In silico identification of GPI-anchored proteins in Paracoccidioides

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ABSTRACT

Glycosylphosphatidylinositol-anchored proteins (GPI-proteins) are widely found in eukaryotic organisms. In fungi, GPI-proteins are thought to be involved in diverse cellular mechanisms such as cell wall biosynthesis and cell wall remodeling, adhesion, antigenicity, and virulence. The conserved structural domains of GPI-protein allow the utilization of *in silico* prediction approach to identify this class of proteins using a genome-wide analysis. We used different previously characterized algorithms to search for genes that encode predicted GPI-proteins in the genome of *P. brasiliensis* and *P. lutzii*, thermal dimorphic fungi that causes paracoccidioidomycosis (PCM). By using these methods, 98 GPI-proteins were found in *P. brasiliensis* with orthologs in *P. lutzii*. A series of 28 GPI-proteins were classified in functional categories (such as glycoside hydrolases, chitin-processing proteins, and proteins involved in the biogenesis of the cell wall). Furthermore, 70 GPI-proteins exhibited homology with hypothetical conserved proteins of unknown function. These data will be an important resource for the future analysis of GPI-proteins in *Paracoccidioides spp.*

Keywords: Paracoccidioidomycosis, *Paracoccidioides brasiliensis*, *Paracoccidioides lutzii*, Cell wall, Glycosylphosphatidylinositol-anchored proteins

1. Introduction

In pathogenic fungi, cell wall components mediate the contact, adhesion, and invasion of the host tissue (Levitz, 2010) which is characterized by a distinctive composition depending on the morphological phase. The covalently linked glycosylphosphatidylinositol (GPI)-proteins represents the main class of proteins in the cell wall. Members of this group are pivotal players in cell wall biogenesis and remodeling and in essential pathogenic processes such as adhesion to and degradation of host tissues (Hube and Naglik, 2001; Martchenko et al., 2004; Richard et al., 2002; Richard and Plaine, 2007; Sundstrom, 2002). All GPI-proteins show common characteristics such as a C-terminal consensus sequence for GPI modification and an N-terminal signal peptide for translocation across the membrane of the endoplasmic reticulum. In addition, many GPI-proteins are heavily glycosylated. These well-defined characteristics allow the use of *in silico* approaches for screening and identification of GPI-proteins in the genome (De Groot et al., 2003; Eisenhaber et al., 2004).

The *Paracoccidioides* genus includes species of thermodimorphic pathogenic fungi that cause paracoccidioidomycosis (PCM) which is the most prevalent systemic human mycosis occurring in Latin America. Multilocus and genomic sequencing studies indicate the existence of two distinct species within the *Paracoccidioides* genus, *P. brasiliensis* and *P. lutzii* (4). *P. brasiliensis* is comprised of distinct lineages (S1, PS2, PS3, and PS4) that cause most of PCM cases throughout South America (4–6). *Paracoccidioides lutzii* isolates are found predominantly in north, central and southwest of Brazil and Ecuador (Teixeira et al., 2009; Theodoro et al., 2012).

The complete genome sequence of three strains (Pb18, Pb03 and Pb01-like) from the *Paracoccidioides* genus (Desjardins et al., 2011a; Munoz et al., 2014) have been recently available. The genomic information has allowed the identification, in *P. brasiliensis* and *P. lutzii*, of a group of 62 predicted GPI-proteins based on an *in silico* analysis using one program of prediction (Desjardins et al., 2011a, b). Based in the current genome sequence of *P. brasiliensis* and *P. lutzii* we used five algorithms to look for additional GPI-proteins that may not have been detected by the previous genome analysis. The identification of new *P. brasiliensis* GPI-proteins may reveal targets to be used in the future for the development of new diagnostic tools, drugs, and immunologic tests.

