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ORIGINAL ARTICLE

Proteome analysis of *Candida albicans* cells undergoing chlamydosporulation

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Abstract

Chlamydospore though considered as a unique and rare morphological form of *Candida albicans*, regulation and significance of chlamydosporulation is not very clear. SWATH-MS analysis of chlamydosporulation specific proteins revealed that 319 (137-Up regulated and 182-Down regulated) proteins expressed differentially. Functional annotation showed significant modulations in proteins involved in cellular architecture (30), carbohydrate (29), amino acid (17), fatty acid (3), Nucleic acid (14), vitamins (1) metabolism as well as signaling (6), stress response (26), transport (cytoplasmic-21, mitochondrial-6 and nuclear-1), gene expression (transcription-12, RNA processing-6, translation-53, PTM-18), proteolysis (15) etc. Enhanced mannan, β1, 3-glucan and chitin contribute in thickening of cell wall while Hyr1 (218-fold) and Als3 (38.16-fold) dominates the cell surface chemistry of chlamydospores. In addition to ergosterol, enhanced sphingolipids, phospholipids and fatty acids make chlamydospore membrane more sturdy and rigid. Up-regulation of maltase (64-fold) followed by enhanced glycolysis and tricarboxylic acid cycle under nutrient-limiting condition is indicative of chlamydosporulation. Glyoxylate and fermentative pathway reported to facilitate survival of *C. albicans* under glucose limiting and microaerophilic condition was up-regulated. Enhanced biosynthesis of glutathione, trehalose homeostasis, and inhibition of NAD+ generation ,etc., potentiate oxidative, osmotic and nitrosative stress tolerance. Up regulation of Rsr1 (8.83-fold) and down regulation of Bcy1 (4.20-fold), Tfs1 (negative regulator of RAS) indicates cAMP-PKA pathway activates chlamydosporulation through Efg1 (a morphogenic regulator) in our study. In general, morpho-physiological modulations in *C. albicans* is a result of different sets of transcriptional programs that facilitate survival under nutrient and oxygen limiting condition.

Keywords *C. albicans* · Chlamydospore · LC–MS/MS · Metabolism · Cell wall

Introduction

Candida albicans, a polymorphic opportunistic pathogen associated with superficial to life-threatening systemic infections among immunocompromised individuals is included in the list of organisms with potential antibiotic resistance threat, recently (CDC report 2013; Kullberg and Arendrup 2015). Morphogenic plasticity is considered as a survival strategy that enables *C. albicans* to colonize and invade host tissues by evading host defense mechanisms under a wide range of extreme micro-environments (Brown et al. 2014; Cutler 1991; Ernst 2000; Lim et al. 2012; Ruhnke 2006). Different morphological forms viz. yeast, hyphae, pseudohyphe, chlamydospore, opaque cells and biofilms exhibit differential responses towards host defense mechanisms as well as antifungal agents (Cutler 1991; Lim et al. 2012; Ruhnke 2006; Tyc et al. 2014). Among these, the hyphal form is a prerequisite for tissue invasion and invasive candidiasis and biofilms on indwelling medical devices are considered as difficult-to-treat infections with very high mortality (Mc Manus and Coleman 2014; Mun et al. 2016; Neville et al. 2015; Williams 2011). Considering the significance in virulence, these morphological and growth forms were studied exhaustively in recent years while chlamydospore, considered as a non-virulent form is neglected by scientific community (Bottcher et al. 2016; Citiulo et al. 2009).

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Chlamydospores are thick-walled spherical cells (6–10 µm) considered as dormant form induced under unfavorable environmental conditions like oxygen limitation and embedded growth in matrix (Giosa et al. 2017; Nobile et al. 2003; Sonneborn et al.1999). The presence of chlamydospores in clinical specimens is reported but it may be unlikely to play any role in pathogenesis (Citiulo et al. 2009). Chlamydospores are more specialized and relatively rare morphological states formed to survive under harsh conditions (Nobile et al. 2003; Sosinska 2012). It could be an adaptive response towards exposure to reactive oxygen species of host cells or towards co-existing microorganisms (Berman and Sudbery 2002; Douglas et al. 2005). Chlamydospores are metabolically active, can germinate under favorable condition and produce daughter chlamydospores, blastospores, pseudohyphae and true hyphae (Citiulo et al. 2009; Staib and Morschhäuser 2007). Chlamydospores thick wall is providing protection against the adverse micro-environments. However, not much study is available on structure and composition of chlamydospore cell wall (Jansons and Nickerson 1970).

Though various environmental, nutritional and genetic factors are implicated, regulation and significance of chlamydospore formation are not very clear (Bottcher et al. 2016). In present study, we have made an attempt to identify chlamydospore specific proteins using LC–MS/MS analysis. This is the first attempt at identifying chlamydospore specific proteins. Proteomic analysis revealed morphophysiological modulations responsible for altering cellular architecture that enables *C. albicans* to survive under extreme micro-environments.

Material and methodology

Candida albicans strain and growth condition

Candida albicans ATCC 10231 a quality control strain was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India and maintained on yeast extract peptone dextrose (YPD) agar at 4 °C. Rice extract agar with 1% Tween 80 was used for chlamydospore induction. Yeast Extract Peptone Dextrose (YEPD) broth and Rice extract agar were purchased from Hi-media Laboratories, Pvt. Ltd. Mumbai (India). All the chemicals used in this study were purchased from Sigma-Aldrich Pvt. Ltd., Bangalore (India) and solvents from Qualigens and SD Fine Chemicals Ltd, Mumbai (India).

Induction of chlamydospores

Inoculums were prepared using *C. albicans* cells grown for 48 h at 30 °C in YEPD broth. Cells were harvested by centrifugation at 448 g for 2 min, washed thrice with sterile distilled water and re-suspended in 1 ml of sterile distilled water (Holmes and Shepherd 1987; Odds 1988).

Chlamydosporulation was induced using rice extract agar (Rice extract 1.3 g, Tween 1%, pH 7.1). In brief, sterile rice extract with low melting agar plates were inoculated with 1×10^3 cells per plate (Kelly and Funigiello 1959). Plates were overlaid with sterile, polyethylene sheets of 7.5 cm diameter for anaerobiosis and invasive growth necessary for chlamydospores induction. Plates were wrapped with parafilm and incubated at 30 °C for 14 days (Miller et al. 1974). Chlamydospore formation was monitored by observing the plates under dissecting microscope. Chlamydospores were stained using Lactophenol cotton blue, observed microscopically (OLYMPUS CX21i) and photographed using microscope attached camera (OLYMPUS digital camera E-PL1) (Kim et al. 2002).

Harvesting of chlamydospores

Chlamydospores were harvested from 12-day-old plates grown at 30 °C. In brief, chlamydospores plates were washed with sterile distilled water to remove colonies of yeast phase cells growing on the agar surface. Rice extract agar containing chlamydospores was molten using warm (45 °C) sterile distilled water, centrifugation at 112 g for 1 min. Chlamydospore pellets were washed thrice with sterile distilled water and used for protein extraction. However, chlamydospores as harvested from semisolid agar, traces of hyphae producing chlamydospores were present along with chlamydospores in the sample. Cells from the colonies growing on the rice extract agar surface were harvested by centrifugation, washed with sterile distilled water and used as a control.

Cell surface hydrophobicity

Cell surface hydrophobicity of yeast phase cells and chlamydospores of *C. albicans* was analyzed using a method developed by Rosenberg, et al. (1980) and Hazen and Hazen (1987). Briefly, yeast cells and chlamydospores were re-suspended in PBS until the OD (at 620 nm) reaches to 0.5. 1.3 ml from each of these suspensions was distributed in three test tubes. 100 µl from each test tube were again added into the wells of 96 well micro titer plates and initial OD (at 620 nm) was recorded using Thermoscan-Ex micro plate reader (Thermo Fisher Scientific Inc., 168 3rd Ave, Waltham, MA 02451, USA). 0.3 ml of octane was added to remaining cell suspension (1.2 ml) separately, mixed vigorously (3 min), and allowed to separate for 15 min. From these suspensions 100 µl were cautiously transferred to the wells of 96-well micro-titer plates and final OD was recorded. Triplicates were used for each samples and experiment was repeated thrice. Percentage CSH was

calculated using following formula and compared with control. Results were presented as percentage of CSH ± SD (standard deviation) (Hazen and Hazen 1987; Rosenberg et al. 1980).

$$\left[\text{CSH percentage} = (1 - \text{final OD of aqueous phase/initial OD of cell suspension}) \times 100\right].$$

Estimation of adhesion

Adhesion of yeast phase cells and chlamydospores of *C. albicans* was determined using method described by Panagoda et al. (2001) and He et al. (2006). Briefly, 100 µl of cell suspension (1×10^7 cells/chlamydospores/ml) was inoculated in 96 wells micro titer plates and incubated at 30 °C for 90 min with moderate shaking (50 rpm) on orbital shaker for adhesion. After incubation, wells were washed thrice with PBS to remove un-adhered ones and the numbers of adhered cells/chlamydospores in each well were counted, microscopically (Metzer Make inverted Microscope) and compared. The average number of yeast phase cells adhered is considered as 100% adhesion. Experiment was repeated thrice and triplicates were used for each sample and results were shown as adhesion percentage ± SD (standard deviation) (He et al. 2006; Panagoda et al. 2001).

Ergosterol extraction

Ergosterol content of yeast phase cells and chlamydospores of *C. albicans* was estimated as per Arthington-Skaggs et al. (1999). Briefly, 0.1 g of washed cell/chlamydospore pellets were suspended into 300 µl of ethanolic KOH (25%) and incubated at 85 °C for 1 h and 2 h, respectively. Samples were cooled to room temperature and ergosterol was extracted using n-heptane [75% (v/v)] with vortexing. Layers were allowed to separate and n-heptane layer was transferred to new vial, cautiously. 200 µl of n-heptane layer was diluted to fivefold in ethanol (100%) and spectrum was recorded in the wavelength range of 230–300 nm using a UV–Visible spectrophotometer (Shimadzu Analytical (India) Pvt. Ltd. Mumbai- 400 059, India).

