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Comparative Proteomic Analysis of *Candida albicans* and *Candida glabrata*

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Abstract
Introduction *Candida albicans* and *Candida glabrata* are the two most common opportunistic pathogens which are part of the normal flora in humans. Clinical diagnosis of infection by these organisms is still largely based on culturing of these organisms. In order to identify species-specific protein expression patterns, we carried out a comparative proteomic analysis of *C. albicans* and *C. glabrata*.

Methods We used "isobaric tag for relative and absolute quantitation" (iTRAQ) labeling of cell homogenates of *C. albicans* and *C. glabrata* followed by LC-MS/MS analysis using a quadrupole time-of-flight mass spectrometer. The MS/MS data was searched against a protein database comprised of known and predicted proteins reported from these two organisms. Subsequently, we carried out a bioinformatics analysis to group orthologous proteins

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across *C. albicans* and *C. glabrata* and calculated protein abundance changes between the two species.
Results and Conclusions We identified 500 proteins from these organisms, the large majority of which corresponded to predicted transcripts. A number of proteins were observed to be significantly differentially expressed between the two species including enolase (Eno1), fructose-bisphosphate aldolase (Fba1), CCT ring complex subunit (Cct2), pyruvate kinase (Cdc19), and pyruvate carboxylase (Pyc2). This study illustrates a strategy for investigating protein expression patterns across closely related organisms by combining orthology information with quantitative proteomics.

Keywords Biomarker · Candidiasis · Candidemia · Medical mycology · Molecular diagnostics · Fungal infection · Quantitative proteomics

Abbreviations

MTCC	Microbial type culture collection
ORF	Open reading frame

Introduction

Candida albicans and *Candida glabrata* are the commonest opportunistic pathogens of human mucosa and blood [1–3]. Clinical disorders with immunosuppression including those with HIV and patients on chemotherapy provide an opportunity for the otherwise harmless *C. glabrata* to cause candidiasis. As a result, *C. glabrata* is now second to *C. albicans* among the various *Candida* species isolated from patients with candidiasis [4, 5]. High prevalence of *C. glabrata* in the oral flora of patients with diabetes mellitus, advanced cancers, and HIV infection has been reported in several studies [6–11]. Often, high mortalities are associated with *C. glabrata* infections especially among cancer patients and bone marrow transplant patients [12–16]. The majority of *C. glabrata* isolates are reported to be resistant to both fluconazole and itraconazole [11], whereas most isolates of *C. albicans* are sensitive to these antifungal agents [9, 10]. Thus, it is even more important to diagnose *C. glabrata* infections early and accurately so that the appropriate therapy can be initiated [17, 18].

Although *C. albicans* and *C. glabrata* are often co-isolated from mucosal lesions, not many investigations have focused to study these organisms together. When together, they exhibit more severe symptoms and pose a greater difficulty in treatment [19, 20]. Molecular diagnostic procedures described thus far to distinguish between these two species cannot easily be incorporated as routine diagnostics as they require specialized equipment and expertise [21–25]. Although these organisms cannot be distinguished based on the clinical presentation of the patients, they differ in many aspects of genetic and physiological attributes. *C. albicans* thrives as a diploid [26, 27] and exhibits considerable heterozygosity while *C. glabrata* exists as a haploid genome [28]. Unlike *C. albicans*, *C. glabrata* cannot form true hyphae [29]. *C. glabrata* does not produce extracellular proteinases including secreted aspartyl proteinases [30] which are considered as virulent factors of *Candida* species [31–35]. *C. albicans* lacking superoxide dismutases are unable to thrive in the presence of macrophages [36]. Phospholipases that have been implicated in the virulence of *C. albicans* [37–41] also do not seem to play a major role in *C. glabrata* infection [42, 43]. *C. albicans* and *C. glabrata* are also known to differ in their host response profiles. Infection by *C. glabrata* induces expression of high levels of granulocyte monocyte colony-stimulating factor in the cells of oral mucosa, while *C. albicans* is not known to elicit such a response. On the other hand, *C. albicans* infection produces a strong IL-8 response, whereas it is elicited only weakly in the case of *C. glabrata*-infected cells [44–46]. *C. glabrata* is often resistant to several antimicrobial proteins secreted by the host such as beta defensins [47–49], histatins, and magainins [50–52]. A substantial variation among gene families involved in cell wall formation, transport, and sexual reproduction has been reported by comparing genomes of different pathogenic species of *Candida* and related yeasts [53]. Interestingly, a phylogenetic comparison of sequenced yeast genomes showed that *C. glabrata* belongs to "*Saccharomyces* clade" while most other *Candida* species including *C. albicans* belong to "*Candida* clade" [53]. The existence of distinct variation in diverse clinical features indicates the probable differences in the mechanisms of infection and adaptations to overcome host defense mechanisms and antifungal drugs. The availability of genome sequence information for both *C. albicans* [26] and *C. glabrata* [28] now provides a scaffold for proteomic investigations to understand distinct biological traits of these organisms at the molecular level. A comparative proteomic profile of *C. albicans* and *C. glabrata* should also provide information on differentially expressed proteins in these organisms that could serve as candidate molecules to distinguish these two species by protein-based tests such as ELISA. We describe a quantitative proteomic investigation using isobaric tag for relative and absolute quantitation (iTRAQ) reagents and mass spectrometry to identify the differentially expressed proteins in *C. albicans* and *C. glabrata*.