2. Material and Methods

In silico identification of predicted GPI-proteins and transcription analysis

The pipeline for the identification of GPI-protein coding genes consisted of the following steps: (1) A proteome file containing 8741 ORF sequences of *P. brasiliensis* strain Pb18 and 9.132 ORF sequences of *P. lutzii* (Desjardins et al., 2011a, b) were downloaded from the Broad Institute website (<http://www.broad.mit.edu>) last accessed on March 2013. These proteomes were searched for proteins carrying a GPI anchor signal peptide (omega site) using five different methods: one developed by de Groot and collaborators (De Groot et al., 2003), Big-PI ([http:// Eisenhaber et al., 2004](http://Eisenhaber%20et%20al.,%202004)), GPI-SOM ([http:// Fankhauser and Maser, 2005](http://Fankhauser%20and%20Maser,%202005)), PredGPI ([http://Pierleoni et al., 2008a, b](http://Pierleoni%20et%20al.,%202008a,%20b)) and FragAnchor ([http:// Poisson et al., 2007](http://Poisson%20et%20al.,%202007)). (2) predicted-GPI proteins detected by at least two methods were selected for further analysis. Protein sequences from *P. brasiliensis* and *P. lutzii* that presented mismatches after alignment by ClustalW were reannotated (see the following section) (3) Proteins were searched for the presence of an N-terminal ER-import signal by SignalP V3.0 software available at (<http://www.cbs.dtu.dk/services/SignalP-3.0/>). The standardized threshold value of 0.5 was applied for signal peptides in the two algorithms [SignalP-NN (Smean) and SignalP-HMM (Sprob)]. (4) The presence of internal membrane domains and protein localization was investigated using TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>) and PSORTII (<http://psort.nibb.ac.jp>).

Predicted GPI proteins were used as queries in BLASTp searches (Altschul et al., 1990) to identify sequence similarities in 17 other fungal genomes (Cuomo and Birren, 2010). After the alignment with *ClustalW* (<http://www.genome.jp/tools/clustalw>), we considered orthologues when their protein sequence presents > 30 % of amino acid identity in a region encompassing > 60 % of the whole sequence. BLASTP searches were performed at NCBI (<http://www.ncbi.nlm.nih.gov/blast>).

For transcription analysis, ESTs were manually searched for hits in the Broad Institute database.

Annotation and manual curation of GPI-protein coding genes

Genomic DNA sequences encompassing ORFs coding for GPI-anchoring signals – from 1000 bp upstream the start codon to 1000 bp downstream the stop codon - were analysed by five programs to determine the exon-intron map: *Augustus* ([http://bioinf.uni-](http://bioinf.uni-greifswald.de/augustus/)
greifswald.de/augustus/), *Fgenesh* (<http://linux1.softberry.com>), *GeneMark*

(<http://opa.biology.gatech.edu/GeneMark/>), *Geneid* (<http://genome.crg.es/geneid.html>) and *Genscan* (<http://genes.mit.edu/GENSCAN.html>),. The consensus gene sequence from all prediction strategies was aligned by *ClustalW* with the orthologues of *P. brasiliensis* (Pb18, Pb03) and *P. lutzii* (Pb01) whose sequences are from the NCBI fungal data bank. The annotated sequences were re-analyzed to confirm the presence of GPI-anchoring signal and signal peptide, and absence of transmembrane domains as described in the previous section.

3. Results and Discussion

In silico identification of GPI proteins in *P. brasiliensis*

Five different *in silico* methods were used to comprehensively identify putative GPI proteins in the *P. brasiliensis* and *P. lutzii* genome (De Groot et al., 2003; Eisenhaber et al., 2004; Fankhauser and Maser, 2005; Pierleoni et al., 2008a; Poisson et al., 2007). The resulting genomic sequences were reannotated when there was a discrepancy in the exon-map structure after alignment with conserved genomic sequences from close relatives (e.g *P. brasiliensis* X *P. lutzii*; see materials and methods and supplementary data). We identified 28 GPI-proteins that can be classified into 5 functional categories, 70 conserved proteins with unknown functions, in a total of 98 GPI-proteins (Table 1). The ratio of predicted GPI-proteins (1.12%) to the proteome is similar to that observed in filamentous fungi and yeasts (de Groot et al., 2009; de Groot et al., 2003; Desjardins et al., 2011; Eisenhaber et al., 2004). We did not find differences in the number of GPI-proteins homologs between *P. brasiliensis* and *P. lutzii*.

