Proteomic analysis of a *Candida albicans pga1* Null Strain

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A R T I C L E   I N F O

A B S T R A C T

We previously characterized Pga1, a *Candida albicans* (*C. albicans*) cell wall protein necessary for proper virulence, adhesion, and resistance to oxidative stress. By utilizing tandem mass spectrometry coupled with bioinformatics to investigate cell wall proteome expression in a *pga1* null fourteen and 36 proteins were identified in the wild type grown under filamentous and non-filamentous conditions respectively, but were not detected in the mutant, including members of the PGA GPI anchored family. Virulence and adhesion proteins such as Hsp 90, Sap10, Cdc11, Int 3 and members of the lipase family were also identified exclusively in the wild type.

1. Introduction

The yeast *Candida albicans* is frequently inoffensive in humans, but has the ability to cause death, especially in immunocompromised individuals [1]. Known as the leading causative factor of fungal infection, *C. albicans* is the fourth most isolated pathogen in hospitalized patients [2]. Moreover, in the genus *Candida*, *C. albicans* is known to be the ultimate virulent species [3] since half of all candidiasis infections have *C. albicans* as their agent. These infections range from treatable superficial to lethal systemic infections [4]. At certain disease state, and once the patient is immunocompromised, *C. albicans* has the ability to overwhelm host defenses. Many physiological states like immunosuppressive therapy, diabetes, neutropenia, and pregnancy predispose individuals to severe candidiasis [5].

Various factors are responsible for the success of *C. albicans* as a pathogen. For instance, *C. albicans* is able to undergo a phenotypic switching between two main morphologies: round, yeast, cells and filamentous, hyphal cells. These two morphologies are responsible for distinct functions since the yeast form is needed for clonal expansion, while the virulent hyphal form is needed for invasive growth and dissemination. Both are thus necessary for successful infection [3]. Moreover, *C. albicans* has developed a wide arsenal of genes coding for virulence factors such as proteases, phospholipases, lipases and dismutases [6,7]. Many of these virulence elements are cell wall immunogenic factors. These factors are involved in distorting or digesting the membranes of the host to allow for successful invasion as well as in fighting reactive oxygen species produced by the host immune system [8]. Cell wall constituents are responsible for adhesion which is considered the first and the most significant step while establishing

infection, besides stress tolerance, morphological switching, antigenicity, and other virulence linked processes [9]. The moldable nature and organization of the cell wall is fundamental for infection. For example, hyphal cells show an increase in cell wall thickness reflected as an increase in chitin deposition, and expression of many hypha specific cell wall proteins that are not present in yeast cells [10]. In order to attach to the cell wall, most characterized adhesins such as Hwp1, Hwp2 and members of the agglutinin like sequence-Als protein family [11] utilize preformed glycosylphosphatidylinisotol anchor (GPI-anchor) joined post translationally to the C-terminus to attach to the cell surface and extend outward to interact with the host. Numerous examined adhesins have common structural characteristics: an N-terminal globular immunoglobulin-like, lecithin domain, serine-threonine-rich domain including preserved repeats and a C-terminal glycosylated trunk region that spread the active from the surface of the cell wall [12]. At least, 70 of the 115 GPI genes recognized in *C. albicans* have been mutated [13], and of the 15 mutant strains tested for virulence 12 have exhibited an obvious reduced virulence compared to the wild-type strains [14].

Putative GPI-anchored protein 1 (Pga1) is a 133 amino acid long cell wall protein induced during cell wall regeneration previously characterized in our lab by creating a *pga1* null mutant strain through homologous marker cassette recombination [15]. Pga1 was found to be involved in virulence, adhesion, biofilm formation, chitin deposition, and resistance to oxidative stress.

