Screening of new secretory cellulases from different supernatants of white rot fungi from Misiones, Argentina

Romina Olga Coniglio, María Isabel Fonseca, Laura Lidia Villalba and Pedro Darío Zapata

Laboratorio de Biotecnología Molecular, Instituto de Biotecnología de Misiones, CONICET, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Posadas, Argentina

ABSTRACT
Cellulases hydrolyse the cellulose chain into single sugars efficiently. These sugars can be fermented in the bioethanol process, a source of renewable energy. Misiones rainforest is one of the most biodiverse systems on the planet subtropical ecoregions, which is the most probable site to find new fungal strains with potential for degrading cellulose through cellulases. The aim of this work was to find an efficient cellulolytic microorganism through the exploration of native white rot fungi from Misiones. From the qualitative screening 11 fungal strains were selected. The quantitative analysis revealed that the isolated LBM 033 was the best cellulases producer, reaching 57,226 and 387 U/l of cellobiohydrolase, \(\beta\)-glucosidase and endoglucanase activity, respectively. The zymograms showed that the molecular mass of most of the endoglucanases ranged from 69 to 88 kDa and the molecular mass of most of the cellobiohydrolases was 45 kDa. The search of new cellulases of secretory organisms should lead to an efficient degradation of cellulosic materials, and thus facilitating potential applications in the production of bioenergy from lignocellulosic biomass.

ARTICLE HISTORY
Received 11 July 2016
Accepted 28 November 2016

KEYWORDS
Endoglucanase; cellobiohydrolase; \(\beta\)-glucosidase; white rot fungi; screening

Introduction
Cellulose, a linear-polymer composed by \(\beta\)-D-glucopyranosyl units linked by \(\beta\)-1,4-D-glucosidic bonds, a main component of plant cell wall, represents the most abundant renewable biomass available on earth and the principal waste from the agriculture (Lee et al. 2011).

Lignocellulosic biomass obtained from agricultural residues, herbaceous grasses and forest products provide an abundant source of carbohydrates (Bhattacharya et al. 2015).

A promising strategy for the use of this renewable resource is the microbial hydrolysis of the carbohydrates fraction of the lignocellulosic biomass and fermentation of the resultant reducing sugars for the production of biofuel and other value added products. The growing concerns about shortage of fossil fuels, the emission of green house gases and air pollution by incomplete combustion of fossil fuels has also resulted in an increased focus in the possibility to use cellulases for the enzymatic hydrolysis of the lignocellulosic waste in the process of producing bioethanol (Sukumaran et al. 2005).

Microbial hydrolysis of the cellulosic waste is performed through cellulase hydrolysing enzymes characterised as cellulases. Cellulases are divided into three major groups, namely 1,4-\(\beta\)-D-endoglucanase (EG, EC 3.2.1.4), 1,4-\(\beta\)-D-cellobiohydrolase (CBH, EC 3.2.1.91) and \(\beta\)-glucosidase (BGL, EC 3.2.1.21). These enzymes act synergistically and hydrolyse cellulose chain into single sugars in an efficient manner. The EGs and the CBHs act together to hydrolyse cellulose to small cello-oligosaccharides. The oligosaccharides (mainly cellobiose) are subsequently hydrolysed to glucose by the BGLs (Toda et al. 2008; Manavalan et al. 2015).

A large number of bacterial and fungal strains have been reported to produce multiple cellulolytic enzymes. Among these, white rot fungi are known as decomposers of wood components (Toda et al. 2008; Fonseca et al. 2014).

In the production of bioethanol, the costs of the enzymes used for the hydrolysis of the raw material need to be reduced and the efficiency increase in order to make the process economically feasible (Kuhad et al. 2011; Kubicek & Kubicek 2016).

In this sense, the search for new organisms with cellulolytic potential becomes essential.
Tropical and subtropical rainforests in Argentina are restricted to small areas that do not reach 2% of the land area, and yet, are home to more than 50% of the biodiversity (Brown et al. 2002). Misiones rainforest is one of the most biodiverse systems on the planet subtropical ecoregions, which is why we expect to find new fungal strains with potential for degrading cellulose in order to apply their cellulases in biotechnological processes including the production of biofuels.