Synthesis and processing of glycan, chitin and other enzymatic processes of the cell wall

Sixteen of the predicted GPI-proteins have homology with glycosyl hydrolases (GH), (Cantarel et al., 2009; Lairson et al., 2008). Three proteins belong to the GH72 family or Gel/Gas/Phr of 1,3- β - glucanosyltransferases (Castro Nda et al., 2009; Mouyna et al., 2000; Popolo et al., 2008). Four GPI-proteins belong to the Crh (GH16) family which consists mainly of 1,3- β -glucanases and 1,3-1,4- β - glucanosyltransferases (Cabib et al., 2008). Three members of the Dfg5 (GH76) family were also identified, and they are endomannanases with a putative role in the incorporation of GPI-proteins to the cellular wall (Maddi et al., 2012). An α -amylase (GH13) is present and has a putative role of in the processing of 1,3- α -glucana/1,4- α -glucana (Camacho et al., 2012). The PbPga1 is a cell wall protein with immunogenic properties and is recognized specifically by serum of patients with paracoccidioidomycosis (Valim et al., 2012).

The presence of a lysozyme (GH23) is interesting. The biological function of this enzyme in fungi has not yet been determined. It can be speculated that the molecule can serve as a tool in the breakage of the bacterial peptidoglycan for nutritional purposes (Korczynska et al., 2010). An N-acetyl- β -D-glicosaminidase (GH20) was identified (Santos et al., 2004) and is a member of the GH92 family. Finally, it was identified a member of the GH3 family, a glucosyl-transferase (GT1) and a cutinase (CE5). Cutinases are extracellular enzymes secreted by microorganisms to break the cellular wall of plants (Lin and Kolattukudy, 1980). The protein is possibly a false positive because these enzymes are secreted (de Vries, 2003).

Cell wall biogenesis

One of the proteins identified (Ecm33p) is thought to play an important role in cell wall biogenesis. This protein shares homology with the Ecm33p family, also a ubiquitous fungal GPI protein family. Deletion of *ECM33* in *S. cerevisiae* results in swollen cells that secrete increased levels of 1,6- β -glucosylated CWPs into the culture medium and are hypersensitive to drugs that interfere with cell wall biosynthesis (De Groot et al., 2001).

Proteins with varied functions

Four proteins share homology with fungal proteins not directly associated with the cell wall. We identified two superoxide dismutases (SOD). SOD converts superoxide radicals into hydrogen peroxide and molecular oxygen and is thought to be important to the pathogenesis of fungal infection. Two proteins share homology with hydrophobins, which in filamentous fungi play a role in the formation of aerial hyphae and in the attachment of hyphae to hydrophobic surfaces. The protein PbPga1 was first identified by homology with a predicted GPI-protein of *Histoplasma capsulatum*. PbPga1 has been characterized as a cell surface protein recognized by sera from patients with paracoccidioidomycosis (Valim, 2012)

Conservation of *Paracoccidioides brasiliensis* GPI- proteins

We analyzed the conservation of predicted GPI proteins of known function in 17 different fungal genomes. These proteins represent the most conserved proteins (Fig 1) and are involved in different biosynthetic and processing pathways (glycosylases, proteases and lipases). On the other hand, the less conserved proteins (Fig 2) are those with unannotated function. These proteins may not have catalytic roles in the cell walls. Many of the identified proteins with unknown function are expressed as evidenced by EST hits in databases (Table 1)

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Table 1 – Predicted GPI proteins of *Paracoccidioides*