Since Pga1 is a cell surface protein we hypothesized that a cell lacking Pga1 would exhibit multiple defects mainly in the cell wall proteome and cell wall related processes. As such the aim of this study was to perform a proteomic analysis of the cell surface of a *pga1* mutant

using Tandem-MS to determine changes in cell surface protein expression that might explain the above-mentioned phenotypes. Accordingly, after cell wall isolation from the wildtype and mutant strains grown under filamentous and non-filamentous conditions, various enzymes and chemicals were used to fractionate the isolated proteins depending on the type of their cell wall incorporation into non-covalently linked and disulfide bound proteins, alkali labile proteins, and beta glucan associated proteins. Tryptic digestion was applied to the obtained fractions followed by Tandem-MS to the cleaned-up digests and subsequent analysis thus enabling the determination of cell wall proteins exclusive to each strain under each growth condition. Similar methods have previously been utilized to determine cell wall proteins of *C. albicans* and *C. glabrata* [16,17]. Secretome, plasma membrane proteome, and the effect of surface stress on the cell wall of *C. albicans* have also been studied by exploiting such techniques [18–20]. In addition, we have recently successfully applied this approach to identify expressed cell wall proteins from a *C. albicans DSE1* mutant strain [21].

2. Methods

2.1. Strain utilized

The *C. albicans* wild type strain RM1000 (*ura3Δ:_imm434/ ura3Δ:_imm434his1:hisG/his1:hisG*) [22] histidine and uridine auxotroph, and *pga1:URA3/pga1:HIS1* null strains were used in this study [15].

2.2. Media preparation and culture conditions

The *C. albicans* strains were full-grown on rich potato dextrose broth (PDB) liquid media (Hi Media, India) until exponential phase. Strains were incubated at 28 °C under aerobic conditions for non-filamentous growth. Filamentous culture utilized the same PDB media supplemented with 10% FBS and were incubated at 37 °C.

2.3. Cell wall isolation and protein extraction

Twelve independent cell wall extractions from each strain and for each condition (filamentous, non-filamentous) were performed. Cells were spun at 4000 rpm for 5 min, and were re-suspended in 5 mL Tris (5 mM, pH = 7.8). Protease Inhibitor Cocktail (6 μL, abcam ab65621) and cold glass beads were added. Vortexing (30 cycles) was performed for breakage as follows: 30 s on vortex then 30 s on ice. Samples turned orange due to a reaction of acidic cytosol with the Protease inhibitor. The efficiency of breakage was monitored under the microscope. The supernatant was poured off and pellet re-suspended in NaCl (40 mL, 1 M) and spun. The wash step was repeated 3–4X. SDS extraction buffer (50 mM Tris, 2% SDS, 100 mM Na-EDTA, 150 mM NaCl, pH 7.8) with β-ME (8 μL per 1 mL SDS extraction buffer) was added (0.5 mL buffer per 100 mg wet weight walls) and the pellet re-suspended. Tubes were boiled for 10 min and spun for 5 min at 3000 rpm. The supernatant was collected and analyzed for SDS extractable proteins. SDS extraction buffer and β-ME were added as before to re-suspend pellet. Samples were boiled, cooled, and spun for 5 min at 3000 rpm and suspended in water. Wash steps with water (Type 2+) were performed to remove excess SDS. The final pellet was frozen in liquid N_2 and freeze-dried. Lyophilized cell walls were stored at −20 °C until use.

2.4. Extraction of alkali labile cell wall proteins

The cell wall pellets were incubated with NaOH (30 mM) at 4 °C overnight. The mixture was then neutralized with aqueous acetic acid (30 mM) [23]. Samples were spun, supernatant was collected and subjected to tryptic digestion followed by MALDI-TOF-TOF analysis.

2.5. Glucanase treatment of cell wall pellets

To maximize the extraction of cell wall proteins, 10^8 cells were treated with 1 mg of Glucanase in sodium acetate buffer (1 mL, 150 mM, pH 5) at 37 °C overnight [24]. Cell numbers were estimated using spectrophotometric analysis. Supernatants were collected and subjected to tryptic digestion.

