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ORIGINAL ARTICLE

# Use of water hyacinth as a substrate for the production of filamentous fungal hydrolytic enzymes in solid-state fermentation

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## Abstract

The objective of the present work was to evaluate the water hyacinth (WH) as a substrate for the production of hydrolytic enzymes (cellulases and hemicellulases) of 100 strains of filamentous fungi under conditions of solid growth. Five fungal strains, identified as *Trichoderma harzianum, Trichoderma atroviride, Penicillium griseofulvum, Penicillium commune* and *Aspergillus versicolor*, were selected and studied for their ability to grow on water hyacinth as a substrate and carbon source only, evaluating hydrolytic enzymatic activities (α-L-arabinofuranosidase, cellulase, xylanase and β-D-xylopyranosidase) and extracellular protein per g of water hyacinth dry matter (gdm). The five strains selected were able to produce the four enzymes studied; however, *T. harzianum* strain PBCA produces the highest xylanase (149.3 ± 14.3 IU/gdm at 108 h), cellulase (16.4 ± 0.6 IU/gdm at 84 h) and β-D-xylopyranosidase (127.7 ± 14.8 IU/gdm at 48 h). In contrast, the fungus with the highest α-L-arabinofuranosidase activity was *A. versicolor*, with 129.8 ± 13.3 IU/gdm after 108 h. In conclusion, *T. harzianum* showed the best production of the hydrolytic enzymes studied, using as a matrix and carbon source, water hyacinth. In addition, catalytic activities of arabinofuranosidase and xylopyranosidase were reported for the first time in *T. versicolor* and *T. harzianum*.

**Keywords** α-L-Arabinofuranosidase · *Aspergillus* · *Penicillium* · *Trichoderma* · Water hyacinth · Xylanase · β-D-Xylopyranosidase

## Introduction

Water hyacinth (WH) (*Eichhornia crassipes*) is an aquatic plant and due to its high growth rate and efficiency to produce seeds, is commonly considered an aquatic weed. The presence of WH reduces the amount of dissolved oxygen and the amount of sunlight that penetrates into water bodies, thus affecting the naturally occurring biota and increasing the pollution in several water environments. This aquatic weed is a persistent worldwide problem in more than 80 countries (Jafari 2010). However, a number of potential applications exist for WH, which include as an agent in phytoremediation, cellulose nanocrystals, composting, animal feed and even as a carbon source to produce biofuels, such as biodiesel, biogas or bioethanol (Jafari 2010; Das et al. 2016). It has been described that carbohydrates present in WH lignocellulose hydrolysates are xylose, cellulose, arabinose, galactose and mannose, mainly (Kumar et al. 2009) with lignocellulose being around 36% of the total dry weight of

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lignocellulose of WH. Due to the complexity of the substrate, it is necessary for the combined action of several hydrolytic enzymes such as cellulases, xylanases, arabinofuranosidases and xylopiranosidases for the generation of monosaccharides and it can be destined to biotechnological processes such as the production of biofuels (Nigam 2002). Additionally, WH can be used for the production of xylan, xylooligosaccharides or xylose for food and nonfood applications (Samanta et al. 2012) because WH possesses a significant hemicellulose content, even more than cellulose (Balasubramanian et al. 2012) which can be converted into saccharides, which are of industrial interest, using xylanases, β-D-xylopyranosidases and α-L-arabinofuranosidases.

WH has been used as a growth medium to produce β-glucosidases and endoglucanases from *Rhizopus oryzae* (Karmakar and Ray 2011), cellullases and xylanases from *Trichoderma reesei, Aspergillus niger* and *Myrothecium roridum* (Okunowo et al. 2010), and cellobiohydrolases from *A. niger*. However, there are currently no reports studying the production of β-D-xylopyranosidases and α-L-arabinofuranosidases by fungi grown on WH.

In this work, we report the selection and identification of five filamentous fungal strains that produce cellulase, xylanase, β-D-xylopyranosidase and α-L-arabinofuranosidase activities when they are grown using solid-state fermentation (SSF) and WH as the only carbon source.