<i>P. brasiliensis</i> ORF ID	<i>P. lutzii</i> ORF ID	Size (aa - Pb/PI)	Identity (%)	Big-PI (Pb/PI)	FragAnchor (Pb/PI)	PredGPI (Pb/PI)	DeGroot (Pb/PI)	GPI-SOM (Pb/PI)	SignalP (Pb/PI)	EST	function/family
Carbohydrate modifying enzymes and resident proteins											
04499	07670	370/389	95.7	+	H	H	+	+	-/+	45/47	ECM33
02460	04708	225/225	79.2	+/+	H/H	H/H	+/+	+/+	+	9/7	PbPga1
04918	05068	529/529	97.1	+	H	H	+	+	+	29	GH2
05076	05245	471/471	98.1	+	H	H	+	+	+	6	GH72 (Gel)
04328	06867	224/224	94.6	+	L/H	W/P	+	+	-	-	GH16 (Crh)
04416	06779	436/435	77.7	+/-	L/-	H/-	+/-	+/-	+	5	GH16 (Crh)
06728	05380	754/743	96.9	+/-	L/-	H/-	+	+	+	-	GH20
03691	01139	432/431	92.1	+/+	-	H/P	+/+	+/+	+	-	GH16 (Crh)
04432	06764	532/531	92.8	+/-	H/H	P/H	+/+	+/+	+	3	GH13 (Alpha-amilase)
04693	04534	459/459	97.6	-/+	H	-/W	+	+	+	8	GH76 (DCW1)
05063	05192	443/506	92.5	-	P/P	-	-	+	-	-	GT1
01494	06960	470/471	88.7	-/-	-	H/H	+/+	+/+	+	-	GH76 (DCW1)
03286	03782	445/443	79.4	-/+	-/P	H	-	+	+	3	GH72 (Gel)
07673	05717	323/323	95.4	-	-	H/W	-	-	+	-	GH76 (DCW1)
00483*	01798	862/852	95.6	-	-	-	-/-	-/+	+/-	-	GH3
00795	02100	446/446	97.3	-	-	-	+	+	+	3	GH72 (Gel)
03601	01224	265/363	25.7	-	-	-	+/-	-	-	-	GH16 (Crh1)
04617	04461*	234/237	93.6	-	-	-	+	-/+	+/-	1	CE5 (cutinase)
06344	03482	796/785	96.5	-	-	-	+	-	+	5	GH92
06465	03359	230/230	90.0	-	-	-	+	-	+	-	GH23 (lysozyme)
Aspartic proteases											
06207	03619	513/469	70.4	-/+	-/P	-/H	-/+	-/+	+	2	Pepsin-like proteinase
08282	07583	481/481	95.6	+/-	P/HP	P/HP	+/+	+/+	-	2	Pepsin-like protease
Phospholipases											
01064	06610*	1067/1004	96.3	-	-	-	+	+	-	7/9	Phospholipase D Active site
03299	03796	1424/1398	93.3	-	-	-	+	+	+	4/4	phospholipase C X domain
Hydrophobins											
08524	-	419		+	L	H	+	+	+	-	Hydrophobin
07472	04235*	133/133	100	-	-	P/P	+	-	+/+	2829	Hydrophobin 2

Table 1 (cont.)