2.6. Tryptic digestion

The cell wall extracts were placed at 55 °C for 1 h in reducing buffer (10 mM DTT, 100 mM NH_4HCO_3). Samples were cooled to room temperature and spun. Pellets were incubated for 45 min at room temperature in the dark with an alkylating buffer (65 mM iodoacetamide, 100 mM NH_4HCO_3). A quenching solution (55 mM DTT, 100 mM NH_4HCO_3) was subsequently added to the samples for 5 min at room temperature. Samples were washed 5 times with ammonium bicarbonate buffer (50 mM). Pellets were re-suspended in ammonium bicarbonate (50 mM) and a trypsin solution was added (1 μg/μL). Samples were incubated at 37 °C for 16 h. Then, the samples were spun, and the supernatants were collected and prepped for Zip Tipping by adding TFA (0.1% V/V).

2.7. Peptide concentration

ZipTip C18 clean up tips were wetted in acetonitrile wetting solution and subsequently equilibrated in a 0.1% TFA HPLC water solution. Sample binding was performed by full pressing the pipette at least 10 times in the digest tube. The membrane was then washed in a 0.1% TFA HPLC water solution. The sample was eluted using 10 μL of elution buffer (0.1% TFA (v/v) in HPLC water/acetonitrile (1:1)).

2.8. MALDI TOF/TOF

Digested proteins were spotted on stainless steel target plate and overlaid with CHCA matrix solution and dried. MALDI-TOF-TOF MS spectra were acquired on 4800 MALDI-TOF-TOF analyzer (operated by the 4000 Series Explorer software version 3.7). The instrument was externally calibrated using TOF/TOF Calibration Mixture (Mass Standards Kit for Calibration of AB SCIEX TOF/TOF™ Instruments). The chosen mass range was 499 Da–2500 Da, the focus mass was 1500 Da and a resolution of 4000 was observed. The resulting mass lists were manually scanned for known contaminant mass peaks: keratin, matrix, BSA, and trypsin autolysis. The identified contaminant mass peaks were used to create an exclusion list that applied in the acquisition method for the MS/MS data acquisition. Identical peaks found in both mutant and wild type strains were eliminated and only peaks specific to each strain used to identify peptides.

2.9. Protein identification

Using MASCOT Server, MS/MS Ion Search was carried out using a custom database. The database contained protein sequences of all curated proteins in the Swissprot database (release 2016_07 retrieved on September 18, 2016) filtered for proteins with GO cell wall/membrane localization tags. The peptide and fragment tolerance values were specified at ± 2 Da chosen due to the limited instrument resolution. Carbamidomethyl C was assigned as a fixed modification, while Oxidation at M was chosen as a variable modification. Up to two missed cleavages were allowed for trypsin. A minimum of 2% sequence coverage was imposed for proteins to reduce false positives. Peptide sequences identified by MASCOT but not linked to proteins were blasted on Candidagenome.org. The cut off e-value was < 0.05 in both MASCOT searches as well as BLAST searches.

3. Results

Strains were grown under both filamentous and non-filamentous conditions for cell wall protein extraction Data was acquired using MALDI TOF/TOF with MS/MS acquisition and analyzed using MASCOT for protein identification. Wherever only peptide sequences could be identified on MASCOT the sequences were blasted on the *Candida* genome database (candidagenome.org) for identification.

For growth under filamentous conditions, wild type strain RM1000 allowed the identification of 53 proteins along with 51 unmatched sequences, which resulted in 30 blast hits and for mutant pga1 strain, a total of 84 proteins were identified along with 65 unmatched sequences, which resulted in 41 blast hits. For growth under non-filamentous conditions, wild type strain allowed identification of 78 proteins along with 70 unmatched sequences, which resulted in 45 blast hits. Under the same conditions, the mutant allowed the identification of 90 proteins along with 58 unmatched sequences, which resulted in 40 blast hits. Overall, 14 and 36 proteins were identified in the wild type when grown under filamentous and non-filamentous conditions, respectively. These proteins but were not detected in the mutant.

Herein, we present the identified proteins divided into two categories. Tables 1 and 2 contain proteins that were detected in the wild type strain versus the *pga1* null mutant strain as identified through MASCOT while Tables 3 and 4 contain additional proteins as identified by BLAST.

