Native Macrofungi that Produce Lignin-Modifying Enzymes, Cellulases, and Xylanases with Potential Biotechnological Applications

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With the aim of identifying and exploiting the mycological resources available in the Mexican Sierra Madre Oriental, the lignocellulolytic and pectinolytic potential of autochthonous fungi were evaluated in the present work. A solid media selection system was established in which 74 isolated strains were tested and compared to six international reference strains. The macrofungi *Xylaria* sp CS121, *Inonotus* sp CU7, Basidiomycete CH32, Basidiomycete CH23, *Xylaria poitei*, and *Trametes maxima* CU1 showed the highest cellulolytic and pectinolytic potential. The greatest lignolytic capability was exhibited by *T. maxima* CU1 and *Pycnoporus sanguineus* CS43. Under stirred submerged culture, *T. maxima* CU1 (cellulases, cellobiose dehydrogenase, manganese peroxidase (MnP), and laccase, with 200, 359, 51, and 267 U/L, respectively) and *Xylaria* sp CS121 (198 U/L of xylanases) were the highest enzymatic producers. Under stationary conditions, the best producers were *Inonotus* sp CU7 for cellulases, *P. sanguineus* CS43 for cellobiose dehydrogenase and laccase, and *T. maxima* CU1 for xylanases and MnP (242, 467, 35, 165, and 31 U/L, respectively). These results demonstrate the efficiency of enzymatic profiling as a tool for enzyme discovery with Mexican native fungi.

Keywords: Cellulolytic potential; Enzyme screening; Lignin modifying enzymes; Mexican fungi; Pectinolytic potential

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INTRODUCTION

Lignocellulose is the most plentiful component of photosynthetic plant biomass in the world. Representing almost 50% of total biomass, it is the most abundant organic renewable resource on earth. Plant residues increase slowly in natural ecosystems as trunks, timber, branches, and fallen leaves, while in agricultural ecosystems these accumulations occur over short periods of time and in considerable amounts. For this reason, lignocellulosic-rich residues are seen as a good source of valuable raw matter for paper and fuel production, composts, feed, fodder, and others (Sánchez 2009).

Because of the chemical bonds between plant cell wall components, the disposal of cellulose, hemicellulose, and lignin through artificial degradation is difficult and

expensive in terms of the energy costs and pollutant production. Natural decomposition is slow, and in the case of lignin, only occurs with certain microbiota.

Therefore, research on efficient microorganisms able to degrade plant cell wall components is an investigative interest shared by many throughout the world. At first glance, most explorations have been focused on microorganisms such as bacteria and filamentous fungi, due to their ease of cultivation and enzyme concentration (Khokhar *et al*. 2013). However, a promising source of diverse and efficient enzymatic activities has been discovered in some other fungi, mainly basidiomycetes (Sánchez 2009; Elisashvili *et al*. 2011). Research on the hydrolytic and oxidative activities of plant cell wall components by this type of fungus involves the exploration of native flora and collection of strains. The basidiomycetes have been used as models to understand plant cell wall degradation processes, but just few of them have been applied in industrial practices.

Therefore, in the last decade, a great number of papers have been published in which native mycoflora are explored. Scientists have been searching for fungi of different physiologies in order to obtain their enzymatic profiles. The objectives of these activities are the research of basic useful information on biosystematics and biodiversity (Xavier-Santos *et al*. 2004; Atri and Sharma 2011). This adds to the understanding of saprophytic action by this group over fallen leaves and helps explain how chemical composition changes during degradation (Valášková and Baldrian, 2006), creating humic compounds (Steffen *et al*. 2007).

In the same way, microflora's potential as enzyme producers has been explored in different areas, which include: degradation of lignocellulose for fertilizers production or bioremediation of dyes (Nazareth and Sampy 2003); bio-bleaching and fibers improvement in the paper industry (Sigoillot *et al.* 2002; Elisashvili *et al*. 2011); and on the development of integral biorefineries using agro-industrial wastes (Elisashvili *et al*. 2009).

In northeastern México, the exploration of the biotechnological potential of native fungi from mountain zones surrounding the city of Monterrey and its metropolitan area has led to a collection of 85 macrofungal strains that have been characterized for their capacity at decolorizing synthetic dyes that are commonly used in industry (Hernández-Luna *et al*. 2008). From this work, the laccases from *Trametes maxima* CU1 (Gutiérrez-Soto *et al*. 2011) and *Pycnoporus sanguineus* CS2 were purified, and their ability to degrade dyes with high demand in the region were characterized (Salcedo-Martínez *et al.* 2013).