Materials and Methods

Culturing of *C. albicans* and *C. glabrata*

C. albicans (MTCC 1637) and *C. glabrata* (MTCC 6507) cultures were obtained from the Microbial Type Culture

Collection & Gene Bank resource in Chandigarh, India. The cells were cultured in identical conditions in 2% YPD broth at 30°C with shaking for 6 h. The cells were harvested at the same density by centrifuging at 2,000×*g* for 10 min, and pellets were washed using sterile distilled water. Cell pellets were stored at −80°C until further analysis.

iTRAQ Labeling and Strong Cation Exchange Fractionation

Cell pellets were dissolved in 0.5% SDS and homogenized. The samples were subsequently sonicated using ultrasonicator (Branson Ultrasonics) and centrifuged at 10,000×*g* for 10 min at 4°C. Clear supernatant was collected in fresh vials. Protein estimation was carried out using Lowry's assay. Further, two samples were normalized based on total protein amount. Normalization was verified by SDS-PAGE. Protein samples were reduced, cysteine-blocked, and digested with trypsin. iTRAQ (Applied Biosystems Catalog No. 4352135) labeling of peptides was carried out according to manufacturer's protocol essentially as described earlier [54]. Briefly, 80 μg of protein extract from each sample was treated with 2 μl of reducing agent (tris(2-carboxyethyl) phosphine) at 60°C for 1 h and alkylated with 1 μl of methyl methanethiosulfonate (MMTS)) for 10 min at room temperature. Proteins were then digested using sequencing grade trypsin (Promega) (1:10) overnight at 37°C. Peptides in duplicate from *C. albicans* sample were labeled using iTRAQ reagents containing reporters 114 and 115 and peptides in duplicate from *C. glabrata* sample were labeled using iTRAQ reagents containing reporters 116 and 117 (Fig. 1). Labeling was carried out for 2 h at room temperature. After labeling, the peptides from the two samples were pooled and fractionated using strong cation exchange chromatography on Polysulfoethyl A column (PolyLC, Columbia, MD) (100×2.1 mm, 5 μm particles with 300 Å pores) using an LC Packing HPLC system connected to a Probot fraction collector. Thirty SCX fractions were collected at a flow rate of 0.2 ml/min using a 70-min gradient of KCl from 0 to 350 mM concentration in 10 mM potassium phosphate buffer, 25% acetonitrile (pH 2.85). The fractions were dried and reconstituted in 10 μl of 2% trifluoroacetic acid before mass spectrometric analysis.

Mass Spectrometry and Data Analysis

Mass spectrometry analysis was carried out using a reversed-phase liquid chromatography system (RP-LC) interfaced with a quadrupole time-of-flight mass spectrometer (QSTAR/Pulsar, Applied Biosystems). MS/MS data was acquired by online analysis of peptides eluted using 5–40% acetonitrile in 0.1% formic acid for 30 min with a flow rate of 300 nl/min. The MS/MS spectra were acquired in a data-dependent manner from *m*/*z* 350 to 1,200 Da targeting three most abundant ions in every survey scan for MS/MS with a dynamic exclusion time of 45 s. Twenty percent higher collision energy was applied during MS/MS scan with a charge state-dependent collision energy selection criteria.

