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Hydrolytic enzymes in *Paracoccidioides brasiliensis* - ecological aspects

Bruno Benoliel, Fabrício B.M. Arraes, Viviane Castelo-Branco Reis, Saulo J.L. de Siqueira, Nádia S. Parachin and Fernando A.G. Torres

Laboratório de Biologia Molecular, Departamento de Biologia Celular, Universidade de Brasília, 70910-900 Brasília, DF, Brasil
Corresponding author: F.A.G. Torres
E-mail: ftorres@unb.br

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ABSTRACT. *Paracoccidioides brasiliensis* is a thermally dimorphic fungus that causes paracoccidioidomycosis. The yeast form of this pathogen is found in the animal host whereas the mycelial form is recovered from living and non-living organic material. The sole carbon source available in these habitats is represented by polysaccharides from the plant cell wall. Hydrolytic enzymes are necessary to convert these polymers into simple sugars for fungal metabolism. We report on the presence of ortholog genes of hydrolytic enzymes identified in the *P. brasiliensis* transcriptome and on hydrolytic activities in supernatants of induced *P. brasiliensis* cultures of mycelium and yeast cells. Enzymatic assays have shown cellulase and xylanase activities, both being higher in mycelium than in the yeast form. Amylase and chitinase activities were detected only in mycelium. Data so far reinforce the idea that mycelial *P. brasiliensis* is a saprobe.

Key words: *Paracoccidioides brasiliensis*, Hydrolytic enzymes, Saprophytic, Ecology

INTRODUCTION

Paracoccidioides brasiliensis is a dimorphic fungus that causes paracoccidioidomycosis (PCM), a systemic mycosis characterized by granulomatous inflammation, suppression of cellular immunity and high antibody titers (Rodrigues and Travassos, 1994; Dixon et al., 1998). It is considered a primary pathogen of humans capable of establishing infections in an immunocompetent host (Restrepo et al., 1976). Infection typically occurs by inhalation of dry airborne spores, fungal propagules or mycelium fragments, which settle in the airways, followed by the thermally regulated transition to the parasitic yeast phase (San-Blas et al., 2002). It has been estimated that as many as 10 million individuals are infected by *P. brasiliensis* in some endemic areas of Latin America, mainly in rural regions where forests and agriculture abound (Brummer et al., 1993; Borges-Walmsley et al., 2002).

Microorganisms, such as filamentous and dimorphic fungi, are ubiquitous and are known for their decomposition potential (saprophytes) or parasitic behavior. These fungi possess an efficient hydrolytic system capable of performing several roles, such as conversion of lignocellulosic material to essential metabolites for growth. Usually, these fungi secrete a pool of enzymes, including amylases, cellulases (cellobiohydrolases, endoglucanases), hemicellulases (xylanases), β-glycosidases, and lignin-peroxidases. Some fungal species of the genus *Aspergillus*, *Neurospora*, *Humicola*, *Candida*, and *Trichoderma* are of special interest due to their ability to produce hydrolytic enzymes, such as cellulases, amylases and chitinases, which are of special interest due to their importance in biotechnological processes.

Data from the *P. brasiliensis* transcriptome (Felipe et al., 2003, 2005) revealed orthologs to genes related to many hydrolytic enzymes involved in substrate degradation, cell wall metabolism, and other cell functions (Table 1). The goal of the present study was to detect and assess the activity of these hydrolytic enzymes in mycelium and yeast cultures of *P. brasiliensis*, and also examine whether these activities differ between the two fungal forms.

Table 1. Hydrolytic enzymes found in *Paracoccidioides brasiliensis* transcriptome.

EC number	Orthologue name	e-value	Specific phase
3.2.1.14	Chitinase	6e-78	-
3.5.1.41	Xylanase/chitin deacetylase	e-114	Y
3.1.1.41	Acetylxylan esterase (chain A)	3e-14	Y
3.2.1.1	α-amylase	7e-26	-
3.2.1.33	Glycogen debranching enzyme	4e-66	-
3.2.1.20	Putative α-glucosidase II	1e-92	Y
3.2.1.21	β-glucosidase 3	e-115	-
3.2.1.58	β-glucosidase 4 (1,3-β-glucosidase)	e-132	M
3.2.1.4	β-glucosidase 6 (endoglucanase)	2e-54	-

MATERIAL AND METHODS

Microorganism and culture conditions

Paracoccidioides brasiliensis - isolate Pb01, was grown on modified McVeigh Morton

media (Restrepo, 1980), in which the glucose was replaced by other carbon sources at 0.5% (w/v) concentration (sigmacell (SIG), carboxymethylcellulose (CMC), xylan, potato starch, and chitin). Mycelium and yeast cells were cultivated at 23° and 37°C, respectively. The supernatants of cultures were filtered through a 0.2-µm Millipore filter before being used for enzyme assays.