## Materials and methods

### Strain screening

More than 100 strains of filamentous fungi from the collections of the IBT-UNAM (Instituto de Biotecnología-Universidad Nacional Autónoma de México, México), UAM-I (Universidad Autónoma Metropolitana-Iztapalapa, México), CBMSO-CSIC (Centro de Biología Molecular Severo Ochoa-Consejo Superior de Investigaciones Científicas, Spain) and IRD (Institut de Recherche pour le Développement, France) were studied. Their ability to grow on agar plates (1.5%) containing 0.2% carboxymethylcellulose (CMC-sodium salt) or 0.2% birchwood xylan was determined. Solid media were supplemented with 0.2% $NaNO_3$, 0.1% $K_2HPO_4$, 0.05% $MgSO_4$, 0.05% KCl, 0.2% and 0.2% peptone. After 5 days of incubation at 28 °C, the plates containing the fungal strains were flooded with the Gram's iodine reagent (2 g of KI and 1 g of iodine in 300 mL of distilled water) for 3–5 min and the activity halo, a relative method to estimate the cellulase or xylanase activity, was measured (Kasana et al. 2008). The size ratio of the hydrolysis halo/size of the colony was used during the growth of the microorganisms during 5 days (Cruz-Ramirez et al. 2012) to select the performance of the strains to hydrolyze CMC or xylan. The five strains with the highest ratio (above 1.5) were selected for the subsequent studies.

### Strain identification

Identification was performed using molecular methods and internal transcribed spacer (ITS) sequences as molecular markers. The fungal strains with the highest enzymatic activities were grown at 28 °C in liquid culture with 20 g malt extract/L and 10 g peptone/L to obtain mycelium and then used for DNA extraction with the method described by Raeder and Broda (1985). The ITS region was amplified using universal primers, ITS4 and ITS5 (White et al. 1990), and the sequenced products were compared with those reported in GenBank; the obtained partial sequences were deposited in GenBank with the accession number reported in the results section.

### Substrate preparation

Fresh WH was collected from the Xochimilco water canals (Mexico City, Mexico) in collaboration with the TEMA S.A. Company. The roots were manually removed, and the remainder of the plant was dried in a rotary oven.

### Characterization of the substrate

#### Moisture determination

10 g of WH was weighted and subjected to drying in an oven at 60 °C for 48 h until constant weight. The moisture content was determined by weight difference.

#### Extractable material in organic solvents

The TAPPI T204 om-88 was used. 10 g of water hyacinth with a particle size of 0.425 mm was used (TAPPI T204 om-88 1987).

#### Extractable material in hot and cold water

The TAPPI T207 om-93 standard was performed, using the material previously subjected to extraction of organic solvents (TAPPI T207 om-93 1993).

#### Ash content

To estimate the ash content, the TAPPI standard T211 om-93 was performed and 1 g of WH was utilized with a particle size of 0.425 mm (TAPPI T211 om-93 1993).

### Determination of lignin insoluble in acid

The TAPPI T222 om-88 standard was performed using 1 g of WH (TAPPI T222 om-88 1988).

### Holocellulose content

The method described by Browning (1967) was performed using 2 g of WH.

### Culture conditions for solid-state fermentations

Three hundred milligram batches of WH (prepared as described above) were used for solid-state fermentation. The initial pH was adjusted to a value of 6 through the addition of 0.25 mM $H_2SO_4$ prior to the sterilization of the WH; subsequently, the substrate was sterilized at 15 lb of pressure for 20 min. The WH was supplemented with modified Pontecorvo medium (1953) composed of 1.8 g $(NH_4)_2SO_4$/L, 0.5 g urea/L, 0.5 g $KH_2PO_4$/L, 0.2 g KCl/L, 0.2 g $MgSO_4 \cdot 7\ H_2O$/L and 0.3 mL of trace metals/L, containing 100 mg $Na_2B_4O_7 \cdot 10H_2O$/L, 50 mg $MnCl_2 \cdot 4H_2O$/L, 50 mg $Na_2MoO_4 \cdot 2H_2O$/L, 250 mg $CuSO_4 \cdot 5H_2O$/L, 85 g $FeCl_3$/L, 100 mg $ZnSO_4$/L. To harvest the spores, each one of the fungi was grown on 30 mL of PDA contained in 250-mL Erlenmeyer flasks; after 7 days of fungal growth at 28 °C, to each flask was added 30 mL of a solution of Tween 20 (0.01%) sterile; and with the help of an orbital shaker, the spores were detached from the mycelium and harvested which were subsequently counted using a Neubauer chamber. The WH was inoculated with the Pontecorvo medium containing $2 \times 10^7$ spores per gram of dry matter until the humidity of the WH was 70%. Solid cultures were incubated at 28 °C; humidity was adjusted to 70%. Samples were taken and analyzed every 12 h during 5 days determining extracellular protein, and enzymatic activities of xylanases, cellulases (CMCase), xylopyranosidases and arabinofuranosidases, as described below. The analyses were performed in triplicates for each sample.