The aims of this work were determine the isoenzyme profile of different fungal strains and find a highly cellulolytic microorganism through the exploration in a sample of native white rot fungi from Misiones, Argentina.

Materials and methods

**Fungal strains and maintenance**

Fourteen fungal strains isolated from the Misiones rainforest (Argentina) were used in this study; eight belong to the genus *Trametes* (LBM 002, LBM 010, LBM 017, LBM 018, LBM 029, LBM 030, LBM 031, LBM 033) and six belong to the genus *Pycnoporus* (LBM 014, LBM 020, LBM 021, LBM 023, LBM 024, LBM 038). All fungal strains were procured from the culture collection of the Instituto de Biotecnología Misiones ‘María Ebe Reca’, Universidad Nacional de Misiones, Argentina, and were maintained on MEA solid medium (12.7 g/l malt extract, 15 g/l agar) plates at 4°C and periodically subcultured.

**Qualitative screening of cellulase-producing strains**

Initial screening of endoglucanase-producing fungi was carried out on agar plates containing Czapek-agar medium (2 g/l NaNO₃, 1 g/l KH₂PO₄, 0.5 g/l KCl, 0.5 g/l MgSO₄.7H₂O, 0.01 g/l FeSO₄.7H₂O, 20 g/l agar) supplemented with 0.1% carboxymethylcellulose (CMC). The pH of the medium was adjusted to 4.5 with 100% glacial acetic acid. Plugs of 5 mm diameter were placed in the centre of Petri dishes. A non-inoculated plate was used as negative control. After incubation at 28°C for 4 days, Petri dishes were incubated at 50°C for 1 h. Then, plates were flooded with an aqueous solution of Congo red (0.1% Congo red in distilled water) and shaken at 80 rpm for 15 min on a rotary shaker. The Congo red solution was then poured off, and plates were flooded with 1 M NaCl to reveal the degradative halo. The colony diameter and the degradative halo were then measured. The cellulose degradation coefficient (CDC) was determined as follows:

\[
\text{CDC} = \frac{\text{dh}}{\text{dc}}
\]

Where, dh: degradative halo
dc: colony diameter

Initial screening of β-glucosidase-producing fungi was carried out on agar plates containing 0.1 mM 4-methylumbelliferyl glucoside (Mu-g; Sigma, MO, USA), 0.1% carboxymethylcellulose and 0.1% yeast extract. The pH was adjusted to 4.5. The strains showing fluorescence after 5 days at 28°C were considered as positive. A non-inoculated plate was used as negative control.

Initial screening of cellobiohydrolase-producing fungi was carried out on agar plates containing 0.1 mM 4-methyl umbelliferyl cellobioside (Mu-c; Sigma, MO, USA), 0.1% carboxymethyl cellulose and 0.1% yeast extract. The pH was adjusted to 4.5. Strains showing fluorescence after 5 days at 28°C were selected for the quantitative analysis. Inoculated plates in medium without substrate and a non-inoculated plate in medium with substrate (data no show) were used as negative controls.

**Culture conditions to quantitative screening**

The incubation trials were performed in 250 ml Erlenmeyer flasks containing 50 ml of Czapec minmal medium supplemented with 1% microcrystalline cellulose as carbon source, 2 g/l peptone as nitrogen source and 5 mm agar plugs removed from each isolated fungus. Cultures were grown at 28°C and maintained at 80 rpm in a rotary shaker. The culture medium was collected at 4, 8, 12, 16, 20, 24, 28 and 32 days of incubation, aliquots were centrifuged at 4°C (15 min, 10,000 g) and supernatant were used to determine enzyme production. The CBH, EG and BGL activities present in the extracellular fraction were assayed.

**Enzyme assay**

The CBH activity of the culture broth was assayed following a modified method described by Wu et al. (2006). The enzymatic reaction mixture containing 200 μl of enzyme solution and 800 μl of 500 μM
p-nitrophenyl-β-D-cellubioside (pNPC; Sigma, MO, USA) in 0.05 M sodium acetate buffer (pH 4.8) were incubated for 30 min at 50°C. The amount of p-nitrophenol released was measured at 410 nm after addition of 10% Na₂CO₃ to the reaction mixtures. One unit (U) of pNPC-hydrolysing activity was defined as the amount of enzyme necessary to release 1 µmol of p-nitrophenol per minute.