<i>P. brasiliensis</i> (Pb)	<i>P. lutzii</i> (PI)	Size (aa - Pb/PI)	Identity (%)	Big-PI (Pb/PI)	FragAnchor (Pb/PI)	PredGPI (Pb/PI)	DeGroot (Pb/PI)	GPI-SOM (Pb/PI)	SignalP (Pb/PI)	EST	function
Superoxide dismutases											
02842	02971	227/228	74.4	+	P/P	H	-/+	+	+	-	Superoxide dismutase
07418	04164	154/154	95.4	-	-	-	+	-	+	-	Superoxide dismutase
Unknown											
05000	05158	184/184	90.2	+	H	H	+	+	+	3	CFEM superfamily
06374	02225	241/435	19.1	+	H/P	H/P	+	-	+	46	CFEM superfamily
03909	00918	135/135	82.2	+	P/-	H	+	+	+	154/9	CFEM superfamily
05363	02225	289/281	84.1	+	P/P	H/H	+	+	+/+	-	CFEM superfamily
00802	02093	728/723	92.2	+	-	H/P	-	+	+	-	CFEM superfamily
03914	00912	189/189	96.3	+	H	H/-	+	+	+	9/154	unknown; CRoW
04649	04490	228/231	95.6	+/+	H/H	H/+	+/+	+/+	+	239	Conserved unknown
06613	05264	245/248	91.8	+/+	H	H	+	+	+	-	Conserved unknown
02867	02996	251/237	47.6	+	H	H	+	+	+	856	Conserved unknown
07354	04085	244/244	98.3	+	H	H	+	+	+	4	Conserved unknown
04509	07681	156/156	84.0	+	H/P	H	+	+	+	56	Conserved unknown
02770	07767*	328/328	93.6	+/+	H/H	H/H	+/+	+/+	+	6	Conserved unknown
00497	01813	219/229	81.3	+	H	H	+	+	+	31/16	Conserved unknown;
07758	05636	158/161	75.9	+	P/H	H	+	+	+	72	Conserved unknown
03600	01225	196/195	83.6	+/+	P/H	H/P	+/+	+/+	+	-	Conserved unknown
00984	07227	233/153	90.2	+	P/H	H	+	+	+	2	Conserved unknown
06677	05334	233/227	84.6	+	P	P	+	+	+	78	Conserved unknown
03799	01033	202/208	95.0	-	P	P	+	+	+	20	Conserved unknown
02955	03079*	281/281	91.4	+/-	L/-	-	+/-	+/-	+	0/5	Conserved unknown
03262	03757	282/309	82.9	+	L/P	H	+	+	+	3/4	Conserved unknown
05482	02344	224/232	93.7	+	L/H	P	+	+	+	10/2	Conserved unknown
00773	02119	350/168	70.2	+	H	H/P	-	+	+	8/3	Conserved unknown; proline rich
02569	04829	217/220	90.7	+	H	H	-	+	+	76	Conserved unknown
01837	02807	182/172	88.3	-/+	-/H	-/P	-/+	+	+	36	Conserved unknown
00450	06528	224/229	90.6	+/-	L	H/-	-	+	+	1	Conserved unknown
07141	00119	534/534	96.0	-	-	-	-	+	+	-	Conserved unknown

Table 1 (cont.)											
<i>P. brasiliensis</i> (Pb)	<i>P. lutzii</i> (Pl)	Size (aa - Pb/Pl)	Identity (%)	Big-PI (Pb/Pl)	FragAnchor (Pb/Pl)	PredGPI (Pb/Pl)	DeGroot (Pb/Pl)	GPI-SOM (Pb/Pl)	SignalP (Pb/Pl)	EST	function
02604	04863	367/389	87.1	-	H/P	H	+	+	+	1/1	Conserved unknown
01394	06493	273/272	89.3	-	H	P	-	+	+	10	Conserved unknown
02487	04745	441/436	89.9	-	H	P	-	+	+	-	Conserved unknown
02535	04795	121/121	86.8	-	L	-	+	+	-	32/43	Conserved unknown
06557	09093	727/724	85.6	-	-/P	H	+	+	+	-	Conserved unknown
06704	05361	600/628	95.0	-	-	P/(W)	+	+	+	-	Conserved unknown
04289	08312	204/204	97.0	-/+	-	P	+	+	+	86/85	Conserved unknown
04623	03197	308/492	37.3	-	-	W/-	-	-	+	-	Conserved unknown
07877	00250	126/126	100.0	-	-	-	+	-	+	-	Conserved unknown
00455	06522	509/508	95.6	-	-	-	+	U/+	+	-	Conserved unknown; DUF185
06524*	08951	437/437	89.2	-	-	-	-/-	-/-	-/-	-	Conserved unknown
04816	00701	149/322	23.4	-	-	-	+/-		+	-	Conserved unknown; DUF3435
03732	01098	423/337	89.0	-	-	-	+	+	+	9/8	Conserved unknown
00118	04530	162/329	16.0	-	-	-	+/-	U/-	+	-	Conserved unknown
02815	07721	128/190	23.4	-	-	-	+/-	-	+	-	Conserved unknown
00406	01364	125/127	65.6	-	-	-	-/-	-/-	-/-	-	Conserved unknown
08385	08073	139/139	96.4	+	H	H	+	+	-	72	Conserved unknown
03926	00898	178/178	93.2	+	P	W	-	+	-	-	Conserved unknown
03750	01075*	517/505	96.2	+ /P	P/P	-	+ /+	-	-	3	Conserved unknown
06896*	02575	398/450	94.4	+ /+	-	-	+ /+	+ /+	-	-	Conserved unknown; DUF3722
07778	05614*	396/289	95.1	+ /+	-	- /-	+ /+	+ /+	-	11/13	Conserved unknown
00058	01719	98/435	68.4	+ /-	+ /-	+ /-	+ /-	+ /-	-	1	Conserved unknown
05186*	07493	518/429	90.6	+	-	-	-	+	-	-	Conserved unknown
03942	00890	304/304	96.0	-	P	P		+	-	1	Conserved unknown; DUF1640
03510	01314	97/97	96.9	-	P	-	+	+	-	-	Conserved unknown
01219	06319*	723/697	91.8	-	L/(L)	-	+ /+	+ /+	-	-	Conserved unknown
03777	01053	686/686	95.3	-	H	-	-	+	-	-	Conserved unknown