## Preparation of enzymatic extracts

Enzymatic extracts were obtained by adding 6 mL of distilled water to 300 mg of fermented substrate, which was then stirred in a vortex at room temperature for 2 min and then centrifuged for 5 min at 15,000×*g* to obtain liquid extracts free of fungal biomass; these extracts were utilized for protein analyses and extracellular enzymatic activity. The extracts were analyzed immediately. The extracellular protein content was determined using the Bradford assay (Bradford 1976) and a standard curve of bovine serum albumin in the range of 0 to 25 mg/L.

## Enzymatic activity measurements

### Xylanases

The crude extract was incubated with 0.2% birchwood xylan for 10 min at 40 °C (100 mM citrate buffer, pH 5.5). The activity was then calculated by quantifying reducing sugars according to Quintanar et al. (2012). One international unit (IU) was defined as the amount of enzyme required to release 1 µmol of reducing sugar (expressed as xylose) per min under the assay conditions. All activities are referred to as initial grams of WH dry matter (IU/gdm).

### Cellulases as CMCases

Samples were incubated with 0.2% carboxymethylcellulose for 10 min at 40 °C (100 mM citrate buffer, pH 5.5), and the enzymatic activity was calculated by quantifying the reducing sugars according to Quintanar et al. (2012). One international unit was defined as the amount of enzyme required to release 1 µmol of reducing sugar (expressed as glucose) per min under the assay conditions.

### β-D-Xylopyranosidases

One hundred fifty microlitres of crude extract was incubated with 150 µL of *p*-nitrophenyl-β-D-xylopyranoside (1 mg/mL in distilled water) at 30 °C for 10 min, 900 µL of $Na_2CO_3$ 0.1 M was added to stop the reaction, and the activity was calculated to quantify the *p*-nitrophenyl according to the method of Tagawa and Kaji (1988). One international unit was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenyl per min under the assay conditions.

### α-L-Arabinofuranosidases

One hundred fifty microliters of crude extract was incubated with 150 µL of *p*-nitrophenyl-α-L-arabinoside (1 mg/mL in distilled water) at 30 °C for 10 min, 900 µL of $Na_2CO_3$ 0.1 M was added to stop the reaction, and the activity was calculated by quantifying the *p*-nitrophenyl according to Tagawa and Kaji (1988). One international unit was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenyl per min under the assay conditions.

### Analysis of results

The results were analyzed using a variance analysis and comparison between means with the Statistica 7 software.

## Results and discussion

### Screening and identification of fungal strains with cellulolytic and xylanolytic activities

More than 100 fungal strains were selected and studied from four laboratories (from the IBT-UNAM (México), UAM-I (México), CBMSO-CSIC (Spain) and IRD (France) collections). Five strains with the highest specific xylanolytic and cellulolytic activities were selected. All the tests were carried out in Petri dishes and the strains selected were showed the highest halo of enzymatic activity (Fig. 1). This method of selection has been utilized before in several studies with reliable results (Reader and Broda 1985; Runa et al. 2009). For the identification of the strains, the molecular marker ITS (internal transcribed spacer sequences—ITS4 and ITS5) was utilized, reported by White et al. (1990); After amplification and sequencing of extracted DNA, it was compared with the GenBank databases and these five strains were identified: *Trichoderma harzianum, Trichoderma atroviride, Penicillium commune, Penicillium griseofulvum* and *Aspergillus versicolor* (Table 1).

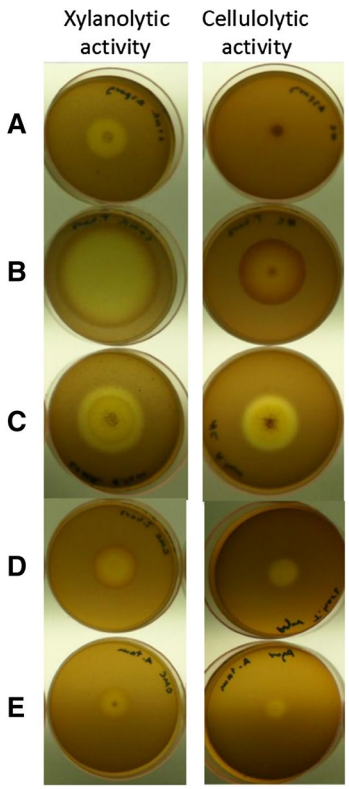


**Fig. 1** Halos of hydrolytic activity of selected filamentous fungi grown on bacteriological agar supplemented with Pontecorvo salts, and carboxymethylcellulose and birch xylan as carbon source for cellulolytic and xylanolytic activities, respectively. A = *P. commune*; B = *T. harzianum*; C = *T. atroviride*; D = *A. versicolor*; E = *P. griseofulvum*

