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Gene expression analysis of *Paracoccidioides brasiliensis* transition from conidium to yeast cell

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Paracoccidioides brasiliensis infectious process relies on the initial expression of virulence faactors that are assumed to be controlled by molecular mechanisms through which the conidia and/or mycelial fragments convert to yeast cells. In order to analyze the profile of the thermally-induced dimorphic gene expression, 48 h C-L transition cultures which had been incubated at 36°C were studied. By this time approximately 50% of the conidial population had already reverted to yeast form cells. At this transition time, an EST-Orestes library was constructed and characterized. As a result, 79 sequences were obtained, of which 39 (49.4%) had not been described previously in other libraries of this fungus and which could represent novel exclusive C-Y transition genes. Two of these sequences are, among others, cholestanol delta-isomerase, and electron transfer flavoprotein-ubiquinoneoxidoreductase (ETF-QO). The other 40 (50.6%) sequences were shared with Mycelia (M), Yeast (Y) or Mycelia to yest transition (M-Y) libraries. An important component of this group of sequences is a putative response regulator receiver SKN7, a protein of high importance in stress adaptation and a regulator of virulence in some bacteria and fungi. This is the first report identifying genes expressed during the C-Y transition process, the initial step required to understand the natural history of *P. brasiliensis* conidia induced infection.

Keywords *Paracoccidioides brasiliensis*, conidia, yeast cells, transition, genes, EST-Library

Introduction

The thermo-dimorphic fungus *Paracoccidioides brasiliensis* is the causative agent of paracoccidioidomycosis (PCM). During the infectious process, the initial expression of the fungus' virulence is assumed to be dependent upon the cellular differentiation and molecular mechanisms by which the infectious particles – conidia and/or mycelial fragments – convert to yeast cells due to temperature changes [1]. Thus, the characterization of this thermally-induced process may allow a better understanding of the host-fungus interaction.

P. brasiliensis produces more than one type of asexual conidia. One of these is the microconidium, produced by the strains when they are cultured under conditions of nutritional deprivation such as in media with reduced carbohydrate content [2] and is a variable process depending on the isolates and the environmental conditions [3]. They are produced in reduced quantities, are rather small – approximately 4–5 μm – and represent a dormant cell stage [4]. Additionally, conidia take a long time to be produced

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(up to 3 months in appropriate culture medium). As a result, collecting the number of conidia necessary for transition evaluation and molecular studies requires a prolonged incubation and the use of a large number of Petri dishes with the infectious mycelial form.

Several studies have described the *in vivo* and the *ex vivo P. brasiliensis* transition process from conidium to yeast (C-Y), showing that in general this process was microscopically visible at approximately 24 h at 37°C, reaching the transition peak (80%) at 72–92 h post-inoculation or co-culture [5–15].

Prior to 2003, little was known about the molecular machinery involved in *P. brasiliensis* morphogenesis, apart from a SDS-PAGE analysis of the expression of cytosolic proteins during *P. brasiliensis* transitional M-Y stages [16]. Later on, a differential display approach designed to detect divergent transcripts in *P. brasiliensis* mycelial, yeast, and transitional forms was carried out [17]. However, after 2003 authors undertook diverse, broad-range approaches aimed at determining transition genes by such methods as a biochip [14], construction of ESTs libraries [18] and quantitative real-time reverse transcriptional PCR [11]. Since then, several studies have aimed at characterizing *P. brasiliensis* complete genome and gene expression profile, publishing the yeast (Y) and the mycelial (M) phase transcriptomes, performing transition analysis based in microarray technology [14], constructing ESTs libraries from the early steps of the M-Y morphogenesis [18], and describing, in all of them, genes related to putative virulence factors, possible therapeutic targets and proteins related to the transition process and reporting new, non-previously described sequences [10,11,14,18]. These studies identified certain proteins and genes that constituted the first approaches to deal with the differences in expression patterns found during the transitional process. They have brought forth important information on the transitional M-Y process at the molecular level, but none of them have addressed the idiosyncrasies of the C-Y morphogenesis.