00231	01547	263/263	97.3	-	H	-	-	-	-	-	-	Conserved unknown
06057*	05937	424/421	94.0	-	-/-	-	-	-/-	-	-	-	Conserved unknown
04535	04363	142/370	40.8	-	L/-	-	-	+/-	-	-	-	Conserved unknown
00551	01876	756/736	90.4	-	L/-	-	-	+/-U	-	-	-	Conserved unknown
05519	-	67		-	L	-	+	-	-	-	-	Conserved unknown
08345	08031	303/266	86.8	-	L/-	-	-	+/-	-	-	-	Conserved unknown
02992	03117*	451/484	90.0	-/+	-	H/-	+	+	-	-	-	Conserved unknown
01591	07061	591/568	92.9	-	-	-	+	-/+	-	-	-	Conserved unknown; DUF3480
04859	05011*	142/142	87.3	-	-	-	+	-	-	-	-	Conserved unknown
05013	05173	214/210	93.3	-	-	-	+/-	+/-	-	-	-	Conserved unknown
05708	05555	974/1226	88.9	-	-	-	+	-	-	-	-	Conserved unknown;
07277	08671	488/503	40.1	-	-	-	+/-	+/-	-	-	-	Conserved unknown
04284	07517	59/130	22.0	-	-	-	+	-	-	-	-	Conserved unknown
07635	05753	565/565	94.3	-	-	P/W	-/-	+	-	-	-	Conserved unknown
07754	-	78		-	-	-	+	-	-	-	-	Conserved unknown
08596*	09050	336/333	87.3	-	-	-	-/-	+	-	-	-	Conserved unknown

HP= Highly Probable; P = Probable; L = Low Probable; W = Weakly Probable

SignalIP: + secretion signal, – no secretion signal not present

GH: glycosylhydrolase

<i>Pb</i>	<i>Pl</i>	Name/function	<i>B. dermatitidis</i>	<i>H. capsulatum</i>	<i>C. immitis</i>	<i>M. canis</i>	<i>T. rubrum</i>	<i>A. nidulans</i>	<i>P. marneffei</i>	<i>U. reesii</i>	<i>N. crassa</i>	<i>F. oxysporum</i>	<i>M. grisea</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>	<i>S. pombe</i>	<i>C. neoformans</i>	<i>U. maydis</i>	<i>R. oryzae</i>
ORF																			
PADG_07418	PAAG_04164	SOD																	
PADG_06688	PAAG_05346	peptidase																	
PADG_00400	PAAG_01372	GH3																	
PADG_00483	PAAG_01798	GH3																	
PADG_02814	PAAG_07722	PLC																	
PADG_03306	PAAG_03802	GT62																	
PADG_02190	PAAG_00495	GT2																	
PADG_05076	PAAG_05245	GH72																	
PADG_05063	PAAG_05192	GT1																	
PADG_04299	PAAG_06899	GH47																	
PADG_01954	PAAG_02926	SOD																	
PADG_06700	PAAG_05357	GH28																	
PADG_06728	PAAG_05380	GH20																	
PADG_03299	PAAG_03796	PLC																	
PADG_03286	PAAG_03782	GH72																	
PADG_04918	PAAG_05068	GH2																	
PADG_06207	PAAG_03619	Aspartyl protease																	
PADG_04693	PAAG_04534	GH76																	
PADG_04499	PAAG_07670	ECM33																	
PADG_04416	PAAG_06779	GH16																	
PADG_04328	PAAG_06867	GH16																	
PADG_04617	PAAG_04461	CE5																	
PADG_04435	-	cutinase																	
PADG_04432	PAAG_06764	α -amilase																	
PADG_00795	PAAG_02100	GH72																	
PADG_06344	PAAG_03482	GH92																	
PADG_07472	PAAG_04235	Hydrophobin2																	
PADG_01494	PAAG_06960	GH76																	
PADG_02842	PAAG_02971	SOD																	
PADG_08282	PAAG_07583	Aspartyl protease																	
PADG_07673	PAAG_05717	GH76																	
PADG_00012	PAAG_01763	Carboxypeptidase																	
PADG_04195	PAAG_00636	Dioxygenase																	
PADG_07620	PAAG_05768	Unknown?																	
PADG_01014	PAAG_06556	Poly (ADP-ribose) glycohydrolase																	
PADG_08524	-	hydrophobin																	
PADG_02460	PAAG_04708	Pga1																	