Ergosterol estimation

Ergosterol content was determined using the values of absorbance at 230 nm and 281.5 nm and the formula by Arthington-Skaggs et al. (1999):

$$\text{Percentage ergosterol} + \%24(28)\text{DHE} = \left[(\text{A}281.5/290) \times F\right]/\text{pellet weight}$$

$$\%24(28)\text{DHE} = \left[(\text{A}230/518) \times F\right]/\text{pellet weight},$$

$$\text{So, percentage ergosterol} = \left[\text{percentage ergosterol} + 24(28)\text{DHE}\right] - \%24(28)\text{DHE}]$$

where F is the dilution factor in ethanol and 290 and 518 are the E values (percent/centimeter) determined for crystalline ergosterol and 24(28) DHE, respectively. Results were showed as ergosterol percentage ± SD (standard deviation).

Cell lysis and protein extraction

Proteins (chlamydospore and yeast phase cells) were extracted using the protocol optimized by Haar (2007). Briefly, chlamydospores and yeast phase cells ($1x10^8$ equivalent cells) were resuspended in 200 µl of freshly prepared lysis buffer (0.1 M NaOH, 0.5 M EDTA, 2% SDS and 2% β-mercaptoethanol) containing (10 µl/ml) PIC (protease inhibitor cocktail) and vortexed. Samples were incubated at 90 °C for 15 min and neutralized using 5 µl of 4 M acetic acid after incubation. Samples were further incubated at 90 °C for 15 min and centrifuged at 2800 g for 5 min. Supernatants were transferred to new vials containing 5 µl of PMSF (Phenyl methane sulphonyl fluoride). Proteins were precipitated using 4 volumes of methanol, 1 volume of chloroform and 3 volumes of sterile distilled water with vortexing. Precipitated proteins were centrifuged at 2800*g* for 5 min, pellets were washed using 3 volumes of methanol, centrifuged at 2800 g for 5 min and air dried. Air dried pellets were re-suspended in rehydration buffer (6 M urea, 2 M Thiourea, 2% CHAPS, 1% DTT, pH 8.75) (Haar 2007) and protein concentrations were determined as per Bradford method (Bradford 1976).

Sample preparation

Proteins (50 µg) were dissolved in ammonium bicarbonate buffer (50 mM) containing rapigest (0.1%). Proteins were reduced with 3 µl of DTT (100 mM) at 60° C for 15 min, alkylated using 3 µl of Idoacetamide (200 mM) at room temperature for 30 min, and digested at alkaline pH using 2 µg of trypsin per 50 µg of proteins (i.e. 2:50). Digestion was stopped by adding 2 µl of concentrated HCL after 18 h of digestion. Peptides were separated by spinning at high speed (1500 rcf) for 15 min at 4 °C, washed several times with 0.1% TFA, size fractionated (3 kDa) using Zip tip C_{18} chromatography columns (Millipore; Billerica, MA) and eluted in 100% Acetonitrile. Samples were reconstituted in 15 µl of Acetonitrile (3%) and formic acid (0.1%) with continuous vortexing and used for further analysis (Gillet et al. 2012).

Liquid chromatography and mass spectrometry analysis

Peptides (4 µg) were separated and mass was determined using Micro LC 200 (Eksigent; Dublin, CA) coupled with Triple- TOF 5600 (AB Sciex; Concord, Canada) mass spectrometer in high-sensitivity mode. Equal amounts of samples of chlamydospores and control were spiked to generate the SWATH (sequential window acquisition of all theoretical fragment ion spectra) spectral library of fragment ions and analyzed using Information Dependant acquisition (IDA) (Collins et al. 2013; Gillet et al. 2012; Liu et al. 2006, 2013).

SWATH MS analysis

Swath MS analysis was carried out using the instrument setting as described in Ingle et al. (2017), Collins et al. (2013); Gillet et al. (2012); Liu et al.(2006); Liu et al. (2013). The mass spectral data acquired in triplicates was searched against *Candida* databases, Uniprot ids were searched using Protein Pilot software and differentially expressed proteins were identified using markerview software. Subsequently SWATH-MS was performed for relative quantification of differentially expressed proteins as mentioned in Ingle et al. (2017).

Statistical analysis

The student *t* test and probability were performed for statistical analysis. Samples with probability (*p*) value ≤ 0.05, number of matching peptides ≥ 2 and fold change ≥ 2, were considered for further analysis.

Validation of proteomic data using real-time qPCR analysis of selected genes

Expression of selected genes during chlamydosporulation was evaluated at mRNA level using real-time qPCR analysis. Gene-specific primers were designed using primer3 plus software (Tm 58–60 °C, product size 120–150 bp preferred for primer pairs) (Table 5). Total RNAs were prepared using RNeasy Mini kit (50 reactions) (Cat. No. 74104, Qiagen Pvt. Ltd) by lysing Chlamydospores using lyticase and purified using RNA Sure Mini Kit, Nucleo-pore (Genetix) according to the manufacturer's instructions. cDNAs were prepared using purified RNA (2 µg) as a template and High-Capacity cDNA Reverse Transcription Kit as per the manufacturer's instructions (Green et al. 2004). RNA level was measured using KAPA SYBR® FAST qPCR Kit as per manufacturer's instructions and parameters and CFX96 Touch TM Real-Time PCR Detection System (Biorad Pvt. Ltd). Samples were analyzed in triplicates using biological replicates and data are reported as mean ± SD. Using ANOVA, statistical significance was calculated and *p*-values less than 0.05 were considered significant. Gene expression was normalized with GAPDH levels and with control cells.

Results

Induction of chlamydosporulation

Embedded and microaerophilic growth on rice extract agar medium at 30 °C induced invasive growth in *Candida albicans*, initially. The invading hyphae later developed suspensor cells that produced thick-walled chlamydospores, terminally within 4 days of incubation. Further incubation up to 12 days leads to increase in the number of chlamydospores (Fig. 1).

Modulation in cell surface hydrophobicity (CSH), adhesion and ergosterol during chlamydosporulation

In present study, in response to diverse morphology cell surface hydrophobicity, adhesion as well as ergosterol significantly modulated. Cell surface hydrophobicity of *C. albicans* yeast phase cells and chlamydospores (with filaments) at 30° C were found (37.92 ± 4.98, 31.70 ± 3.64, 27.61 ± 3.12) and (9.12 ± 1.29, 11 ± 3.33, 10 ± 4.05) respectively (Fig. 2a). This showed that CSH of yeast phase cells more as compare to chlamydospores (with filaments). However, adhesion of yeast phase cells and chlamydospore (with filaments) at 30° were found (82 ± 3.61, 79.67 ± 4.51, 77.67 ± 3.79) and (2.33 ± 0.58, 3 ± 2, 2.33 ± 2.08), respectively (Fig. 2b). Yeast pahse cells are more adhesive than chlamydospores (with filaments). In addition to this, ergosterol content was analyzed through spectrophotomer, which showed that chlamydospore (with filaments) contain more ergosterol as compare to yeast phase cells (Fig. 2c). Percent ergosterol content of yeast phase cells and chlamydospores (with filaments) was found (0.0073 ± 0.00076, 0.0087 ± 0.0013, 0.0076 ± 0.001) and (0.124 ± 0.06, 0.0207 ± 0.002, 0.0196 ± 0.0018) respectively (Fig. 2c).

Identification of chlamydosporulation specific proteins using LC–MS/MS analysis

LC–MS/MS analysis identified 1177 proteins out of which, 319 were modulated significantly (Ingle et al. 2017). MS/MS data is submitted to Peptide Atlas and data set is publically available with the data set identifier PASS01061 at http://www.peptideatlas.org/PASS/PASS01061. Differentially expressed proteins were identified; functionally annotated and grouped into different categories according to their functions using databases like CGD, SGD, KEGG and Uniprot

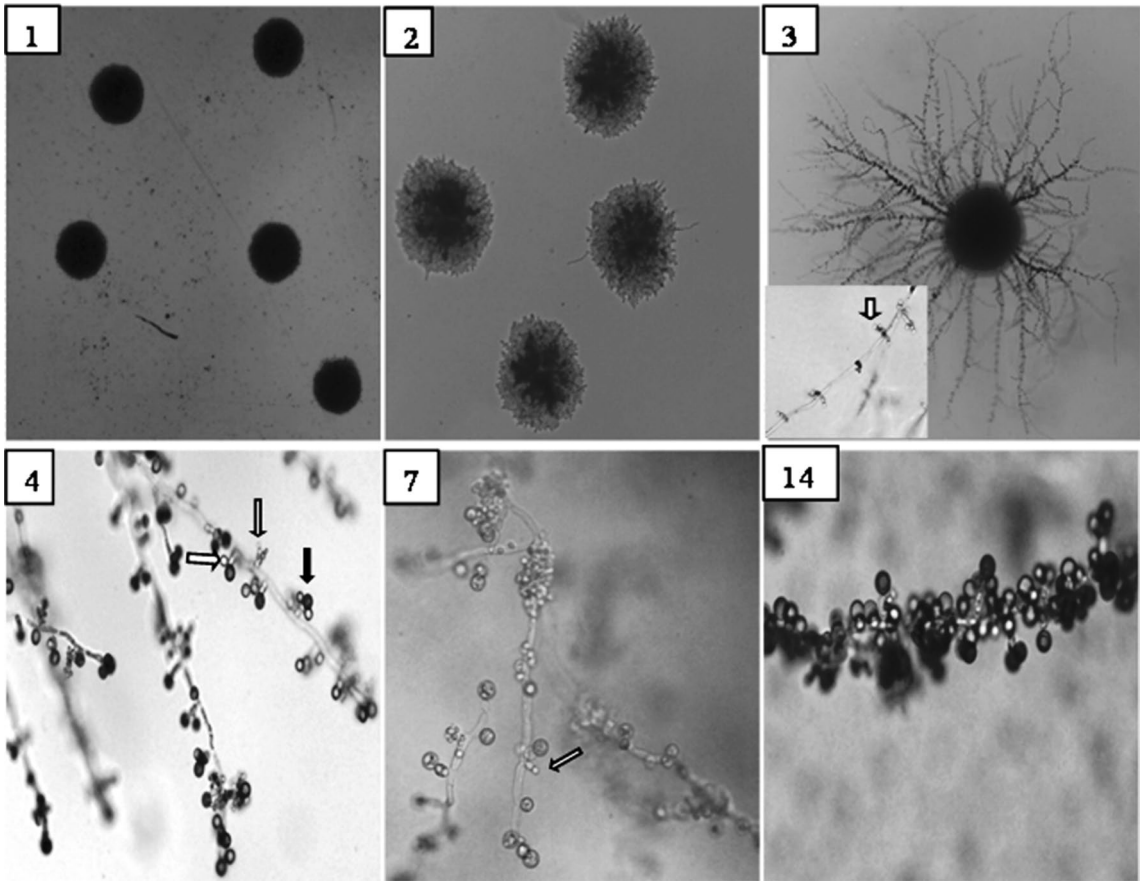

Fig. 1 Light microscopy images of Chlamydospore (with hyphae) formation of *C. albicans,* ATCC 10231. Numbers indicates the days of incubation, white arrow indicates developing chlamydospores, and black arrow indicates mature chlamydospores