In the present work, 74 native macrofungi newly isolated from different ecotypes (timber, plant litter, and dung) in northeastern México were explored. The aim was to find new enzyme sources capable of degrading cell wall components of plants, laying a foundation for their subsequent operative and functional characterization relative to the development of digestive aids to improve animal nutrition.

EXPERIMENTAL

Materials

A collection of 74 macrofungal strains from the Enzymology Laboratory at Facultad de Ciencias Biológicas, UANL and six reference strains were used in this work. Isolates were obtained from carpophores collected in oak forests and scrubland around Monterrey, N.L., México, from different ecotypes, mainly lignocellulosic substrates. The

reference strains were kindly donated by M. Pickard from the University of Alberta, Canada. Their corresponding codes are: COL1 *Berkjandera adusta* UAMH 8258, COL2 *Coprinus cinereus* UAMH 4303, COL3 *Coprinus* sp UAMH 130509, COL4 *Phanerochaete chrysosporium* ATCC 24785, COL6 *Trametes hirsuta* UAMH 8165, and COL7 *T. versicolor* UAMH 8272. Native and collection strains consisting of vegetative mycelia were preserved at 4 ºC in YMGA medium (10 $g \cdot L^{-1}$ malt extract, 4 $g \cdot L^{-1}$ glucose, 4 $g \cdot L^{-1}$ yeast extract, and 15 $g \cdot L^{-1}$ agar) as reported by Hernández-Luna *et al.* in 2008 and were transferred to new plates every three months for conservation and studies.

Methods

Plate screening for degrading enzymes of plant cell wall components

Identification of the main enzymatic activities involved in the modification of the components of plant cell walls was carried out using the medium previously reported by Sin *et al*. (2002). The base composition of the medium was: 0.1% peptone, 0.01% yeast extract, and 1.6% agar, which was supplemented with different carbon sources, dyes, or chromogenic substrates for revealing the presence of various enzyme activities through degradation of carbon source, discoloration of dye, or color generation. Corresponding supplements to these activities were: 2% carboxymethyl cellulose (CM-cellulose, Sigma) to detect cellulases, xylan (birch wood xylan, Sigma) for xylanases, 0.5% pectin (pectin from citrus peel, Sigma) for pectinases, 0.02% Poly R-478 dye for lignin modifying enzymes (LME), 0.015% Azure B dye for lignin peroxidase (LiP), and 0.02% syringaldazine for laccases. For dye decolorizing and laccase tests, 0.2% glucose was added as a carbon source as well. All media were sterilized by autoclaving at 121 ºC and 1.05 $kg.cm^{-2}$ for 15 min. Autoclaved media were poured in three division plates, allowed to solidify, and each division was inoculated with a 0.5 cm diameter cylinder of mycelium taken from the periphery of a 5 day growth colony of each strain growing in YMGA . All media were incubated at 28 ºC; colony growth as well as the existing areas of reaction or discoloration was registered daily as the diameter increased (mm) measured with a vernier caliper. Detection of cellulase, xylanase, and pectinase was conducted on the third day of growth (in most isolates), revealing their degradation by the addition of iodine solution. All assays were performed in triplicate, and a scale was established as support for the recording of results and the selection of the best enzyme producers.

Scale for the interpretation of results

In order to establish a scale for interpretation of the results for carbohydrolases, the ratio considering hydrolysis reaction and the growth diameters was used (Table 1). In this way, the hydrolysis area on the third day of the assay was revealed in the medium supplemented with CM-cellulose, xylan, or pectin.

Table 1. Scale for Carbohydrolases Results Interpretation

Symbol	Ratio	Description
+	≤ 1	Reaction area smaller or equal to the growth area.
++	1.1 - 1.9	Reaction area greater than the growth area without doubling the ratio.
+++	≥ 2	Reaction area greater than the double of the growth area.

A value of one plus symbol (+) was given when the ratio was smaller than 1. Two plus symbols (++) corresponded to a ratio between 1 and 2. Finally, three plus symbols (+++) were assigned when a ratio greater than 2 was achieved (Fig. 1).