Fig 1. Presence of homologs for GPI proteins with known function.

GT62 = α -1-6 mannosyltransferase

GT2 = dolichyl-phosphate beta-glucosyltransferase

ORF(Pb)	ORF (PI)	<i>B. dermatitidis</i>	<i>H. capsulatum</i>	<i>C. immitis</i>	<i>M. canis</i>	<i>T. rubrum</i>	<i>A. nidulans</i>	<i>U. reesii</i>	<i>F. oxysporum</i>	<i>N. crassa</i>	<i>M. grisea</i>	<i>P. marneffei</i>	<i>C. neoformans</i>	<i>S. cerevisiae</i>	<i>R. oryzae</i>	<i>U. maydis</i>	<i>S. pombe</i>	<i>C. albicans</i>
PADG_03942	PAAG_00890																	
PADG_03417	PAAG_03924																	
PADG_03750	PAAG_01075																	
PADG_07354	PAAG_04085																	
PADG_04289	PAAG_08312																	
PADG_04649	PAAG_04490																	
PADG_05000	PAAG_05158																	
PADG_01394	PAAG_06493																	
PADG_03691	PAAG_01139																	
PADG_03732	PAAG_01098																	
PADG_01219	PAAG_06319																	
PADG_05482	PAAG_02344																	
PADG_06704	PAAG_05361																	
PADG_06613	PAAG_05264																	
PADG_07778	PAAG_05614																	
PADG_02535	PAAG_04795																	
PADG_03015	PAAG_03134																	
-	PAAG_07679																	
PADG_00119	PAAG_01656																	
PADG_07758	PAAG_05636																	
PADG_6374	-																	
PADG_02955	PAAG_03079																	
PADG_04717	PAAG_04558																	
PADG_03277	PAAG_03775																	
PADG_02569	PAAG_04829																	
PADG_04509	PAAG_07681																	
PADG_02867	PAAG_02996																	
PADG_08385	PAAG_08073																	
PADG_02604	PAAG_04863																	
PADG_03909	PAAG_00918																	
PADG_01837	PAAG_02807																	
PADG_00497	PAAG_01813																	
PADG_02487	PAAG_04745																	
PADG_03799	PAAG_01033																	
PADG_06677	PAAG_05334																	
PADG_06418	PAAG_03418																	
PADG_05363	PAAG_02225																	
PADG_02992	PAAG_03117																	
PADG_00802	PAAG_02093																	
PADG_00984	PAAG_07227																	
PADG_03926	PAAG_00898																	
PADG_00450	PAAG_06528																	
PADG_00776	PAAG_02117																	
PADG_03914	PAAG_00912																	
PADG_06557	PAAG_09093																	
PADG_05279	PAAG_08515																	
PADG_02770	PAAG_07767																	
PADG_03262	PAAG_03757																	
PADG_03673	PAAG_01156																	
PADG_03600	PAAG_01225																	
PADG_00058	PAAG_01719																	
PADG_00773	PAAG_02119																	

Fig 1. Presence of homologs for GPI proteins with unknown function.