etc., using David software (Functional annotation Bioinformatics Microarray). Among these differentially expressed proteins, 137 were up-regulated while 182 were Down-regulated under chlamydospore inducing condition (Ingle et al. 2017). The number of up-regulated and down-regulated proteins in different biological processes viz. metabolism, cell wall and membrane composition, stress response and signaling, gene expression, transport etc. were shown in Tables 1, 2, 3 and 4, Fig. 2a, b.

Modulation of proteins involved in metabolism

A total of 108 proteins involved in metabolism were modulated significantly, out of which 51 were involved in carbohydrate metabolism and energy generation (Table 1). The maximum up regulation of 259.37-fold was observed in case of Pgk1 followed by 64.83 (CAWG_04860/Mal32), 35.99 (Ald4), 17.93 (CAWG_01786/Hxk2), 13.85 (LELG_01826/Icl1) fold while maximum down-regulation of 46.59-fold was observed in case of (CaO19.285/Ctn1) followed by 32.83 (Ams1) and 10.48 (Glo2) fold etc. (Table 1). Five proteins involved in fermentation were up-regulated and maximum up regulation of 12.10-fold was observed in case of Ife2 (Table 1).

Eleven out of the thirteen proteins involved in lipid, sterol and fatty acid biosynthesis were up-regulated and maximum up-regulation of 36.85 was observed in case of Scs7 (Sphingolipid alpha-hydroxylase), while two were down-regulated (Table 1). Seventeen proteins expressed differentially indicating significant modulation in amino acid metabolism, wherein Gcv1 was up-regulated by 10.66-fold while maximum down-regulation of 14.55-fold was observed in case of Gln1 (Table 1). Similarly, differential expression of fourteen proteins indicates significant modulation in nucleotide metabolism i.e. Ysa1 was down-regulated by 18.19-fold while Ura4 was up-regulated by 6.25-fold (Table 1). Heme (Hem13, Hem15) and Vitamin (Snz1) biosynthesis and fatty acid degradation were enhanced under chlamydospore inducing condition (Table 1).

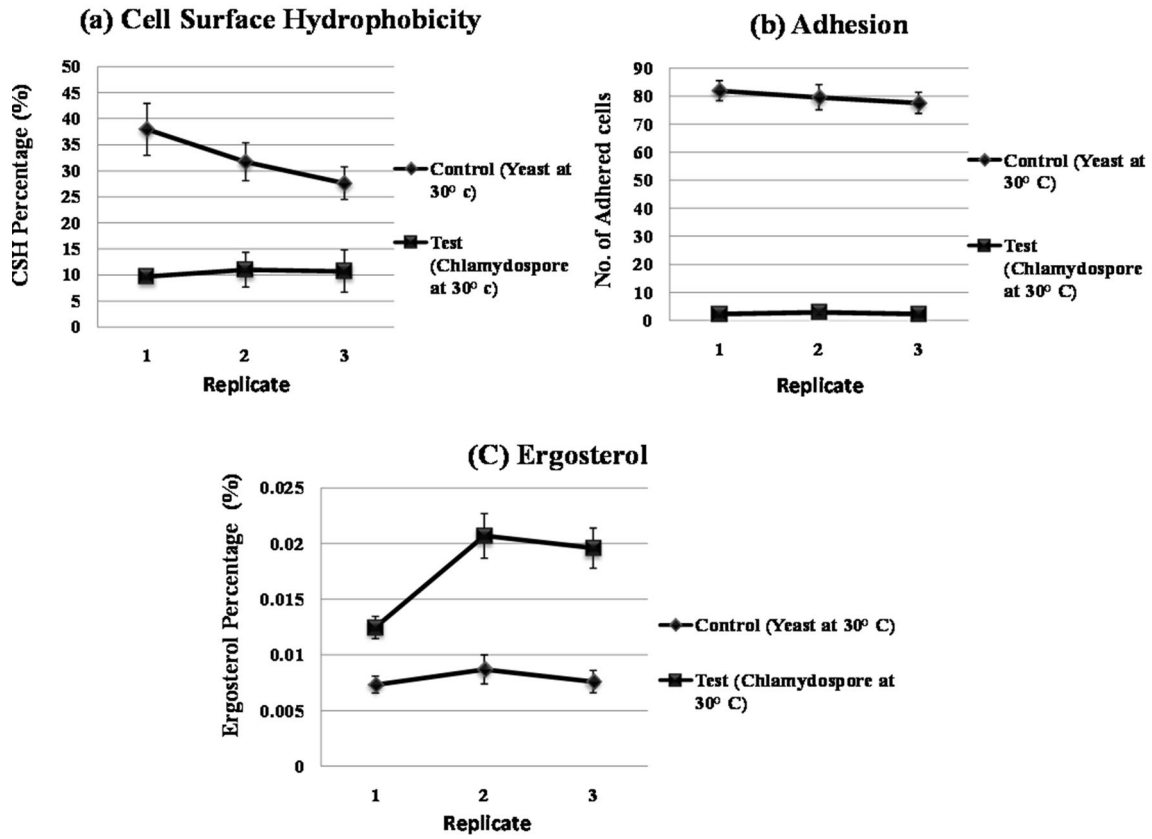

Fig. 2 Determination of **a** cell surface hydrophobicity **b** adhesion and **c** ergosterol content of yeast phase cells and Chlamydospore (with filaments) of *C. albicans* (ATCC 10231)

Modulation of proteins involved in cell wall and membrane biosynthesis

A total of eighteen proteins involved in biosynthesis of cell wall components viz. mannan (2), beta 1, 3-glucan (1), beta 1, 6-glucan (2), chitin (1), cell wall proteins (7) and membrane (5) were modulated significantly during chlamydosporulation (Table 2). The cell wall proteins viz. Hyr1, Als3 (hyphae specific) and Ldg1 were up-regulated maximally by 218.24, 38.16 and 38.43-fold respectively while maximum down-regulation of 49.15-fold was observed in case of Kre9 involved in biosynthesis of beta 1,6-glucan (Table 2). Among the proteins involved in membrane structure and biosynthesis like Fmp52, Scs7, Ino1 etc., were up-regulated by 82.65 36.85 and 12.82-fold respectively (Table 2). It indicates that cell wall of chlamydospore is rich in mannan, chitin and beta 1, 3-glucan while membrane is enriched with sphingolipids, phospholipids, sterols etc.

Modulation of proteins involved in stress response and signal transduction

Twenty out of the twenty-six proteins modulated during chlamydospore growth were involved in oxidative stress response while four in heat stress and one each in metal ion and drug-induced stress (Table 2). Among these, ten were up-regulated while sixteen were down-regulated and maximum up-regulation of 242.48-fold was observed in case of Yhb1 (Nitric oxide dioxygenase), and down-regulation in case of Whs11 (176.48-fold) (Table 2).

Among the six proteins involved in signal transduction, maximum up-regulation of 8.83-fold and down-regulation of 16.7-fold was observed in case of Rsr1 (CLUG_ 03767) and Asr2 (CAWG_02167), respectively (Table 2).

Table 1 Modulation of proteins involved in metabolism during chlamydosporulation in *Candida albicans* (ATCC 10231)

	Protein name	Function	Fold change	
			Yeast	Chlamydospores
Metabolism				
Starch and Xylose degradation	CAWG_04860 (Mal32)	Maltase	+	+64.83
	Xyl2	Xylitol dehydrogenase	+	+5.62
	Ifr2	Zinc-binding dehydrogenase	+	−3.17
Glycolysis	Pgk1	3-Phosphoglycerate kinase	+	+259.37
	CAWG_01786 (Hxk2)	Hexokinase II	+	+17.93
	Cdc19	Pyruvate kinase	+	+9.62
	G210_5347 (Eno1)	Enolase I Ortholog(s) C. *dubliniensis* CD36	+	+5.47
	Glk1	Glucokinase	+	+5.45
	CAWG_03619 (Fba1)	Fructose-bis-P aldolase	+	+5.40
	CaO19.1946	Similar to an aldose 1-epimerase-related protein	+	+4.95
	CAWG_00576 (Eno1)	Enolase I Ortholog(s) *Candida maltose*	+	+4.06
	CANTEDRAFT_116913 (Tim)	Triosephosphate isomerase	+	+2.95
	Tpi1	Triose-phosphate isomerase	+	+2.36
	CD36_08010 (Eno1)	Enolase I	+	+2.32
	CAWG_04094(Gpm1)	Glycerate phosphoMutase	+	+2.21
	CAWG_01943 (Pgi1)	Glucose-6-P isomerise	+	+2.20
	Tdh3	Triose phosphate dehydrogenase	+	+2.07
Pyruvate catabolism	CAWG_03171(Pdc11)	Pyruvate decarboxylase	+	+2.85
	Pyc2	Pyruvate carboxylase	+	−4.19
	CaO19.285 (Ctn1)	Carnitine acetyl transferase	+	−46.59
Galactose and glycogen metabolism	CAWG_05451 (Gph1)	Glycogen phosphorylase	+	+3.03
	Pgm2	Phosphoglucomutase	+	+2.73
	Glc3	Cytoplasmic glyoxalase II	+	−2.76
Trehalose metabolism	CD36_30070 (Ugp1)	UTP-glucose-1-phosphaturidyl transferase	+	+2.74
	Ugp1	UTP-glucose-1-phosphaturidyl transferase	+	+2.67
	CAWG_06130 (Tps3)	Trehalose-phosphate synthase regulatory subunit	+	+2.61
Oligosaccharide degradation	Ams1	Putative 1,4-glucan branching enzyme	+	−32.83
Acetyl CoA and Acetaldehyde Formation	Ald4	Mitochondrial aldehyde dehydrogenase	+	+35.99
	CAWG_05515 (Lat1)	Dihydrolipoamide acetyltransferase (E2) of (PDC)	+	−2.87
TCA cycle	CAWG_00467 (Idh1)	Isocitrate dehydrogenase subunit 1	+	+4.45
	CAWG_02112 (Aco1)	Aconitase	+	+2.51
	CD36_28700 (Cit1)	Citrate synthase	+	+2.19
	Lsc1	Putative succinyl-CoA ligase	+	+2.15
	Mdh1-3	Malate dehydrogenase	+	+2.03
Glyoxylate and Methylglyoxal	LELG_01826 (Icl1)	Isocitrate lyase	+	+13.85
	Glo2	Cytoplasmic glyoxalase II	+	−10.48

