Chlamydospore Specific Proteins of *Candida albicans*

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Abstract: Polymorphic yeast, *Candida albicans*, forms thick-walled structures called chlamydospores in order to survive under adverse conditions. We present proteomic profile changes occurring during chlamydospore formation. Chlamydospores were induced by inoculating *C. albicans* cells (grown for 48 h) on rice extract and semisolid agar containing tween 80 (1%), and were overlaid by a polyethene sheet to induce microaerophilic conditions at 30 °C. Proteins extracted from chlamydospores and hyphae (producing chlamydospores) were identified by LC-MS/MS analysis. Present datasets include proteomic data (Swath spectral libraries) of chlamydospores and yeast phase cells, as well as methodologies and tools used for the data generation. Further analysis is expected to provide an opportunity to understand modulations in metabolic processes, molecular architecture (i.e., cell wall, membrane, and cytoskeleton) and stress response pathways leading to chlamydospore formation and thus facilitating survival of *C. albicans* under adverse conditions.

Data Set: http://www.peptideatlas.org/PASS/PASS01061 (Username: PASS01061, Password: PF8546i)
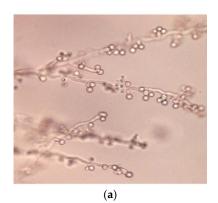
Data Set License: There is no specific license.
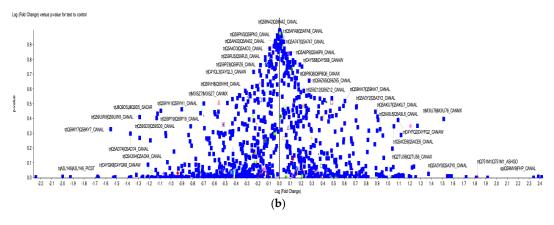
1. Introduction

Morphophysiological plasticity enables *Candida albicans* to survive under a variety of extreme microenvironments [1–11]. It exists in the form of yeast, hyphae, pseudohyphae, chlamydospore, white/opaque cells, according to the microenvironment [1–11]. Cells in different morphological forms respond differently towards host defense mechanisms, antifungal agents, stress conditions [8]. It makes *C. albicans* one of the most successful opportunistic pathogens of humans [1,2]. Among these morphological forms, chlamydospore is one of the most interesting structures, and develops under unfavorable conditions such as oxygen limitation and embedded growth in matrix (Figure 1a) [5–7]. The significance and regulation of chlamydospore formation are not yet fully understood, though Böttcher et al. (2016) provides a detailed account of signaling pathways regulating chlamydospore formation in response to different environmental and nutritional factors [1,3,4]. Chlamydospores are a relatively unusual morphological form that facilitates survival of *C. albicans* under extreme micro environments by significantly lowering metabolism; however, they can germinate once conditions become favorable [3,4,6,8,9]. The thick walls could provide protection against the adverse microenvironment; however, not much study is available on the structure and composition of chlamydospore cell walls [9–14]. In the present study, a proteomic profile of chlamydospores of *C. albicans* generated using LC-MS/MS provides insights into the regulation of chlamydospore formation and survival strategies.

2. Results

Proteomic data of chlamydospore (test) and yeast phase cells (control) of *C. albicans* were generated through processed peptides using Micro LC-Triple TOF 5600 SWATH MS analysis. Specifically, SWATH MS mode was utilized for optimization of quadrupole settings in the instrument [15–20]. Samples were spiked from each treatment (chlamydospores and yeast phase cells) to produce the SWATH spectral library, and were analyzed via LC-MS/MS by information dependent acquisition (IDA) (Figure 1b, Supplementary Tables 1–17). Data was uploaded to PeptideAtlas and is available at the Official URL for this dataset: http://www.peptideatlas.org/PASS/PASS01061. Functional annotation (using David software, *Candida* genome database, *Saccharomyces* genome database, Kyoto Encyclopedia of Genes and Genomes and UniProt databases) revealed significant modulations in metabolism, cellular architecture, gene expression and stress response pathways required for survival of *Candida albicans* under adverse microenvironments [11,12].

(**a**)

(**b**)

Figure 1. (**a**) Light microscopy images of chlamydospores with hyphae (chlamydospore-producing) formation of *C. albicans* (ATCC 10231); (**b**) Scatter plot of differentially expressed proteins during Chlamydospore formation.

3. Discussion

Candida albicans is one of the most important opportunistic pathogens in humans, worldwide [21]. Morphological plasticity (i.e., yeast, hyphae, pseudohyphae, chlamydospore, white and opaque cells) enables *C. albicans* to survive and grow in extreme niches by evading host immune responses [21,22]. Among these morphological forms, chlamydospores (dormant spores), though rarely observed in tissues [23], exhibit different responses towards the host immune system, and germinate and form hyphae once conditions becomes favorable through multiple, interlinked signaling pathways [1–3,8]. Our proteomic data will provide an opportunity to understand the regulation of morphogenesis (i.e., chlamydospores formation) and survival under adverse environmental conditions in addition to modulation of molecular architecture (i.e., cell wall, membrane, and cytoskeleton of chlamydospore).