In this report, an EST-Orestes library [19] of genes expressed during 48 h of the thermally-induced C-Y transition process was constructed, followed by its identification, clustering and characterization. Seventy-nine sequences were found, 39 of which were not present in any of the previously described *P. brasiliensis* libraries. This is the first report identifying genes expressed during the C-Y transition process.

Materials and methods

Fungus inoculum and conidial to yeast (C-Y) transition process

The mycelia form of *P. brasiliensis* ATCC 60855 was used to produce the conidia that were collected from 3 month incubation. For RNA extraction during the transition process, conidia were purified using the glass-wool filtration protocol [20]. Their concentration and viability was determined through Neubauer chamber counts and by ethidium bromide-fluorescence staining [21], respectively. To induce the C-Y transition process, harvested conidia were incubated for 48 h under constant agitation in 250 ml Erlenmeyer flasks at 36°C in 100 ml of Brain Heart Infusion broth (BHI: BD) plus 1% glucose. Subsequently, conidia were harvested and their transition percentages evaluated in accord with the methods originally described by Restrepo *et al*. [15].

EST-Orestes library construction and sequencing

Total RNA was obtained by maceration of the conidia with liquid nitrogen followed by the addition of Trizol (Invitrogen, Carlsbad, CA, USA). Total RNA was purified with the Picopure kit (Arcturs, Mountan View, CA, USA), treated with DNAse I (Invitrogen, Carlsbad, CA, USA) and tested through a conventional PCR using β-tubulin primers [11] so as to confirm lack of chromosomal DNA contamination. Amplification and production of the cDNA were made with the RiboAmp kit (Arcturs, Mountan View, CA, USA) and the cDNA was employed in the PCR-Orestes using the random primer 31-PROR (5'CGCAAATAGGCGAAGGA CTT 3') [19]. The PCR-amplified cDNA was purified by elution from an agarose gel with QiaexII (Qiagen, Stanford Valencia, CA, USA), selecting fragments between 500 and 2000 and cloned and transformed using the TOPO TA cloning Kit (Invitrogen, Carlsbad, CA, USA). Individual colonies were selected and stored at 70°C in 96-well plates with LB-glycerol broth.

Two strategies were used to obtain the DNA intended for sequencing. Plasmid extraction [22] or PCR amplification of the inserted fragments were done using the TOPO® TA cloning Kit procedure (Invitrogen Carlsbad, CA, USA). Plasmid sequencing was conducted in an ABI 3100 fluorescence automated sequencer (ABI, Foster City, CA) and PCR amplification products were studied with MACROGEN (Seoul, South Korea). In both cases, single run sequencing was performed.

Library analysis

Analysis, clustering and cleaning of the EST-Orestes sequences were made using a pipeline built by employing the Phrap [23] and Cap3 [24] software (Molecular Biology Group of the Faculty of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, Brazil). Sequences were automatically edited, cleaned from the vector and clustered in singlets or contigs as described previously [11]. Similarity of the sequences to those from *P. brasiliensis* and other organisms was searched using the BlastX and BlastN

algorithms against the NCBI, (http://www.ncbi.nlm.nih.gov/BLAST/), the EMBL-EBI-fungal EST, (http://www.ebi.ac.uk/blast2/nucleotide.html) and Goldman's EST (http://143.107.203.68/pbver2/default.html) databases.

Reverse Transcription PCR (RT-PCR) and analysis of the PCR products

After analysis of the library and based on the functionality and features of the possible genes found, some sequences were selected in order to determine their presence during the conidia to yeast transition. Two primers, which were useful not only for RT-PCR but also for qPCR were designed for each gene using Primer Express (Applied Biosystems). cDNA was produced with the Superscript III platinum (Invitrogen Carlsbad, CA, USA) using RNA from the 48h C-Y transition and processed as previously described. This sample was used to check the expression of each of the genes and, additionally, the PCR products obtained were sequenced and their identities confirmed by BlstX.

