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General metabolism of the dimorphic and pathogenic fungus *Paracoccidioides brasiliensis*

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ABSTRACT. Annotation of the transcriptome of the dimorphic fungus *Paracoccidioides brasiliensis* has set the grounds for a global understanding of its metabolism in both mycelium and yeast forms. This fungus is able to use the main carbohydrate sources, including starch, and it can store reduced carbons in the form of glycogen and trehalose; these provide energy reserves that are relevant for metabolic adaptation, protection against stress and infectivity mechanisms. The glyoxylate cycle, which is also involved in pathogenicity, is present in this fungus. Classical pathways of lipid biosynthesis and degradation, including those of ketone body and sterol production, are well represented in the database of *P. brasiliensis*. It is able to synthesize *de novo* all nucleotides and amino acids, with the sole exception of asparagine, which was confirmed by the fungus growth in minimal medium. Sulfur metabolism, as well as the accessory synthetic pathways of vitamins and co-factors, are likely to exist in this fungus.

Key words: *Paracoccidioides brasiliensis*, Metabolism, Transcriptome, Expressed sequence tags, Paracoccidioidomycosis, Dimorphic fungus

INTRODUCTION

Paracoccidioides brasiliensis is a soil-borne and thermo-regulated dimorphic fungus that causes paracoccidioidomycosis (PCM), the most prevalent fungal systemic disease in Latin America (San-Blas and Niño-Vega, 2001). PCM attacks the skin, lymph nodes and various internal organs, including the lungs and the central nervous system (Restrepo, 1985). Its clinical presentations range from a localized and benign disease to a progressive and potentially lethal systemic infection (Montenegro and Franco, 1994). All patients from whom the fungus is isolated must be treated, and despite new antifungal drugs, pulmonary fibrosis is still the most frequent sequel. The outcome of infection depends on several factors, including host responses and the virulence of the infecting isolate.

Onset of infection depends on a dimorphic transition from saprophytic mycelium to virulent yeast form, which based on *in vitro* experiments is triggered by a temperature shift from the average 26°C that the fungus encounters in soil and/or plants to 37°C in the human host. Many aspects of *P. brasiliensis* ecology remain unclear, especially those that concern its environmental niche (McEwen et al., 1995; Restrepo et al., 2001).

The biochemical events regulating dimorphic transition in *P. brasiliensis* are poorly defined, and several important questions remain unanswered. Aiming at a better knowledge of phase transition in *P. brasiliensis*, a scientific network named PbGenome has been initiated in Midwest Brazil (https://www.biomol.unb.br/Pb). As an initial step, this group sequenced expressed sequence tags (ESTs) of both phases (Felipe et al., 2003, 2005). Six thousand and twenty-two expressed genes (PbAESTs - *P. brasiliensis* assembled TAGs) were identified and half were annotated in the project, yielding a comprehensive view of *P. brasiliensis* metabolism. This provided sufficient information to allow us to detect differentially expressed sequences by means of cDNA microarray and/or electronic subtraction experiments (data not shown).

As expected for a unicellular saprobe, representative enzymes from all classical metabolic pathways were found in the EST gene set. By comparing the metabolism of *P. brasiliensis* with that of the well-known budding yeast, *Saccharomyces cerevisiae*, and with other fungi, it was observed that 33% of the PbAESTs are implicated in metabolism. Most of them are involved in processes that affect fungal survival, such as carbohydrate metabolism, β-oxidation and lipid biosynthesis, the urea cycle and amino acid processing, purine and pyrimidine metabolism, and accessory pathways involving sulfur, vitamins and co-factors (Figure 1).

We examined the different metabolic pathways inferred from *P. brasiliensis* transcriptome annotation by comparing them with data from *S. cerevisiae*, the biological characteristics of which have already been thoroughly investigated, with *Homo sapiens*, for the sake of host-parasite interactions, and with those of other dimorphic pathogens, including *Candida albicans*, *Histoplasma capsulatum* and *Coccidioides immitis*. These findings should help us develop novel investigative approaches concerning the natural course of PCM in the host, thus elucidating the molecular mechanisms that are involved in the dimorphism, virulence and pathogenicity of this fungus.

CORE CARBOHYDRATE METABOLISM

Sugars are carbon sources for all yeasts, which convert glucose-6-phosphate or fructose-6-phosphate to pyruvate, through the glycolytic pathway. Under aerobic conditions, pyru-

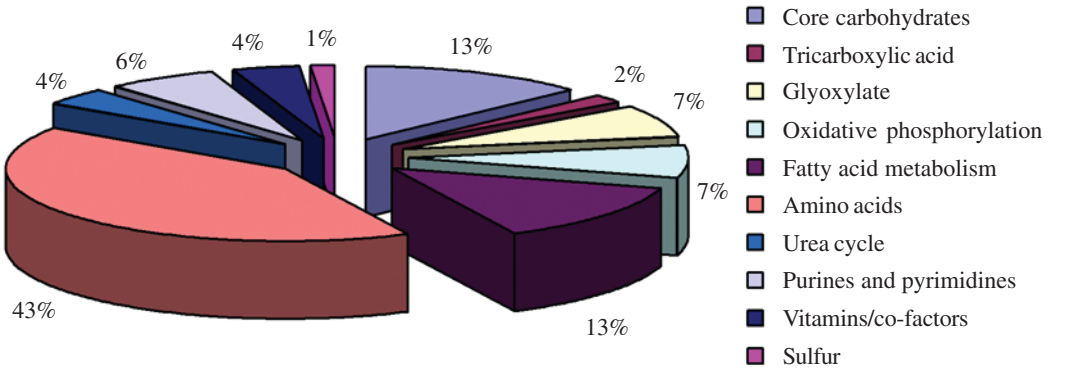

Figure 1. Distribution of PbAESTs in the main metabolic pathways, according to *Paracoccidioides brasiliensis* transcriptome database.

vate is usually oxidized to CO_2 through the tricarboxylic acid (TCA) cycle. Glycolysis and the TCA cycle perform a dual role in organisms; they produce energy in the form of ATP, NADH or NADPH and provide "building blocks" to synthesize other biomolecules (Flores et al., 2000). *Paracoccidioides brasiliensis* has both the glycolytic and gluconeogenic pathways, and all enzymes are present in both the mycelial and yeast forms.