Table 1 (continued)

	Protein name	Function	Fold change	
			Yeast	Chlamydospores
Electron Transport chain	Piso0_005603 (Sdh2)	Iron-sulfur protein subunit	+	+52.15
	CaO19.1682	Membrane protein of complex I	+	+5.47
	Nuc2	Putative NADH-ubiquinone oxidoreductase	+	+4.48
	Qcr7	Putative ubiquinol-cytochrome-c reductase	+	+4.21
	Cor1	Core protein of QH2 cytochrome c reductase	+	+2.38
	Atp16	Subunit of the mitochondrial F1F0 ATP synthase	+	−2.51
	CD36_63930 (Atp15)	Predicted proton-transporting ATP synthase activity	+	−2.76
	Cqr1	Potential reductase	+	−2.76
	CD36_64885 (Cox12)	OrthologsVIb Subunit, cytochrome c oxidase activity	+	−2.85
	Pst2	Putative, NADH: quinone oxidoreductase	+	−3.32
	CaO19.287 (Nuo2)	Nadh:Ubiquinone oxidoreductase	+	−3.53
	Atp5	Putative F0-ATP synthase FO subunit B	+	−3.75
	Cyt1	Cytochrome c1 respiratory	+	−3.79
	CORT_0B04410 (Qcr6)	Ortholog 6 subunit ubiquinol cytochrome-c reductase	+	−5.57
	CAWG_03509 (Qcr9)	Putative ubiquinol cytochrome c reductase Subunit 9	+	−9.75
	CAWG_03893 (Cox17)	Cytochrome c Oxidase	+	−9.89
	CAWG_04373 (Atp14)	Putative mitochondrial F1F0 ATP synthase subunit h	+	−11.77
Fermentative pathway	Ife2	Putative alcohol dehydrogenases	+	+12.10
	CAWG_04871 (Adh1)	Alcohol dehydrogenase	+	+4.87
	CAWG_04871 (Adh1)	Alcohol dehydrogenase	+	+4.87
	Adh5	Alcohol dehydrogenase	+	+3.75
	CAWG_00592 (Adh2)	Alcohol dehydrogenase	+	+3.31
Fatty acid metabolism	Pox1-3	Acyl-coenzyme A oxidase	+	+2.95
Phospholipid	Ino1	Inositol-1-phosphate synthase	+	+12.82
Sterol	Ach1	Acetyl CoA hydrolase	+	+5.95
	Erg10	Acetyl-CoAC-acetyltransferase	+	+5.24
	Erg9	Farnesyl-diphosphate farnesyl transferase	+	+4.42
	CAWG_03271 (Cbr1)	Putative cytochrome B5 reductase	+	+2.94
	CAWG_01359 (Mvd)	Mevalonate diphosphate decarboxylase	+	+2.26
	Ncp1	NADPH-cytochrome P450 reductase	+	−3.41
Sphingolipid	Scs7	Sphingolipid alpha-hydroxylase	+	+36.85
	Cfa1	Sphingolipid C9-methyltransferase	+	−7.76
Fatty acid	CAWG_02796 (Fas2)	Alpha subunit of fatty acid synthetase complex	+	+8.17
	CORT_0C01740 (Fas2)	Alpha subunit of fatty acid synthetase complex	+	+4.61
	Acp12	Acyl carrier protein domains	+	−3.28

Table 1 (continued)

	Protein name	Function	Fold change	
			Yeast	Chlamydospores
Amino acid metabolism	Gcv1	Glycine decarboxylase	+	+10.66
	CAWG_04997 (Shm2)	Serine hydroxymethyltransferase	+	+8.28
	CAWG_03282 (Lys1)	Mitochondrial C1-tetrahydrofolate synthase precursor	+	+5.64
	Mis11	Saccharopine dehydrogenase	+	+4.87
	Cys4	Cystathionine beta-synthase	+	+4.61
	Ape2	Aminopeptidases yscII	+	+3.48
	Aat1	Aspartate aminotransferase	+	+2.43
	Gdh3	NADP (+)-dependent glutamate dehydrogenase	+	−2.00
	Leu42	Cobalamin-independent methionine synthase	+	−2.59
	CAWG_01512 (Met6)	Putative alpha-isopropylmalate synthase	+	−3.06
	Hom6	Homoserine dehydrogenase	+	−3.38
	Ser1	3-phosphoserine aminotransferase	+	−4.25
	Pro3	Δ1-pyrroline-5-carboxylate reductase	+	−4.55
	Arg1	Argininosuccinate synthase	+	−4.83
	CAWG_04309 (Kti11)	Zn-ribbon protein	+	−10.00
	Gcv3	Glycine decarboxylase	+	−8.10
	Gln1	Glutamine synthetase	+	−14.55
Nucleotide Metabolism	Ura4	Dihydroorotase	+	+6.25
	CAWG_03916 (Apt1)	Adenine phosphoribosyltransferase	+	+5.32
	CaO19.5054 (Bna6)	Putative quinolinate phosphoribosyl transferase	+	+5.09
	Fca1	Cytosine deaminase	+	+3.07
	CAWG_00458 (Ura1)	Dihydroorotate dehydrogenase	+	+2.65
	Ade17	Adenine	+	−2.08
	CAWG_05175 (Adk1)	Putative adenylate kinase	+	−2.66
	Fur1	Uracil phosphoribosyltransferase	+	−3.23
	CaO19.12103 (YdfG)	Carbonyl reductase (NADPH) activity	+	−4.39
	CD36_51380 (Ura4)	Dihydroorotase activity	+	−4.60
	Ura2	Bifunctional carbamoylphosphate synthetase CPSase	+	−5.28
	CaO19.12418 (Pof1)	Promoter of filamentation	+	−7.59
	CaO19.11368 (Apa2)	Putative ATP adenylyltransferase II	+	−15.06
	Ysa1	Predicted Nudix hydrolase family member	+	−18.19
Heme	Hem13	Coproporphyrinogen III oxidase	+	+9.25
	Hem15	Ferrochelatase	+	+2.61
Vitamin	Snz1	Snooze	+	+2.64
Other Metabolism	Osm1	Osmotic sensitivity	+	+5.45
	CD36_02580	Predicted dienelactone hydrolase domain	+	+5.33
	Paa11	Polyamine N-acetyl tranferase	+	−2.47
	CAWG_03805 (Stf2)	Stabilizing factor	+	−2.95

+ Up-regulated, − down-regulated

Table 2 Modulation of proteins involved in cell wall/membrane biosynthesis, cell signaling, stress response and transport during chlamydosporulation in *Candida albicans* (ATCC 10231)

	Protein name	Function	Fold change	
			Yeast	Chlamydospores
Cell wall				
Surface	CAWG_00991 (Csh1)	Cell surface hydrophobicity	+	−6.79
Mannan	Psa2	Mannose-1-phosphate guanyltransferase	+	+6.53
	CD36_87780 (Srb1)	GDP-mannose pyrophosphorylase	+	+3.41
GPI	Hyr1	Hyphally regulated cell wall protein	+	+218.24
	Als3	Agglutinin like sequence	+	+38.16
	Als1	Agglutinin like sequence	+	−11.75
	CAWG_03911 (Plb)	Phospholipase B	+	−20.93
β1,6-Glu	CAWG_01343 (Phr2)	Glycosidase	+	−2.49
	Kre9	Killer resistant protein	+	−49.15
β1,3-Glu	CaO19.7214	Gucan-1,3-glucosidase	+	+5.99
Chitin	Uap1	UDP-N-acetylglucosamine pyrophosphorylase	+	+3.49
Wall protein	Ldg8	Secreted protein	+	+38.43
	Png2	Putative peptide:*N*-glycanase	+	−6.11
Cell membrane				
Membrane protein	Fmp45	Predicted membrane protein	+	+11.66
	Lsp1	Long chain bases stimulate phosphorylation	+	−2.95
	CAWG_00926 (Pil1)	Eisosome component	+	−4.73
Phospholipid	Ino1	Inositol-1-phosphate synthase	+	+12.82
Sterol	Ach1	Acetyl CoA hydrolase	+	+5.95
	Erg10	Acetyl-CoAC-acetyltransferase	+	+5.24
	Erg9	Farnesyl-diphosphate farnesyl transferase	+	+4.42
	CAWG_03271 (Cbr1)	Putative cytochrome B5 reductase	+	+2.94
	CAWG_01359 (Mvd)	Mevalonate diphosphate decarboxylase	+	+2.26
	Ncp1	NADPH-cytochrome P450 reductase	+	−3.41
Sphingolipid	Scs7	Sphingolipid alpha-hydroxylase	+	+36.85
	Cfa1	Sphingolipid C9-methyltransferase	+	−7.76
Fatty acid	CAWG_02796 (Fas2)	Alpha subunit of fatty acid synthetase complex	+	+8.17
	CORT_0C01740 (Fas2)	Alpha subunit of fatty acid synthetase complex	+	+4.61
	Acp12	Acyl carrier protein domains	+	−3.28
Organelle	Fmp52	Found in mitochondrial proteome	+	+82.65
	Rtn1	Reticulon-like	+	+3.39
Signal transduction				
CLUG_03767 (Rsr1)		RAS-related protein	+	+8.83
CaO19.12544 (Ofr1)		Opaque formation regulator	+	−2.10
Asr3		Adenylyl cyclase	+	−3.72
CAWG_03886 (Bcy1)		Bypass of cyclic-AMP requirement	+	−4.20
Cmd1		Calmodulin	+	−5.12
CAWG_02167 (Asr2)		Adenylyl cyclase	+	−16.73
Stress responsive				
Oxidative	Yhb1	Nitric oxide dioxygenase	+	+242.48
	CAWG_02572 (Ccp1)	Cytochrome c peroxidase	+	+11.04
	Lys7	Copper chaperone for SOD1	+	+5.74
	CaO19.1682 (Nce103)	Nonclassical export,carbonic anhydrase	+	+5.47
	CAWG_02689 (Gtt11)	Glutathione S-transferase	+	+3.95
	Gcs1	Gamma-glutamylcysteine synthetase	+	+3.34
	Trr1	Thioredoxin reductase	+	+3.11
	Piso0_000124	Protein involved in zinc ion binding	+	+2.11