Enzyme assays

Hydrolytic activity was calculated by measuring the amount of reducing sugars released from xylan (Tuohy and Coughlan, 1992), potato starch (Bernfeld, 1955), CMC, or filter paper (Mandels et al., 1976). The filtered supernatant of cultures was incubated with the substrate solutions [1% (w/v) xylan, 1% (w/v) potato starch, 1% (w/v) CMC or Whatman No. 1 filter paper (3.0 x 0.5 cm), all in 50 mM acetate buffer, pH 6.5] at 37°C for 1 h. Dinitrosalicylic acid (0.5 ml) was added to halt the reaction. The mixture was boiled for 5 min and allowed to cool naturally. Reduced sugars were determined by measuring the absorbance at 550 nm. One unit of enzymatic activity was defined as the amount of enzyme that produced 1 µmol of reducing sugars per min at 37°C, by hydrolyzing substrates. Chitinase activities were measured with colloidal chitin as a substrate. The reaction mixture, consisting of 150 µl supernatant of culture, 150 µl 0.02 M phosphate buffer and 300 µl 0.3% colloidal chitin, was incubated at 37°C. This mixture was centrifuged and the amount of reducing sugar produced was measured by the DNS method (Miller, 1959), with N-acetyl-glucosamine (GlcNAc) as the sugar standard. One unit of activity was defined as the amount of enzyme that released 1 µmol GlcNAc per minute. All assays were performed in triplicate.

Cellulose hydrolysis

Fungi play a major part in recycling cellulose, which is a β-1,4-linked glucose polymer. An important feature of this molecule is its crystalline structure; elementary fibrils are stiffened by both inter- and intra-chain hydrogen bonds, which result in a sufficiently packed structure that prevents penetration, not only by enzymes, but also by small molecules, such as water. However, some regions, called amorphous, are sufficiently spacious to permit penetration by larger molecules, including cellulases (Lynd et al., 2002).

The complete process of biodegradation of cellulose into soluble glucose monomers requires the concerted action of three enzymatic activities: endoglucanase, which cleaves β-1,4 internal sites in amorphous regions, generating oligosaccharides of various lengths; exoglucanase, which liberates cellobiose from reducing and nonreducing ends of the polysaccharide, and β-glycosidases, which hydrolyze cellobiose to glucose (Wood and McCrae, 1978). Many fungi are capable of growing on cellulose as the sole carbon source, including those normally found on wood.

The enzyme α-glycosidase (EC 3.2.1.20) catalyses the liberation of α-glucose from the nonreducing ends of substrates, such as malto-oligosaccharides, α-glycosides and α-glucans. Many α-glycosidases hydrolyze not only synthetic α-glycosides and oligosaccharides but also α-glucans, such as soluble starch and glycogen (Chiba, 1988). These enzymes are classified into families I and II, based on substrate specificity and amino acid sequence (Chiba, 1997). Family I enzymes hydrolyze such heterogeneous substrates as sucrose and *p*-nitrophenyl α-glycoside more rapidly than homogenous substrates, such as malto-oligosaccharides, and they show

little or no activity against α-glucans. Family II enzymes are more active against homogenous than heterogeneous substrates, and some of them are capable of hydrolyzing α-glucans. A yeast-specific, putative α-glycosidase II was found in the *P. brasiliensis* transcriptome (Table 1).

Saccharomyces cerevisiae contains a wide range of endo- and exo-1,3-β-glucanases, and it seems likely that approximately 15 genes in this yeast encode polypeptides with glucanase or related enzymatic activities (Baladrón et al., 2002). Some of these have roles during cell separation, while others exhibit transglycosylase activity; they also may be involved in extending and rearranging 1,3-β-glucan chains and cross-linking these polymers to other wall components. Several glucanolytic activities have been detected in the cell walls of the respiratory pathogens *Aspergillus fumigatus*, *Coccidioides posadasii* and *Coccidioides immitis*. Some of the implicated enzymes exhibit both glucanase and transglycosidase activities, and like their yeast counterparts, they may have roles in cell wall remodeling during morphogenesis. The cell wall of *C. immitis* contains a 120-kDa β-glycosidase with 1,3-β-glucanase activity. A considerable body of evidence has accumulated suggesting that this enzyme has a morphogenetic role during the parasitic growth phase of this pathogen (Cole and Hung, 2001; Hung et al., 2001). Disruption of the corresponding gene led both to a reduction in the rate of development of the parasitic growth phase and to a reduction in the mycelium growth rate. Furthermore, its knockout appeared to cause a marked decrease in the virulence of this organism in mice (Cole and Hung, 2001). Related sequences were found with high similarity to *C. immitis* β-glycosidases 3 (EC 3.2.1.21), 4 (EC 3.2.1.58) and 6 (EC 3.2.1.4) in the *P. brasiliensis* transcriptome (Table 1).