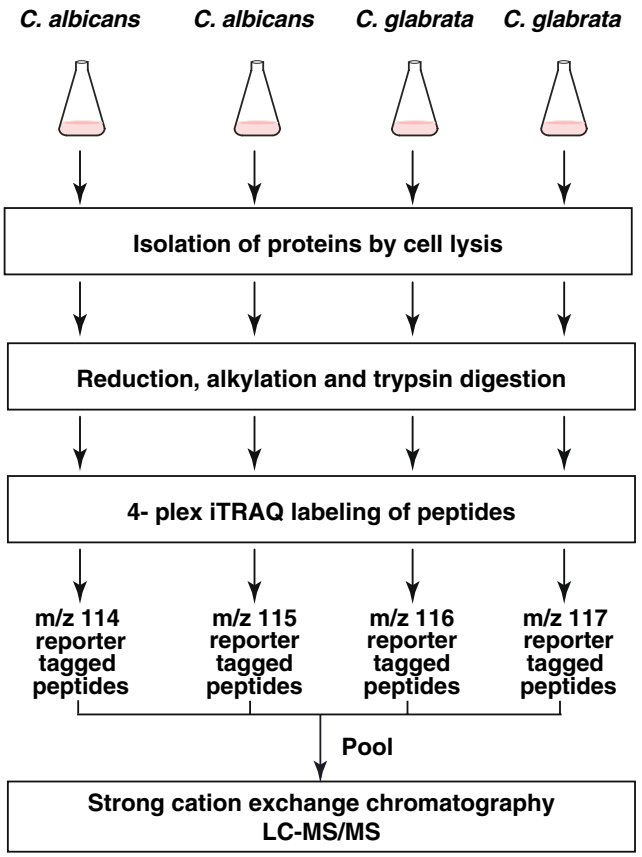
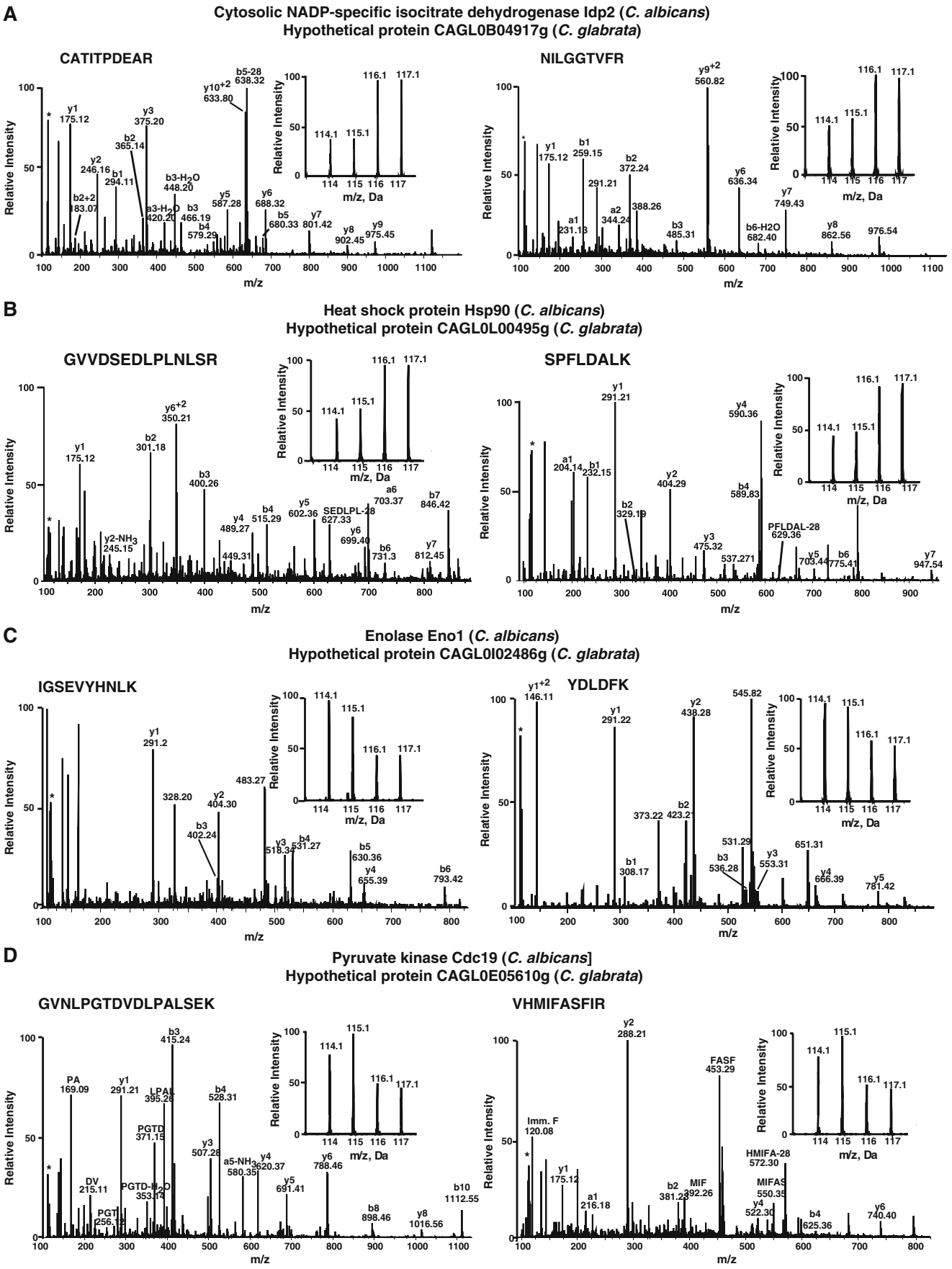