Glucose, fructose and mannose are phosphorylated by hexokinases (EC: 2.7.1.1). Enzymes for hexose phosphorylation vary among the different yeasts. While *S. cerevisiae* and *Candida tropicalis* possess two hexokinases (Hxk1p and Hxk2p) and a glucokinase (Glk1p - EC: 2.7.1.2), *Schizosaccharomyces pombe*, *Kluyveromyces lactis* and *Yarrovia lipolytica* have only one of each (Flores et al., 2000). PbAESTs related to the two hexokinase genes were found in *P. brasiliensis*; these have high similarity to the genes from *S. cerevisiae* (*HXK1* and *HXK2*). Pyruvate kinase (EC: 2.7.1.40) catalyses the second ATP forming reaction in the glycolytic pathway. In *S. cerevisiae*, two genes exist that encode different proteins. One of these, encoded by the *PYK1* gene, is strongly activated by fructose-1,6-bisphosphate, while the other, encoded by *PYK2*, does not respond to this compound (Pronk et al., 1996). Two PbAESTs, whose sequences resemble the budding yeast genes, were found.

Quantitatively, the two major fates of the pyruvate produced in glycolysis are either its oxidation to CO_2 or its conversion into ethanol or lactate. Under aerobic conditions, oxidation will predominate, whereas transformation to ethanol prevails under anaerobic conditions. Oxidation of pyruvate to CO_2 occurs via the TCA cycle, the first step of which is oxidative decarboxylation catalyzed by pyruvate dehydrogenase (EC: 1.2.4.1), a mitochondrial multienzymatic complex that is comprised of three different components: pyruvate dehydrogenase, dihydrolipoamide acetyl transferase (EC: 2.3.1.12) and dihydrolipoamide dehydrogenase (EC: 1.8.1.4), all of which were identified in *P. brasiliensis*, as expected. An alternative route for regenerating NAD^+ in *P. brasiliensis* is through lactate dehydrogenase (EC: 1.1.1.28), which catalyses the reduction of pyruvate to lactate and can replace alcoholic fermentation.

Paracoccidioides brasiliensis is able to perform the so-called pyruvate-bypass, which involves the synthesis of acetyl CoA through the concerted action of pyruvate decarboxylase (EC: 4.1.1.1), acetaldehyde dehydrogenase (EC: 1.2.1.10) and acetyl CoA synthetase (EC:

6.2.1.1). These reactions, and the transport of the newly formed acetyl CoA into the mitochondria, could in principle "bypass" the action of pyruvate dehydrogenase.

During growth on non-fermentable carbon sources, cells require sugar phosphates for the synthesis of essential cellular components. The process by which these compounds are synthesized is known as gluconeogenesis. Most of the glycolytic reactions are reversible under physiological conditions, with two exceptions. Phosphofructokinase (EC: 2.7.1.11) and pyruvate kinase have to be bypassed due to unfavorable thermodynamic balances. In *P. brasiliensis*, this is achieved by phosphoenolpyruvate carboxykinase (EC: 4.1.1.49) and fructose-1,6-bisphosphatase (EC: 3.1.3.11), just as in *S. cerevisiae* and *C. albicans* (Eschrich et al., 2002) and in all other eukaryotes. Gluconeogenesis and the glyoxylate cycle (see below) may be important for providing carbohydrates for cell wall biosynthesis, which could be involved in the morphological switch from mycelium to yeast, as is proposed for *C. albicans* (Eschrich et al., 2002).

Ethanol can be used as a carbon and energy source by many fungi, and all the enzymes needed to produce it were found in *P. brasiliensis.* Assimilation occurs via the intermediary metabolites, acetaldehyde, acetate and acetyl-CoA. Central to the production or the utilization of ethanol are alcohol dehydrogenases (types I and II - EC: 1.1.1.1), enzymes that catalyze the reversible reduction of acetaldehyde to ethanol. Alcoholic fermentation is probably important for the survival of *P. brasiliensis* in the host.

The analysis of 19 PbAESTs yielded 11 genes involved in galactose metabolism, none of them differentially expressed (Table 1), 10 of which were also found in baker's yeast. In this pathway, galactokinase (EC: 2.7.1.6), a type of hexokinase, converts D-galactose to α-D-galactose-1P, from which uridine diphosphate (UDP)-galactose is formed through the action of galactose-1-phosphate uridylyltransferase (EC: 2.7.7.10). UDP-galactose can enter the nucleotide sugar pathway, or be converted to galactinol by galactinol synthase 1. Galactinol can also derive directly from D-galactose, in a reaction catalyzed by α-galactosidase (EC: 3.2.1.22). The same process can also generate melibitol, epimelibiose, galactosylglycerol, glycerol, D-mannose, D-sorbitol, and D-myo-inositol. One possible fate of UDP-galactose is conversion by UDP-glucose 4-epimerase (EC: 5.1.3.2) to UDP-glucose, which can either enter the pentose pathway, via glucose-6P, or be transformed by uridine 5'-triphosphate (UTP)-glucose-1-phosphate uridylyltransferase (EC: 2.7.7.9) into D-glucose, which follows the glycolysis pathway.

Fructose and mannose are also metabolized by *P. brasiliensis*. Based on the analysis of 28 PbAESTs, 12 genes related to this branch of sugar metabolism were identified, one of which, aldolase (EC: 4.1.2.13), is differentially expressed by the yeast form (Table 1). *Saccharomyces cerevisiae* expresses 10 of those genes. *Paracoccidioides brasiliensis* is able to convert D-fructose into D-fructose-1,6 biphosphate, which is cleaved by aldolase into dihydroxyacetone phosphate and glyceraldehyde-3P. Dihydroxyacetone-P is isomerized into glyceraldehyde-3P and enters glycolysis. Although it was not identified as a PEP-dependent phosphotransferase-like transport system, this fungus is likely to take up the extracellular substrates D-fructose, D-mannose, L-sorbose, D-sorbitol, and D-mannitol. It is known that some of these, including D-fructose, L-sorbose and D-sorbitol, can arise from other metabolic pathways, such as glycolysis and aminosugar metabolism.

Paracoccidioides brasiliensis possibly accumulates two types of glucose compounds, glycogen and trehalose, since we have found all the necessary enzymes for its metabolic synthesis in the transcriptome. Both play crucial roles in metabolic adaptation, including energy storage and protection against stress. Another potential function of glycogen and trehalose is the

Table 1. Some enzymes involved in general metabolism of *Paracoccidioides brasiliensis*.

