Journal of Proteomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Serum responsive proteome reveals correlation between oxidative phosphorylation and morphogenesis in *Candida albicans* ATCC10231

Radfan Ahmed[a], Santosh Kodgire[a], B. Santhakumari[b], Rajendra Patil[c], Mahesh Kulkarni[b], Gajanan Zore[a,*]

[a] School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded 431606, MS, India
[b] CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008, MS, India
[c] Department of Biotechnology, Savitribai Phule Pune University, Ganeshkhind, Pune 411007, MS, India

ARTICLE INFO

Keywords:
Candida albicans
Fetal bovin serum
Morphogenesis
Pathogenicity
Oxidative phosphorylation
Liquid chromatography–mass spectrometry analysis

ABSTRACT

To understand the impact of fetal bovine serum (FBS) on metabolism and cellular architecture in addition to morphogenesis, we have identified FBS responsive proteome of *Candida albicans*. FBS induced 34% hyphae and 60% pseudohyphae in *C. albicans* at 30 °C while 98% hyphae at 37 °C. LC-MS/MS analysis revealed that 285 proteins modulated significantly in response to FBS at 30 °C and 37 °C. Out of which 152 were upregulated and 62 were downregulated at 30 °C while 18 were up and 53 were downregulated at 37 °C. Functional annotation suggests that FBS may inhibit glycolysis and fermentative pathway and enhance oxidative phosphorylation (OxPhos), TCA cycle, amino acid and fatty acid metabolism indicating a use of alternative energy source by *C. albicans*. OxPhos inhibition assay using sodium azide corroborated the correlation between inhibition of glycolysis and enhanced OxPhos with pseudohyphae formation. *C. albicans* induced hyphae in response to FBS irrespective of down regulation of Ras1,Asr1/Asr2, indicates the possible involvement of MAPK and cAMP-PKA independent pathway. The Cell wall of cells grown in presence of FBS at 30 °C was rich in mannan, Beta 1,3-glucan and chitin while membranes were rich in ergosterol compared to those grown at 37 °C.

Significance of the study: This is the first study suggesting a correlation between OxPhos and morphogenesis especially pseudohyphae formation in *C. albicans*. Our data also indicate that fetal bovine serum (FBS) induced morphogenesis is multifactorial and may involve MAPK and cAMP-PKA independent pathway. In addition to morphogenesis, our study provides an insight in to the modulation of metabolism and cellular architecture of *C. albicans* in response to FBS.

1. Introduction

Candida albicans is an opportunistic pathogen that causes candidiasis in healthy as well as immune-compromised patients, however it is deadly among the latter [1–3]. Candidiasis could be superficial (skin, nails and hair), mucocutaneous (oral cavity, esophagus, gastrointestinal tract and vagina) to life-threatening systemic infections [4–6]. It is one of the most frequent pathogens associated with bloodstream infections where it ranks fourth with significantly high mortality [7, 8]. In addition to this, it was reported to colonize body implants (medical devices heart valve, stent, catheters etc.) in the form of difficult-to-treat biofilms due to drug resistance [9]. Considering its clinical status, *Candida* is included in the list of pathogens with potential drug resistance threat against already limited therapeutic options by CDC [10]. Thus the understanding mechanism of pathogenesis to identify novel targets for potent antifungal agents is being explored with heightened interest in recent years.

Virulence factors, like hyphae induction, hydrolytic enzymes, cell surface adhesin etc., are known to facilitate tissue specific infections however among these, ability to form hyphae is considered as a prerequisite for invasive candidiasis [2]. Morphogenesis in addition to virulence enables *C. albicans* to respond differentially towards antifungal agents, microenvironment as well as host defense mechanisms [11]. A constant interplay among the host-pathogen-microbiome interaction determines the fate of *C. albicans* growth commensal or pathogen [12]. A slight modulation in balance in host immune status (immunocompromised condition, immunosuppressive drugs) and or microbiome (prolonged use of antibiotics) enable *C. albicans* to become pathogenic and invade host tissues [13]. Considering the significance of hyphae in survival and virulence, attempts are being made to understand physiological modulations under hyphal form that transform a commensal *C. albicans* into an opportunistic pathogen and survive [14,