Paracoccidioides brasiliensis has been grown on CMC and microcrystalline cellulose as the sole carbon source in both mycelium and yeast forms, and it is able to convert cellulose to simpler sugars that can be assimilated. These data are corroborated by enzymatic assays, whereby total cellulase activity was assessed in the supernatants of cultures (Table 2). The enzymatic activities are low when compared with fungi described as cellulolytic (Lynd et al., 2002), but are sufficient for *P. brasiliensis* to thrive on cellulosic sources.

Xylan hydrolysis

Xylan is a component in plant cell walls, being the second most abundant polysaccharide found in nature. It consists of a heteropolysaccharide containing substitute groups of acetyl, 4-O-methyl-d-glucuronosyl and α-arabinofuranosyl residues linked to the backbone of β-1,4-linked xylopyranose units (Subramaniyan and Prema, 2002). The xylan layer is covalently linked to lignin and interacts non-covalently with cellulose and thus protects the fibers against degradation by cellulases (Beg et al., 2001). Its selective removal increases fiber parasitism (Johri and Ahmad, 1991; Biely and Tenkanen, 1998).

Due to the heterogeneity and complex structure of plant xylan, its complete degradation to constituent sugars requires the action of a complex of hydrolytic enzymes with diverse modes of action (Beg et al., 2001). The most important xylanolytic enzyme is endo-β-1,4-xylanase, which cleaves mainly in regions of the main chain that lack substitute groups (Biely and Tenkanen, 1998). The other enzymes work synergistically with it, and this multifunctional xylanolytic system is quite widespread among fungi (Beg et al., 2001). In spite of their multiplicity, however, these systems are not sub-classified, as are, for example, cellulases (Johri and Ahmad, 1991).

The regulation of xylanase secretion is still not fully understood, but these enzymes are produced during fungal growth both on cellulose and xylan (Biely and Tenkanen, 1998). Xylanase

Table 2. Enzymatic assays of the supernatant of *Paracoccidioides brasiliensis* cultures grown in different carbon sources.

Carbon source	Assay	Phase	Activity (U/ml)
CMC	CMCase	M	-
	CMCase	Y	-
	FPase	M	1.59×10^{-3}
	FPase	Y	0.86×10^{-3}
SIG	CMCase	M	-
	CMCase	Y	-
	FPase	M	6.08×10^{-3}
	FPase	Y	4.61×10^{-3}
Xylan	Xylanase	M	48.60×10^{-3}
	Xylanase	Y	8.35×10^{-3}
Starch	Amylase	M	1.56×10^{-3}
	Amylase	Y	-
Chitin	Chitinase	M	5.10×10^{-5}
	Chitinase	Y	-

production is stimulated by low-molecular weight xylan fragments produced by constitutive enzymes (Beg et al., 2001), and it is likely to be important for the degradation of cellulose to glucose by fungi, since xylan enzymatic removal allows cellulases to reach cellulose in vegetal sources. Some microorganisms have a xylanosome, a discrete multifunctional enzymatic complex found on their surface that plays an important role in the degradation of hemicelluloses (Beg et al., 2001).

The *P. brasiliensis* transcriptome has an enzyme for xylan degradation, which is expressed in the yeast form. Acetylxylan esterases (EC 3.1.1.41) are enzymes that hydrolyze the ester linkages of the acetyl groups at position 2 and/or 3 of the xylose moieties of natural acetylated xylan from hardwood. These enzymes integrate xylanolytic systems, together with xylanases, β-xylosidases, α-arabinofuranosidases, and methylglucuronidases; these are all required for the complete hydrolysis of xylan. Many xylan-degrading bacteria and fungi produce acetylxylan esterases and modular xylanases, with both glycosidase and esterase activities, in order to overcome the inhibitory effects of naturally occurring acetylated xylan in plant cell walls (Dupont et al., 1996; Laurie et al., 1997). Minimal media containing xylan as sole carbon source was sufficient for *P. brasiliensis* growth, mainly in mycelial cultures, where the resulting biomass was greater than that obtained with yeast cultured on xylan or other substrates (data not shown). Enzymatic assays of the supernatants of these cultures have shown considerable xylanase activity, about six times as high as with yeast culture. Activity values of xylanases in *P. brasiliensis* are presented in Table 2.