Results and discussion

Despite the fact that mycelial fragments are capable of causing infection [25], data arising from experimental models suggest that conidia are more likely to be the infecting propagules in nature [5,6] and consequently, observing the transition from conidia to yeast cells represents a more natural approach to the study of the infectious process. This transition could be a fundamental step in the pathogenesis of PCM and could represent a suitable target for hindering the infectious process [1].

The present study is the first attempt to correlate *P. brasiliensis* C-Y morphological changes with the gene expression modifications which occur concomitantly. An EST-Orestes library was obtained and after its pipeline screening, cleaning of undesirable sequences and clustering, 276 sequences were obtained that corresponded to 79 different sequences, distributed in 39 contigs and 40 singlets (GenBank accession codes available in http://www.ncbi.nlm.nih.gov/sites/entrez). Blast analyses with the different databases, showed that 42 (53%) of the sequences were either unknown (E-value higher than 10^{-3}) or hypothetical (previously recognized in other organism but without known function). Nevertheless, in the remaining 37 (47%) sequences, a function could be assigned. Of interest is the fact that a high percentage (27%) corresponded to signal transduction sequences (Fig. 1). Of relevance is the fact that a major proportion of the sequences with known functions present in the C-Y library (21 or 43%), had been described previously in both monomorphic (*Aspergillus terreus*, *A. fumigatus*, *A. oryzae*) and dimorphic fungi (*Coccidioides immitis*, *P. brasiliensis*). This finding further supports the hypothesis of structural and functional homology in different fungal genera sharing common genes controlling key biologic phenomena.

The comparative evaluation of the sequences in our library with genes obtained in other libraries was directed to better understand the gene expression profiles of different morphological stages, such as the M, Y and M-Y [10,11,18] through the EMBL – EBI databases. This analysis revealed a group of 39 novel and exclusive C-Y sequences that had not been detected previously in these libraries and that, as such, could represent important effectors directing the C-Y transition. Some of these sequences are, among others, cholestanol delta-isomerase, and electron transfer flavoprotein-ubiquinoneoxidoreductase (ETF-QO), 7 hypothetical proteins, and 13 unknown proteins (Table 1).

In fungi, the enzyme cholestanol delta-isomerase is involved in the biosynthesis pathway required to transform lanosterol into the major sterol in the plasma membrane, ergosterol [26], but it also appears to affect cell lipoprotein internalization [27]. In yeasts, ergosterol biosynthesis comprises at least 39 components of the already known cholesterol pathway [28]. Although, 16 of these 39 gene components have been identified in *P. brasiliensis* transcriptome, this particular enzyme has not been described [29]. Because of its function, cholestanol delta-isomerase could be important in the cell membrane remodeling process during the C-Y transition process, but its significance remains unclear and this hypothesis should be further studied.

The electron transfer flavoprotein-ubiquinoneoxidoreductase (ETF-QO) is an iron-sulphur flavoprotein that is bound to the inner mitochondrial membrane and is a component of an electron-transfer system in eukaryotic organisms in the mitochondrial cytochrome bc1 (complex III).

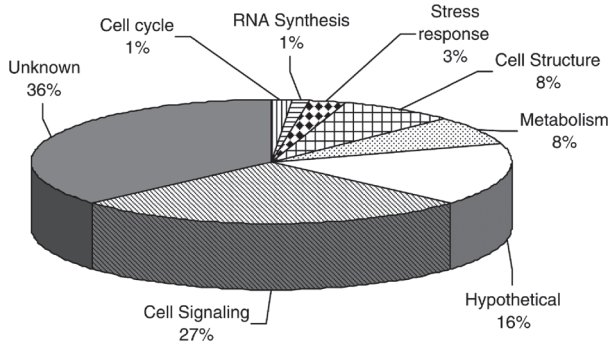

Fig. 1 *Paracoccidioides brasiliensis* C-Y transitional sequences ($n = 79$) distributed by possible function, showing the percentage of predicted genes in 8 different groups of sequences [42] according to their biological function. Hypothetical genes refer to sequences that show similarity to predicted proteins or DNA sequences having no assigned cellular role in databases. Unknown refers to new sequences with no significant similarity with other proteins or DNA sequences in the databases used as references.