### WH characterization

The results obtained from the characterization of the WH are shown in Table 2. A 5.22% lignin content was observed which is slightly lower than that described by Tham (2012); the low lignin content can be an advantage and may favor the hydrolysis of the biopolymers present in WH such as cellulose and hemicellulose (Juárez-Luna et al. 2011). The concentration of holocellulose found in the present work (around 30%) varies considerably with that described by other authors; this variation may be due to climatic differences, age of the plant, height above sea level, geographical area, etc., which can influence the variation observed. The carbohydrates present in the holocellulose of the WH are of great interest since they can be used for a great variety of biotechnological processes, mainly for the production of bioethanol (Guragain et al. 2011). Abraham and Kurup (1996) reported a content of 53.3% of holocellulose, Nigam in 2002 reported 66.9%, Gunnarsson and Petersen (2007) reported 52.9%, Kumar et al. (2009) reported 67.6%, and Sornvoraweat and Kongkiattikajorn (2010) reported 51.7% holocellulose in samples of *E. crassipes*. It is, therefore, of utmost importance to take into account the edaphic and geographical factors to estimate sugar yields that can be destined to biotechnological processes.

### Protein production by the selected filamentous fungi grown on WH

The selected fungal strains were grown on WH as a solid matrix and only carbon source. The extracellular protein was quantified as an indicator of microbial growth. All of the analyzed strains were able to use the lignocellulosic material as a carbon source, generating mycelia and extracellular

**Table 1** Strain identification and GenBank accession numbers

| Original code | Collection origin | Strain | GenBank accession number |
|---|---|---|---|
| CBMSO 56 | CBMSO-CSIC | *Aspergillus versicolor* | JX436463 |
| 108A | IBT-UNAM | *Penicillium commune* | JX436464 |
| CBMSO 7 | CBMSO-CSIC | *Penicillium griseofulvum* | JX436465 |
| L892 | IRD | *Trichoderma atroviride* | JX436466 |
| PBCA | IBT-UNAM | *Trichoderma harzianum* | JX436467 |

**Table 2** Chemical composition of the water hyacinth

| | Components | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ash | Moisture | Extractables in hexane | Extractables in cold water | Extractables in hot water | Lignin | Holocellulose |
| Content (%) | 17.43 ± 0.4 | 11.66 ± 2.84 | 7.35 ± 0.59 | 15.1 ± 0.73 | 26.51 ± 5.26 | 5.22 ± 0.66 | 30 ± 0.34 |

The results represent the mean ± standard derivation

protein (Table 3). *T. harzianum* was the strain that produced the highest amount of extracellular protein, whereas *A. versicolor* had the lowest. *T. atroviride, P. griseofulvum* and *P. commune* displayed a similar behavior with similar protein concentrations. In all cases, the production of extracellular protein was evident after 36 h of cultivation.

Five fungal strains were selected during the screening as they showed high cellulolytic and xylanolytic activities relative to growth during the plate assays. Also, the five selected strains were able to grow using WH as an only carbon source which is indicative of the production of saccharifying enzymes. The molecular identification of strains indicated the presence of *Trichoderma, Aspergillus* and *Penicillium* species; all of these species have been previously reported as capable strains of producing cellulases and xylanases. However, there have been few studies about β-D-xylopyranosidase and α-L-arabinofuranosidase production.