Chitin hydrolysis

Chitin in a linear homopolymer of β-1,4-linked N-acetyl-D-glucosamine, and its fiber structure is similar to that of cellulose. It is the main component of insect exo-skeletons and of

fungal cell walls and is possibly the second most abundant polysaccharide in nature (Nelson and Cox, 2000). Chitinases are endoglucanases that cleave the internal β-1,4-acetylglucosamine linkages in chitin polymers (Wattanalai et al., 2004). Bacteria produce chitinase to assimilate chitin as a carbon and nitrogen source; plants produce chitinases in response to invasion by fungi, many of which also produce these enzymes (McCreath et al., 1995).

The cell wall, which is involved in many aspects of fungal physiology, is a β-glucan polymer linked by 1,3-β-bonds with some occasional 1,6-β-branching (Gonzales et al., 1997). However, this cell wall is a highly dynamic structure subject to changes, for example, during cell expansion and division in yeasts, and during spore germination and septum formation in filamentous fungi (Adams, 2004). The maintenance of this plasticity in situations of morphogenetic changes such as occur in dimorphic fungi depends upon the activities of various enzymes that are associated with the cell wall. Ghormade et al. (2000) detected chitinase and N-acetylglucosaminidase activities in cell wall-bound and free fractions in the dimorphic fungus *Benjaminiella poitraisii*. These enzymes were found to be involved in the yeast-to-mycelium transition.

Chitinases are also associated with the biology of insect mycopathogens. Fungal chitinases can disrupt the cuticle barrier, providing access to nutrients (Wattanalai et al., 2004). At a late stage of infection, internal fungal cells must emerge from the insect to produce conidiophores. At this stage the insect endocuticle is digested, suggesting that exocellular chitinases play a major role in infection. Chitinases can also inhibit the development of other microbial competitors. Lorito et al. (1998) showed that certain fungal endochitinases, such as the one isolated from *Trichoderma harzianum*, can act as potent anti-fungal enzymes.

Paracoccidioides brasiliensis yeast cells have a highly differentially expressed xylan/chitin deacetylase (EC 3.5.1.41), which was also identified by the high number of expressed sequence tags (ESTs) in the transcriptome. This deacetylase catalyses the hydrolysis of the acetamide group of GlcNAc units in chitin and in chito-oligosaccharides. Two types of chitin deacetylase have been investigated to date in Zygomycetes and Deuteromycetes. Chitin deacetylase from *Mucor rouxii*, a Zygomycete, removes N-acetyl groups from the non-reducing GlcNAc units of the substrate. This enzyme is involved in the biosynthesis of chitosan (Davis and Bartnicki-Garcia, 1984), a linear homopolymer of α-(1-4)-linked GlcN units with much higher water solubility and broader applications than chitin (Rha et al., 1984; Knorr, 1984; Hirano, 1989). In contrast, the extracellular chitin deacetylase from *Colletotrichum lindemuthianum*, a Deuteromycete, was shown to have an endo-type pattern of action, in which the chito-oligosaccharide substrates with a degree of polymerization (*n*) equal to or greater than 4 are eventually fully deacetylated via a specific pathway (Tokuyasu et al., 2000). Chitotriose is also fully deacetylated, but through a random deacetylation process, in which either of the three GlcNAc units can be deacetylated first, while the smallest substrate, chitobiose, is only deacetylated at the non-reducing GlcNAc residue. Deuteromycetes that produce chitin deacetylase are all plant pathogens and secrete the enzyme during penetration into host cells (Kauss et al., 1982; Siegrist and Kauss, 1990; Deising and Siegrist, 1995). The role performed by this enzyme in the process remains unclear. Mycelium and yeast forms from *P. brasiliensis* were able to grow on minimal media containing chitin as the sole carbon source. Enzymatic assays were performed to detect total chitinase activity in the supernatants, which was observed only in mycelium cultures of *P. brasiliensis* grown on chitin (Table 2).