3.1. MASCOT identified proteins under non filamentous growth

Table 1 summarizes the identified proteins extracted under non filamentous growth conditions found in the wild type but lacking in the mutant. A total of 13 proteins were identified with protein coverage ranging from 1 to 10%, Note the presence of Hsp90 a highly antigenic virulence factor, Sod5 a superoxide dismutase and multiple cell wall proteins of the Pga1 family.

3.2. MASCOT identified proteins under filamentous growth

Table 2 summarizes the identified proteins extracted under filamentous growth conditions found in the wild type but lacking in the mutant. Six proteins were identified. Note the presence of Sap 10, a protease involved in virulence, and Pga5 involved in cell wall structure

3.3. BLAST identified proteins under filamentous growth

As can be seen in Table 3, eight proteins were found upon BLAST searching the *C. albicans* database with MASCOT generated peptide sequences. These proteins include heat shock proteins, and proteins involved in virulence such as Cdc1 and Int1, and proteins involved in cell structure such as Pga4.

3.4. BLAST identified proteins under non filamentous growth

A total of 23 proteins were found to be exclusively detected in the wild type upon blasting MASCOT peptides onto the *C. albicans* database. As can be seen in Table 4, proteins involved in virulence, chitin deposition, and cell wall structure belong to this category.

4. Discussion

The purpose of this study was to further characterize the *C. albicans* cell wall protein Pga1. It has previously been found that *pga1* plays a role in the virulence and filamentation of *C. albicans* [15]. The *Pga1* null strain (*pga1:URA3/pga1:HIS1* null) was previously found to be defective in adhesion and biofilm formation and exhibited a decrease in cell wall chitin content, and a decreased resistance to oxidative stress. MALDI mass spectrometry allowed us to identify a number of proteins expressed in the wild type but not detected in the mutant strain. It is worth noting that failure to detect a protein in a sample does not necessarily imply the protein is absent; as the possibility exists that the

Table 1
MASCOT identified proteins under non filamentous growth.

RM1000 exclusive							
Gene Name	Protein Accession	Protein Description	Protein Matches	Protein Coverage (%)	Protein Sequences	Peptide Sequence	# Missed Cleavages
CDC11	G1UB61	Septin Cdc11	28	10.5	3	EEQIKLEEERLR	2
						KFEERVHQDLINK	2
						KSINFSIMIIGESGSGR	1
EXG2	Q5AIA1	Glucan 1,3-beta-glucosidase 2	7	3.8	1	EAGLNMVRIPIGYWSFEK	1
HSP90	P46598	Heat shock protein 90 homolog	29	9.2	6	DSGIGMTK	0
						DEEDEKKEK	2
						KNNIKLYVR	2
						FTVTLDETNERLGR	1
						DTTMSSYMSSKK	1
						AVEKSPFLDALK	1
LIP9	Q9P4E6	Lipase 9	1	3.3	1	QSGQAVLNSIRASLK	1
MP65	Q59XX2	Cell surface mannoprotein MP65	21	2.9	1	SNQQAAISSIK	0
PGA31	Q5A5U9	Cell wall protein Pga31	2	2.4	1	DSYAVVK	0
PGA32	Q5ADQ7	Probable GPI-anchored adhesin-like protein Pga32	11	6	2	TTLGQVTTPSR	0
						LSSSKSIYSNSTTSR	1
PGA52	Q59L72	GPI-anchored protein 52	4	6.3	1	QAGADVATTLATVTGQSATTTSKK	1
PGA55	Q59SG9	Probable GPI-anchored adhesin-like protein Pga55	23	0.8	1	ATGENGEEITK	0
PIR1	Q59SF7	Cell wall mannoprotein Pir1	2	6.6	1	ACSSANNLEMTLHDSVLKDTHER	1
PNS1	Q5AB93	Protein Pns1	7	2.3	1	SAKDTFDLIRFK	2
SLA1	Q5ALV2	Actin cytoskeleton-regulatory complex protein Sla1	24	4.4	6	DWWMVENIATRR	1
						SKDWWMVENIATR	1
						LTDGPLR	0
						LSVEDLEYVER	0
						DWWMVENIATR	0
						EGDEVYIIDQKK	1
SOD5	Q5AD07	Cell surface Cu-only superoxide dismutase 5	23	7	1	FTPANNGTVSVSVDLK	0

Table 2
MASCOT identified proteins under filamentous growth.