EC number	Ortholog name	Differential expression by EST analysis
Main carbohydrates and glyoxylate metabolism		
3.2.1.1	α-amylase	-
3.2.1.21	β-glucosidase	-
3.2.1.58	1,3-β-glucosidase***	M
6.2.1.1	Acetyl-CoA synthetase***	Y
1.1.1.1	Alcohol dehydrogenase***	Y
4.1.2.13	Aldolase***	Y
3.2.1.33	Amylo-α-1,6-glucosidase	-
4.1.3.6	ATP citrate lyase	Y
2.3.3.8	Citrate synthase***	Y
3.2.1.4	Endoglucanase	-
2.7.1.2	Glucokinase***	M
2.4.1.11	Glycogen synthase	-
2.7.1.1	Hexokinase	-
4.1.3.1	Isocitrate lyase***	-
1.1.1.28	Lactate dehydrogenase	-
1.1.1.40	Malate dehydrogenase	-
2.3.3.9	Malate synthase	-
2.7.2.3	Phosphoglycerate kinase***	Y
2.4.1.15	Putative α,α-trehalose-phosphate synthase***	Y
1.2.4.1	Pyruvate dehydrogenase (E1 component)***	Y
2.7.1.40	Pyruvate kinase***	Y
6.2.1.5	Succinyl-CoA synthetase (α subunit)***	Y
Oxidative phosphorylation		
3.6.3.14	ATP synthase	-
1.9.3.1	Cytochrome C oxidase	Y
1.3.5.1	Fumarate reductase (flavoprotein subunit)	-
3.6.3.6	H(+)-transporting ATPase	-
3.6.1.1	Inorganic pyrophosphatase	-
3.3.2.1	Isochorismatase	Y
1.6.5.3	NADH-ubiquinone oxidoreductase***	Y
1.3.99.1	Succinate dehydrogenase	-
1.10.2.2	Ubiquinol-cytochrome C reductase***	Y
Fatty acid metabolism		
1.1.1.34	3-hydroxy-3-methylglutaryl-CoA	-
1.1.1.35	3-hydroxyacyl-CoA dehydrogenase	-
2.8.3.5	3-oxoacid CoA-transferase	-
2.3.1.41	3-oxoacyl-[acyl-carrier-protein] synthase[1]	Y
6.4.1.2	Acetyl-CoA carboxylase	-
1.14.15.3	Alkane 1-monooxygenase	-
1.14.14.1	Cytochrome P450	-
4.2.1.17	Enoyl-CoA hydratase	-
2.7.8.1	Ethanolaminephosphotransferase	-
2.5.1.29	Farnesyltranstransferase	-
2.7.1.30	Glycerol kinase	-

Continued on next page

Table 1. Continued.

EC number	Ortholog name	Differential expression by EST analysis
1.1.1.8	Glycerol-3-phosphate dehydrogenase (NAD^+)	-
4.3.1.4	Hydroxymethylglutaryl-CoA lyase	-
3.1.3.27	Phosphatidylglycerol phosphatase	-
2.7.8.8	Phosphatidylserine synthetase	-
3.1.4.4	Phospholipase D	-
Amino acid metabolism and urea cycle		
2.6.1.11	Acetylornithine aminotransferase	Y
6.3.4.5	Argininosuccinate synthase***	Y
4.1.1.28	Aromatic-L-amino-acid decarboxylase***	Y
2.4.2.17	ATP phosphoribosyl transferase	-
2.7.7.4	ATP-sulfurylase***	Y
2.7.2.11	Glutamyl kinase	Y
2.1.3.3	Ornithine carbamoyltransferase	Y
4.1.1.17	Ornithine decarboxylase	-
2.1.1.-	Putative methyltransferase	Y
2.5.1.16	Spermidine synthase	Y
Purine and pyrimidine metabolism		
2.7.1.20	Adenylate kinase	Y
2.1.2.3	AICAR transformylase/IMP cyclohydrolase	-
3.5.3.4	Allantoicase	-
3.5.2.3	Dihydroorotase	-
6.3.5.2	GMP synthase	-
1.1.1.205	IMP dehydrogenase	-
2.7.4.6	Nucleoside diphosphate kinase	-
4.1.1.23	Orotidine-5'-phosphate decarboxylase	-
6.3.5.3	Phosphoribosylformyl glycinamidine synthetase	-
2.4.2.14	Phosphoribosylpyrophosphate amidotransferase	-
2.7.6.1	Ribose-phosphate pyrophosphokinase	-
3.5.1.5	Urease	-
2.7.1.48	Uridine kinase	M
Sulfur, vitamin and co-factor metabolism		
3.1.3.7	3'(2'),5'-bisphosphate nucleotidase	-
1.8.4.8	3'-phosphoadenosine-5'-phosphosulfate reductase***	Y
2.7.1.25	Adenylylsulfate kinase***	Y
2.7.7.4	ATP-sulfurylase	Y
1.3.3.3	Coproporphyrinogen oxidase	Y
4.4.1.8	Cystathionine β-lyase	-
2.5.1.47	Cysteine synthase	-
2.3.1.31	Putative homoserine O-acetyltransferase	-
2.3.1.30	Putative serine O-acetyltransferase	-
1.4.3.5	Pyridoxamine 5'-phosphate oxidase***	Y
1.8.3.1	Sulfite oxidase	-
1.8.1.2	Sulfite reductase	-

***Enzymes differentially expressed detected in cDNA microarray experiment and electronic subtraction (data not shown). M = mycelial phase, Y = yeast phase. EST = expressed sequence tag.

progression of cell division. Mobilization of storage carbohydrates temporarily increases the sugar flux, thus enabling the cells to go through the next round of division (Guillou et al., 2004).

Glycogen synthesis in *P. brasiliensis* can be initiated by glycogenin (EC: 2.4.1.186), producing an α(1,4)-glucosyl chain that is elongated by glycogen synthase (EC: 2.4.1.21). The chains are ramified by the branching enzyme that creates α(1,6)-linkage. Glycogen degradation occurs by the combined action of glycogen phosphorylase (EC: 2.4.1.1), which releases glucose-1-P, and a debranching enzyme, which transfers a maltosyl unit to the end of an adjacent linear α(1,4)-chain and releases glucose. A two-step reaction catalyzed by phosphoglucomutase (EC: 5.4.2.2) and UDP-glucose pyrophosphorylase (EC: 2.7.7.9) leads to the synthesis of UDP-glucose (Wilson et al., 2004).

Trehalose is a non-reducing disaccharide composed of two α-(1,1)-linked glucose molecules; it is absent in vertebrates but common in fungi and plants (Jules et al., 2004). Trehalose is now being recognized as a crucial defense component that stabilizes proteins and biological membranes under various stress conditions, including increased temperature, hydrostatic pressure, desiccation, nutrient starvation, osmotic or oxidative stress, and exposure to toxic chemicals (François and Parrou, 2001). Trehalose is produced in a multistep process, whose prime substrate is glucose. Glucose is converted into glucose-6-phosphate (G6P), which, together with UDP-glucose, leads to the formation of trehalose-6-phosphate (T6P), and subsequently trehalose. The enzymes catalyzing the process are T6P synthase (*Tps1* - EC: 2.4.1.15) and T6P phosphatase (*Tps2* - EC: 3.1.3.12). Trehalose can also be split into two molecules of glucose, thus closing the *trehalose cycle*: 2 glucose → G6P + UDPG → T6P → trehalose → 2 glucose (Schiraldi et al., 2002; Jules et al., 2004). With the exception of α,α- trehalase (EC: 3.2.1.28), all enzymes of the trehalose cycle were found in the *P. brasiliensis* transcriptome. The trehalose biosynthetic pathway is a potential specific target for the development of antifungal drugs, due to its absence in vertebrates. The effects of the *tps1* mutation have been studied in some pathogenic yeasts, and this pathway seems to be implicated in infectivity in *C. albicans*, *Criptococcus neoformans* and *Magnaporthe grisea* (Gancedo and Flores, 2004).