Table 1 Novel Exclusive *Paracoccidioides brasiliensis* C-Y transition sequences (n=39)*. This table describes 26 genes with known functions

Cluster	Function	Possible protein	Organism	I	E	S	Blast
Contig 26	Structural	Membrane protein mp19-3 mRNA	*Danio rerio*	96	4,00e-15	M	N
Singlet 02	Structural	Membrane protein mp19-3 mRNA	*Danio rerio*	88	6,00e-12	M	N
Singlet 30	Structural	rolA gene for hydrophobin putative	*Aspergillus oryzae*	96	2,00e-05	L	N
Contig 05	Signal transduction	RAB 43	*Homo sapiens*	100	2,00e-09	L	N
Contig 06	Signal transduction	SH3 and PX domain containing 3	*Mus musculus*	94	3,00e-15	M	N
Contig 36	Signal transduction	SH3 and PX domain containing 3	*Mus musculus*	93	1,00e-08	L	N
Singlet 19	Signal transduction	SH3 and PX domain containing 3	*Mus musculus*	97	2,00e-08	L	N
Contig 09	Signal transduction	Phospholipase C-like 2	*Mus musculus*	97	4,00e-13	M	N
Contig 34	Signal transduction	Phospholipase C-like 2	*Aspergillus oryzae*	45	1,00e-08	L	N
Contig 16	Signal transduction	Regulator of G-protein signalling 13	*Homo sapiens*	100	1,00e-08	L	N
Singlet 22	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	87	2,00e-12	M	N
Contig 33	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	92	3,00e-25	H	N
Singlet 32	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	9	1,00e-21	H	N
Singlet 35	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	88	3,00e-15	M	N
Singlet 37	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	87	8,00e-19	M	N
Contig 01	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	93	3,00e-17	M	N
Contig 40	Signal transduction	RAC3, RAS-related C3 botulinum substrate 3	*Mus musculus*	93	1,00e-26	H	N
Contig 04	Metabolism	Cholestanol delta-isomerase	*Aspergillus fumigatus*	60	2,00e-09	L	X
Singlet 46	Metabolism	ETF-QO†	*Aspergillus terries*	92	7,00e-24	H	X
Singlet 14	Hypothetical	Unknown	*Asperfillus oryzae*	100	1,00e-04	L	X
Singlet 31	Hypothetical	Unknown (Af293 hypothetical protein)	*Aspergillus fumigatus*	94	3,00e-22	H	X
Singlet 27	Hypothetical	Unknown (hypothetical protein 112.t00017)	*Coccidioides immitis*	61	5,00e-25	H	X
Contig 27	Hypothetical	Unknown (Filament-forming protein Tpr/p270)	*Entamoeba histolytica*	43	6,00e-04	L	X
Contig 29	Hypothetical	Unknown hypothetical protein CIMG_01064)	*Coccidioides immitis*	97	5,00e-14	M	X
Singlet 42	Hypothetical	Unknown (unnamed protein product)	*Aspergillus oryzae*	65	1,00e-19	M	X
Singlet 20	Hypothetical	Unknown (contig sequence: AoEST3076)	*Aspergillus oryzae*	97	9,00e-10	L	N

I, Identity Percentage; E, Error Probability; S, Similarity level (H, high; M, Moderate; L, Low) *Characterization of the sequences was done using BLAST analysis against Fungi-EST data base in EMBL (http://www.ebi.ac.uk /blast2/nucleotide.html). The remaining 13 sequences did not shown homology with known sequences and are not described here because lack of valuable information about them. †Electron transfer flavoprotein-ubiquinone oxidoreductase.