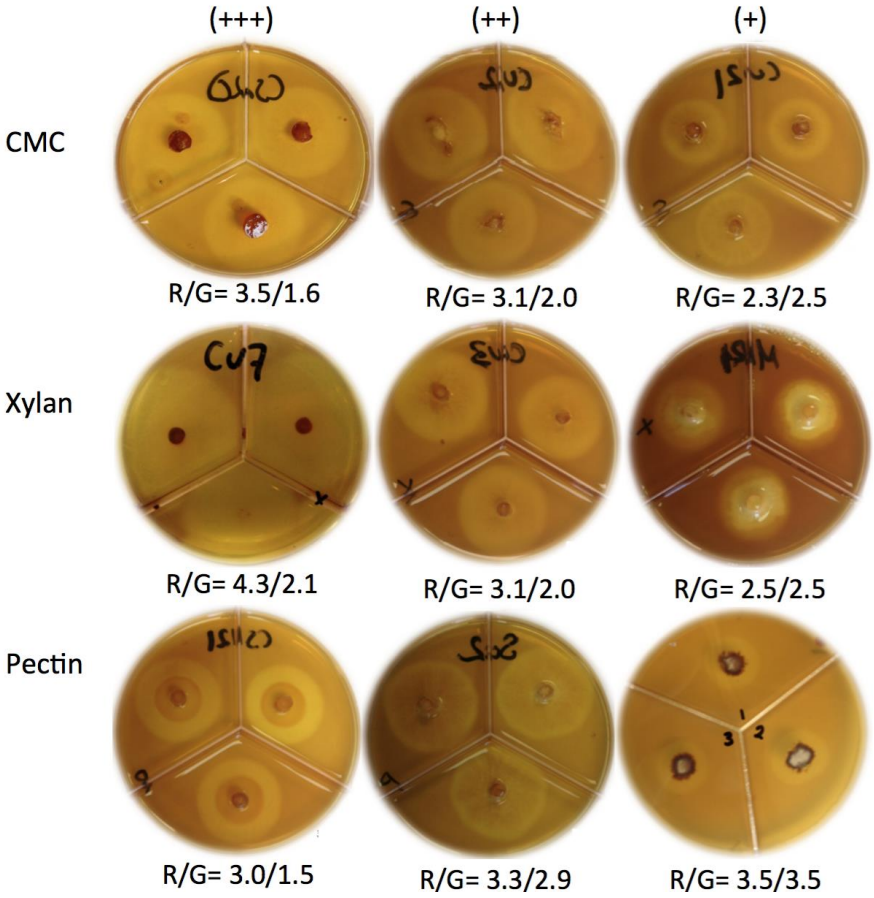

Fig. 1. Hydrolysis scale used for cellulases, xylanases, and pectinases detection. Enzyme activity was revealed at the third day of growth with iodine solution. Values given considered the difference between growth and hydrolysis areas.

In the case of Poly R-478, if decolorization was only partial from the seventh day or later, the assigned value was (+). If complete decolorization appeared in the area after the seventh day of growth, the corresponding value was (+ +). Only when decolorization area appeared on the fifth day of or before, a value of (+ + +) was assigned. For Azure B if complete decolorization become evident showing a light blue color on day 7 or before, the value of (+ + +) was given. When decolorization occurred after day 9, the value (+ +) was assigned. In most isolates only a zonal transformation appeared after day 5, showing a pink color; in those cases, a value of (+) was assigned. For laccase activity, just one plus symbol was assigned (+) to strains that showed a mild pink reaction appearing on the syringaldazine after the fifth day, a two plus symbols (+ +) for strains that showed a reaction at the fifth day, and a three plus symbols (+ + +) corresponded only to strains whose reaction was a deep pink before the fifth day of growth; all these are shown graphically in Fig. 2.

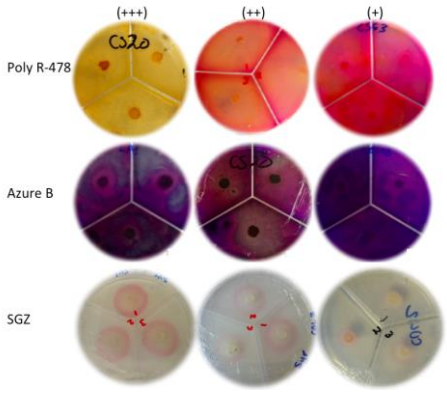

Fig. 2. Detection scale used for selection of lignin modifying enzymes producer strains. Poly R-478 decolorization was associated with lignolytic activity of isolates; Azure B decolorization with LiP production; and pink reaction of syringaldazine with laccase activity.