The BGL activity was assayed by the method described by Herr et al. (1978). One unit (U) of β-glucosidase activity was defined as the amount of enzyme necessary to release 1 µmol of p-nitrophenol per minute.

The EG activity was measured following the method described by Ghose (1987). One unit (U) of endoglucanase activity was defined as the amount of enzyme which liberated 1 µmole of glucose/min under standard conditions.

Statistical analysis

All experiments were completed in duplicate. Cellulase activities for the analysis of variance and significance tests were evaluated using Graph Pad Prism 5th version.

Zymography

Electrophoresis was performed in gels containing 7.5% (w/v) acrylamide. Depending on the enzyme assay, 2% (w/v) of either CMC or crystalline cellulose was added into the separating gel. About 5 mU/l were applied to the gel. Electrophoresis was conducted at 100 V for 2 h. After the electrophoresis period, the gels were fixed with a solution methanol: acetic acid: water (4: 1: 5), immersed in 0.05 mM sodium acetate buffer pH 4.8 and incubated for 60 min at 50°C. The gels were then stained with 0.1% (w/v) Congo red solution and washed with tap water and 1 M NaCl. Light yellowish activity bands were visible on a deep red background.

SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in gels containing 7.5% (w/v) acrylamide. Samples were applied to the gel in the same manner as in the zymogram technique, except that 10% (w/v) SDS was added. Electrophoresis was conducted at 100 V for 2 h adding 1 g/l SDS to the running buffer. At the end of the electrophoresis, SDS was removed by washing with buffer 50 mM sodium acetate pH 4.8 containing 25% (v/v) isopropanol. The gels were fixed and stained as previously described. Light yellowish activity bands were visible on a deep red background.

The molecular weight of the isoenzymes was compared with a molecular weight marker (Kaleidoscope, BioRad).

Results

Preliminary screening and selection of cellulase-producing strains

In this study 14 fungi autochthonous of the province of Misiones (Argentina) were analysed for EG, CBH and BGL production (Table 1). All the tested fungi produced zones of hydrolysis in CMC agar plates (Figure 1). Particularly the strain belonging to the Trametes genus named LBM 033, showed high activity (P < 0.001) against CMC. The cellulose degradation coefficient of LBM 033 was greater than 3, while the coefficients of the rest of the strains tested were about 1, which means that no difference could be observed between the degradation and growth halos in those strains.

Table 1. Qualitative screening for EG, BGL and CBH activities on plates.

| Fungal strain | CDC | BGL activity | CBH activity |
|---------------|-----|--------------|--------------|
| LBM 014       | 0.98| +            | +            |
| LBM 020       | 0.82| +            | +            |
| LBM 018       | 0.84| +            | −            |
| LBM 024       | 0.93| +            | +            |
| LBM 023       | 0.84| +            | +            |
| LBM 010       | 0.91| +            | −            |
| LBM 021       | 0.92| +            | +            |
| LBM 029       | 1.06| +            | +            |
| LBM 030       | 0.97| +            | +            |
| LBM 033       | 3.02| +            | +            |
| LBM 031       | 1   | +            | +            |
| LBM 038       | 0.94| +            | +            |
| LBM 002       | 0.98| +            | −            |
| LBM 017       | 1.61| +            | +            |

*CCellulose degradation coefficient.
+ Indicate positive reaction.
− Implies no detectable reaction.
LBM 029) and some strains showed fluorescence across the plate (LBM 017, LBM 031, LBM 020, LBM 021, LBM 014 and LBM 023). Nevertheless, the isolates LBM 018, LBM 010 and LBM 002 showed opaque zones in most of the plate, with a little halo on the edge of the colony. That is why they were considered as positive, but discarded for the quantitative assay (Figure 2).