* Corresponding author at: Research Laboratory 1, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded 431606, MS, India.
E-mail addresses: b.santhakumari@ncl.res.in (B. Santhakumari), rpatil@unipune.ac.in (R. Patil), mj.kulkarni@ncl.res.in (M. Kulkarni), gbzsrtmun@gmail.com (G. Zore).

https://doi.org/10.1016/j.jprot.2018.06.018
Received 10 April 2018; Received in revised form 5 June 2018; Accepted 18 June 2018

15]. Several factors viz. serum, *N*-acetylglucosamine, proline, glucose, alkaline pH and body temperature 37 °C etc., are known to induce hyphae [16, 17]. Fetal bovine serum (FBS) is the first and the most potent inducer of filamentous growth in *C. albicans* and thus a germ tube test was developed and still being used to differentiate isolates of *C. albicans* from other yeasts [18, 19]. Considering the complexity of FBS, morphogenesis induced by FBS is multifactorial and thus mediated through multiple signaling pathways like cAMP-PKA and MAPK pathway involving crosstalk [20, 21]. However very little is known about the *C. albicans* hyphae induced by FBS especially its virulence potential determined by cell surface chemistry (CSH, adhesion), membrane properties and cellular metabolism. Thus in the present study, we have made an attempt to understand morphophysiological modulations in *C. albicans* cells in response to FBS and its impact on pathogenicity and survival using a proteomic approach.

2. Materials and methods

The Standard strain of *C. albicans* ATCC 10231 was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTech), Chandigarh, India. The strain was cultured on YEPD (1% yeast extract, 2% peptone, 2% dextrose and 2.5% agar) agar slants with pH 6.5 and maintained at 4 °C.

2.1. Inoculums preparation

C. albicans ATCC 10231, yeast cells, grown on YEPD broth for 24 h at room temperature were harvested by centrifugation (1000 rpm for 2 min) at room temperature and cells were washed thrice with sterile distilled water, and starved byre-suspending in 1 mL distilled water for 1 h at 30 °C. Cell density was determined microscopically using hemocytometer and adjusted to 2×10^6 cells/mL and used in further studies.

2.2. Induction of filamentous growth

The starved cells (2×10^6 cells/mL) were added to the flasks containing pre-warmed (37 °C) induction medium (YEPD broth containing 10% fetal bovine serum). The flasks were incubated at 30 °C and 37 °C and induction of hyphae was monitored after 90 min incubation. However, incubation was continued and cells and hyphae were harvested after for 6 h for protein and RNA extraction. Induction was carried out in triplicate flasks [22].

2.3. Sample preparation for LC-MS/MS analysis

2.3.1. Protein extraction

Cells were lysed by suspending 3×10^9 cells in 2000 µL lysis buffer containing protease Inhibitor Cocktail (PIC) (10 µl/mL) and then incubated at 90 °C. After 10 min, 50 µL of 4 M acetic acid was added and incubated at 90 °C for 10 min. The suspension was centrifuged for 5 min at 5000 rpm, and the supernatant was transferred to new tubes containing 5 µL/mL of Phenyl Methane Sulphonyl Fluoride (PMSF). The proteins were precipitated using 4 volumes of methanol, 1 volume of chloroform and 3 volumes of water (under chilled condition) with simultaneous vortexing. Protein pellets were isolated by centrifugation (5 min at 5000 rpm) washed with. Pellets were dried and re-suspended in rehydration buffer (composition: 6 M Urea, 2 M Thiourea, 2% CHAPS and 1% DTT pH adjusted at 8.75) and stored at −80 °C until used [23].