Identification of enzymatic profiles in liquid media

The determination of production curves for cellulases, xylanases, and ligninases was performed in a rich medium for basidiomycetes as reported by Pozdnyakova *et al.* (2006). Its composition per liter was: 10 g glucose; 0.5 g yeast extract; 10 g peptone; 0.72 g NH_4NO_3; 1.0 g $MgSO_4$; 0.5 g KCl; and 1 mL of trace solution (1 g/L $FeSO_4$; 2.8 g/L $ZnSO_4$; 3.3 g/L $CaCl_2$). Production was accomplished in 500 mL Erlenmeyer flasks containing 200 mL of medium and 2% wheat straw (m/v). After autoclaving (121 ºC, 1.05 $kg \cdot cm^{-2}$) for 15 min and allowing to cool at room temperature, each flask was inoculated with three 0.5 mm diameter cylinders of mycelium taken from the periphery of a 5 day growth fungal colony in YMGA. Flasks were incubated at 28 ºC under stirring (150 $rev \cdot min^{-1}$) and stationary conditions; every two days a 2 mL aliquot was taken for the assays of cellulases, xylanases, laccase, LiP, and manganese-dependent peroxidase (MnP). The determination of cellulase (CMCases, Avicelases, and β-D-glucosidase) and xylanase activities was made following the method reported by Miller (1959). The reaction mixture consisted of 0.5 mL of sodium citrate (50 mM at pH 5.0), 0.3 mL of the respective substrate (2% CM-cellulose, Avicel, D-cellobiose, or D-Xylan), and 0.2 mL of sample aliquot. The mixture was incubated for 30 min at 50 °C. For the quantification of glucose or xylose, 0.1 mL of the reaction mixture and 0.1 mL of dinitrosalicylic acid (DNS) were added. After boiling for 5 min, the combination was placed in an ice bath and 1 mL of double distilled water was added. Photometric measurement was performed at 540 nm with a Shimadzu UV-Vis 1240 mini-spectrophotometer. A standard curve of glucose and xylose was used for cellulase and xylanase determinations, respectively. All assays were performed in duplicate. Laccase determinations were conducted using the method described by Abadulla *et al*. (2000), using 2,6-dimethoxyphenol (DMPO) as a

substrate in sodium acetate buffer (200 mM at pH 4.5), reading at 468 nm in the spectrophotometer. For LiP, the method proposed by Tien and Kirk (1988) was used. The reaction mixture consisted of veratryl alcohol (3 mM) and hydrogen peroxide (2 mM) in sodium succinate buffer (50 mM at pH 4.5) and read at 310 nm. The assay for MnP was carried out following the protocol reported by Elisashvili *et al*. (2011), following the formation of malonate-Mn II complex at 270 nm. The reaction was started with 10 μL of sample, and all assays were made at room temperature per triplicate.

RESULTS AND DISCUSSION

Northeast México is a region with a great biodiversity that has not been well studied yet. Similarly, mycological resources present in this region have been underutilized as study models or novel sources of biomolecules and functional metabolites of biotechnological interest. The white-rot basidiomycetes (WRF) produce two extracellular enzyme systems responsible for degrading polysaccharide and lignin in the plant wall cell by the action of hydrolases and oxidoreductases (Baldrian and Valášková 2008; Sánchez 2009). These fungi obtain their carbon source from lignin (Pointing 2001; Mtui 2010), an evolutionary advantage that allows them to easily access metabolizable carbon sources, such as cellulose and hemicellulose, due to the presence of cellulases and xylanases. Enzyme production, as well as its operational and functional properties, varies depending on the strain and environment (Elisashvili *et al*. 2011). This has led to the continuous search for new WRF strains able to produce cell wall component degrading enzymes, isolated from different lignocellulose-rich biomass (Xavier-Santos *et al*. 2004; Valášková and Baldrian, 2006; Elisashvili *et al*. 2011; Ben Younes *et al*. 2011; Isikhuemhen *et al*. 2012). Previous reports of Sin and coworkers (2002), compare the diversity of lignocellulose degrading enzymes from basidiomycetes and filamentous fungi obtained from different substrates, finding a higher lignocellulosic potential in basidiomycetes.