Among the 14 fungal strains tested for cellobiohydrolase activity, 11 strains were selected based on the fluorescence observed when the agar plates were exposed under UV light (Figure 3). The strains LBM 017, LBM 029, LBM 030, LBM 031, LBM 033, LBM 014, LBM 020, LBM 021, LBM 023, LBM 024 and LBM 038 presented a positive reaction showing fluorescence across the plate while the negative controls, consisting in inoculated plates without substrate, showed no fluorescence in any case. It is why these strains were chosen to continue with the quantitative studies of enzymatic activity in liquid medium.
Figure 3. Screening of cellobiohydrolases on Mu-c agar plates. Assay were done by duplicate with the 14 studied basidiomycete strains. For each pair of plates, strains inoculated in medium with substrate are observed to the left and strains inoculated in medium without substrate are observed to the right (c-).
Production of β-1,4-endoglucanase, cellobiohydrolase, and β-glucosidase

The cellulase activity (CBH, BGL and EG) produced by the 11 selected isolates grown in liquid Czapek medium with microcrystalline cellulose as a carbon source and peptone as a nitrogen source are shown in Figure 4.

CBH, BGL and EG activities were low or absent in all strains studied on the first two tested periods, 4 and 8 days of incubation (data not shown).

LBM 033 strain had significantly increased CBH activity ($P < 0.001$) compared with all other strains tested on days 12, 16, 20 and 28. LBM 033 strain showed the highest values of enzyme activity at 12, 20 and 28 days (57; 51 and 46 U/l, respectively), with a sharp activity drop on the 32th day (6 U/l) where the LBM 031 strain reaches a value of 46 U/l, significantly higher ($P < 0.001$) than the value of enzyme activity achieved by the LBM 033 strain that day, but still less than that obtained by the strain LBM 033 on day 12. No enzyme activity was observed in LBM 029 ($P < 0.001$).

Regarding BGL activity, the isolate LBM 033 showed highest levels (226 U/l) at 28 days and little or no activity was observed in LBM 029 at all times studied. The LBM 033 strain had higher activity values ($P < 0.05$) with respect to all other strains tested on days 16, 20, 24 and 28. LBM 033 showed a maximum value (226 U/l) on day 28, which dramatically decline on day 32, but no significant differences were observed between 28 and 24 (191 U/l) days of incubation.

Figure 4. EG, BGL and CBH activities corresponding to the 11 isolated selected at 12, 16, 20, 24, 28 and 32 days.
The highest EG activities were found in the isolates LBM 033 (387 U/l) and LBM 038 (315 U/l) at 24 days \((P < 0.01)\), while the lowest EG activity was found in the isolate LBM 029 (65 U/l) at 20 days. The strains LBM 033 and LBM 038 showed a peak of EG activity on day 24 followed by a decrease, however, on day 32 LBM 033 strain showed a value significantly greater \((P < 0.05)\) than the value of LBM 038 enzyme activity.

**Zymograms and SDS-PAGE analysis of supernatants**

To verify the possible presence of EG and CBH isoforms, no denaturing-polyacrylamide gel electrophoresis (ND-PAGE) and SDS-PAGE analyses were carried out. Zymograms of β-glucosidase could not be analysed, since the maximum enzymatic activity achieved was lower than the detection limit of the technique.

**Figure 5** shows non-denaturing gel (A) and SDS-PAGE (B) corresponding to the 11 strains tested for EG enzyme. The LBM 029 strain showed no bands on polyacrylamide gel due to the low enzymatic activity of this strain, while the remaining 10 strains showed degradation zones on the polyacrylamide gels. Most of the strains showed bands in the 69–88 kDa range.

Among the strains belonging to *Trametes* genus, LBM 033 isolate presented a single isozyme, with a molecular weight of about 80 kDa; in the case of LBM 031 and LBM 030 strains, one single isoenzyme of 69 kDa was detected, whereas for LBM 017 isolate three isoenzymes of approximately 30, 39 and 69 kDa were observed. Among the strains of the genus *Pycnoporus*, LBM 020 and LBM 038 isolates provided a band corresponding to 88 kDa isozyme (although LBM 020 had shown two isoenzymes in the denaturing gel). When LBM 014 strain was analysed in the zymogram two bands were observed, however, when the denaturing polyacrylamide gel was revealed three bands corresponding to isozymes of 30, 39 and 88 kDa were detected. A similar pattern was also observed in LBM 017 isolate. LBM 021, LBM 023 and LBM 024 strains showed a similar profile bands on polyacrylamide gel, which consisted of one isoenzyme of 84 kDa, while with no-denaturing gels test, the same strains showed two bands.