Another possibly relevant metabolic pathway in *P. brasiliensis* is starch degradation. Starch constitutes the most abundant rapidly renewable source of energy for living organisms. This heterogeneous polysaccharide is composed of two high-molecular weight components: linear amylose (α-1,4-linked D-glucose residues) and branched amylopectin (containing both α-1,4- and α-1,6-linked D-glucose residues). It is predominantly degraded by hydrolytic enzymes (Ostergaard et al., 2000). *Paracoccidioides brasiliensis* presents at least three of these, namely α-amylase (EC: 3.2.1.1 - which cleaves α-1,4-glycosidic bonds), amylo-α-1,6-glucosidase (EC: 3.2.1.10 - debranching enzyme that hydrolyses α-1,6-glycosidic bonds), and starch phosphorylase (EC: 2.4.1.1). In *S. cerevisiae*, starch degradation increases pseudohyphal formation and invasive growth, a response that has been implicated in the search for nutrients (Vivier et al., 1997). Moreover, in some pathogenic fungi, like *C. albicans* and *P. brasiliensis*, this dimorphic behavior is key to pathogenesis.

GLYOXYLATE CYCLE

The glyoxylate cycle is an alternative pathway to the citric acid cycle for acetate (acetyl CoA) oxidation to dicarboxylic acids (succinate, malate, and oxaloacetate). It is not present in *H. sapiens* and consequently presents presumptive drug targets, such as isocitrate lyase (EC:

4.1.3.1) and malate synthase (EC: 2.3.3.9). In this cycle, isocitrate is cleaved into succinate and glyoxylate by the enzyme isocitrate lyase, and the glyoxylate is subsequently converted to malate by the enzyme malate synthase. This pathway is common in plants and some fungi, and isocitrate lyase is the main controlling protein in the glyoxylate shunt pathway in yeasts (Rude et al., 2002). All enzymes of the glyoxylate cycle were found in *P. brasiliensis.*

The glyoxylate pathway has also been linked to fungal pathogenesis. A cDNA microarray analysis of *S. cerevisiae* was performed to compare transcripts from yeasts that had been internalized by macrophages to those from extracellular yeasts. Many of the most highly induced genes in the former group encoded proteins related to the glyoxylate cycle (Lorenz and Fink, 2001). Also, it seems that glyoxylate is required for the intracellular survival of *Mycobacterium tuberculosis* after phagocytosis by macrophages (McKinney et al., 2000).

The glyoxylate cycle enables microorganisms to grow on acetate or fatty acids as sole carbon sources and may be an important adaptation of pathogens like *C. albicans* and probably *P. brasiliensis*. Recently, two key studies have examined the role of the glyoxylate cycle in the fungal phytopathogens, *M. grisea* and *Leptosphaeria maculans*. In *M. grisea*, mutants lacking isocitrate lyase activity were unable to germinate and cause disease (Solomon et al., 2004).

OXIDATIVE PHOSPHORYLATION

Electron transfer from NADH to molecular oxygen occurs via a linear respiratory chain, which is essential for ATP synthesis downstream of most catabolic pathways, such as carbohydrates and lipids. The mitochondrial chain includes four electron-transferring oligomers, namely complexes I-IV, which are located in the inner membrane of the organelle. The energy released from electron transfer reactions is used to pump protons across the membrane outwards, thus generating an electrochemical gradient that can be used to synthesize ATP by ATPase or complex V. The whole process is known as oxidative phosphorylation and is largely conserved (Hatefi, 1985). *Paracoccidioides brasiliensis* seems to perform oxidative phosphorylation by classical means, in that genes corresponding to components of complexes I, II, III, and IV were found (Felipe et al., 2003). In this pathway, 87.5% of the related genes expressed by *P. brasiliensis* are also expressed by *S. cerevisiae*; some of them are differentially expressed in the yeast phase (Table 1).

FATTY ACID METABOLISM

The complete pathway for fatty acid biosynthesis and metabolism includes 50 genes, distributed in fatty acid biosynthesis path 1 (14 genes), fatty acid biosynthesis path 2 (8 genes) and degradation (28 genes), according to the KEGG website (http://www.genome.jp/kegg/kegg2.html). Eighteen PbAESTs were detected in the transcriptome of *P. brasiliensis*, showing that this microorganism is able to perform 78 enzymatic reactions. This represents 76% of the total observed for the complete pathway, since one specific enzyme is able to catalyze more than one step of the reaction, strongly suggesting that *P. brasiliensis* can indeed carry out all pathways in fatty acid metabolism, as expected.

Several enzymes that have putative homologs in *P. brasiliensis* are related to important industrial products, such as enoyl-CoA hydratase (EC: 4.2.1.17), epimerase and 3-hydroxyacyl-CoA dehydrogenase (EC: 1.1.1.35), which are involved with polyhydroxyalkanoate (PHA)

and γ-decalactone. PHAs are a family of polyesters comprised primarily of (R)-3-hydroxyalkanoic acids, whose chemical profile is similar in many ways to those of thermoplastics and elastomers, and they are regarded as an attractive source of renewable and biodegradable polyesters (Anderson and Dawes, 1990). The major precursors of PHAs include enoyl-CoA, 3-ketoacyl-CoA, (S)-3-hydroxyacyl-CoA, and 3-hydroxyacyl-acyl carrier protein (ACP). These intermediates are converted to (R)-3-hydroxyacyl-CoAs by various enzymes, such as enoyl-CoA hydratase, 3-ketoacyl-CoA reductase (EC: 1.1.1.36), epimerase, and 3-hydroxyacyl-ACP-CoA transferase (EC: 2.8.3.8) (Park et al., 2002). An important biotechnological product from 3-hydroxy-acyl-CoA dehydrogenase activity is γ-decalactone, which is used in the formulation of peach, apricot and strawberry flavors (Wache et al., 2001).

Dicarboxylic acids are chemical intermediates used as raw materials for the preparation of perfumes, polymers, adhesives, and macrolide antibiotics. Several species of yeast belonging to the genus *Candida* excrete diacids as a by-product when cultured on n-alkanes or fatty acids as the carbon source. In *Candida* spp., n-alkanes and fatty acids are metabolized by enzymes from β- and ω-oxidation pathways (Craft et al., 2003). Two essential genes of these pathways were found in the annotation of the *P. brasiliensis* transcriptome: cytochrome P450 (EC: 1.14.14.1) and alkane 1-monooxygenase (EC: 1.14.15.3), further reinforcing the possibility of employing this microorganism to produce dicarboxylic acids useful to industry.