Potato starch hydrolysis

Starch is a polysaccharide that has glucose as a structural unit; it is one of the most abundant polymers found in plant cells. It is comprised of two glucose polymers, amylose and amylopectin. Amylose consists of long, linear chains of D-glucose residues connected by α-1,4-linkages, while amylopectin is branched by α-1,6-linkages. Both of them vary in molecular weight from a few thousand to over a million kilo-Daltons (Nelson and Cox, 2000).

Amylases are able to perform starch hydrolysis and have been reported to occur most widely in microorganisms, although they are also found in plants and animals. Two major classes of amylases have been identified, namely α-amylase and glucoamylases (Pandey et al., 2000). α-Amylases (endo-1,4-α-D-glucan glucohydrolase, EC 3.2.1.1) are extracellular enzymes that randomly cleave inner 1,4-α-D-glucosidic linkages between adjacent glucose units in the linear amylose chain and are classified according to their action and properties. An α-amylase was identified in the *P. brasiliensis* transcriptome and was found to be expressed in both forms (Table 1) by EST analysis.

Glucoamylases (EC 3.2.1.3) hydrolyze single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise fashion. Unlike α-amylase, most glucoamylases are also able to hydrolyze the α-1,6-linkages at the branching points of amylopectin, although at a lower rate than 1,4-linkages. Thus, glucose, maltose and limit dextrins are the end products of glucoamylase action.

O-glycosyl hydrolases (EC 3.2.1.-) are a widespread group of enzymes that hydrolyze the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. A classification system for glycosyl hydrolases, based on sequence similarity, has led to the definition of 85 different families. These enzymes can be classified as isoamylase, pullulanase and branching variants. Isoamylase hydrolyses 1,6-D-glucosidic branch linkages in glycogen, amylopectin and dextrin; 1,4-glucan branching enzyme functions in the formation of 1,6-glucosidic linkages of glycogen, and pullulanase is a starch-debranching enzyme. An enzyme of this family, a glycogen debranching enzyme (EC 3.2.1.33), was found in *P. brasiliensis* transcriptome.

Both forms of *P. brasiliensis* were able to grow on minimal media containing potato starch as the sole carbon source but amylolytic activity was only detected in the supernatants of mycelial cultures of this fungus (Table 2).

DISCUSSION

Paracoccidioides brasiliensis, the etiological agent of PCM, is a dimorphic fungus whose yeast form has a well-established habitat as a parasite of animals, including humans, armadillos and penguins (Camargo and Taborda, 1993; Bagagli et al., 1998). The habitat of the mycelial form of *P. brasiliensis* has not yet been determined; this has proven to be a difficult task for mycologists (Borelli, 1971a). Several factors have contributed to this difficulty, such as the rarity of isolation of the fungus from the environment, the large number of negative reports in attempts involving soil samples and the low repeatability of isolation of the fungus from the same area. These aspects indicate that the specific growth conditions of the pathogen in the soil have not been fully clarified (Franco et al., 2000).

Associations of ecological aspects and reported clinical cases have suggested that the

incidence of PCM is not uniform. This disease is restricted to people who have had close contact with rainforests or agriculture areas (Calle et al., 2001). Moreover, other studies showed that certain ecological variables, such as altitude (800-2100 m), temperature (17-24°C) and minimal precipitation rates of about 2000 mm/year are related to the incidence of PCM (Borelli, 1964; Borelli, 1971b; Calle et al., 2001).

Armadillos are considered a natural reservoir of PCM. In their constant digging activity they are in deep contact with soil, both to search food and to build burrows ranging in depth from four to six meters, where they make nests with vegetal material (Bagagli et al., 1998). The fungus was isolated from armadillos in the yeast form, whereas infection was probably via airborne *P. brasiliensis* conidia, as in humans. The animals from which *P. brasiliensis* was isolated were in the same endemic areas as humans, indicating that probably both are infected by a common environmental source, most likely located in gallery forests or rural areas (Bagagli et al., 1998).

In the environment, *P. brasiliensis* is found in the infective mycelial form, which is considered to be a saprobe. It has to be versatile in using diverse resources that can become temporarily available. Saprophytic fungi, therefore, produce the broadest spectrum of hydrolytic enzymes (St. Leger et al., 1997). The natural habitat of *P. brasiliensis* is thought to be located in vegetation sites disturbed by humans near water sources, where it was found in soil with vegetal debris and living plants (Conti-Diaz and Rilla, 1989; Restrepo-Moreno, 1994). These data indicate that *P. brasiliensis* probably produces enzymes related to the hydrolysis of plant cell wall components. To corroborate this hypothesis, particles of mycelium and yeast forms of *P. brasiliensis* were found in the digestive tract of fruit-eating bats, in which mycelial particles were more susceptible than the yeast form and were killed before passing to the rectum, suggesting the presence of *P. brasiliensis* in fruits (Greer and Bolanos, 1977).