**Figure 6** shows non-denaturing gels (A) and SDS-PAGE (B) for cellobiohydrolase enzyme for the 11 strains tested.

In the non-denaturing gels the unique band visualised in LBM030 and LBM 038 strains could be resolved in two bands, while the band observed in the lane corresponding to LBM 014 strain was resolved into three bands in the denaturing gels.

All the studied strains, except LBM 029 and LBM 033 showed a band corresponding to a 45 kDa isozyme. The LBM 030, LBM 031 and LBM 038 strains also showed a band of 36 kDa. The isolate LBM 014 showed profile bands in polyacrylamide gel consisting of three isoenzymes of approximately 36, 45 and 65 kDa.

**Discussion**

The Paranaense forest region (Misiones) is characterised by its biodiversity and within this environment a large number of microorganisms can be isolated. Cellulase secretion ability of the
microorganisms isolated is of upmost importance for the application in biotechnological process. The screening results obtained in this study, showed that among the white-rot basidiomycetes tested, a native fungi belonging to a Trametes genus labelled LBM 033 appeared to be very promising as cellulases producer in liquid media.

Screening of endoglucanase-producing fungi, which may be considered semiquantitative, revealed that the LBM 033 strain had the highest value of CDC. At the same time, the LBM 033 strain had the highest value of EG activity in submerged culture.

We described easy and efficient methods for the screening of CBH and BGL-producing microorganisms.

Screenings of β-glucosidase and cellobiohydrolase-producing fungi may be considered exclusively qualitative and the strains were classified as positive or negative. The strains that tested positive for CBH and BGL activities were chosen to continue with the quantitative studies of enzymatic activity in liquid medium. In the tested plates for the CBH enzyme, no differences between positive strains were observed. Instead, for tested plates for the BGL enzyme, some differences were found but these differences are not correlated with the results found in the quantitative assay.

We selected the 11 strains that tested positive for CBH activity. The fungal CBH in particular is of great interest as powerful cellulolytic fungi are capable of producing two different forms of the CBH enzymes: CBHI and CBHII (Chukeatirote et al. 2012). It should be noted however that the CBHI is the principal component of the cellulases enzymes as reported in Trichoderma reesei (Uusitalo et al. 1991) and in Volvariella volvácea (Jia et al. 1999).

Among all the enzymatic activities, the EG activity was higher than CBH for all strains, as mentioned above (Falkoski et al. 2012).

Regarding endoglucanase activity, the LBM 020 strain showed the maximum activity within the strains of the Pycnoporus genus tested (335 U/l). This value was higher than cited in the literature, which was 116 U/l when Pycnoporus sanguineus was cultured in a medium supplemented with CMC (Gutiérrez-Soto et al. 2015).

The LBM 033 strain showed the maximum activity within the strains of the Trametes genus tested (387 U/l). For Trametes versicolor, endoglucanase activity was found similar to that found in this work, in solid-state fermentation of mandarin peelings (Kachlishvili et al. 2012). When the same species was cultured in submerged fermentation (Irbe et al. 2014), enzymes activities of 100 U/L (medium supplemented with glycerol) and 700 U/l (medium supplemented with wheat bran) were found.

Pycnoporus coccineu recorded a maximum activity of cellulase (CMCase) of 649 U/l on day 60, while T. versicolor cellulase activity was 104 U/l on day 40 as reported by Liew et al. (2011). In the present work the maximum peaks were reached at 12, 24 and 28 days for cellobiohydrolase, endoglucanase and β-glucosidase, respectively. Moreover, when P. sanguineus was cultured under submerged fermentation using corn cobs as carbon source (Falkoski et al. 2012), β-glucosidase activity was 250 U/l. This value was similar to the value found in this work.
The comparison of cellulase-producing strains is very important for industrial characterisation of an entire secretome, as well as for the more fundamental study of cellulose expression. Each cellulase producer has its own cellulase profile that is significant from an industrial point of view (Dojnov et al. 2015).