Plate Screening for Degrading Enzymes of Plant Cell Wall Components

The lignocellulolytic, amylolytic, and pectinolytic potential of 74 native isolates obtained from different ecotypes of subtropical regions was determined. Table 2 shows the general results of the lignocellulolytic, pectinolytic, and amylolytic potentials of all strains. Ninety percent of the isolates were cellulase producers, while 96% were xylanase producers. Almost all of the isolates were able to act upon the cellulose and xylan, while 38.5% and the 18.7% showed the greatest hydrolytic activity, respectively. These results are very similar to those reported by Sin *et al*. (2002), who isolated fungi from different substrates rich in lignocellulose, which resulted in better degradation of cellulose rather than lignin. However, the activity shown by the isolates reported by Sin *et al*. (2002) was less compared to the positive control, while the best isolates in the present work showed greater activities than the international reference strains (Fig. 4).

In the analysis of the pectinolytic potential of the isolates, 68.7% of these acted on pectin, where 13.7% showed the greatest pectinase activity. Within the species with high pectinolytic potential, strains *Pycnoporus sanguineus* CS2 and *P. sanguineus* CS43 stood out. There are few reports about pectinases produced by basidiomycetes (Pericin *et al*. 1992; Pericin *et al*. 1997; Levin and Forchiassin 1998; Xavier-Santos *et al*. 2004), indicating that these results contribute to the validation of basidiomycetes as a potential

source of pectinases with more robust operative and functional properties than those commercially available.

In the analysis of the lignolytic potential of the native strains, it was shown that 37.9% of the strains were able to produce LME, from which 9.5% completely discolored Poly R-478 dye on the fifth day of growth. In the test for the detection of LiP producing fungi, only the LA1 fungus was able to discolor Azure B on the fifth day of growth, showing a better result than *P. chrysosporium* ATCC 24785. Despite this, 40% of the isolates were able to transform Azure B from blue to pink. These same isolates showed a strong reaction over the syringaldazine, suggesting that laccase might be involved in the transformation of Azure B (Arantes and Milagres 2009). In a previous study (Sin *et al*. 2002), a pair of strains (*Periconia* sp. 1 y *Piricaudia* sp.) also showed this partial discoloration of Azure B. The possibility that this transformation of Azure B is associated with the production of high redox potential laccases, the production of MnP-laccase, or the presence of mediators of low molecular weight will be addressed in future investigations.

Table 2. Lignocellulolytic, Pectinolytic, and Amylolytic Potential of Native Macrofungi

Enzyme	High	Medium (%)	Weak	No activity
Cellulases	38.5	28.7	23.7	9.1
Xylanases	18.7	26.3	51.3	3.7
Pectinases	13.7	22.5	32.5	31.3
Amylases	40.9	34.8	21.2	3.1
LME	9.5	13.5	14.9	62.1
Laccases	39.2	4.0	1.3	55.5

The determination of cellulases, xylanases, pectinases, and amylases was conducted on the third day of culture, while the laccase was incubated until the seventh day and the LME for 30 days.

A total of 96.9% of the isolates had the capacity to hydrolyze starch, while 40.9% presented the significant activity over the substrate. Amylases are widely distributed enzymes in plants, animals, and microorganisms, but bacterial enzymes are in the greatest demand at the industrial level (Pandey *et al*. 2000; Gupta *et al*. 2003), even though there are reports that indicate that certain fungi are good amylase producers (Das and Sen-Mandi, 1992; Pal *et al*. 1980). There are studies where amylases of basidiomycetes have been exploited in order to increase the nutritional value of agroindustrial residues in the livestock industry (Han 2003; Han *et al*. 2005). Thus, knowing the amylolytic potential of the isolates makes it possible to obtain new producers of amylases that can be used for the development of digestive adjuvants.

From this system in solid media, strains *Xylaria* sp. CS121, *Inonotus* sp. CU7, Basidiomycete CH32, Basidiomycete CH23, and *X. poitei* were selected as the candidates with the highest cellulolytic and pectinolytic potential. Additionally, strains *T. maxima* CU1, *P. sanguineus* CS2, and *P. sanguineus* CS43 had lignolytic and amylolytic potential, while the Basidiomycetes CS 52, RS 9 and CH37 only displayed cellulolytic ability (Table 3). *T. maxima* CU1 showed higher activity than the international reference strains *P. chrysosporium* ATCC 24785, *T. hirsuta* UAMH 8156, or *T. versicolor* UAMH 8272 (Fig. 3).