## Saccharifying enzyme production by the filamentous fungi grown on WH

Finally, the enzymatic extracellular activity for cellulases, xylanases, β-D-xylopyranosidases, and α-L-arabinofuranosidases was analyzed. CMCase activity (Fig. 2a) was detected in the five strains; starting to increase at 36 h, with a maximum at 84 h. *T. harzianum* had the highest activity (16.4 ± 0.6 UI/gdm at 84 h). A similar tendency was evident for the xylanase activity (Fig. 2b), i.e., *T. harzianum* produced the highest activity (149.3 ± 14.3 IU/gdm in 108 h), and this result is very similar to that described by López-Ramírez et al. (2018) who reported up to 100 IU/gdm produced by *T. harziamum* grown on pine sawdust but in conditions of aeration and agitation, Rezende et al. (2002) detected 36 IU/mL of xilanolytic activity of *T. harzianum* using bagasse from sugarcane as a substrate; on the other hand, Rahnama et al. (2013) reported 433 IU/gms using rice straw as a substrate. It is clear that the substrate is essential to induce xilanolytic activity since both rice straw and WH have a high content of hemicelluloses, whereas for β-D-xylopyranosidase activity (Fig. 3b) was 127.7 ± 14.8 IU/gdm at 48 h of cultivation time, respectively. The values obtained for these enzymatic activities are consistent with the extracellular protein production levels reported in Table 2. Even though *A. versicolor* produced the lowest levels of extracellular protein, this strain showed the highest activity of α-L-arabinofuranosidase, with 129.8 ± 13.3 IU/gdm after 108 h of cultivation time (Fig. 3a); in addition, this strain produced 71.7 ± 0.2 IU/gdm of β-D-xylopyranosidase activity at 108 h.

*Trichoderma* species are well known for cellulase and xylanase production (Zhang et al. 2017). López-Ramírez et al. (2018) detected 9 IU/gdm of cellulolytic activity in *T. harzianum* using as substrate, pine sawdust, Ikram-Ul-Haq et al. (2005) reported up to 0.427 IU/ mL of cellulolytic activity using cotton fibers as a substrate, and Pathak et al. (2014) detected 3.96 IU/gdm of cellulolytic activity of *T. harzianum* using wheat grains. Comparing what

**Table 3** Production of extracellular protein (mg/mL) during the growth of fungi on water hyacinth

| Time (h) | Fungal strain | | | | |
|---|---|---|---|---|---|
| | *A. versicolor* | *T. atroviride* | *T. harzianum* | *P. griseofulvum* | *P. commune* |
| 12 | 41 ± 5 | 92 ± 1 | 60 ± 6 | 93 ± 0 | 83 ± 0 |
| 24 | 33 ± 0 | 43 ± 17 | 54 ± 8 | 83 ± 1 | 90 ± 17 |
| 36 | 56 ± 12 | 148 ± 12 | 180 ± 2 | 189 ± 12 | 157 ± 8 |
| 48 | 94 ± 5 | 155 ± 24 | 254 ± 6 | 229 ± 46 | 176 ± 14 |
| 60 | 97 ± 9 | 181 ± 18 | 674 ± 95 | 356 ± 99 | 219 ± 55 |
| 72 | 167 ± 3 | 420 ± 32 | 514 ± 16 | 298 ± 31 | 355 ± 23 |
| 84 | 187 ± 2 | 365 ± 8 | 639 ± 1 | 401 ± 25 | 453 ± 58 |
| 96 | 122 ± 7 | 371 ± 35 | 496 ± 7 | 308 ± 10 | 321 ± 8 |
| 108 | 131 ± 16 | 399 ± 35 | 650 ± 33 | 334 ± 23 | 325 ± 7 |
| 120 | 216 ± 9 | 562 ± 17 | 712 ± 26 | 457 ± 54 | 483 ± 14 |