4. Materials and Methods

4.1. Candida albicans Strain and Growth Conditions

Candida albicans (ATCC 10231), a quality control strain, was obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India and maintained on yeast extract peptone dextrose (YPD) agar slants at 4 °C. Yeast Extract Peptone Dextrose (YEPD) Broth and Rice extract agar were purchased from Hi-media Laboratories, Pvt. Ltd. (Mumbai, India). All the fine chemicals used in this study were purchased from Sigma-Aldrich Pvt. Ltd (Bangalore, India), Bangalore, and solvents used were purchased from Qualigens and SD Fine Chemicals Ltd. (Mumbai, India).

4.2. Test: Induction of Chlamydospore Formation

Candida albicans cells (grown for 48 h in YEPD broth at 28 °C) were grown on a nutrient-limiting rice extract agar (rice extract 1.3%) containing 1% tween 80 (for exerting surface stress). The inoculum was overlaid with circles of sterile polyethylene transparent sheets for microaerophilic conditions under embedded growth necessary for chlamydospore production. Plates were further incubated at 30 °C for 14 days [13,14].

4.3. Control Experiment

Candida albicans cells were grown on nutrient-limiting rice extract agar (rice extract 1.3%) plates containing 1% tween 80 (without microaerophilic conditions and embedded growth) at 30 °C for 14 days. Surface-grown cells were used as control.

4.4. Extraction of Proteins

Chlamydospores and hyphae (chlamydospore producing) from test and yeast phase cells (from control) were harvested from twelve-day old plates. Chlamydospores were lysed and proteins were extracted as per the protocol optimized by Haar (2007), and concentration was estimated by using the Bradford method [15,16].

4.5. Sample Preparation for LC-MS

Extracted proteins were subjected to trypsin digestion and peptides generated were washed using Zip tip C_{18} chromatography columns (Millipore; Billerica, MA, USA) and concentrated.

4.6. Liquid Chromatography and Mass Spectrometry

LC-MS/MS was carried out using a Triple-TOF 5600 (AB Sciex; Vaughan, ON, Canada) mass spectrometer coupled with a Micro LC 200 (Eksigent; Dublin, CA, USA) in high-sensitivity mode. Samples were spiked from each treatment to produce the SWATH spectral library and analyzed via LC-MS/MS by information-dependent acquisition (IDA) in duplicate [17–20]. In brief, a digested protein sample (4 μg) was injected into a Eskigent C18-RP HPLC column (100 × 0.3 mm, 3 μm, 120 Å) and then separated using a 95-min gradient of 3 to 35% ACN (acetonitrile) for IDA run and (1 μg) for SWATH run [17–20].

4.7. SWATH MS Analysis

Datasets were acquired (in triplicate) on Micro LC-Triple TOF 5600 SWATH MS. The instrument was specifically tuned in SWATH-MS mode to optimize the quadrupole settings for the selection of precursor ion selection windows 25 *m/z* wide. Using an isolation width of 25 *m/z* (containing 1 *m/z* for the window overlap), a set of 34 overlapping windows was constructed covering the precursor mass range of 400–1250 *m/z*. From 100 to 2000 *m/z* SWATH MS/MS spectra were collected. According to the calculation for a charge 2+ ions, for each window, the collision energy was optimized, centered upon the

window with a spread of 15 eV. For all fragment-ion scans in high-sensitivity mode accumulation time (dwell time) of 100 ms was used. For 100 ms each SWATH-MS cycle a survey scan in high-resolution mode was also acquired resulting in a duty cycle of 3.4 s [17–20]. A spectral library was generated from IDA run using Protein pilot software version 4 by analyzing data against yeast database. Paragon algorithm is used in Protein pilot software. Approximately 1000 proteins were identified, with 5% FDR. The generated spectral library was used for SWATH analysis with 20 ppm error. SWATH runs were acquired in triplicate. SWATH runs of control and test were uploaded in SWATH processing window with 5 min RT window, 50 ppm mass error and 99% confidence. Processed SWATH file was exported to marker view software [17–20].

Exported files were saved as ion, peptide and protein files format. Protein file format was used for further analysis in Marker view. Data was normalized by Total sum normalization. Processed SWATH runs were grouped as control and test and compared using *t*-test [17–20]. Protein expression data was generated, and proteins with *p*-value < 0.05 and fold change ≥2 fold were considered as differentially expressed proteins in test with respect to control [11,12]. Uniprot ID of all differentially expressed proteins were retrieved and functionally annotated using David software [11,12].

4.8. Statistical Analysis

Statistical analysis was carried out using *t*-test. Probability p value less than 0.05 were considered to be significant and proteins with a number of matching peptides (≥2) and fold change (≥2) were considered for further analysis (samples were acquired in triplicate).

5. Conclusions

Proteomic profile of chlamydospores of a polymorphic fungus *C. albicans* were generated using LC-MS/MS analysis. Approximately 1000 proteins identified were characterized using different databases and tools. The dataset presented in this paper indicates significant modulation in proteins associated with morpho-physiological (chlamydospore formation) transition and stress responses. This data will be useful in understanding underlying mechanisms that facilitate survival of eukaryotic cells under extreme micro-environments.

Supplementary Materials: The following are available online at https://figshare.com/s/2c6887e058245d0b612c. DOI: 10.6084/m9.figshare.5286097.

Author Contributions: Gajanan Zore and Sujata Ingle designed experiment, Sujata Ingle performed microbiological work and fixed samples for protein preparation. Rajendra Patil prepared the protein extract, Santosh Kodgire and Asha Shiradhone performed LC-MS/MS analysis and Gajanan Zore and Sujata Ingle wrote the MS.

Conflicts of Interest: The authors declare no conflict of interest.

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