Acetyl-CoA carboxylase (EC: 6.4.1.2) marks commitment to fatty acid synthesis by carboxylation of acetyl-CoA to malonyl-CoA and is essential for yeast survival. Soraphen A, a polyketide isolated from the myxobacterium *Sorangium cellulosum*, strongly inhibits fungal growth by suppressing the catalytic activity of this enzyme (Vahlensieck et al., 1994).

Experiments using cDNA microarrays (data not shown) demonstrated that 3-oxoacyl-[acyl-carrier-protein] synthase (EC: 2.3.1.41) is up regulated in the *P. brasiliensis* yeast phase (Table 1). This enzyme appears to be specific for ACP derivatives of various lengths, catalyzing their condensation with malonyl-ACP, thus forming a β-ketoacyl-ACP in the first elongation reaction of fatty acid synthesis (Lai and Cronan, 2003). Taken together, these data suggest that biosynthesis of long chain fatty acids is a distinctive feature of the yeast form.

Ketone bodies are formed from acetyl CoA when oxaloacetate is limiting. Six enzymes, four of which have been identified in the *P. brasiliensis* transcriptome, carry this out. Two genes coding for hydroxymethylglutaryl-CoA lyase (EC: 4.3.1.4) and 3-oxoacid CoA-transferase (EC: 2.8.3.5), which participate in acetoacetate production, were identified in *H. sapiens* and *P. brasiliensis*, but neither are described in *S. cerevisiae* or *C. albicans*.

Many essential cellular molecules are derived from products of the mevalonate pathway, including sterols and ergosterol synthesis, co-enzyme Q, phenyl groups and dolichol. Yeasts accumulate ergosterol, the equivalent of cholesterol in animals and sitosterol in plants, as the end product; these are structural components of membranes (Bagnat et al., 2000; Umebayashi and Nakano, 2003). Thirty-nine enzymes, 16 of which were identified in *P. brasiliensis*, are responsible for ergosterol biosynthesis. The 14-α sterol demethylase (cytochrome P450 or *Erg11* in *C. albicans* - EC: 1.14.14.1) is important in ergosterol biosynthesis (White et al., 2002). It takes part in flavin or flavoprotein reductions, with incorporation of molecular oxygen, and it is the primary target for azole antifungals. A corresponding gene was found in the *P. brasiliensis* transcriptome. Sterol synthesis by the mevalonate pathway is partially modulated through feedback-regulated degradation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC: 1.1.1.34), which catalyzes the rate-limiting, irreversible step of the process. *Saccharomyces*

cerevisiae has two isozymes, Hmg1p and Hmg2p. In aerobic growth, the proportion of Hmg1p in the cell is high and that of Hmg2p low (Szkopinska et al., 2000; Nash et al., 2002). *Hmg* genes were identified in *P. brasiliensis*, in *H. sapiens* and in several *Candida* species, but not in *C. albicans*. 2,3-oxidosqualene-lanosterol cyclase (EC: 5.4.99.7), which was also identified in the transcriptome of *P. brasiliensis*, is a key enzyme in the biosynthesis of sterols in animals and fungi in that it performs the connection step between the acyclic and cyclic precursors of sterols. The 22,23-epoxy-2-aza-2,3-dihydrosqualene and its N-oxide derivative are good antifungal candidates because they are efficient inhibitors of oxidosqualene-lanosterol cyclase in sterol biosynthesis in *S. cerevisiae* and *C. albicans*.

Heptaprenyl pyrophosphate synthetase (EC: 2.5.1.30) was detected in *P. brasiliensis*, but not in other yeasts. It is involved in sterol and terpenoid biosynthesis and catalyzes the synthesis of all-trans C35 prenyl pyrophosphate from isopentenyl pyrophosphate and farnesyl or geranylgeranyl pyrophosphate, but it does not catalyze a reaction between isopentenyl pyrophosphate and either dimethylallyl or geranyl pyrophosphate (Takahashi et al., 1980).

AMINO ACID METABOLISM

Amino acids act as the "building blocks" of proteins, and they contribute to the synthesis of various biologically important molecules, such as nitrogenous bases of nucleic acids, coenzyme electron carriers, and the oxygen-binding pyrrole ring of hemoglobin, and they play roles as hormones and neurotransmitters. When these molecules are deaminated, their carbon skeletons can be fed into the citric acid cycle to be oxidized to carbon dioxide and water. Alternatively, they may be used as precursors of other biomolecules, including glucose. In many forms, amino acids can be viewed as connectors of metabolic pathways.

Except for asparagin (Asp), all amino acids are synthesized by *P. brasiliensis*. This information is confirmed by fungus growth in modified McVeigh-Morton medium. Both forms of this pathogenic fungus can be grown on this medium without cysteine (as reported previously by Restrepo and Jimenez, 1980) when supplied only with Asp. This fact corroborates *P. brasiliensis* transcriptome data (Felipe et al., 2005).

Biosyntheses of phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) share a common start. The first reaction is the condensation of phosphoenolpyruvate and D-erythrose-4-phosphate to form 7(P)-2-dehydro-3-deoxy-D-arabino-heptonate, progressing from this to chorismate formation, where two divergent paths arise, one leading to Trp and the other to Phe and Tyr. An important point to be considered is the absence in humans of the upstream diverging branch that leads to shikimate formation, which makes this sub-pathway an attractive target for the development of drugs. In *P. brasiliensis* we found three enzymes involved in these steps: chorismate synthase (EC: 4.2.3.5), phospho-2-dehydro-3-deoxyheptonate aldolase (EC: 2.5.1.54), and enolase (EC: 4.2.1.11).

In Tyr metabolism, an important end product is melanin, a negatively charged, hydrophobic pigment of high molecular weight (Hill, 1992). Melanization has been recently detected in *P. brasiliensis*. Both conidia (infectious propagules) and yeasts (systemic form) appear to produce it (Gómez et al., 2001). The complete pathway of DOPA-melanin biosynthesis was found in *P. brasiliensis* during our analysis. It begins with the conversion of Tyr to L-DOPA and is composed of five different enzymes. Melanin is associated with fungal virulence, and the best-described model is *C. neoformans*, an agent of life-threatening meningitis. Therapeutic

strategies that inhibit melanization were found to prolong the survival of lethally infected mice (reviewed by Gómez and Nosanchuk, 2003). The systemic herbicide glyphosate, which acts on chorismate synthase, interrupts the common part of Phe, Tyr and Trp biosynthesis pathways. This enzyme is a possible target for antifungal therapy, since it has already been demonstrated that it can prevent melanization in *C. neoformans* (Nosanchuk et al., 2001).