Table 2 (continued)

	Protein name	Function	Fold change	
			Yeast	Chlamydospores
	Glr1	Glutathione reductase	+	−2.23
	Trp99	Potential peroxiredoxin	+	−2.32
	Gps2	Putative glutathione peroxidase	+	−2.98
	Pst3	Protoplasts-secreted	+	−3.79
	Ca35A5.08	Rehydrin-like protein	+	−4.19
	Grp2	Methylglyoxal reductase	+	−4.21
	Dot5	Disruptor of telomeric silencing	+	−4.33
	Ttr1	Putative glutaredoxin	+	−4.68
	CAWG_01338 (Hbr1)	Haemoglobin response gene	+	−7.14
	Mxr1	Putative methionine sulfoxide reductase	+	−13.33
	Sod3	Cytosolic manganese-containing superoxide dismutase	+	−13.68
	Trx1	Thioredoxin	+	−18.65
Metal ion	Crd2	Copper resistance determinant	+	−12.34
Heat Shock	CaO19.822 (Hsp21)	Small heat shock protein	+	+3.80
	Hsp12	Heat shock protein 12	+	−8.41
	Hsp12	Heat shock protein 12	+	−8.81
	Whs11	White colony protein	+	−176.48
Stress	CAWG_02881(Rct1)	Required for caspofungin tolerance	+	+3.52
Transport				
Cytoplasmic	Vma5	Putative vacuolar H(+)-ATPase	+	+35.08
	Gdi1	Putative Rab GDP-dissociation inhibitor	+	+9.04
	Ypt521	Yeast protein two521	+	+7.79
	CaO19.12543 (Het1)	Putative sphingolipid transfer protein	+	−2.18
	CaO19.5689 (Sec28)	Secretory 28	+	−2.75
	CaO19.2304 (Prk1)	Putative protein serine/threonine kinase	+	−2.82
	CaO19.1544 (Bug1)	Binder of USO and GRH1	+	−2.83
	CAWG_05907 (Yop1)	YIP One Partner	+	−2.96
	Clc1	Clathrin light chain	+	−3.44
	Gsp1	Genetic suppressor of Prp20-1	+	−3.98
	Pma1	Plasma membrane H(+)-ATPase	+	−4.56
	Hmt1	HnRNP methyltransferase	+	−4.57
	CAWG_03253 (Sec23)	Secretory	+	−4.71
	Ycf1	Yeast cadmium factor	+	−5.55
	CD36_09500 (Ntf2)	Nuclear transport factor	+	−6.20
	Gim3	Gene involved in microtubule biogenesis	+	−12.77
	CAWG_02936 (Erv25)	Protein ERV25	+	−14.45
	CAWG_05431(Acb1)	Acyl-CoA binding	+	−15.10
	CaO19.10437 (Sbe22)	Suppressor of BEm4	+	−23.44
	Vma10	Subunit G V1 membrane domain of V-ATPase	+	−5.59
	Vma4	Vacuolar membrane Atpase		−15.33
Mitochondrial	Fcj1	Formation of crista junctions protein 1	+	+6.22
	CAWG_00983 (Por1)	Mitochondrial outer membrane porin	+	+2.86
	CaO19.1236 (Gvp36)	BAR domain protein	+	−2.34
	CAWG_03580 (Tim10)	Translocase of the inner mitochondrial membrane	+	−6.38
	CaO19.7882 (Ymc2)	Yeast mitochondrial carrier	+	−8.81
	CD36_73310 (Tim9)	Translocase of the inner mitochondrial membrane	+	−11.14
Nuclear	Rna1	Rapid cessation of net RNA accumulation	+	−2.97

+Up-regulated, − down-regulated

Modulation of proteins involved in transport

Twenty-eight proteins involved in transport were significantly modulated (23 down-regulated, 5 up-regulated) during chlamydospore form growth (Table 2). Among the twenty-one proteins involved in cytoplasmic transport, six were up-regulated and fifteen were down-regulated (Table 2). Maximum up-regulation of 35.08-folds was observed in case of Vma5 (Putative vacuolar H (+)-ATPase) and down-regulation of 23.44-fold in Sbe22 (CaO19.10437), respectively (Table 2).

Modulation of proteins involved in regulation of cell cycle, cytoskeleton, genome organization, replication and repair

Among the sixteen proteins modulated, two, six, five and three were involved in cell cycle, cytoskeleton, genome organization and replication and repair, respectively (Table 3). One of the two involved in cell cycle regulation, four out of the six in cytoskeleton, all the five in genome organization and one of the three involved in replication and repair were up-regulated respectively (Table 3). Among these, maximum up- regulation of 8.15-fold and down-regulation of 12.5-fold was observed in case of Arp7 (genome organization) and Ssp2 (cell cycle) respectively (Table 3).

Modulation of proteins involved in genome function (transcription, RNA processing, translation and proteolysis)

Twelve proteins involved in transcription, six in RNA processing, 71 in translation and fifteen in proteolysis were significantly modulated under chlamydospore form growth (Table 3). Among the twelve proteins involved, eight were involved in transcription initiation and four in elongation (Table 3). All the eight involved in transcription initiation were down-regulated while three of the five involved in elongation were unregulated (Table 3). Similarly, six proteins involved in RNA processing were down-regulated (Table 3).

Out of the seventy-one proteins involved, thirty-eight (21 up-regulated, 17 down-regulated) were involved in ribosomal assembly, nine in translation initiation (2 up-regulated, 7 down-regulated), three in elongation (2 up-regulated, 1 down-regulated), fifteen in post translational modification (2 up-regulated, 13 down-regulated) and six were involved in mitochondrial ribosome assembly (3) and mitochondrial PTM (3) (Table 3). Maximum up-regulation of 16.23-fold and down-regulation of 16.35-fold was observed in case of Rbp4 and Rtg1 respectively (Table 3). On the other hand, Snu13 (CAWG_02750) was down-regulated maximally by 7.24-fold amongst the six down-regulated proteins involved in RNA processing (Table 3). Similarly amongst the 71 proteins involved in translation and post-translational modifications, maximum up regulation of 11.15-fold observed in case of Rps23 while Tif3 was down-regulated maximally i.e. 24.28-fold (Table 3). Among the fifteen proteins involved in proteolysis (2 up-regulated and 13 down-regulated), maximum up-regulation of 66.85-fold and down-regulation of 84.5-fold was observed in case of Pre7 (AGOS_AGL324 W) and Pbi2 (CaO19.10285), respectively (Table 3). Thirteen modulated proteins were uncharacterized (Table 4).

Discussion

Chlamydospore, the thick-walled resting spores induced in response to stresses (like the nutrient limitation, microaerophilic and osmotic stress etc.), is considered as a survival strategy of *C. albicans* (Citiulo et al. 2009; Staib and Morschhäuser 2005; Nobile et al. 2003). *C. albicans* cells experience most of these stresses (except temperature 37 °C) during growth on rice extract agar containing Tween 80 in our study, citing the biological significance of our data (Berman and Sudbery 2002; Douglas et al. 2005). Though it was found in some of the tissue samples recently, chlamydospore is considered as a non-virulent form of *C. albicans* and thus neglected by the scientific community (Palige et al. 2013; Citiulo et al. 2009; Lim et al. 2012). However, few recent studies have provided some insights into the regulation of chlamydospore formation and survival strategies (Bottcher et al. 2016; Giosa et al. 2017; Palige et al. 2013; Lim et al. 2012). Mutant analysis by Navarathna et al. (2016) revealed significance of a component of chromatin remodeling complex (ISW2) in chlamydosporulation (Navarathna et al. 2016). Our study showed that nutrient limiting and microaerophilic condition enhances activity of Ras related protein Rsr1, the upstream regulator while down regulate Bcy1, a negative regulator of cAMP-PKA pathway (Table 2) (Biswas et al. 2007). It suggests that chlamydospore formation is induced through cAMP-PKA pathway (Table 2) (Biswas et al. 2007). Earlier studies have shown that nutrient starvation activate cAMP-PKA pathway through Ras1 and induce chlamydospore formation through EFG1 in presence of minimal cAMP i.e. surplus cAMP is inhibitory (Bottcher et al. 2016). It was further supported by down regulation of adenyl cyclases (to avoid surplus cAMP concentration) and Tfs1, a negative regulator of Ras protein in our study (Bottcher et al. 2016). It confirms that involvement of cAMP-PKA pathway is essential for chlamydospore formation and thus osmotic, heat shock and cell wall integrity damage induced stress tolerance (Bottcher et al. 2016; Chautard et al. 2004; Harcus et al. 2004). However, Hog1 mitogen-activated protein (MAP) kinase and quorum sensing molecule farnesol were also implicated in chlamydosporulation (Eisman et al. 2006; Martin et al. 2005). Chlamydosporulation need reprogramming of the transcriptional program through chromatin remodeling complexes (ISW2) that leads to chlamydospore specific transcripts including