RM1000 exclusive

Gene Name	Protein Accession	Protein Description	Protein Matches	Protein Coverage	Protein Sequences	Peptide Sequence	# Missed Cleavages
EGD2	Q5ANP2	Nascent polypeptide-associated complex subunit alpha	4	3.9	1	QIKGISR	1
ERG1	Q92206	Squalene monooxygenase	1	2.4	1	GFILLGDSLNMR	0
PGA5	Q59VW6	1,3-beta-glucanosyltransferase Pga5	8	7.6	3	EVQEEEPGVPGLPGSNK	0
						EPHYIDPLANPFTCLR	0
						ELCFKVDCEDINANGR	1
QDR1	Q5A6P6	MFS antiporter Qdr1	4	3.1	2	RIVGNGSIRPK	1
						RIVGNGSIRPKNVLNK	2
SAP10	Q5A651	Candidapepsin-10	3	4.2	1	MDLVIMNFVFLLYLTSVVK	0
SHO1	Q5AQ36	High osmolarity signaling protein Sho1	4	4.7	1	DEILEVDDIDGKWWQARR	2

peptide might be found in too low a concentration to be detected by our machine. However the lack of detection in one strain versus the other is still significant as it implies that a protein is found in higher amounts in one strain versus the other, explaining a previously observed phenotype. Most of the observed proteins are obviously cell wall proteins, however, in BLAST searches we detected a few cytoplasmic proteins. Their presence may be due to inadvertent rupture of the protoplast in the course of cell surface protein extraction.

Many of the detected proteins belong to the large PGA family that consists of cell wall proteins of different functions ranging from immunogenic determinants to virulence related proteins such as adhesins, proteases and lipases but that have a common GPI anchor to attach them to the cell surface [12]. The differential presence of these proteins in the wild type is to be expected since the mutant is defective in many of these attributes. Furthermore, the *pga1* deletion might have affected and destabilized the cell surface resulting in such differential expression of cell wall proteins.

Hsp90 is a well-known cell surface antigenic determinant and a virulence factor that has also been shown to play a role in biofilm formation, drug resistance and filamentous growth [25]. Hsp 90 is an essential protein however. The fact that it was not detected in the mutant implies a strong downregulation of expression. The decrease in virulence and biofilm formation observed in the mutant can be explained through a decrease in Hsp90 expression.

Many of our identified proteins actually play a role in virulence. Cdc 11, a septin, has previously been shown to be involved in invasive growth and virulence, in addition to affecting chitin deposition [26] since a cdc11 deletion has been shown to result in abnormal chitin localization and content by possibly interfering with chitin biosynthesis enzyme localization and function [27]. The mutant suffers from decreased adhesion, virulence and chitin content. As such the lack of

Cdc11 in the mutant can explain these observed phenotypes. Many lipases have also been found to be absent in the mutant such as Lip6, 8, 9 and 10. Lipases and phospholipases are key virulence factors since they contribute to hydrolyzing and degrading key host cellular components such as the cell membrane. Sap 10 is a member of a large family of secreted aspartyl proteases, enzymes that degrade cellular proteins. Sap 10 has been shown to play a role in adhesion and virulence [28]. The lack of detection of such a protein can explain the decrease levels of adhesion in the mutant. Similarly Int1, lacking in the mutant has been tightly linked to the ability of *C. albicans* to adhere [29]. Cfl1, an oxidoreductase protein detected in our study has been shown to be necessary for proper adhesion [30], hence the mutant suffers from decrease in adhesion capabilities. It should be noted that the lack of adhesion can also affect biofilm formation as adhesion is a precursors to biofilm formation, as such the decrease in biofilm formation observed in the mutant can be explained by the decrease in adhesion.