Aspartate is also the common precursor of methionine (Met) and threonine (Thr). We found the main enzymes of this process in *P. brasiliensis*, which begins with phosphorylation of aspartate to aspartyl-β-phosphate. With the exception of homocysteine-methyl-transferase (EC: 2.1.1.13), which performs the last reaction of the Met pathway, all other enzymes involved in the biosynthesis of Met and Thr from Asp were annotated in *P. brasiliensis*. The catabolism of Met involves synthesis of the important biological methylating agent, S-adenosylmethionine, which results from the action of S-adenosylmethionine synthetase (EC: 2.5.1.6) and after successive reactions is converted to succinyl-CoA.

L-cysteine (Cys) is a product of L-serine in a two-step pathway: serine O-acetyltransferase (EC: 2.3.1.30) converts L-serine into acetyl-L-serine, which is converted to Cys by O-acetylhomoserine aminocarboxypropyltranferase (EC: 2.5.1.49). Cys may be also synthesized from pyruvate by cystationine β-lyase (EC: 4.4.1.8) or cystationine γ-lyase (EC: 4.4.1.1). Two enzymes degrade Cys: cysteine dioxygenase (EC: 1.13.11.20) and aspartate aminotransferase (EC: 2.6.1.1).

The biosynthesis of Cys and Met requires sulfur. If this substrate is not available in the environment, the fungus has to increase its take-up by over expressing permeases and some enzymes involved in amino assimilation, mainly Cys and Met, to minimize the synthesis process. Cys and Met pathways are probably functional in *P. brasiliensis*, since most of the corresponding enzymes were found in both phases (yeast and mycelium) grown in culture media supplemented with inorganic sulfur (Felipe et al., 2005).

Two enzymes are differentially expressed in the yeast phase of the fungus and are involved in many pathways of amino acid metabolism. The first one is a putative methyltransferase (EC: 2.1.1.-), involved in histidine, selenoamino acid and tryptophan metabolisms. The second is the aromatic-L-amino acid, decarboxylase (EC: 4.1.1.28), which catalyzes the decarboxylation of 5-hydroxy-kynurenine (Trp metabolism), phenylalanine (Phe degradation), tyrosine (Tyr degradation), and L-histidine (His degradation). We have also identified the PbAEST 1318, which is differentially expressed in the *P. brasiliensis* mycelium phase. It probably encodes a protein with a high degree of sequence similarity to GAP1p, general amino acid permease, of *S. cerevisiae* and NAAP1p, amino acid permease, of *Neurospora crassa*. Amino acid permeases are integral membrane proteins involved in the transport of amino acids into the cell (Walker, 1998). This permease is related to system II of amino acid transport; it has affinity to amino acids with an α amino group, i.e., all except proline. In *N. crassa*, its activation occurs only in old culture medium, or in environments in which nitrate is the only available nitrogen source. This protein is associated with organic substance decomposition (Margolis-Clark et al., 2001). System II, also called the general amino acid transport system, has a high affinity for a broad range of amino acids, but it has a low velocity compared to systems I and III (Pall, 1969).

The biosynthetic pathways of the amino acids taurine and hypotaurine, selanoamino acids, aminophosphonate and cyanoamine were identified in *P. brasiliensis* as well. PbAESTs speculated to be involved in taurine and hypotaurine metabolism show similarity to glutamate decarboxylases, γ-glutamyltransferase, acetate kinase, and cysteine dioxygenase genes in *S.*

cerevisiae. In aminophosphanate and selanoamino acid metabolism, the PbAEST that bore homology with methyltransferase is differentially expressed in the *P. brasiliensis* yeast form. PbAESTs for cyanoamino acid pathways were also found (Table 1).

UREA CYCLE

The urea cycle involves arginine and ornithine synthesis. Two amino groups are introduced in the cycle: the first derives directly from ammonia formed inside mitochondria, by deamination or oxidation. The second group derives from aspartate, also formed by transamination in mitochondria and transported into the cytosol. Arginine is one of the most versatile amino acids in eukaryotes, being used as a precursor for the synthesis of proteins and for a great range of compounds, including nitric oxide, urea, polyamines, proline, glutamate, creatine, and agmatine. Of the enzymes that catalyze rate-limiting steps in arginine synthesis and catabolism, argininosuccinate synthase, the two arginase isoenzymes, the three nitric oxide synthase isoenzymes, and arginine decarboxylase have been recognized in recent years as key factors in regulating novel aspects of arginine metabolism (Wu and Morris, 1998). In *S. cerevisiae*, three enzymes from the arginine synthesis pathway are present: arginase (*Car*1 - EC: 3.5.3.1), argininosuccinate lyase (*Arg*4 - EC: 4.3.2.1), and argininosuccinate synthetase (*Arg*1 - EC: 6.3.4.5). Arginase expression responds to multiple environmental signals; expression is induced in response to the intracellular accumulation of arginine and repressed when readily transported and catabolized nitrogen sources are available in the environment (Smart et al., 1996). The *Arg*4 gene encodes the arginine biosynthetic enzyme (Beacham et al., 1984). The *Arg*1 homolog gene (PbAEST 378) is differentially expressed in the *P. brasiliensis* yeast form. Other enzymes of the urea cycle that were found in *P. brasiliensis* are: ornithine carbamoyltransferase (EC: 2.1.3.3), ornithine decarboxylase (EC: 4.1.1.17), acetylornithine aminotransferase (EC: 2.6.1.11), spermidine synthase (EC: 2.5.1.16), urease (EC: 3.5.1.5), and glutamyl kinase (EC: 2.7.2.11) (Table 1).

PURINES AND PYRIMIDINES

De novo purine synthesis appears to be identical in nearly all organisms, and it begins with their metabolic precursors: amino acids, ribose 5-phosphate, CO_2, and NH_3. Although the free bases are not intermediates in the *de novo* pathways, they are intermediates in some of the salvage pathways. Analyzing data concerning *de novo* purine synthesis enzymes in the *P. brasiliensis* transcriptome, we identified seven enzymes of this pathway out of the 15 that are known from all organisms. Although *P. brasiliensis* may not have all the enzymes, it contains some of the most important for the regulation of purine biosynthesis, such as phosphoribosylpyrophosphate amidotransferase (EC: 2.4.2.14), ribose-phosphate pyrophosphokinase (EC: 2.7.6.1), IMP dehydrogenase (EC: 1.1.1.205), and GMP synthase (EC: 6.3.5.2). Untill now, the data show that *P. brasiliensis* can probably produce GMP and AMP *de novo*.