Table 3 Modulation proteins involved in cell cycle, genome organization, replication, gene expression during chlamydosporulation in *Candida albicans* (ATCC 10231)

	Protein name	Function	Fold change	
			Yeast	chlamydospores
Cell cycle				
Tpd3		tRNA processing deficient	+	+2.68
Ca38F10.10c (Ssp2)		Sporulation specific meiosis	+	−12.51
Cytoskeleton				
Arp7		Actin-related protein	+	+8.15
CAWG_04431 (Cof1)		Putative cofilin	+	+6.16
Tub1		Alpha-tubulin	+	+5.63
Tub2		Tubulin beta chain	+	+5.27
Arc15		Putative ARP2/3 complex subunit	+	−2.08
Act1		Actin	+	−2.75
Genome organization				
Ctn2		Protein with DNA binding domain	+	−2.21
Rpd31		Reduced potassium dependency	+	−4.51
CAWG_03623 (Nhp6a)		Non histone protein 6A	+	−8.69
CANTEDRAFT_115395 (Hat2)		Histone H2A	+	−10.94
CORT_0B08290 (Htb1)		Histone H2B	+	−11.13
Replication and repair				
Pol30		Polymerase	+	+2.13
Ubc13		Ubiquitin-conjugating	+	−5.21
CAWG_00976 (Hta2)		Histone h two A 2	+	−10.34
Gene expression				
Transcription				
Initiation	CaO19.2296 (Rfx1)	Regulatory factor X	+	−2.17
	Hmo1	High mobility group (HMG) family	+	−3.07
	CAWG_01268 (Mbf1)	Multiprotein-bridging factor 1	+	−3.11
	Bdf1	Bromodomain transcription factor	+	−3.44
	CAWG_05532 (Rvb2)	Putative trancription modulator	+	−5.15
	Taf10	TATA binding protein associated factor	+	−8.02
	Ncb2	Negative cofactor B	+	−10.24
	Cbf1	Putative centromer binding factor 1	+	−14.03
Elongation	Rbp4	RNA polymerase II fourth largest subunit	+	+16.23
	CAWG_04831 (Spt4)	Elongation factor SPT4 (Suppressor of Ty's)	+	+15.57
	Rnt1	RNase three	+	+4.94
	Rtg1	Retrograde regulation	+	−16.35
RNA processing				
Nop58		Nucleolar protein of 58 kDa	+	−2.36
CaO19.1862 (Rtc3)		Restriction of telomere capping	+	−3.58
CAWG_00494 (Caf20)		Cap associated factor	+	−3.81
Lhp1		La-homologous protein	+	−4.05
CD36_72250 (Lsm4)		Like SM	+	−5.01
CAWG_02750 (Snu13)		Putative U3 snoRNP protein	+	−7.24
Translation and post translational modification				
Ribosome assembly	Rps23	Putative ribosomal protein23A	+	+11.15
	CD36_44070 (Rpl22B)	Ribosomal 60S subunit protein L22B	+	+6.24
	Rps21	Ribosomal protein of the Small (40S) subunit21	+	+6.04
	Rpl5	Ribosomal 60S subunit protein L5	+	+5.87

Table 3 (continued)

	Protein name	Function	Fold change	
			Yeast	chlamydospores
	Rpl4B	Ribosomal protein L4B	+	+5.60
	Rps1	Putative ribosomal protein 10 of the 40S subunit	+	+5.49
	CAWG_03801 (Rpl38)	Ribosomal 60S subunit protein L38	+	+5.44
	Rpl9B	Ribosomal protein L9Hap43-induced	+	+5.22
	CAWG_05436 (Yae1)	Ortholog(s) have role in ribosomal large subunit biogenesis	+	+3.96
	CAWG_02808 (Rpl35)	60S ribosomal protein L35	+	+3.89
	CD36_50810 (Rps11A)	Ribosomal protein of the small subunit11A	+	+3.85
	CAWG_03325 (Rpl30)	60S ribosomal protein L30	+	+3.44
	Rps8A	40S ribosomal protein S8A	+	+3.25
	G210_5404	60S ribosomal protein L2	+	+2.95
	Rpl3	Ribosomal protein L3	+	+2.79
	Rps5	Ribosomal protein S5	+	+2.62
	Rpl15A	Ribosomal 60S subunit protein L15A	+	+2.59
	CAWG_04143 (Rps9B)	40S ribosomal protein S9-B	+	+2.37
	CAWG_02763 (Rpl19A)	Ribosomal protein L19	+	+2.28
	CAWG_00346 (Rps17B)	40S ribosomal protein S17-B	+	+2.23
	Rpl18	Ribosomal 60S subunit protein L18A	+	+2.15
	CAWG_01150 (Rpl26B)	60S ribosomal protein L26-B	+	−2.03
	CPAR2_807560 (Rps15)	Ribosomal protein of the small subunit15	+	−2.04
	Rps12	Acidic ribosomal protein S12	+	−2.10
	Rps18	Likely cytosolic ribosomal protein S18	+	−2.15
	Rpp0	Ribosomal protein P0	+	−2.37
	CAWG_02832 (Rps21B)	Putative ribosomal protein S19	+	−2.72
	Rpp2A	Ribosomal protein P2 alpha	+	−2.99
	CAWG_01239 (Rps21B)	Ribosomal protein S21	+	−3.06
	Rps20	Ribosomal protein of the small subunit 20	+	−3.21
	CAWG_05170 (Rpl25)	60S ribosomal protein L25	+	−3.35
	Rps0	40S ribosomal protein S0	+	−3.36
	Rpp2B	Ribosomal protein P2 beta	+	−3.45
	Ubi3	Ubiquitin-ribosomal protein fusion S27a	+	−3.50
	CAWG_00923 (Rsa3)	Ribosome assembly	+	−5.08
	Rrs1	Regulator of ribosome synthesis	+	−6.78
	CAWG_04900 (Sot1)	Suppressor of QSR1truncations	+	−7.43
	CTRG_03083 (Rps29-A)	40S ribosomal protein S29-A	+	−8.67
Initiation	Anb1	Anaerobically induced	+	+5.12
	Scd6	Suppressor of clathrin deficiency	+	+3.17
	CAWG_01241 (Tif)	Translation initiation factor	+	−2.26
	Tif6	Eukaryotic translation initiation factor 6	+	−2.79
	CAWG_02919 (Ded1)	Defines essential domain	+	−4.15
	Tif11	Translation initiation factor eIF1a	+	−5.76
	Sgn12	Slower growth on non-fermentable carbon sources	+	−12.28
	Tif3	Translation initiatioin factor 3	+	−13.26
	CAWG_05493 (Gir2)	Genetically interacts with ribosomal genes	+	−24.28
Elongation	Rpp1B	Ribosomal protein P1 beta	+	+4.14
	Cam1-1	Putative translation elongation factor	+	+3.15
	Efb1	Elongation factor 1-beta	+	−2.93

Table 3 (continued)

	Protein name	Function	Fold change	
			Yeast	chlamydospores
Post translation modification	CAWG_02226 (Yke2)	Yeast Ortholog of mouse KE2	+	+12.56
	Cct2	Chaperonin containing TCP-1	+	+4.93
	CAWG_04567 (Sti1)	Stress inducible	+	−2.07
	CAWG_01012 (Fpr3)	Fk 506-sensitive proline rotamase	+	−2.14
	Hsp104	Heat shock protein 104	+	−2.23
	Sti1	Stress inducible	+	−2.49
	CD36_13060 (Hsp10)	Heat shock protein 10	+	−3.10
	CAWG_00045 (Hsp10)	Heat shock protein 10	+	−3.16
	CAWG_05601 (Rbp1)	Rapamycin-binding protein	+	−3.79
	Smt3	Suppressor of Mif two	+	−3.93
	CAWG_05587 (Cyp1)	Cyclophilin peptidyl-prolyl cis–trans isomerase	+	−4.32
	CAWG_00272 (Egd1)	Putative GAL4 DNA-binding enhancer protein	+	−4.53
	CAWG_05553 (Hsp90)	Heat shock protein 90	+	−5.94
	CTRG_00453 (Rub1)	Related to ubiquitin	+	−6.24
	CAWG_02324 (Cpr6)	Cyclosporin-sensitive proline rotamase	+	−9.42
Mitochondrial ribosome assembly	Mrpl10	Mitochondrial ribosomal protein L10	+	+4.40
	Mrpl27	Putative 60S ribosomal protein L27	+	−2.94
	Mrpl12	Likely mitochondrial ribosomal protein	+	−2.08
Mitochondrial post translation modification	Phb12	Prohibitin	+	+4.40
	Cpr3	Cyclosporin A-sensitive proline rotamase	+	−2.94
	Mge1	Mitochondrial GrpE	+	−2.08
Proteolysis				
AGOS_AGL324 W (Pre7)		Proteosome subunit beta type-6	+	+66.85
CAWG_00970 (Rpn13)		Regulatory particle non-ATPase	+	+8.66
Pre5		Alpha6 subunit of the 20S proteasome	+	−2.03
01-Apr (Apr1)		Aspartic proteinase	+	−2.14
Pre9		Alpha3 (C9) subunit of the 20S proteasome	+	−4.07
CAWG_05677 (Hsm3)		enHanced spontaneous mutability	+	−4.33
CAWG_03436 (Tma17)		Translation machinery associated	+	−4.89
Pup2		Putative proteasome subunit	+	−4.97
Fes1		Factor exchange for Ssa1p	+	−6.16
CD36_00370 (Ubp6)		UBiquitin-specific protease	+	−6.28
Tfs1		cdc twenty-five suppressor	+	−8.95
Pre3		Putative beta-1 proteasome subunit	+	−11.20
Pre1		Putative beta 4 subunit of the 20S proteasome	+	−33.97
Prb2		Proteinase B	+	−47.69
CaO19.10285 (Pbi2)		Putative protease B inhibitor	+	−84.51