The mutant has been shown to be defective in proper resistance to oxidative stress as shown by increased susceptibility to hydrogen peroxide. This study has determined that Sod5 is not expressed in the mutant. Sod5 is a member of the superoxide dismutase family that is necessary to protect the cell from free radicals [31]. In a previous study of a *C. albicans DSE1* mutant Zohbi et al. [21] identified Sod1, another member of this family as missing in the mutant, contributing to its decreased resistance to oxidative agents.

A deletion of cell wall proteins might make the cell surface less rigid, more brittle and as such result in an increased overall susceptibility to cell wall disrupting agents. The mutant has been previously shown to exhibit decreased resistance to calcofluor white and Congo red. Calcofluor white interferes in chitin microfibril assembly, while Congo red interferes in both chitin assembly and beta-1,3-glucan assembly [32]. A decrease in chitin would be reflected as an increased

Table 3
BLAST identified proteins under filamentous growth.

RM1000 exclusive

Gene Name	Description/Function	Sequence	Score	E-value	% Match
CDC11	Septin; agar-invasive growth, full virulence and kidney tissue invasion in mouse, but not kidney colonization	KFEERVHQDLINK	51	3.00E − 08	100
CDR1	Multidrug transporter of ABC superfamily; transports phospholipids in an in-to-out direction	EYFEKMGWK	39	6.00E − 05	100
		QTTADFLTSLTNPAER	58	2.00E − 10	100
FMP13	Mitochondrial inner membrane protein	DIESVIGR	30	4.10E − 02	100
		SEIPPPPPPPPPPKAKR	70	8.00E − 14	100
HSP70	Putative Hsp70 chaperone	IDKSQVEEIVLVGGSTR	61	5.00E − 04	100
INT1	Protein structurally similar to alpha-subunit of human leukocyte integrin; role in morphogenesis, adhesion, and mouse cecal colonization and systemic virulence	DLNFANYSNNTNRPR	58	2.00E − 10	100
PGA4	GPI-anchored cell surface protein; beta-1,3-glucanosyltransferase	LPSGLYFNCGDDDMAR	63	8.00E − 12	100
QDR2	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family	SLDWYYR	31	2.00E − 02	100
RBR1	Glycosylphosphatidylinositol (GPI)-anchored cell wall protein; required for filamentous growth at acidic Ph	SGASSVASAAK	38	2.00E − 04	100

Table 4
BLAST identified proteins under non filamentous growth.