Fig. 1 A schematic of quantitative proteomic characterization of *C. albicans* and *C. glabrata*. After homogenization of cells, the proteins were digested with trypsin. The resulting peptides from *C. albicans* and *C. glabrata* were labeled with different iTRAQ reagents in a 4-plex strategy and subsequently pooled. The peptide mixture was analyzed by LC-MS/MS, and the proteins were identified by searching the MS/MS spectra against the custom protein database and quantitated using intensities of reporter ions

We created a custom protein database of non-redundant protein sequences of *C. albicans* and *C. glabrata* by combining ORFs of *C. albicans* from the *Candida* Genome Database (http://candidagenome.org/), ORFs of *C. glabrata* from databases of Genolevures (http://genolevures.org/download.html), and protein sequences of these organisms from NCBI RefSeq database. This customized database contained 39,196 protein sequences with 28,152 protein sequences from *C. albicans* and 11,044 from *C. glabrata*. We used ProteinPilot software (V 3.0 Applied Biosystems) for data analysis, which includes Paragon algorithm for


A
Cytosolic NADP-specific isocitrate dehydrogenase Idp2 (C. albicans)
Hypothetical protein CAGL0B04917g (C. glabrata)
CATITPDEAR
NILGGTVFR
B
Heat shock protein Hsp90 (C. albicans)
Hypothetical protein CAGL0L00495g (C. glabrata)
GVVDSEDLPLNLSR
SPFLDALK
C
Enolase Eno1 (C. albicans)
Hypothetical protein CAGL0I02486g (C. glabrata)
IGSEVYHNLK
YDLDFK
D
Pyruvate kinase Cdc19 (C. albicans]
Hypothetical protein CAGL0E05610g (C. glabrata)
GVNLPGTDVDLPALSEK
VHMIFASFIR
Relative Intensity
m/z
m/z, Da

◀ **Fig. 2** MS/MS spectra of representative shared peptides from differentially expressed ortholog proteins. **a** Peptides CATITPDEAR and NILGGTVFR belonging to cytosolic NADP-specific isocitrate dehydrogenase Idp2 [*C. albicans* SC5314] and CAGL0B04917g [*C. glabrata* CBS138], expressed higher in *C. glabrata*. **b** Peptides GVVDSEDLPLNLSR and SPFLDALK belonging to heat shock protein Hsp90 [*C. albicans* SC5314] and hypothetical protein CAGL0L00495g [*C. glabrata* CBS138] expressed higher in *C. glabrata*. **c** Peptides IGSEVYHNLK and YDLDFK belonging to enolase Eno1 [*C. albicans* SC5314] and CAGL0I02486g [*C. glabrata* CBS138], expressed higher in *C. albicans*. **d** Peptides GVNLPGTDVDLPALSEK and VHMIFASFIR belonging to pyruvate kinase Cdc19 [*C. albicans* SC5314] and CAGL0E05610g [*C. glabrata* CBS138], expressed higher in *C. albicans*. (*Insets*) Relative intensity of reporter ions (*m*/*z*; *C. albicans* 114, 115 and *C. glabrata* 116, 117) from MS/MS fragmentation

peptide identification and Pro Group algorithm for summarizing proteins. Search parameters included iTRAQ labeling at N-terminus and lysine residues, cysteine modification by MMTS as fixed modifications, and trypsin as a protease. Proteins identified with >95% confidence or Protscore >1.3 as determined by Pro Group algorithm were used for further analysis. We used false discovery rate analysis by PSPEP software that is in-built into ProteinPilot 3.0. The data generated by LC-MS/MS analysis of 30 SCX fractions were searched against the custom protein database, which includes protein sequences that belong to *C. albicans* and *C. glabrata* filtered from RefSeq, *Candida* Genome Database and ORF database of Genolvures using ProteinPilot 3.0 software. Peptides identified in this study are catalogued in Supplementary Table 1. A detected protein threshold of 1.3 which corresponds to a confidence of 95% was used in identification and quantitation of proteins.