De novo pyrimidine biosynthesis proceeds differently from purine nucleotide synthesis; the six-member pyrimidine ring is made first and then attached to ribose 5-phosphate, and carbamoyl-phosphate is required in this process. In the *P. brasiliensis* transcriptome, we have found enzymes of the pyrimidine pathway, namely dihydroorotate dehydrogenase (EC: 1.3.3.1), dihydroorotase (EC: 3.5.2.3), orotidine 5'-phosphate decarboxylase (EC: 4.1.1.23), and cytidine

5'-triphosphate synthase (EC: 6.3.4.2). Thus, it is likely that *P. brasiliensis* is able to produce both purines and pyrimidines *de novo*.

Salvage reactions are important in the metabolism of purine nucleotides, because of the energy required for the synthesis of purines. A free purine base that has been cleaved from a nucleotide can produce the corresponding nucleotide by reacting with the compound phosphoribosylpyrophosphate, formed by a transfer of a pyrophosphate group from ATP to ribose-5-phosphate. Two different enzymes with different specificities catalyze salvage reactions: adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase. Analysis of the transcriptome indicates that *P. brasiliensis* can produce nucleotides through the salvage pathway, since one of enzymes required for AMP production (adenine phosphoribosyltransferase - EC: 2.4.2.7) was identified.

Adenosine kinase (EC: 2.7.1.20) is an enzyme of purine biosynthesis that catalyzes the transfer of the γ-phosphate from ATP to the 5'-hydroxyl of adenosine (Ado), generating AMP and ADP. It is partly responsible for maintaining a steady state. A possible adenosine kinase-like (PbAESTs 927) sequence was found in the *P. brasiliensis* transcriptome, which would probably help this fungus to maintain adenosine levels, generating adenylate (AMP) whenever possible. Sequencing of the *S. cerevisiae* genome revealed an open reading frame (YJR105w) encoding a putative protein highly similar to adenosine kinases from other species. Disruption of this gene (renamed *Ado*1) affected the utilization of S-adenosyl methionine (AdoMet) as a purine source and resulted in a severe reduction in adenosine kinase activity in crude extracts (Lecoq et al., 2001).

Uridine kinase (EC: 2.7.1.48) is one of the most important enzymes of the pyrimidine salvage pathway, generating the end-product cytidine 5'-triphosphate. It catalyzes the transfer of two ATP molecules to uridine 5-monophosphate, yielding UTP. *Paracoccidioides brasiliensis* possesses one such enzyme, according to our findings, suggesting a means to utilize CMP through salvage pyrimidine biosynthesis. In *Borrelia burgdoferi*, a uridine kinase was found that probably is involved in the resistance of spirochetes to 5-fluorouracil (5'-FU). Although this study principally correlates species of bacteria, there is a possibility that in fungi like *P. brasiliensis*, resistance to 5'-FU needs to be checked (Boursaux-Eude et al., 1997).

The catabolism of purine nucleotides is initiated by hydrolysis to the nucleoside, and subsequently to the free base, which is further degraded. Deamination of guanine produces xanthine, and deamination of adenine produces hypoxanthine. Hypoxanthine can be oxidized to xanthine, which is therefore a common degradation product of both adenine and guanine. Xanthine is oxidized in turn to uric acid, which in some organisms is oxidized to allantoate. Allantoate is further degraded to glyoxylate and urea by microorganisms and some reptiles. Analyses revealed two enzymes of the latter process in the *P. brasiliensis* transcriptome, urate oxidase (EC: 1.7.3.3) and urease, both present in equal percentages in mycelium (50%) and yeast (50%) forms. On the contrary, the enzyme allantoicase (EC: 3.5.3.4) is found only in the yeast phase. Thus, *P. brasiliensis* can degrade urate to allantoate in both phases of their life cycle, and urea can in turn be oxidized, producing ammonia and glyoxylate. These data are interesting, since the enzymes required for degradation of urea from uric acid (urate) are present in the yeast phase, i.e., the pathogenic phase of the fungus. Taken together, these data have given more evidence about the possible role of urease in patogenicity.

Urease activity has been found in many human fungal pathogens, such as *C. neoformans*, *C. immitis*, *H. capsulatum*, *Sporothrix sckenchii*, and *Trichosporon* and *Aspergillus*

species, implying that this enzyme might have a role in pathogenicity. In the *P. brasiliensis* transcriptome, some enzymes were found that are involved in the deamination of purines and subsequent degradation of urea: urate oxidase, allantoicase and urease. This suggests an important role of the urease pathway for survival of pathogenic *P. brasiliensis* within the host. In this sense, although there have been some rare case reports of urease-negative *C. neoformans* strains causing infection (Bava et al., 1993), most clinical isolates produce large amounts of urease. In fact, rapid detection of urease activity is one means of tentatively identifying *C. neoformans* in clinical specimens (Canteros et al., 1996). A possible explanation for the importance of urease in *P. brasiliensis* is that it creates an appropriate environment for infection, since urea would be degraded to ammonia and the pH would rise, allowing *P. brasiliensis* to survive within the gastric mucosa. However, the role of urease in *P. brasiliensis* can only be assessed after extensive trials, including urease gene disruption experiments with analysis of resulting phenotypes.

VITAMIN AND CO-FACTOR METABOLISM

Vitamins are small biomolecules needed in small amounts in the physiology of all eukaryotes. They can be classified into two main groups: 1) water-soluble and 2) fat-soluble vitamins. The water-soluble vitamins include vitamin C (ascorbate, an antioxidant) and vitamin B complex (components of co-enzymes), while fat-soluble vitamins include vitamins A (a precursor of retinol), D (a regulator of calcium and phosphorus metabolism), E (a membrane antioxidant in membranes), and K (a participant in glutamate carboxylation). In *P. brasiliensis*, 83 PbAESTs were identified that are involved in vitamin and co-factor metabolism. Few enzymes, but at least one involved in biosynthesis and/or degradation in each pathway, were found in this fungus, except for retinol metabolism; none of those enzymes were represented in the data set.

Analysis of the CoA biosynthesis pathway strongly suggests that *P. brasiliensis* does not synthesize CoA. Despite the absence of CoA, by analyzing the pantothenate and CoA biosynthesis in the *P. brasiliensis* transcriptome, it was found that this fungus is able to synthesize 2-dydropantoate from pyruvate and D-4-phosphopantothenate from alanine and aspartate metabolism. In *S. cerevisiae,* the enzyme pantothenase (EC: 1.1.1.169) catalyzes the formation of (R)-pantoate from 2-dydropantoate, whereas this enzyme seems to be absent in *P. brasiliensis*.

In riboflavin metabolism, two enzymes were found in *P. brasiliensis*, GTP cyclohydrolase II (EC: 3.5.4.25) and riboflavin synthase (EC: 2.5.1.9), which synthesize riboflavin from GTP. However, there are two important enzymes that were not found in *P. brasiliensis*, diaminohydroxyphosphoribosyl aminopyrimidine deaminase (EC: 3.5.4.26) and 5-amino-6-(5-phosphoribosylamino) uracil reductase (EC: 1.1.1.193).