Our aim was to contribute with more information about *P. brasiliensis* biochemistry in an ecological context. We, thus, investigated some of the most important polysaccharide hydrolytic activities in the supernatant of induced cultures and the corresponding ortholog genes in the *P. brasiliensis* transcriptome.

We found that *P. brasiliensis* possesses some of the main enzymatic activities necessary to hydrolyze vegetal material. Xylanase activity was present in both forms of this fungus, but it is six times higher in mycelium than in yeast, which was expected since the former is found in the environment as a saprobe, with chiefly vegetal substrates as carbon sources. The xylanase activity was found to be the most important hydrolytic activity, being related to the hydrolysis of hemicellulose. In nature, these carbon sources constitute a group of polysaccharides associated with cellulose fibers in the plant cell wall, producing a complex of carbohydrate polymers. Xylan is the main component of hemicellulose (Thomson, 1993). Hydrolysis of hemicellulose allows both the generation of usable small sugars directly from this compound and the release of cellulose fibers to degradation by cellulases.

Cellulolytic activity from supernantant cultures was determined by their action on filter paper. It was apparent that *P. brasiliensis* secretes cellulases to the culture supernatant, since activity was detected in mycelium and yeast cultures of the fungus grown on SIG (Table 2). Interestingly, the cultures grown on CMC yielded no detectable activities. These data suggest that *P. brasiliensis* probably prefers insoluble (SIG) substrates (similar to those found in nature) over soluble (CMC) sources. Transcripts of β-1,4-glycosidase and endoglucanase homologs

were found in our EST database (Table 1). A cellobiohydrolase and another endoglucanase homolog were also found, although with a very high e-value. More investigation about these transcripts is needed to confirm these results; however, the enzymatic assays indicate the presence of a cellulolytic system in *P. brasiliensis*, which was able to grow in media containing cellulose as the sole carbon source.

Previous plate assays showed that *P. brasiliensis* mycelium growing on solid media containing starch was able to degrade it and form a hydrolytic halo when plates were stained with iodine. The yeast cells do not present this characteristic (data not shown). Enzyme activity on starch was determined for the *P. brasiliensis* culture supernatants, with both forms of the fungus being grown with starch as the sole carbon source. Also, amylase activity was only observed on mycelium cultures, corroborating the plate assays (Table 2). The α-amylase ortholog gene was also found in the *P. brasiliensis* transcriptome. This compound is found in nature as a plant energy reserve, further contributing to the hypothesis that *P. brasiliensis* grows on plant substrates and can hydrolyze vegetal carbohydrates.

We have also detected hydrolytic activity against chitin in *P. brasiliensis*. The secreted chitinase activity was observed only in mycelium grown on chitin as the sole carbon source. The *P. brasiliensis* cell wall has about 34% chitin (Moreno et al., 1969), but the concentration of GlcNAc residues in the yeast form was twice as high as in mycelia (Kanetsuna et al., 1969), suggesting that *P. brasiliensis* chitinases may be involved in the differentiation process. Chitin deacetylase was also found in the transcriptome (Table 1). We found a chitinase ortholog gene in the transcriptome that contains motifs of extracellular localization. Data from enzymatic assays (Table 2) suggest that *P. brasiliensis* uses chitin as a carbon source; this adds to previous evidence of its saprophytic behavior, hydrolyzing vegetal and other organic materials. The observation that both amylase and chitinase activities were not detected in the yeast culture supernatant could be due to the production of an extra-cellular form of these enzymes during the saprophytic phase, while in its pathogenic form these activities are probably associated with the cell wall.

Saprophytic fungi have the broadest spectrum of protein- and polysaccharide-hydrolyzing enzymes to take up available nutrients from environment. This versatility is required since there is a large diversity of substrates in nature, including animal, vegetal and fungus sources. Outside of the host, *P. brasiliensis* is found in mycelial form at room temperature. It was always isolated from organic materials, suggesting that this fungus is able to use complex substrates. We found strong evidence that *P. brasiliensis* mycelium can use some of the most abundant polysaccharides present in nature, corroborating the hypothesis that *P. brasiliensis* is indeed a saprobe in part of its life cycle.

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