+Up-regulated, − down-regulated

Table 4 Modulation of un-characterized proteins during chlamydosporulation in *Candida albicans* (ATCC 10231)

Protein name	Function	Fold change	
		Yeast	Chlamydospores
Uncharacterized proteins			
QRI8	Protein of unknown function	+	+23.60
CaO19.7199	Putative uncharacterized protein	+	+13.57
CaO19.4947	Putative uncharacterized protein	+	+8.79
CD36_25110	Putative uncharacterized protein	+	+6.23
CaO19.7740	Putative uncharacterized protein	+	+4.59
CaO19.13072	Putative uncharacterized protein	+	−2.74
ykr049	Protein of unknown function DUF1687	+	−3.29
CaO19.1394	Putative protein of unknown function	+	−3.95
NDAI0G02900	Uncharacterized protein	+	−4.79
Q9P826(Uni Id)	Putative uncharacterized protein	+	−5.67
CaO19.7531	Uncharacterized protein	+	−7.15
CaJ7.s006	Putative uncharacterized protein CaJ7.s006	+	−7.90
G210_2803	Uncharacterized protein	+	−99.58

+Up-regulated, − down-regulated

components of cytoskeleton (actin, tubulin and cofilin) (Cao et al. 2005; Navarathna et al. 2016). Components of cytoskeleton (actin, tubulin and cofilin) and chromatin remodeling complex (Arp7) were up regulated in our study. Similarly, enhanced levels of components (Yke2 and Cct2) involved in proper folding of alpha, beta tubulin and actin indicate increased strain on PTM machinery, as reported earlier in response to GlcNAc (García-Sánchez et al. 2005; Kamthan et al. 2012). Enhanced Phb12 level in our study contributes in stabilizing newly synthesized proteins (CGD 2010; Schleit et al. 2013). Up-regulation of Pre7 (66.85-fold) a proteasome component indicates that ubiquitin-dependent proteasome activity was enhanced during chlamydospore growth (Table 3) (CGD 2010; Liu et al. 2016; Verma et al. 2000). Up-regulation of Tpd3 in our study confirms enhanced cytokinesis) during chlamydosporulation as reported earlier (Liu et al. 2016; Lorenz et al. 2004; Sarkar et al. 2002).

Nutrient limitation, microaerophilic and osmotic stress modulate metabolism during chlamydospore growth that leads to acquire unique morphological and architectural characteristics in addition to survival using nonconventional and complex carbohydrates (viz. maltose, xylitol etc.) (Fig. 3) (Jamai et al. 2007; Bruno et al. 2006). Significant up-regulation of maltase (64-fold) followed by enhanced glycolysis and tricarboxylic acid cycle in our study confirms earlier hypothesis that states, "Enhanced glycolysis and tricarboxylic acid cycle under glucose limiting condition contribute to link the regulation of chlamydospores production in *Candida"* (Table 1 and Fig. 3) (Brown et al. 2014; Bottcher et al. 2016; CGD 2010; Han et al. 2011). Enhanced glyoxylate cycle reported to facilitate survival of *C. albicans* under glucose limiting condition is enhanced in our study (Fig. 3) (Barelle et al. 2006; Ene et al. 2012). Microaerophilic condition triggers alcohol production leading to hyphae induction required for chlamydospore formation (Fig. 3) (Chauhan et al. 2011; Smith et al. 2004). In addition to energy generation, glycolysis provides precursors for the biosynthesis of lipids (storage molecules) and trehalose required for stress (oxidative, heat, desiccation, osmotic etc.) tolerance (Fig. 3) (Brown et al. 2014; Pereira et al. 2001; Yoda et al.2000). Inhibition of NAD+synthesis increases oxidative stress tolerance and extend lifespan as NAD is an essential cofactor for cellular redox reactions (Table 1) (Pereira et al. 2001; Bedalov et al. 2003; Kato and Lin 2014). Enhanced glutathione biosynthesis, up-regulation of Osm1, Yhb1 and down-regulation of Ysa1 could be the compensatory responses to enhanced oxidative, nitrossative and osmotic stresses during chlamydospore growth (Table 2) (Chen et al. 2008; Cottier et al. 2012; Liu et al. 2000; Michán and Pueyo 2009; Nett et al. 2009; Yadav et al. 2011). Enhanced level of Yhb1 was also validated at RNA level using qRT-PCR analysis (Tables 2, 5 and Figs. 4, 5). Chlamydospore inducing condition further potentiates antioxidant machinery through the maturation of Sod1 and trehalose homeostasis (through up-regulation of Lys7 and Hsp21 respectively) (Dong et al. 2013; Gleason et al. 2014; Mayer et al. 2012). Morpho-physiological and cellular architectural modulations seems to affect cellular and mitochondrial membrane functions like transport and osmotic stability as proteins involved in maintaining these functions like Gdi1, Cof1, Por1 were up-regulated (Cederquist et al. 2012; Curwin et al. 2012; Kamthan et al. 2012; Lin et al. 2010; Cao et al. 2005). However vacuolar transport and vacuolar

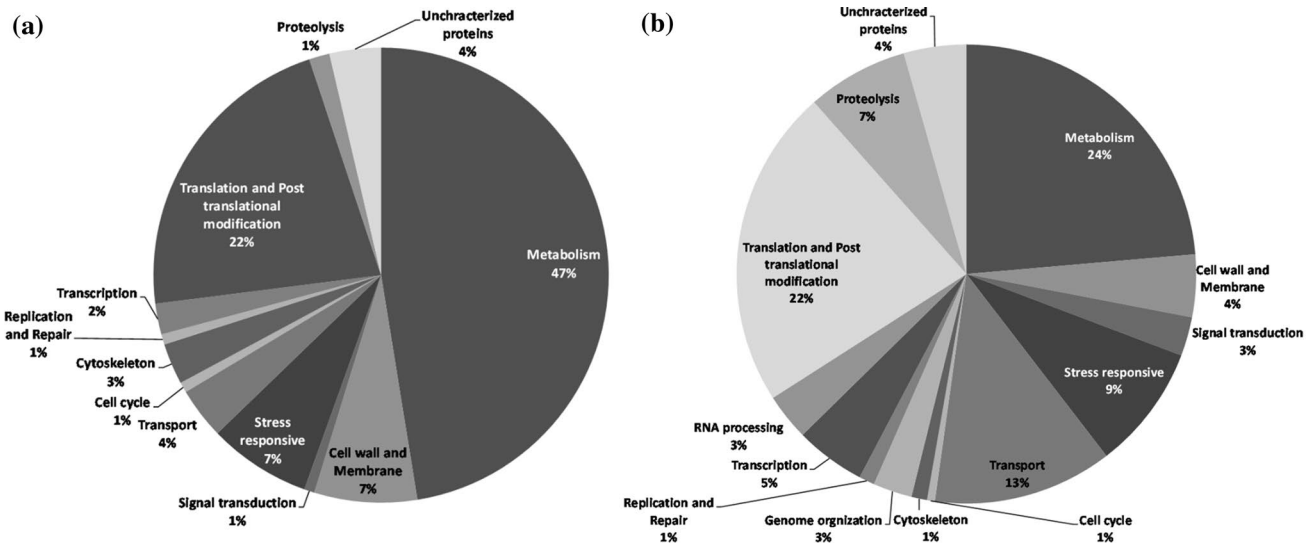

Fig. 3 Chlamydospore specific proteins of *Candida albicans* (ATCC 10231). **a** Up-regulated **b** down-regulated proteins

Table 5 Primers used in real time-qPCR analysis

Sr. no	Gene name	Forward primer (5→3)	Reverse primer (5→3)	Amplicon size
1.	Pgk1	TTGGATGCTGCTGTCAAATC	TGAAGCACCACCACCAGTAG	132
2.	Yhb1	CGTTGCTGGTGGTATTGGTA	TTTGAAAAGGTTGCCGAAAG	130
3.	Hyr1	CAATACCGGTGCTCACACTG	TTTTCCATCAAAGCCAGTCA	136
4.	Kre9	CGGATCAAGCTTCAGGATTT	CATTTGCATTGGTGCGTATC	107
5.	Ald4	ATTGAATGTGGTGGGGTTCA	GAAACTCATCAATCCCCCATT	124

(H)-ATPase activities (viz. endocytosis, tagging of lysosomal enzymes) were modulated in our study (Cabezon et al. 2009; Cabrera et al. 2013; Johnston et al. 2013).

Metabolic modulation enhances biosynthesis of components of cell wall and membrane during chlamydospore growth. The thick cell wall is reported to provide protection against adverse microenvironment, however, structure and composition of cell wall of chlamydospore is not very clear (Hazel and Williams 1990; Citiulo et al. 2009; Jansons and Nickerson 1970). Different environmental stresses/factors reported to modulate cell surface chemistry viz. cell surface hydrophobicity (CSH) and adhesion, the two important virulence factors facilitating survival of *C. albicans* (Odds and Bernaerts 1994). These morphophysiological modulations are results of modulation in central metabolic pathway that leads to modulations in cellular architecture and thus cell surface properties (Ingle et al. 2017). Cell surface properties are defined by cell surface chemistry, cell surface hydrophobicity (CSH) and adhesion etc. (Brown et al. 2014; Hazen et al. 1986; Hazen 1990; Klotz and Penn 1987). Cell surface properties are extremely important in survival and virulence of *C. albicans* (Odds and Bernaerts 1994). CSH determines host-*Candida* cell interaction, i.e. adhesion and colonization followed by tissue invasion at different tissue sites with varied microenvironments (Ener and Douglas 1992). In general, adhesion increases with increase in CSH i.e. CSH and adhesion is directly proportional (Ener and Douglas 1992; Klotz et al. 1985). More numbers of adhesins in *C. albicans* compared to non-virulent yeast *S. cerevisiae* cites the importance of cell surface molecules in pathogenicity (Guthrie and Fink 2002). Csh1 (Cell surface hydrophobicity protein 1) was down regulated in our study (Table 5). Down regulation of Csh1 during chlamydosporulation correlates positively with adhesion (Klotz 1990; Samaranayake et al. 2003). Decreased cell surface hydrophobicity is indicative of decreased virulence in chlamydospores (Fig. 2a) (Guthrie and Fink 2002; Samaranayake et al. 2003).