ETF-QO accepts electrons from the electron-transfering flavoprotein that is located in the mitochondrial matrix and acts at reducing ubiquinone in the mitochondrial membrane. This protein has an important role in fatty acid metabolism, in the catabolism of some amino acids and in the mitochondrial 'one-carbon cycle' [30,31] but its importance *in P. brasiliensis* transition process must be further established.

The evaluation of our library against those corresponding to the EMBL – EBI databases also showed some sequences shared with several of those reported by Bastos *et al*. in their M-Y transition library [18]. Thus, 23 (29.1%) sequences exhibited similarities with those reported in this M-Y transition library (Table 2) and, as such, could be considered exclusive of the biological mechanisms by which conidia or mycelial fragments convert to yeast cells. The most important representative of this group is a guanine nucleotide binding protein 13 (gamma subunit), but among others an array of 11 unknown proteins and 1 hypothetical protein, have also been found.

The G proteins subunits α and β have been described in *P. brasiliensis* as molecules involved in the cAMP signal transduction pathways which is involved in the events needed to control phenotype switching and transition [32,33]. Gγ clones were found in the C-Y transition library. These sequences, not previously described in either the individual M or Y libraries, have also been reported in the M-Y transition library [10,11,18]. Fernandes *et al*. questioned their absence based on the fact that the activity of this protein depends on the existence of the three constituting subunits, Gα, Gβ and Gγ [33]. In addition, Nunes *et al*. found that some genes of this family were up-regulated and appeared related to cell cycle, intracellular signaling and signal transduction, and were included in the Gα and Gβ subunits of the G proteins [14]. Thus, G proteins, as well as all their subunits, seem to play a fundamental role during morphogenesis. Nevertheless, new studies on G proteins subunits and their partners in the signal transduction pathways are needed in order to evaluate at the expression and functional level of their relevance during the transition C-Y and M-Y processes.

In addition to the M-Y shared genes, 17 (21.5%) of our sequences were shared with certain genes described in the M and Y stages [10,11]. From these, three were detected exclusively in Goldman's libraries [11] (Table 3A), eight in Felipe's libraries [10] (Table 2B), while six were present in both (Table 3C). One of the most representative sequences is the putative response regulator receiver SKN7, as well as several unknown sequences. The putative response regulator

Table 2 *Paracoccidioides brasiliensis* C-Y transitional sequences ($n = 23$) sharing similarities with those described in the M-Y transition library [18]*

			EST-Bastos 2006		
Cluster	Function	Possible protein	I	E	S
Singlet 41	Signal transduction	Guanine nucleotide binding protein 13, gamma	73	5.6e-05	L
Contig 17	Signal transduction	Guanine nucleotide binding protein 13, gamma	79	5.6e-08	L
Contig 30	Signal transduction	Guanine nucleotide binding protein 13, gamma	75	5.6e-05	L
Singlet 40	Signal transduction	Guanine nucleotide binding protein 13, gamma	77	3.6e-07	L
Singlet 24	Signal transduction	Regulator of G-protein signalling 13	68	1.6e-05	L
Contig 15	Signal transduction	RIKEN cDNA C030046I01 gene	88	1.5e-05	L
Contig 03	Metabolisms	P17F11 protein – nudix	86	1.1e-04	L
Contig 24	Metabolism	P17F11 protein – nudix	94	3.4e-05	L
Contig 32	Hypothetical	Unknown (Hypothetical protein 112.t00017)	94	3.1e-07	L
Singlet 07	Structural	Ladinin	88	7.6e-05	L
Singlet 23	Structural	Ladinin	90	8.1e-05	L
Contig 19	Cellular cycle	Calcyclin	95	2.2e-06	L
Contig 07	Unknown	Unknown	92	3.7e-05	L
Contig 08	Unknown	Unknown	86	2.5e-10	L
Contig 14	Unknown	Unknown	79	1.4e-05	L
Contig 22	Unknown	Unknown	95	2.8e-05	L
Contig 25	Unknown	Unknown	94	1.3e-05	L
Contig 31	Unknown	Unknown	93	2.6e-04	L
Contig 38	Unknown	Unknown	96	5.1e-05	L
Contig 39	Unknown	Unknown	82	7.8e-06	L
Singlet 01	Unknown	Unknown	81	5.9e-06	L
Singlet 26	Unknown	Unknown	77	6.9e-08	L
Singlet 34	Unknown	Unknown	79	2.9e-08	L