SDS-PAGE is the most commonly used method for judging the apparent molecular weight of enzymes (Sajith et al. 2016). The EGs are monomeric, with molecular masses typically between 22 and 45 kDa but enzymes almost double the size were found in Sclerotium rolfsii and Gloeophyllum sepiarium (Baldrian & Valášková 2008). In this work, the majority of bands for most of the strains were in the 69–88 kDa range for endoglucanases. However, iso-enzymes of 30 and 39 kDa were also detected. The CBH enzymes are usually monomeric with a molecular weight typically ranging between 39 and 65 kDa although Dichomitus squalens-derived CBH is smaller with a CBH II molecular weight of 36 kDa (Manavalan et al. 2015). In this work, the molecular mass of CBH was 45 kDa in all cases, but in some cases a 36 or 65 kDa band was observed.

The association of Congo red and microcrystalline cellulose or carboxymethyl cellulose generates an intense coloration that fades before the depolymerising activity of cellulases (Teather & Wood 1982). The existence of differences between the bands observed in non-denaturing gels and denaturing gels may be explained by differences in this interaction. Chang et al. (2012) showed that the activity of AMCEL5B, a bifunctional cellulase found in xytophagous insects, is generally influenced by chemicals products. Some chemicals, such as metal ions, detergents and reducing/oxidising agents, may affect the dynamic action between enzymes and substrates. The cellulase activity of AmCel5B was enhanced by Ca^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, dithiothreitol and 2-mercaptoethanol, but was inhibited by ethylenediaminetetraacetic acid (EDTA) and SDS. The influence of these chemicals on the cellulase activity suggests that some divalent cations may be interacting with basic (Arg, Lys and His) or acidic (Asp and Glu) amino acid residues of cellulases enzymes.

There are several studies on fungal cellulolytic systems, but these are limited to a few key species such as Trichoderma reesei and Phanerochaete chrysosporium. Considered a key role of fungi in degrading plant biomass in ecosystems, it is important to keep studying new fungal species and exploring the great biodiversity of our region to find organism with a very good cellulases degrading ability.

**Conclusion**

In this work, we described easy and efficient methods for qualitative screening of CBH- and BGL-producing microorganisms. The zymogram results of endoglucanase and cellobiohydrolase enzymes of the 11 strains selected showed different production profiles: the molecular mass of most of the endoglucanases ranged from 69 to 88 kDa and the molecular mass of most of the cellobiohydrolases was 45 kDa, with additional bands of 36 and 65 kDa in some cases. The quantitative analysis revealed that the isolated LBM 033 was the best cellulase producer, reaching 57 U/l, 226 U/l and 387 U/l of cellobiohydrolase activity, β-glucosidase activity and endoglucanase activity, respectively, suggesting that LBM 033 is able to secrete a complete cellulolytic enzymatic complex that could be applied for the hydrolysis during biomass conversion.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This work was supported by the UNaM (16Q560-year 2014–2016). Coniglio is a postgraduate CONICET fellowship holder and Fonseca MI is CONICET investigator.

**References**

Baldrian P, Valášková V. 2008. Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev. 32:501–521.

Bhattacharya AS, Bhattacharya A, Pletschke BI. 2015. Synergism of fungal and bacterial cellulases and hemicellulases: a novel perspective for enhanced bio-ethanol production. Biotechnol Lett. 37:1117–1129.

Brown AD, Grau A, Lomáscalo TGasparri NL. 2002. Una estrategia de conservación para las selvas subtropicales de montaña (yungas) de argentina. Ecotrópicos. 15:147–159.

Chang CJ, Wu CP, Lu SC, Chao AL, Ho THD, Yu SM, Chao YC. 2012. A novel exo-cellulase from white spotted longhorn beetle (Anoplophora malasiaca). Insect Biochem Mol Biol. 42:629–636.

Chukeatiroth E, Maharachchikumbura SSN, Wongkham S, Sysouphanthong P, Phookamsak R, Hyde KD. 2012. Cloning
and sequence analysis of the cellobiohydrolase I genes from some Basidiomycetes. Mycobiology. 40:107–110.

Dojnov B, Grujić M, Vujčić Z. 2015. Reliable simultaneous zymographic method of characterization of cellulolytic enzymes from fungal cellulase complex. Electrophoresis. 36:1724–1727.