2.3.2. Trypsin digestion

The extracted proteins were hydrolyzed by Proteomic Grade trypsin (Sigma T6567). In brief, 100 µg of protein was dissolved in 50 mM ammonium bicarbonate buffer containing 0.1% Rapigest (Waters Corporation, MA, USA). Proteins were reduced and alkylated by treating with 100 mM dithiothreitol for 15 min at 60 °C and 200 mM iodoacetamide for 30 min at room temperature respectively. Denatured proteins were treated with trypsin (1:25) at 37 °C for 16 h and the reaction was stopped by addition of 0.1% formic acid. Digested peptides were desalted by using C18 Zip tips (Millipore, Billerica, MA) and the eluted peptides were concentrated by using vacuum concentrator. The peptides were reconstituted in 3% ACN with 0.1% formic Acid and used for mass spectrometric analysis.

2.3.3. Liquid chromatography and mass spectrometry

Protein identification and relative quantification was performed by SWATH-MS (Sequential window acquisition of all theoretical fragment ion spectra, strategy for high throughput labels free protein quantification) analysis by using Triple-TOF 5600 (Sciex; Concord, Canada) mass spectrometer coupled with Micro LC 200 (Eksigent; Dublin, CA) in high-sensitivity mode. For generation of spectral library equal amounts of digested peptides from each treatment were pooled together and analyzed via LC-MS/MS in an Information dependent acquisition (IDA) mode.

2.3.4. SWATH MS analysis

SWATH MS datasets were acquired (in Triplicate) on Micro LC-Triple TOF 5600. Peptides were directly injected into an Eksigent C18–RP HPLC column (100 × 0.3 mm, 3 µm, 120 Å) then separated using a 90 min gradient of 3% to 35% mobile phase (Mobile phase A: 100% water with 0.1% (v/v) formic acid, Mobile Phase B: 100% acetonitrile with 0.1% (v/v) formic acid) at a flow rate of 8 µL/min. In SWATH-MS mode, the instrument was specifically tuned to optimize the quadrupole settings for the selection of precursor ion selection windows 25 *m/z* wide. Using an isolation width of 26 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. SWATH MS/MS spectra were collected from 100 to 2000 m/z. The collision energy was optimized for each window according to the calculation for a charge 2+ ion centered upon the window with a spread of 15 eV. An accumulation time (dwell time) of 100 ms was used for all fragment-ion scans in high-sensitivity mode, and for each SWATH-MS cycle a survey scan in high-resolution mode was also acquired for 100 ms resulting in a duty cycle of 3.4 s [24–27].

2.3.4.1. ***Protein identification and quantification***. To obtain spectral library from IDA analysis, proteins were searched against UniProt reviewed *Sachharocyces cerevisae* database by using Protein Pilot software 5.0 with following parameters, 1% FDR). The precursor and fragment initial mass error tolerance was set to 0.05 and 0.1 Da,respectively. Search parametes also included carbamidomethylation of cysteine residues as fixed modifications and methionine oxidation as variable modification. The generated spectral library was used as a database to in the SWATH analysis. SWATH data was analyzed with mass error of 20 and 30 ppm for precursors and fragment ions respectively. SWATH files were exported to Marker View to do relative quantification of proteins.

2.3.5. Statistical analysis

The *t*-test was used for statistical analysis, probability p-value < 0.05 were considered to be significant, Number of matching peptides ≥ 2 and ≥ 2 fold change difference of protein expression (biological samples were acquired in triplicate).

2.4. Oxidative phosphorylation (OxPhos) inhibition assay

The starved cells (2 × 106 cells/mL) were added to the flasks containing pre-warmed (37 °C) induction medium (YEPD broth containing 10% fetal bovine serum) with (test) or without (control) 10 mM sodium azide. The flasks were incubated at 30 °C and 37 °C and morphogenesis was monitored after 90 min using hemocytometer, a percentage of cell types were counted and compared with the control. An Experiment was performed in triplicates and repeated six times [28].