathogenesis (Cessna *et al.* 2000). In *Aspergillus niger* the use of ammonium salts in the culture medium promotes the acidification, which is a prerequisite for the production of citric acid (Max *et al.* 2010). There is evidence that the phytopathogenic fungi are capable of modulating the pH of the host as a means to increase its virulence (Eshel *et al.* 2002), which allows the expression of enzymes related to this condition as in the case of extracellular acidic proteases, which are important in the process of penetration and colonization, degrading the structural proteins of the cell wall of plants, and generating an important source of amino acids (Oliver and Osbourn 1995; Annis and Goodwin 1997).

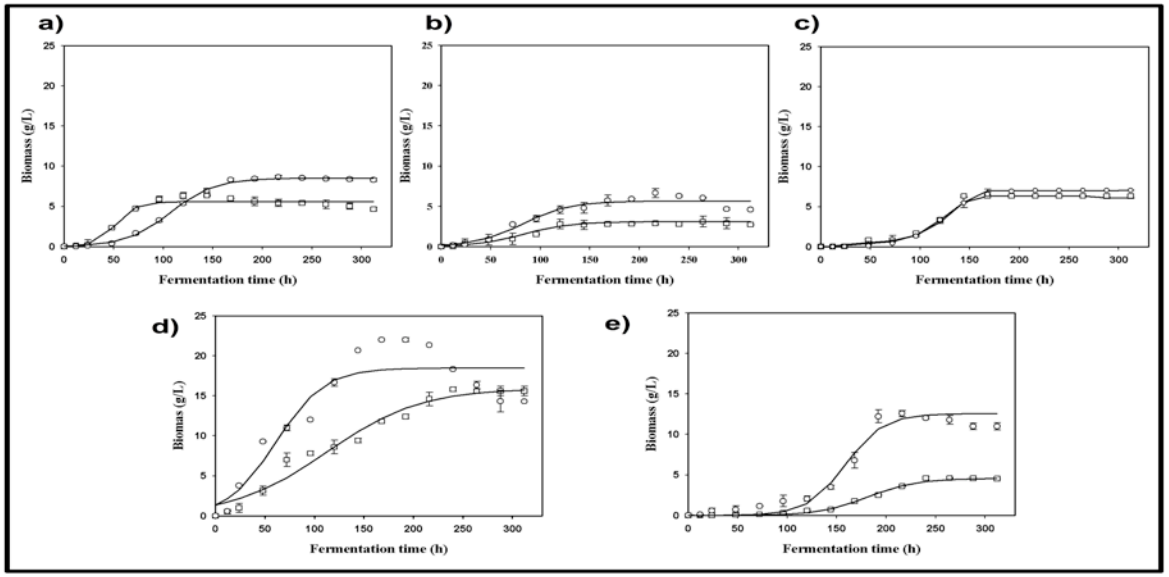

Fig. 8. Growth of *Stenocarpella maydis* in SMF (□) and SSF (○). The error bars represent the standard deviation of three separated replicates for each experiment. a) YEX, b) YEC, c) YEG, d) YPD, e) MMG.

Table 1. Xylanase, Cellulase, and Acid Protease Production in SMF and SSF

System	Medium	X_{max} (g/L)			E_{max} (U/L)			$Y_{E/X}$ (U/gX)			*P* (U/Lh)		
		Xylanase	Cellulase	Acid protease	Xylanase	Cellulase	Acid protease	Xylanase	Cellulase	Acid protease	Xylanase	Cellulase	Acid protease
SMF	YEX	5.59	-	-	18.02	-	-	3,223.61	-	-	125.14	-	-
	YEC	3.10	3.10	-	16.86	7.87	-	5,437.74	2,539.35	-	175.60	32.8	-
	YEG	6.35	-	-	8.46	-	-	1,331.97	-	-	70.483	-	-
	YPD	15.72	-	-	6.74	-	-	4,28.88	-	-	70.229	-	-
	MMG	-	-	4.54	-	-	219.77		-	48.41	-	-	0.70
SSF	YEX	8.52	-	-	19.27	-	-	2,262.27	-	-	267.583	-	-
	YEC	5.65	5.65	-	9.44	9.44	-	1,670.62	1,670.62	-	43.699	43.699	-
	YEG	7.04	-	-	8.59	-	-	1,220.60	-	-	119.347	-	-
	YPD	18.49	-	-	14.16	-	-	765.93	-	-	147.520	-	-
	MMG	-	-	12.56	-	-	806.37	-	-	64.20	-	-	6.72

On the other hand in this study, xylanase and cellulase enzymes were produced when the media pH reached values of 6.5 to 7.0. Fialho and Carmona (2004) found that the xylanases and cellulases produced by *Aspergillus giganteus* occur in a pH range of 6 to 7.