Falkoski DL, Guimaraes VM, De Almeida MN, Alfenas AC, Colodette JL, De Rezende ST. 2012. Characterization of cellulolytic extract from Pycnoporus sanguineus PF-2 and its application in biomass saccharification. Appl. Biochem Biotechnol. 166:1586–1603.

Fonseca MI, Fariña JI, Castrillo ML, Rodríguez MD, Nuñez CE, Villalba LL, Zapata PD. 2014. Biopulping of wood chips with Phlebia brevispora BAFC 633 reduces lignin content and improves pulp quality. Int Biodeter Biodegr. 90:29–35.

Ghose T. 1987. Measurement of cellulase activities. Pure Appl Chem. 59:257–268.

Gutiérrez-Soto G, Medina-González GE, García-Zambrano EA, Treviño-Ramirez JE, Hernández-Luna CE. 2015. Selection and characterization of a native Pycnoporus sanguineus strain as a lignocellulolytic extract producer from submerged cultures of various agroindustrial wastes. BioResources. 10:3564–3576.

Herr D, Baumer F, Dellweg H. 1978. Purification and properties of an extraacellular endo-1,4-β-glucanase from Lenzites trabea. Appl Microbiología Biotechnol. 5:29–36.

Irbe I, Elishashvili V, Asatiani MD, Janberga A, Andersone I, Andersons B, Grinins J. 2014. Lignocellulolytic activity of Coniophora puteana and Trametes versicolor in fermention of wheat bran and decay of hydrothermally modified hardwoods. Int Biodeterior Biodegrad. 86:71–78.

Jia J, Dyer PS, Buswell JA, Peberdy JF. 1999. Cloning of the cbhl and cbhI genes involved in cellulose utilisation by the straw mushroom Volvariella volvacea. Mol Gen Genet. 261:985–993.

Kachlishvili E, Khardziani T, Metreveli E, Kobakhidze A, Elishashvili V. 2012. Screening of novel basidiomycetes for the production of lignocellulolytic enzymes during fermentation of food wastes. Waste Conver J Bioprod Biotechnol. 1:9–15.

Kubicek CP, Kubicek EM. 2016. Fungal enzymes for bio-products from sustainable and waste biomass. Trends Biochem Sci. 41:633–645.

Kuhad RC, Gupta R, Singh A. 2011. Microbial cellulases and their industrial applications. Enzyme Res. 1:10.

Lee K-M, Moon HJ, Kalyani D, Kim H, Kim IW, Jeya M, Lee JK. 2011. Characterization of cellobiohydrolase from a newly isolated strain of Agaricus arvensis. J Microbiol Biotechnol. 21:711–718.

Liew CY, Husaini A, Hussain H, Muid S, Liew KC, Roslan HA. 2011. Lignin biodegradation and ligninolytic enzyme studies during biopulping of Acacia mangium wood chips by tropical white rot fungi. World J Microbiol Biotechnol. 27:1457–1468.

Manavalan T, Manavalan A, Heese K. 2015. Characterization of lignocellulolytic enzymes from white-rot fungi. Curr Microbiol. 70:485–498.

Sajith S, Priji P, Sreedevi S, Benjamin S. 2016. An overview on fungal cellulases with an industrial perspective. J Nutr Food Sci. 6:1.

Sukumaran RK, Singhania RR, Pandey AJ. 2005. Synergism of fungal and bacterial cellulases and hemicellulases: a novel perspective for enhanced bio ethanol production. Sci Ind Res. 64:832–844.

Teather RM, Wood PJ. 1982. Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the Bovine Rument. Appl Environ Microbiol. 43:777–780.

Toda H, Nagahata N, Amano Y, Nozaki K, Kanda T, Okazaki M, Shimosaka M. 2008. Gene cloning of cellobiohydrolase II from the white rot fungus Irpex lacteus MC-2 and its expression in Pichia pastoris. Biosci. Biotechnol. Biochem. 72:3142–3147.

Uusitalo JM, Nevalainen KM, Harkki AM, Knowles JK, Penttilä ME. 1991. Enzyme production by recombinant Trichoderma reesei strains. J Biotechnol. 17:35–49.

Wu B, Zhao Y, Gao PJ. 2006. Estimation of cellobiohydrolase I activity by numerical differentiation of dynamic ultraviolet spectroscopy. Acta Biochim Biophys. 38:372–378.