Results and Discussion

In this study, we applied a quantitative proteomic approach to identify differentially expressed proteins in *C. albicans* and *C. glabrata*. Cell lysates of *C. albicans* and *C. glabrata*

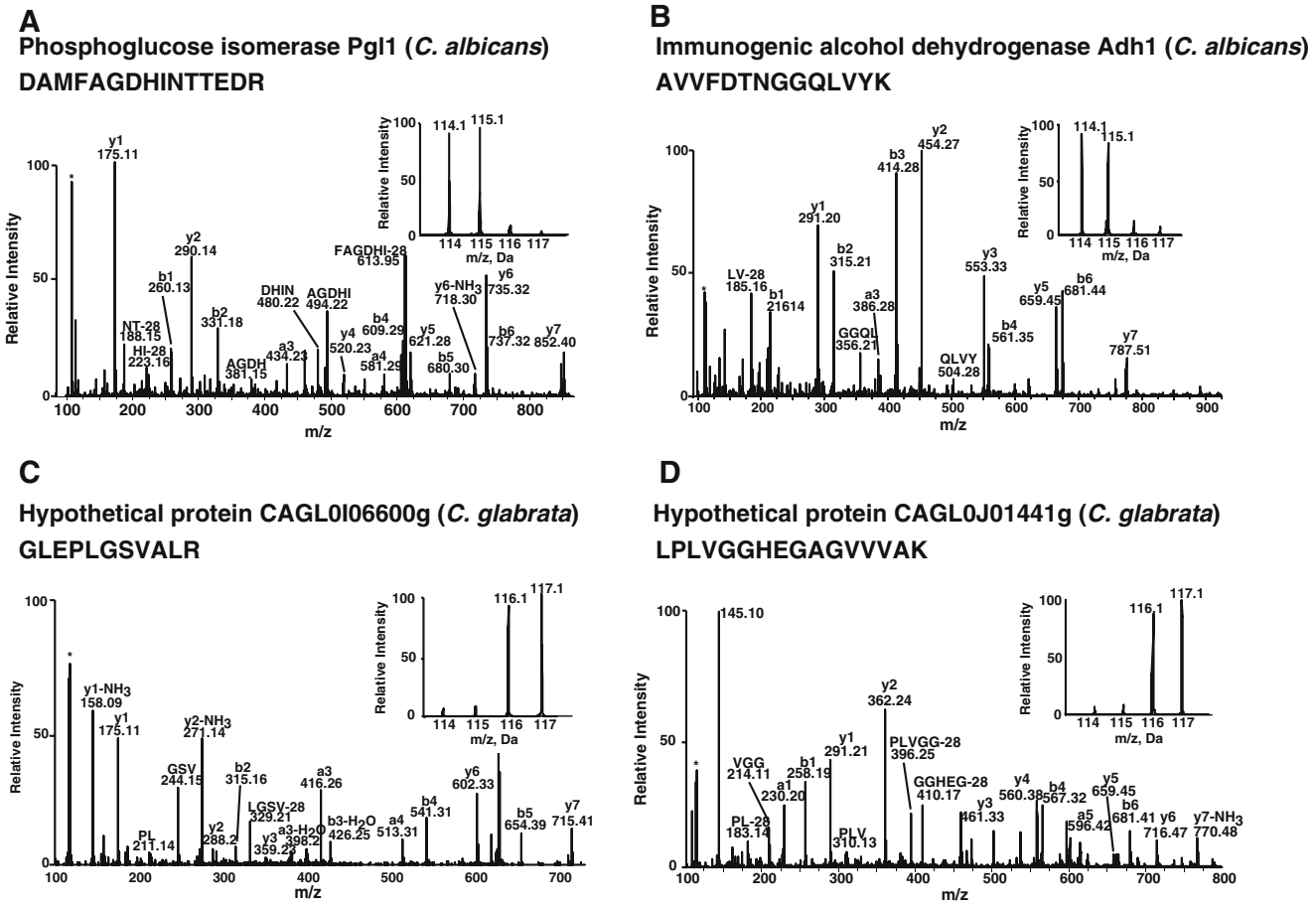


Fig. 3 Representative MS/MS spectra of peptides which are uniquely identified in either *C. albicans* or *C. glabrata.* **a** Peptide DAMFAGDHINTTEDR belongs to phosphoglucose isomerase Pgl1. **b** Peptide AVVFDTNGGQLVYK belongs to immunogenic alcohol dehydrogenase Adh1 of *C. albicans*. **c** Peptide GLEPLGSVALR belongs to hypothetical proteins CAGL0I06600g. **d** Peptide LPLVGGHEGAGVVVAK belongs to CAGL0J01441g of *C. glabrata*. (*Insets*) Relative intensity of reporter ions (*m*/*z*; *C. albicans* 114, 115 and *C. glabrata* 116, 117) from MS/MS fragmentation

were labeled with iTRAQ reagents as shown in Fig. 1. We prepared technical replicates by labeling peptides derived from *C. albicans* with iTRAQ reagents giving rise to reporter ions at *m*/*z* of 114 and 115 and *C. glabrata* derived peptides with those yielding reporter ions at *m*/*z* of 116 and 117. From a total of 24,196 MS/MS spectra, using a ProtScore >1.3 (95% confidence) cutoff, we identified 500 proteins among these organisms, 386 of which belonged to

Table 1 A partial list of proteins differentially expressed in *Candida albicans*

	Gene IDs	Protein name	Relative expression (*C. glabrata*/ *C. albicans*)	Common tryptic peptides (≤95% confidence)	Common tryptic peptides (≥95% confidence)
1	3643055	Translation elongation factor Eft2 [*C. albicans*]	0.39	MVPTSDK, NMSVIAHVDHGK, AYLPVNESFGFTGELR, AVQYLNEIK, AGIISAAK	KFGVDK, KIWCFGPDGNGPNLVVDQTK, FYAFGRVFAGTVK