Two enzymes from biotin metabolism were annotated in *P. brasiliensis*: biotin synthase (EC: 2.8.1.6) and acetyl CoA holocarboxylase synthetase (EC: 6.3.4.15), which are able to degrade dethiobiotin into biotinyl 5'AMP. These enzymes are also present in *C. albicans* and *S. cerevisiae*. The enzyme 8-amino-7-oxononanoate synthase (EC: 2.3.1.47) was found only in *P. brasiliensis*.

Another vitamin synthesized by *P. brasiliensis* is pyridoxine, through the pentose phosphate pathway. This fungus also produces and degrades pyridoxal into pyridoxamine and pyridoxal 5-phosphate (co-enzyme of vitamin B6; Meister, 1965; Metzler, 1977). This pathway is

similar in *S. cerevisiae* and *C. albicans*, but pyridoxal kinase (EC: 2.7.1.35) is absent in the former. Ehrenshaft et al. (1999) showed that the gene *Sor1* is necessary for the synthesis of pyridoxine in *Cercospora nicotianae* and in another filamentous fungus, *Aspergillus flavus*.

In thiamine metabolism, the overall reaction from 2-methyl-4-amino-5-hydroxymethyl-pyrimidine and 4-methyl-5-β-hydroxyethylthiazole is catalyzed by four enzymes found only in *S. cerevisiae* metabolism: 2-methyl-4-amino-5-hydroxymethylpyrimidine kinase (EC: 2.7.1.49), 2-methyl-4-amino-5-hydroxymethylpyrimidine-monophosphate kinase (EC: 2.7.4.7), 4-methyl-5-β-hydroxyethylthiazole kinase (EC: 2.7.1.50), and thiamin phosphate pyrophosphorylase (EC: 2.5.1.3) (Nosaka et al., 1994). However, only two enzymes were found in the same metabolic pathway in *P. brasiliensis*: thiamine biosynthesis protein ThiE (EC: 2.5.1.3), which degrades 4-methyl-5-(2-phosphoethyl)-thiazole to thiamine phosphate and DL-glycerol-3-phosphatase (EC: 3.1.3.-), which synthesizes thiamine. In yeast, thiamine monophosphate is further dephosphorylated to thiamine, which is converted to thiamine pyrophosphate, a co-enzyme form, by thiamine pyrophosphokinase (EC: 2.7.6.2), which is encoded by the *ThI80* gene in *S. cerevisiae* (Nosaka et al., 1993).

Chaffin et al. (1979) reported that *C. albicans* appears to be unique, since it cannot utilize exogenous nicotinate as a precursor for NAD biosynthesis but can efficiently utilize nicotinamide. *Paracoccidioides brasiliensis* has the enzyme nicotinamidase (EC: 3.5.1.19), also found in *S. cerevisiae* and *C. albicans*. It interconverts nicotinamide and nicotinate. Among these three fungi, only *P. brasiliensis* may be able to transform nicotinate and quinolinate resulting from tryptophan metabolism into nicotinamide adenine dinucleotide.

Folate biosynthesis has two different starting substrates: GTP and chorismate (from phenylalanine biosynthesis). With the exception of GTP cyclohydrolase I (EC: 3.1.3.1), the main enzymes involved in this pathway were found in the *P. brasiliensis* transcriptome. Porphyrin metabolism starts with L-glutamate or 5-amino levulinate (from threonine or glycine metabolism). *Paracoccidioides brasiliensis* is able at least to synthesize uroporphyrin I, coproporphyrin I, uroporphyrin III, coproporphyrin III into protoheme and cytochrome c.

SULFUR METABOLISM

In filamentous fungi and yeasts, sulfur metabolism is involved in amino acid biosynthesis and in other major synthetic pathways. Thus, these organisms possess a complex regulatory circuitry that governs the expression of a diverse set of permeases and enzymes, used to take up and assimilate a steady supply of sulfur. Most sulfur in living organisms is present in a reduced form, as organic thiols. For their synthesis, inorganic sulfate is reduced and incorporated into organic compounds in the assimilatory sulfate reduction pathway. After uptake by the cells, inorganic sulfate is first phosphorylated via adenosine triphosphate in two enzymatic steps to generate 32 phosphoadenosine-52-phosphosulfate, reduced to sulfite, and then to sulfide, which is condensed with O-acetyl serine to generate cysteine, which also serves as an intermediate in the synthesis of methionine and S-adenosylmethionine. It is known that a sulfur regulatory circuit operates to insure that the cells maintain an adequate source of sulfur and, conversely, to repress the synthesis of various sulfur catabolic enzymes, whenever cells have an adequate supply of the substance. Regulation may occur both at the level of entry of sulfur from various sources into the assimilatory pathway and via distinct steps within the main pathway itself (Marzluf, 1997; Kopriva et al., 2002).

Paris et al. (1985) demonstrated that the mycelial phase of *P. brasiliensis* can grow with both organic and inorganic sources of sulfur. This sulfur auxotrophy suggests that, in addition to temperature, organic sulfur is an important stimulus for maintaining *P. brasiliensis* in the yeast phase. In support of this hypothesis, Marques et al. (2004) found that several genes involved in the assimilation of sulfur-containing amino acids, such as methionine permease, are more expressed in the yeast phase than in the mycelial phase, indicating a possible role for sulfur metabolism in the maintenance of the yeast state, as in the case of *H. capsulatum*. Ten of the 30 enzymes involved in sulfur metabolism have already been identified in *P. brasiliensis*, three of which, namely 3'-phosphoadenosine-5'-phosphosulfate reductase (EC: 1.8.4.8), adenylylsulfate kinase (EC: 2.7.1.25) and ATP-sulfurylase (EC: 2.7.7.4) are differentially expressed in the yeast form. These data were obtained from the analysis of PbAESTs (Table 1). *Saccharomyces cerevisiae* possesses 70% of the genes related to this pathway in common with *P. brasiliensis.*

REMARKS AND PERSPECTIVES

The transcriptome analysis of the dimorphic and pathogenic fungus *P. brasiliensis* database has allowed the description of its general metabolism. The identification of these metabolic pathways will provide a better understanding of *P. brasiliensis* biology, as well as helping to find more effective drug targets for PCM treatment. *Paracoccidioides brasiliensis* is able to perform the most important pathways of central metabolism, involving carbohydrates, lipids, amino acids, and nucleotides. Also, this pathogenic fungus can obtain various biomolecules, by synthesis and/or assimilation, such as vitamins, co-factors and sulfur. Since ours is an *in silico* study and the transcriptome project presents approximately 80% of *P. brasiliensis* expressed genes, experimental validation of these data will be needed, especially in the pathways where enzymes have not yet been identified.

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