Table 3. Native Fungal Strains with High Cellulolytic Potential

Strain	Cellulases	Xylanases	Pectinases
Mulch isolates with high cellulolytic and pectinolytic potential			
Xylaria sp CS121	+++	+++	+++
Inonotus sp CU7	+++	+++	+++
Basidiomycete CH32	+++	+++	+++
Basidiomycete CH23	+++	+++	+++
Xylaria poitei	+++	+++	+++
Mulch isolates with high cellulolytic potential			
Ps. candollena CU20	+++	+++	-
Basidiomycete CS52	+++	+++	+
Basidiomycete RS9	+++	+++	+
Basidiomycete CH37	+++	+++	++
Wood isolates with high cellulolytic and pectinolytic potential			
T. maxima CU1	+++	+++	+++
P. sanguineus CS2	+++	++	+++
P. sanguineus CS43	+++	++	+++

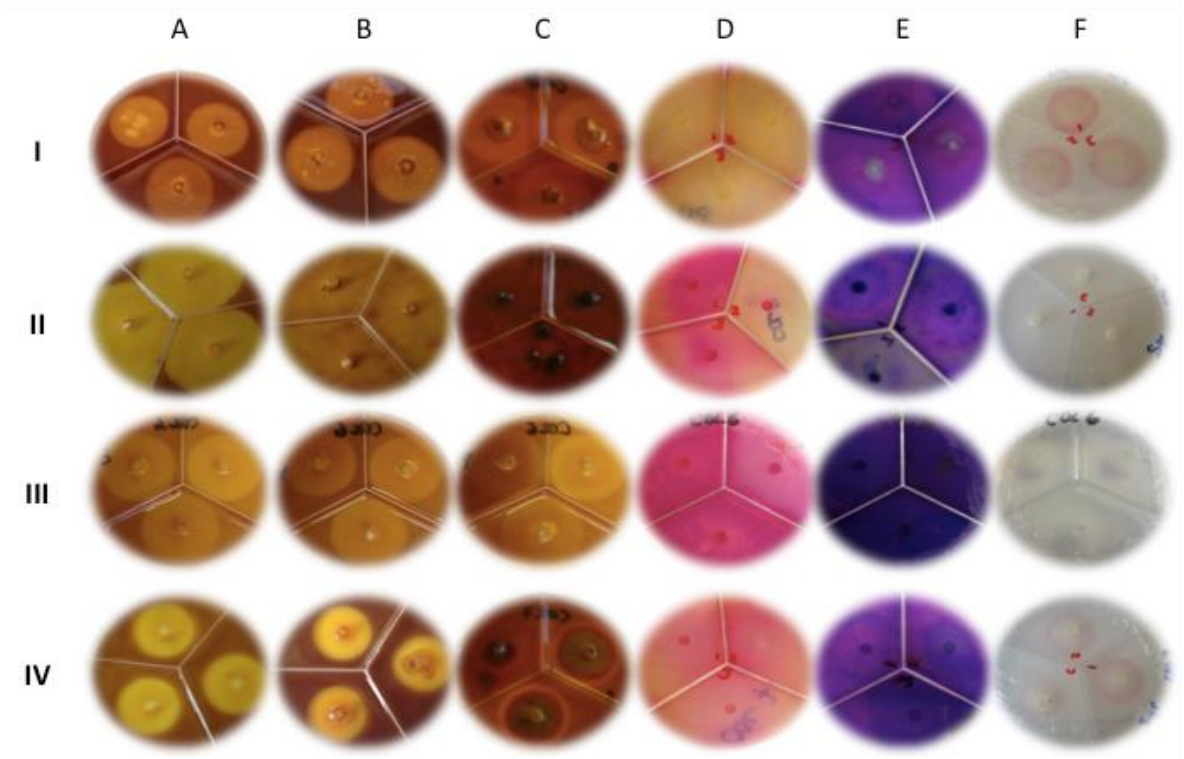

Fig. 3. Enzyme profiles in solid media. Row I corresponds to lignocellulolytic profile of *T. maxima* CU1, row II to that of *P. chrysosporium* ATCC 24785, row III to *T. hirsuta* UAMH 8156, and IV to *T. versicolor* UAMH 8272. The first three panels show the activities revealed with Gram iodine at the third day of growth: panel A corresponds to cellulases, B to xylanases, C to pectinases. Panels D, E and F corresponds to LME, LiP and laccase activities respectively. Discoloration of Poly R-478 (D) and Azure B (E) was observed in cultures along 30 days and laccase results were followed from the third to fifth day of growth.