Xylanase production is slightly favored in SSF, except in the medium with carboxymethylcellulose, where a significant increase was observed in SMF. There are not important differences in the production of cellulase in both types of fermentation, unlike the protease, which is predominant in the SSF. This could be because of the environmental conditions, which are similar to the natural habitat of the fungus. In that sense, the natural supports are a good alternative for producing these enzymes.

Many studies have reported increased production of enzymes by SSF compared with SMF (Antier *et al.* 1993; Acuña-Argüelles *et al.* 1995; Maldonado and Strasser de Saad 1998; Díaz-Godínez *et al.* 2001; Roncero-Gómez *et al.* 2000; Ashokkumar and Gunasekaran 2002; Asther *et al.* 2002; Viniegra-González *et al.* 2003; Hölker *et al.* 2004; Aguilar *et al.* 2004). Specific expression of genes in SSF that are not expressed in SMF has been demonstrated in the case of *Aspergillus oryzae*, where the expression levels of genes in the glycolytic pathway and tricarboxylic acid cycle are low, and decreasing the catabolic repression increases the production of extracellular enzymes (Maeda *et al.* 2004; Akao *et al.* 2005; Oda *et al.* 2006). The fungus was able to grow in media that is natural to corn and produce xylanase and acid protease. It has been reported on the production of xylanases in untreated biomass of corn stalks by some plant pathogens of the genera, *Fusarium*, *Rhizoctonia*, *Sclerotinia*, *Cylindrocarpon*, *Sclerotium*, and *Bipolaris* (King *et al*. 2011).

Several studies have established that phytopathogenic fungi secrete multiple enzymes to infect tissues (Gilbert 2010). Other authors suggest that the pathogenicity may depend on the ability of the fungus to depolymerize the components of the plant cell wall, which may require two or more xylanases, cellulases, and proteases that work together during the infection of the host (Subramaniyan and Prema 2002).

Zymograms showed that *S. maydis* produce two isoforms of xylanase in both SSF and SMF and three isoforms when it was grown on natural substrates. It has been determined that in some cases the number of genes coding for xylanases and cellulases is greater in phytopathogenic fungi that in industrial interest fungal (Martinez *et al.* 2008). This could be related to the ability of these microorganisms to infect and colonize its host, where the action of extracellular hydrolytic enzymes play an important role (Aro *et al*. 2005). For example *Fusarium oxysporum* grown in SSF, with pectin as the sole carbon source, showed 11 xylanase isoenzymes with different optimal production pH, which could be related to its pathogenicity (Gómez-Gómez *et al.* 2002). Álvarez-Cervantes *et al.* (2013) found a xylanase produced by the pathogen of maize *S. reilianum* grown in both SSF and SMF with xylan as the sole carbon source. Subramaniyan and Prema (2002) reported that *Phanerochaete chrysosporium* produced more than 30 isoenzymes of xylanases separated by isoelectric focusing. On the other hand, in the thermophilic fungus *Myceliophthora* sp., 10 isoforms of xylanases, with different functionality were described (Badhan *et al.* 2004).

This is the first report of extracellular xylanase, cellulase, and acid protease from *S. maydis*. These activities could play an important role during the life cycle of the fungus, in addition to various biotechnological applications.

CONCLUSIONS

1. The culture medium obtained the highest values of activity of the three enzymes of *Stenocarpella maydis* studied in this work.
2. The SMF and SSF on PUF with YEX medium produced the highest xylanase activity. Cellulase was produced only in YEC medium in both SMF and SSF on PUF, and the acid protease was observed only in MMG medium at acid pH, but the SSF on PUF produced higher activity than did SMF.
3. Natural supports allowed production of xylanase and acid protease, where the cobs were the best support.
4. Two and three isoenzymes were produced in synthetic medium (both SMF and SSF on PUF) and natural supports, respectively.

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