The results represent the mean ± standard derivation

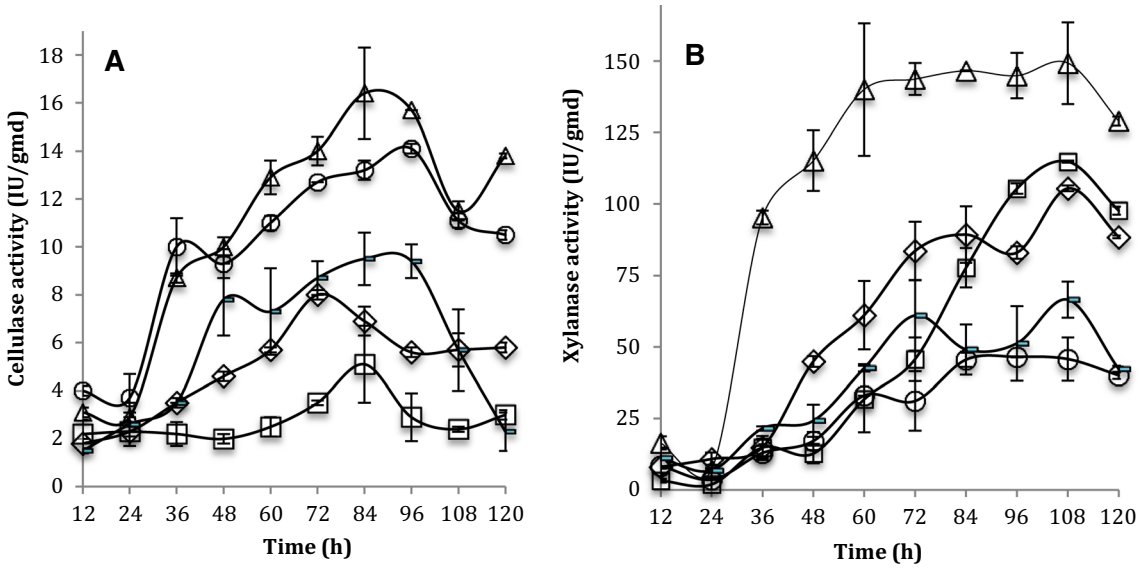


**Fig. 2** Cellulolytic (**a**) and xylanolytic (**b**) extracellular activities detected during growth on water hyacinth of the five fungi under study (◊=*A. versicolor*, □=*T. atroviride*, △=*T. harzianum*, ○=*P. griseofulvum*, − = *P. commune*)

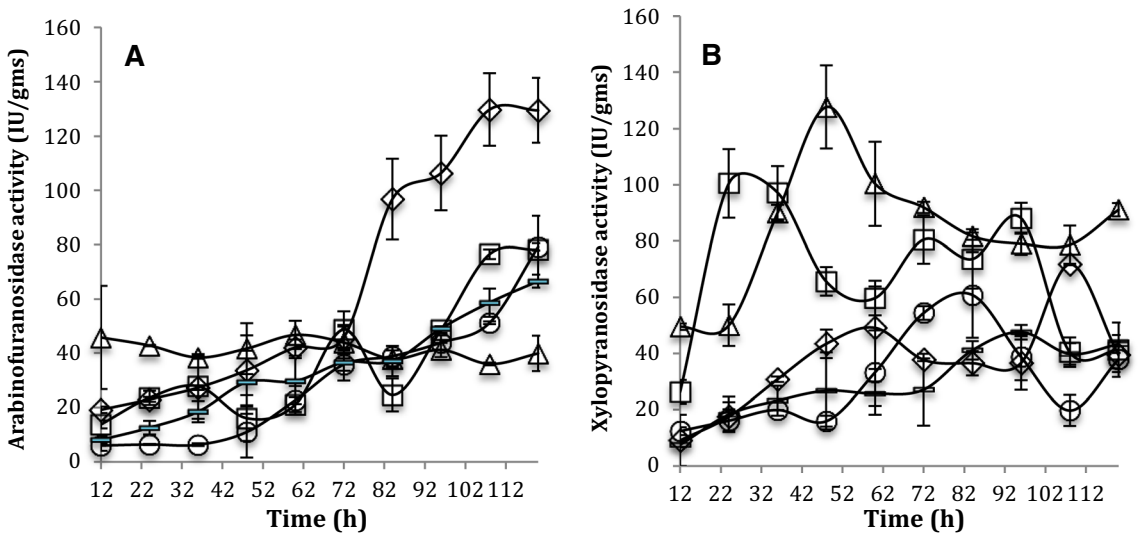


**Fig. 3** Arabinofuranosidase (**a**) and xylopyranosidase (**b**) extracellular activities produced during growth on water hyacinth of the five fungi under study (◊=*A. versicolor*, □=*T. atroviride*, △=*T. harzianum*, ○=*P. griseofulvum*, − = *P. commune*)

has been reported by other authors with the activity value obtained using WH as substrate, it is observed that this can be an excellent substrate for the production of cellulolytic enzymes, contributing to the negative impact of this plant on water bodies. *T. harzianum* is widely utilized as a biological control agent (Saravanakumar et al. 2016), and a thermostable xylanase, endo-β-1,4-xylanase and endo-β-1,3-glucanase have been previously purified and characterized from this fungus (Ahmed et al. 2012). *T. atroviride* has been less studied; but some reports indicate that it possess a higher cellulase activity than *T. reesei* (Kovacs et al. 2008) and it is present in urban stone surface (Paramo-Aguilera et al. 2012). In the literature, there is only one report on xylanase activity being produced by *Penicillium commune* (Baffi et al. 2012) and another report indicating cellulolytic activity (Zyani et al. 2009). *Penicillium griseofulvum* has been the most studied and there are two studies reporting endo-β-1,4-xylanase genes (Cervera-Tison et al. 2009). Finally, the xylanase and cellulose activities of *A. versicolor* have also been studied (Lee et al. 2011).