Screening for Cellulase, Xylanase, and Lignolytic Production in Liquid Medium

From the scrutiny of 74 strains in solid media, strains *P. sanguineus* CS2, *P. sanguineus* CS43, *Armilariella* sp. CS134, *Xylaria* sp. CS121, *T. maxima* CU1, *Inonotus* sp. CU7, and *Ps. candollena* CU20 were selected for the production of cellulases, xylanases, and laccases in submerged cultures with and without stirring, using a wheat straw based medium. Native strain *Armilariella* sp CS134 was used as positive control. In stirring conditions, all of the strains had the CMCases titers, with *T. maxima* CU1 (200 U/flak), *Xylaria* sp. CS121 (199 U/flask), and *Armilariella* sp CS134 (190 U/flask) being the greatest producers. The rest of the strains had a production of less than 100 U/flask. The avicelases showed greater amounts of production at the fourth day, where the majority of the isolates produced over 200 U/flask, except for the strains *P. sanguineus* CS2 (12 U/flask) and *Ps. candollena* CU20 (45 U/flask). The greatest production of xylanases was presented at the fourth day, with *Xylaria* sp. CS121 (198 U/flask), and *Inonotus* sp. CU7 (123 U/flask) being the greatest producers; the rest of the strains produced less than 100 U/flask. β-D-glucosidase showed the greatest production on the fourth day of culture, with *T. maxima* CU1 and *P. sanguineus* CS43 being the greatest producers with 359 and 326 U/flask, respectively. The best laccase producer strain was *P. sanguineus* CS2 (435 U/flask); the rest of the isolates produced less than 10 U/flask. All of these results are shown in Table 4.

Table 4. Lignocellulolytic Enzyme Activity in Wheat Straw Agitated Liquid Medium

Strain	CMCases	Avicelases	Xylanases (U/flask)*	β-D-Glucosidase	Laccase
P. sanguineus CS2	19	12	26	143	435
P. sanguineus CS43	61	221	82	326	22
Armilariella sp. CS134	190	188	57	34	3
Xylaria sp. CS121	199	245	198	210	3
T. maxima CU1	200	209	88	359	21
Inonotus sp. CU7	88	223	123	130	3
Ps. candollena CU20	7	45	18	118	4

CMCases and Xylanases at third day; Avicelases and ß-D-Glucosidase at fourth day, and Laccase at seventh day of growth.
* Liquid media on flask = 200 mL total volume

The results of the enzymatic production of all the strains grown in stationary conditions are shown in Table 5. The isolates *Inonotus* sp. CU7 (242 U/flask), *T. maxima* CU1 (170 U/flask) and *Armilariella* sp. CS134 (152 U/flask), were the best producers of CMCases. Strain *T. maxima* CU1 showed the highest levels of Avicelases (288 U/flask) and xylanases (165 U/flask). For the detection of β-D-glucosidase, strain *P. sanguineus* CS43 was the greatest producer with 476 (U/flask), whereas the rest of the fungi showed levels of less of 200U/flask. Under these culture conditions, the best laccase producer was *P. sanguineus* CS2 (59 U/flask); however, the levels were less than those detected in stirring conditions. The rest of the strains produced less than 10 U/flask.

Table 5. Lignocellulolytic Enzyme Activity in Wheat Straw Static Liquid Medium

Strain	CMCases	Avicelases	Xylanases (U/flask)*	β-D-Glucosidase	Laccase[&]
P. sanguineus CS2	40	12	21	119	59
P. sanguineus CS43	103	254	134	467	5
Armilariella sp. CS134	152	197	87	183	2
Xylaria sp. CS121	102	240	130	216	3
T. maxima CU1	170	288	165	116	2
Inonotus sp. CU7	242	159	63	166	5
Ps. candollena CU20	3	27	22	120	8

CMCases and Xylanases on the third day; Avicelases and -D-Glucosidase on the fourth day, and
* Liquid media on flask = 200 mL total volume
[&] Laccase on the seventh day of growth.