RM1000 exclusive

Gene Name	Description/Function	Sequence	Score	E-value	% Match
APE2	Neutral arginine, alanine, leucine specific metallo-aminopeptidase	YMATTQMEPTDCRR	58	2.00E − 10	100
		TSEGVDESSVLETRSK	56	9.00E − 10	100
CDR2	Multidrug transporter, ATP-binding cassette (ABC) superfamily; transports phospholipids, in-to-out direction	NTNVGNDFVR	39	9.00E − 05	100
CFL1	Oxidoreductase; iron utilization	ATGKYNTR	31	1.30E − 02	100
EGD2	Nascent polypeptide associated complex protein alpha subunit	GISRVTFKQR	39	7.00E − 05	100
ERG11	Lanosterol 14-alpha-demethylase; cytochrome P450 family; role in ergosterol biosynthesis; may contribute to drug resistance	HLTTPVFGKGVIYDCPNSR	75	2.00E − 15	100
		HLTTPVFGK	36	6.00E − 04	100
		ISATYMKEIK	39	7.00E − 05	100
		SLFGDEMRRIFDR	51	2.00E − 08	100
FMP13	Mitochondrial inner membrane protein	DIESVIGR	30	4.10E − 02	100
		QQLEAKLNQK	37	2.00E − 04	100
		LNEERNGRLANLEK	50	3.00E − 08	100
LIP10	Secreted lipase, may have a role in nutrition and/or in creating an acidic microenvironment	DSFYSPPVGFATAKPGDILK	75	3.00E − 15	100
LIP6	Secreted lipase, may have a role in nutrition and/or in creating an acidic microenvironment	SNGHTTETVVGAPAALTWIDAR	82	3.00E − 17	100
LIP8	Secreted lipase, possible roles in nutrition and/or in creating an acidic microenvironment; expressed at all stages of both mucosal and systemic infection	SSKITNIK	26	5.30E − 01	100
MTS1	Sphingolipid C9-methyltransferase; glucosylceramide biosynthesis is important for virulence	ITSVEMAEHVGIR	49	6.00E − 08	100
PAN1	Essential protein involved in endocytosis and polarized growth; role in regulating actin cytoskeleton	AEAQRASQR	33	4.00E − 03	100
PGA28	Putative GPI-anchored adhesin-like protein, has roles in stress- and cell-wall-related processes	TSETGGVSSTANSEAKSGSVTTSK	83	1.00E − 17	100
PGA30	GPI-anchored protein of cell wall	FIGGGKSSSVTK	44	2.00E − 06	100
PGA45	Putative GPI-anchored cell wall protein	LVLSQKNVLLYIFAGVLSK	68	2.00E − 13	100
PGA53	GPI-anchored cell surface protein of unknown function	LINDDELMVQDAQFDYPAIVNLK	86	2.00E − 18	100
PHR1	Cell surface glycosidase; may act on cell-wall beta-1,3-glucan prior to beta-1,6-glucan linkage; role in systemic, not vaginal virulence	MTDVWSGGIVYMYFEEANK	76	1.00E − 15	100
QDR1	Putative antibiotic resistance transporter	RIVGNGSIRPK	42	9.00E − 06	100
RIM9	Protein required for alkaline pH response via the Rim101 signaling pathway	FFESEYRYANDDMRIMR	69	1.00E − 13	100
RPS1	Putative ribosomal protein 10 of the 40S subunit; elicits host antibody response during infection	KKMIEIMQR	37	2.00E − 04	100
STP1	Transcription factor; regulates *SAP2*, *OPT1* expression and thereby protein catabolism for nitrogen source	ALHFIYPAGVKASQR	57	3.00E − 08	100
TIM21	Component of the Translocase of the Inner Mitochondrial membrane (TIM23 complex); for protein import into mitochondria	LIEKNEQAQK	37	2.00E − 04	100
TSA1B	Putative peroxidase; role under oxidative/reductive stress	MAPVVQQPAPSFKK	54	3.00E − 07	100
VMA2	Vacuolar H(+)-ATPase; protein present in exponential and stationary growth phase yeast cultures	LSLEFLEKFEKNFISQGAYENR	82	3.00E − 17	100

susceptibility to these agents. Our results support this as many of the identified proteins play a role in cell wall structure, rigidity or assembly. Exg2 for example is a glucan-1,3-betaglucosidase induced during cell wall regeneration [33]. Pga4 and Pga5 are beta 1–3 glucanosyltransferases that are involved in cell wall assembly and cross-linking the cell wall glucans [34]. Phr1 is required for proper cross linking of beta 1,3-, and 1,6-glucans [35]. Its absence generates a wide array of phenotypes similar to the ones observed in the mutant notably decreased adhesion, biofilm formation, and resistance to cell wall disrupting agents. Finally Pir1, an essential cell wall component involved in rigidity, has been detected in the wild type but not in the mutant implying downregulation of expression, similar to Hsp 90 described above. Pir1 is a 1,3-beta-glucan-linked cell wall protein that is N-mannosylated, O-glycosylated by Pmt1 with observable cell wall defects in the heterozygous mutant [36].

5. Conclusion

By comparing the proteins present in the cell wall of the wild type and the *pga1 null* strain, we conclude that *pga1* performs an important role on the *C. albicans* cell surface since *pga1* mutant strains display several phenotypes and a significantly defective and altered cell wall proteome. The lack of detection of many of these proteins that were only present in the wild type go a long way in explaining the previously observed *pga1* null mutant phenotypes discussed above.

Conflict of interests

None.

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