The present work constitutes the first report on the capacity of the *P. commune* and *A. versicolor* to produce β-D-xylopyranosidases. In the case of the α-L-arabinofuranosidase enzyme, it has been detected mainly in *T. harzianum* (Do Vale et al. 2012; Da Silva-Delabona et al. 2013; de Souza et al. 2018) and in *Trichoderma* sp. with catalytic activity similar to α-L-arabinofuranosidase enzyme (Zhou et al. 2011). Both β-D-xylopyranosidases and α-L-arabinosidases enzymes have important biotechnological applications and, therefore, need to be studied using WH as a substrate to produce these enzymes (Sedlmeyer 2011).

Referring to the results using WH in solid-state fermentations, the presence of xylanase and cellulase activities can be ascribed to the xylan and cellulose contained in the aquatic plant. The xylanolytic activity was higher than the cellulolytic activity for the five studied strains. We suggest that this phenomenon may be due to two reasons. First, the $K_M$ values reported for the *T. harzianum* cellulase are 2.4 $gL^{-1}$ (de la Cruz et al. 1995) and 3.8 $gL^{-1}$ (Ahmed et al. 2012), whereas the $K_M$ for its xylanases is 3.3 $gL^{-1}$ (Chutani and Sharma 2015) and 4.8 $gL^{-1}$ (El-Katatny et al. 2001). The values reported by these authors suggest that xylanases have a greater affinity for their substrate than cellulases since they are capable of releasing a greater amount of monosaccharides. Second, glucose metabolism produces more ATP than xylose metabolism. Thus, fungi require a lower cellulase activity for a similar ATP yield compared to xylose metabolism. Also, a higher amount of β-D-xylopyranosidase activity was obtained before α-L-arabinofuranosidase activity increased. Seiboth and Metz (2011) reported that the L-arabinose requires five enzymes, whereas D-xylose only requires three enzymes for its conversion to D-xylulose 5-phosphate and to be catabolized through the pentose phosphate pathway. Thus, fungi first consume the carbohydrate that is easier to metabolize, in this case the xylose produced by D-xylopyranosidase.

The lignocellulosic degradation using the enzymes studied in this work produced oligosaccharides and monosaccharides with potential biotechnological applications, such as nutraceuticals (Sedlmeyer 2011) or for biofuel production such as bioethanol using microorganisms capable of metabolized pentoses and hexoses present in the substrate (Muñoz-Gutierrez and Martínez 2013). The results indicate that strain PBCA identified as *T. harzianum* in this work present the greatest potential for the use of WH in biorefineries. On the other hand, it has been reported that oligosaccharides of the arabinoxylan have nutraceutical effects in the human intestine (Grootaert et al. 2007); hence, *A. versicolor* by the extracellular enzymatic activity detected may have a potential application to generate nutraceuticals from this polysaccharide.

## Conclusions

WH showed good qualities to be used as a support for the production of hydrolytic enzymes since the five fungi were able to grow on it. This suggests that WH can be used as a support for the production of hydrolytic enzymes being a new alternative for the use of this plant considered invasive. All strains showed hemicellulolytic activity. In general, *T. harzianum* was the fungus that produced the highest amounts of cellulase, xylanase and xylopyranosidase enzymes. *A. versicolor* was the fungus with the highest production of the enzyme arabinofuronasidase. It should be noted that they are the first reports of *A. versicolor* and *T. harzianum* as producers of the enzymes arabinofuranosidase and xylanopyranosidase, respectively. *T. atroviride* may also be a good candidate for the production of the enzymes arabinofuranosidase and xylanopyranosidase since under the conditions of the assay it was the second microorganism producing these enzymes. Likewise, *P. commune* also showed capacity to produce these last enzymes, so the WH can be considered as a substrate that can induce the production of hemicellulases and cellulases.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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