Regarding the production of LiP, in none of the isolates was the presence of LiP detected in the studied culture conditions. For the quantification of MnP in stirring conditions, *Trametes maxima* CU1 was the greatest producer with 10.2 U/flask at the seventh day of culture. Strains *Armilariella* sp CS134, *Xylaria* sp. CS121 and *Inonotus* sp. CU7 produced less than 1 U/flask between day 1 and 7 of growth. In stationary conditions, *Trametes maxima* CU1 presented the lowest concentration (6.2 U/flask) at a shorter time (third day), followed by *Inonotus* sp. CU7 with 4.3 U/flask. *Xylaria* sp. CS121 and *Armilariella* sp CS134 produced 1.6 and 1.0 U/flask, respectively, at day 5.

These results allow one to know the metabolic plasticity of the isolates as a function of the culture conditions, since the same fungus presented differences in the levels of enzymatic production. Isolate *T. maxima* CU1 was the greatest producer in stirring and stationary conditions, presenting high levels in the majority of the detected activities.

The levels of enzymatic production of the native isolates were similar to those reported for different strains of *Lentinus polychrous*, *L. squarrosulus*, and *L. sajor-caju* (Pukahuta *et al.* 2004), where differences were observed in the titers of the same strain. In the case of the laccase production, for these strains, greater titles from 0.43 to 3.15 U/mL were reported at day seven, while the native strains studied here had a range from 0.03 to 0.3 U/mL, with the exception of *P. sanguineus* CS2, which produced 2.17 U/mL. In the present work, low production levels of laccase were also observed in *P. sanguineus* CS43 (0.11 and 0.025 U/mL). Although these titers are low, it was found in previous reports that the laccases from *P. sanguineus* CS2 and *P. sanguineus* CS43 are thermostable enzymes (Salcedo-Martínez *et al*. 2013; Ramirez-Cárdenas *et al.* 2014). In addition, remarkably higher production levels (143,000 U/L) have been obtained for *P. sanguineus* CS43 through Central Composite Design (Ramirez-Cárdenas *et al.* 2014). Therefore, the exploration of new native strains can drive us to obtain new sources of lignocellulolytic enzymes with operational and functional properties more robust than those reported, despite the low levels of production.

Regarding the production of cellulases and hemicellulases in the work of Pukahuta *et al*. (2004), the majority of the strains were greater producers of xylanases than cellulases, while in the present work the fungi studied were greater producers of cellulases than xylanases. In the case of the work by Elisahvili and coworkers (2008), the production of CMCases and xylanases was higher than the total of cellulase activity,

laccase, and MnP; but also there were differences observed in the titers of enzymatic production in function of the strain, culture conditions, as well as the composition of the medium. This has been reported by different authors, who have used substrates rich in lignocellulose of different origins for the enzymatic production of lignocellulases (Elisahvili *et al.* 2008, 2009, 2011; Isikhuemhen *et al*. 2012). These differences can be seen in all these works as a function of the medium and the culture conditions; therefore the selection of native strains with high lignocellulolytic potential can lead to the finding of new sources of novel enzymes, with robust operational and functional properties, despite their low levels of production, as it is the case of the thermostable laccase of *P. sanguineus* CS2, mentioned above. This possibility will be addressed in further studies of optimization of the enzymatic production, as well as its application in the development of new digestive adjuvants for the livestock industry.

CONCLUSIONS

1. Nuevo Leon has a great diversity of macromycetes that possess high lignocellulolytic, pectinolytic, and amylolytic potentials that can be exploited in diverse industrial processes.
2. Seven native strains with cellulolytic, pectinolytic, and amylolytic capacities greater than those showed by six international reference strains were obtained. Two of these native strains (*T. maxima* CU1 and *P. sanguineus* CS43), presented the greatest lignolytic potential as well.
3. In function of the media, as well as the culture conditions, the enzymes detected here can be obtained, or these conditions can be adjusted to obtain one in particular.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the Programa del Mejoramiento del Profesorado (PROMEP 103.5/12/7884) and the Programa de Apoyo a la Investigación Cientifica y Tecnológica de la UANL (CA953-11). We also thank Ing. Olivia Gaona Quintanilla for her critical revision and comments on the manuscript.

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Article submitted: January 23, 2015; Peer review completed: July 6, 2015; Revised version received: August 1, 2015; Accepted: August 10, 2015; Published: August 19, 2015.
DOI: 10.15376/biores.10.4.6676-6689