I, Identity Percentage; E, Error Probability; S, Similarity level (H, high; M, Moderate; L, Low).
*Characterization of the sequences was done using BLAST analysis against Fungi-EST data base in EMBL (http://www.ebi.ac.uk /blast2/nucleotide.html).

receiver SKN7, serves as a transcriptional factor in a two-component regulators signaling pathway [34,35]. It is believed that in eukaryotic microorganisms, SKN7 is a possible transcription factor for heat shock proteins essential for stress adaptation and regulation of virulence in bacteria and certain fungi. In *Saccharomyces cerevisiae* some functions had been proposed such as a role in adaptation to oxidative and thermal stresses [36]. These features postulate this protein as a candidate for an important role in the development of the C-Y transitional process.

Of interest, a comparison of our result with those reported by Nunes *et al*. indicate that three sequences in our C-Y library exhibit homology with those reported in Nunes's microarray studies, and from these only one, singlet 04 (VP1-Pb30001-211E01) – corresponding to an unknown protein – had differential expression levels during the M-Y transition [14] and these sequences have to be further studied to demonstrate its importance.

The evaluation of *P. brasiliensis* early transcriptional gene responses after host interaction *in vitro* [37], and early transcriptional responses after host interaction *in vivo* and with human blood [38,39] have also been done. These studies have allowed the identification of an important number of possible genes associated with pathogenesis. Comparing the above approaches with our C-Y library results, similarities can not be found among our sequences and those found by Tavares *et al.,* Bailao *et al*., and Costa *et al*. [37–39], suggesting that the mechanisms linked to the C-Y transition and those involved in survival inside macrophages and other pathogenic process could be distinct. However, further assays mimicking these host-pathogen interacting environments are necessary to clarify this issue.

As a first proof to confirming the transcription of the found genes during the transition process, some sequences from the library were selected to perform RT-PCR. Those were cholestanol delta-isomerase (Contig 04), putative response regulator receiver (SKN7) (Singlet 52), electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO) (Singlet 46) and one hypothetical protein (Singlet 44). All

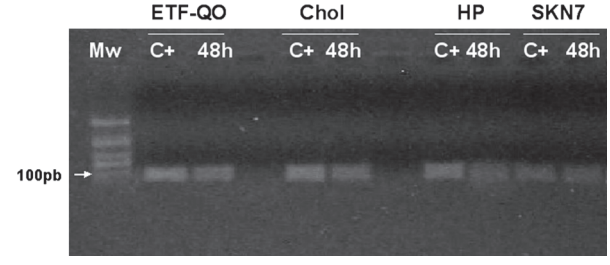

Fig. 2 Amplification products from RT-PCR of Electron Transfer Flavoprotein-Ubiquinone Oxidoreductase – ETF-QO (Siglet 46), Cholestanol delta Isomerase – Chol (Contig 04), Hypothetical Protein – HP (Singlet 44), and Putative Response Regulator Receiver – SKN7 (Singlet 52). Positive amplification control (C+) corresponds to the amplification with each primer of genomic *Paracoccidioides brasiliensis* DNA form yeast cells. 48 h corresponds to cDNA produced from mRNA from 48 h C-Y transition process. MW: molecular weight marker (Ladder markers 100pb – CIB, Medellín Colombia).