Our result suggests that enhanced mannan, β1, 3-glucan and chitin biosynthesis thicken and strengthen the cell wall of chlamydospore (Table 2) (CGD 2010). Increased components viz. β-glucan (β 1, 3-glucan) and chitin are reported to strengthen cell wall under hypo-osmotic and hypoxic stress (Table 2) (Ene et al. 2012; Hall 2015; Smits et al. 2001). Interestingly, down regulation of Kre9 (at protein and RNA level) and Phr2 indicates lack of β 1,6-glucan leading to abnormal cross-linking, however

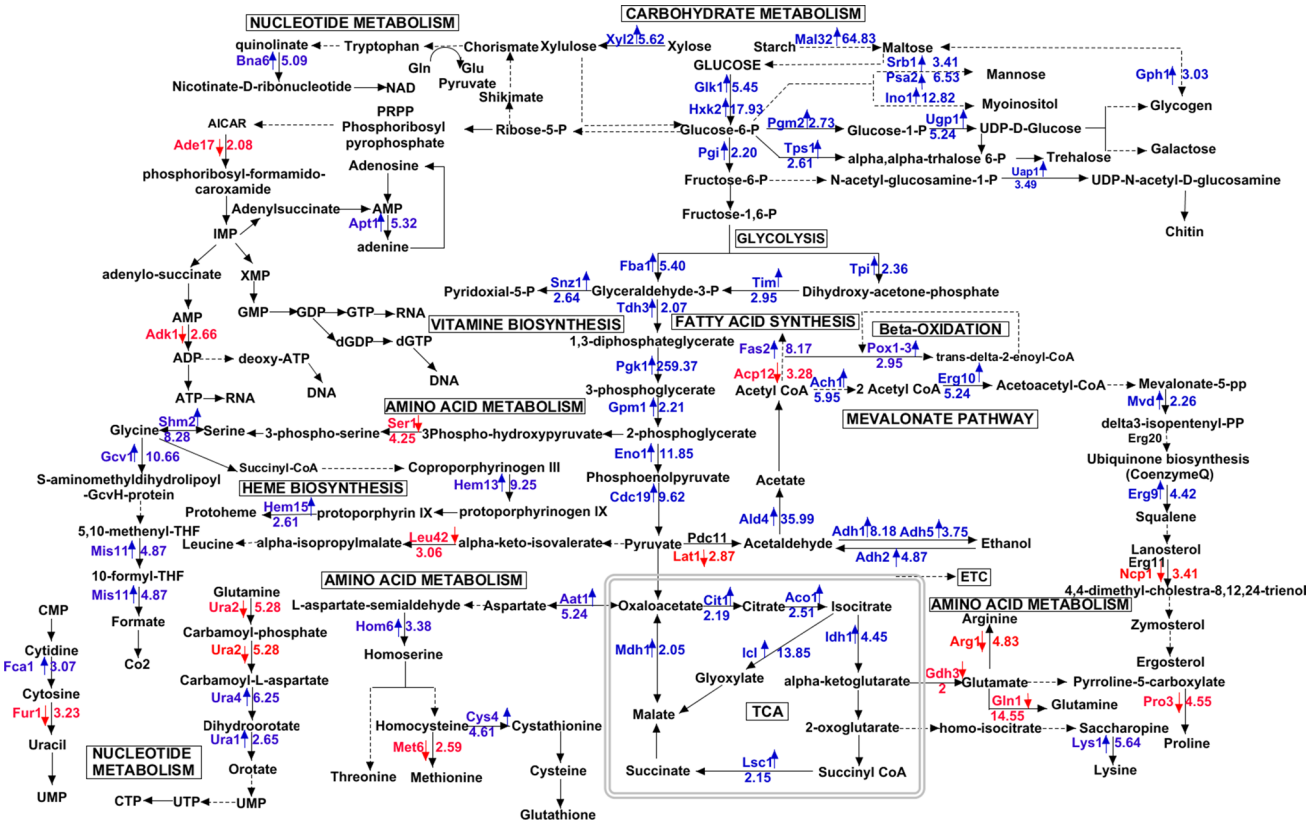

Fig. 4 Hypothetical model based on proteomic analysis showing modulation of metabolism in *C. albicans* ATCC 10231 during chlamydosporulation

enhanced chitin content could be a compensatory response to maintain cell wall strength (Tables 2, 5 and Figs. 3, 4, 5) (Aimanianda et al. 2009; Lee et al. 2012; Smits et al. 2001). Over expression of two hyphae specific GPI (glycosylphosphatidylinositol) anchored proteins (Als3, Hyr1) involved in adhesion, indicates that sample exhibit hyphae producing chlamydospores and those were highly adhesive (Table 2, Fig. 5) (Heilmann et al. 2011; Klis et al. 2009; Richard et al. 2002). However, reduced hydrophobicity (down-regulation of Csh1) as well as down regulation of potential virulence factors (Pst3 and Ttr1) known to affect redox state of target proteins of host cells confirms attenuated virulence in chlamydospores (Table 2) (Collinson et al. 2002; Karababa et al. 2004; Seneviratne et al. 2008). Enhanced biosynthesis confirms that lipids (ergosterol, sphingolipid, phospholipids and fatty acids) are the storage molecules of chlamydospores in addition to providing more strength and rigidity to membranes (Table 2) (Fu et al. 2012; Walther et al. 2006; Young et al. 2002).

Our proteomic data confirms that nutrient limiting and microaerophilic microenvironment is sensed by *C. albicans* through RAS mediated cAMP-PKA pathway that modulate metabolism to use complex carbohydrates like maltose (Fig. 6). Enhanced degradation of complex carbohydrates

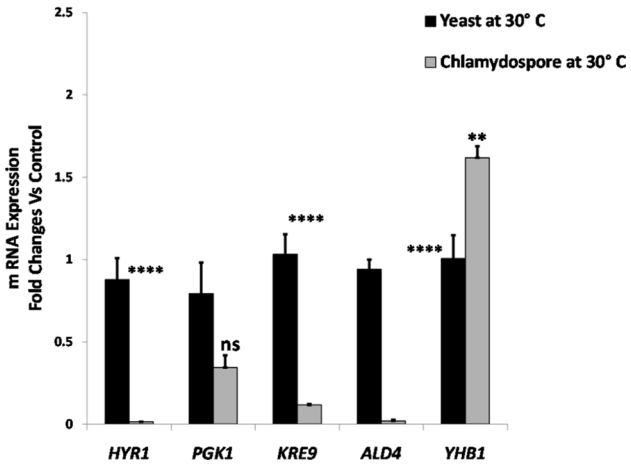

Fig. 5 Real Time qPCR analysis of selected genes. Data is shown as mRNA copies in cells, where significance refers to the difference between chlamydospores and control (yeast form cells), ($n=3$), $^{****}P<0.001$, $^{**}P<0.01$, Bar indicates mean and error bars indicates SD

releases simple sugars and enhances glycolysis and TCA cycle, significantly. In addition to energy, enhanced glycolysis and TCA cycle provides precursors for biosynthesis of

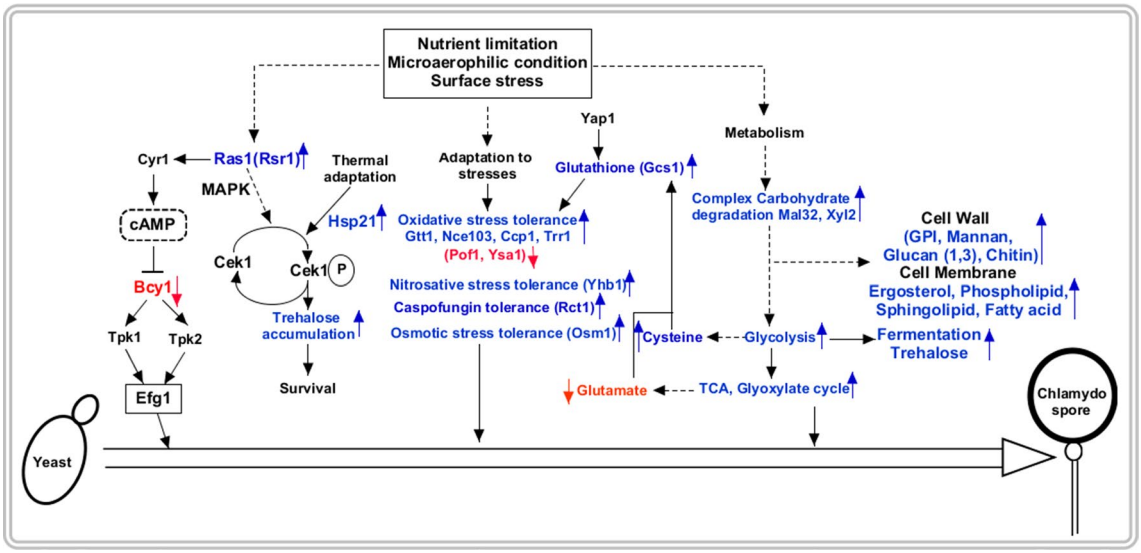

Fig. 6 Hypothetical model based on proteomic analysis showing modulation of cellular processes during chlamydosporulation

lipids and glycogen (energy reserve), sterol and fatty acids (strengthen membrane), chitin, mannan, Beta, 1–3, glucan (strengthen cell wall architecture) and glutathione and trehalose (potentiate stress tolerance) (Fig. 6). In general, we conclude that chlamydosporulation confer tolerance towards hostile microenvironment by strengthening cellular architecture and stress responses through modulations in metabolic pathways and thus survival of *C. albicans*.

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