Table 3 *Paracoccidioides brasiliensis* C-Y transitional process: Sequences sharing similarities with those described in mycelia and yeast libraries*. (A) Sequences present in Y library (11). (B) Sequences present in either M or Y libraries (10). (C) Sequences present in both libraries (10,11).

(A)

			EST-Goldman 2003		
Cluster	Function	Possible protein	I	E	S
Contig 42	Metabolism	Glycine dehydrogenase	98	8.6e-64	H
Singlet 36	Stress Response	HSP 70	77	4.0e-20	H
Singlet 04	Unknown	Unknown	96	1.8e-13	M

(B)

			EST-Felipe 2003		
Cluster	Function	Possible protein	I	E	S
Singlet 48	Hypothetical	Unknown (Hypothetical protein CIMG_10240)	90	1.1e-28	H
Contig 18	Hypothetical	Unknown (Hypothetical protein ATEG_08779)	93	9.9e-50	H
Singlet 44	Hypothetical	Unknown (Hypothetical protein CIMG_09427)	94	5.7e-76	H
Singlet 45	Hypothetical	Unknown (Hypothetical protein CIMG_01739)	94	2.4e-52	H
Singlet 49	Hypothetical	(Unnamed protein product)	96	9.2e-92	H
Singlet 52	Signal transduction	Putative response regulator receiver SKN7p	94	2.2e-54	H
Contig 21	Structural	MFS peptide transporter	94	4.5e-47	H
Singlet 43	Unknown	Unknown	81	3.4e-09	L

(C)

			EST-Goldman 2003 (11)			EST-Felipe 2003 (10)		
Cluster	Function	Possible protein	I	E	S	I	E	S
Contig 35	RNA synthesis	Arginine/serine-rich splicing factor, RSp40	99	9.9e-20	H	90	1.8e-15	M
Contig 28	Stress Response	HSP 90	92	1.0e-33	H	91	3.5e-33	H
Singlet 47	Signal transduction	Putative protein tyrosine phosphatase (Pyp1)	94	1.8e-58	H	95	7.9e-78	H
Contig 23	Unknown	Unknown	95	1.6e-11	M	88	6.1e-09	L
Contig 43	Unknown	Unknown	96	5.2e-38	H	81	1.6e-50	H
Contig 41	Unknown	Unknown	96	6.0e-65	H	84	7.0e-25	H

I, Identity Percentage; E, Error Probability; S, Similarity level (H, high; M, Moderate; L, Low).
*Characterization of the sequences was done using BLAST analysis against Fungi-EST data bases in EMBL (http://www.ebi.ac.uk /blast2/nucleotide.html).

genes studied were expressed during the 48 h C-Y thermal transition since the PCR products were visible in the cDNA samples (Fig. 2). The BlastX analysis of the sequenced amplicons confirms the identity of all of them (data not show). Also, these sequences corresponded to specific genes in the *P. brasiliensis* genome annotated by the Broad Institute (http://www.broad.mit.edu/annotation/genome/paracoccidioides_brasiliensis/MultiHome.html). These findings suggest that all the screened genes are actively expressed at least during the 48h of the C-Y transitional process. Nevertheless a deeper analysis of this genes and other found in this research have to be done to confirm their implications in this process.

Conclusion

In conclusion, 79 different gene sequences were identified during the C-Y transition and were classified. Within them, 39 novel exclusive sequences associated to the dimorphic C-Y transitional stages were described. Even though the identification and demonstration of the presence of these sequences during this process, their relevance and specific function in this biological phenomenon remain to be elucidated. New experiments, currently under study in our laboratory using reverse transcription real time PCR (qPCR), would allow the determination of the specific expression of particular sequences at various moments during the transitional C-Y process to further ascertain their importance. Additionally, the future genetic manipulation of *P. brasiliensis* such as generation of stable mutant knockouts of these genes or the modulation of gene expression levels using RNA interference (RNAi) or antisense RNA [40,41], will be fundamental to understanding their role in the dimorphic process itself or their relevance as virulence factors.

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Conflict of interest: None

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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