	2886326	Hypothetical protein CAGL0A03234g [*C. glabrata*]			
2	3646484	Enolase Eno1 [*C. albicans*]	0.42	DGKYDLDFK, IGSEVYHNLK	YDLDFK
	2889384	Hypothetical protein CAGL0I02486g [*C. glabrata*]			
3	3643438	Pyruvate kinase Cdc19 [*C. albicans*]	0.50	GVNLPGTDVDLPALSEK, VHMIFASFIR, GRPLAIALDTK, AEVSDVGNAILDGADCVMLSGETAK	–
	2887537	Hypothetical protein CAGL0E05610g [*C. glabrata*]			
4	3635585	Fructose-bisphosphate aldolase Fba1 [*C. albicans*]	0.18	VNLDTDCQYAYLTGIR	–
	2890854	Hypothetical protein CAGL0L02497g [*C. glabrata*]			
5	3635345	Fatty acid synthase alpha subunit CaO19.13370 [*C. albicans*]	0.62	AVSVTSFGFGQK	–
	2887355	Hypothetical protein CAGL0E06138g [*C. glabrata*]			
6	3640691	Likely cytosolic ribosomal protein Rps24 [*C. albicans*]	0.47	ANVSKDELR, DAVSVFGFR	–
	2889732	Hypothetical protein CAGL0J03234g [*C. glabrata*]			
7	3647882	Cytoplasmic threonyl-tRNA synthetase Ths1 (Cao19.5685) [*C. albicans*]	0.64	VADFGVIHR	VADFGVIHRNEFSGALSGLTRVR
	2891198	Hypothetical protein CAGL0M12991g [*C. glabrata*]			
8	3646925	RAN-like GTP binding protein Gsp1 [*C. albicans*]	0.74	VCENIPIVLCGNK, NLQYYDISAK, LVLVGDGGTGK, FDVWDTAGQEK	–
	2889320	Hypothetical protein CAGL0I00594g [*C. glabrata*]			
9	3645696	Histone H2B Htb1 [*C. albicans*]	0.46	LILPGELAK, AMSIMNSFVNDIFER, QTHPDTGISQK	–
	2890073	Hypothetical protein CAGL0K11462g [*C. glabrata*]			
10	3636428	Dihydroxyacid dehydratase Ilv3 (cao19.4040) [*C. albicans*]	0.42	GGPGMPEMLKPSSALMGYGLGK, DVALLTDGR	
	2886651	Hypothetical protein CAGL0B03993g [*C. glabrata*]			

C. albicans and 114 proteins belonged to *C. glabrata* (Supplementary Tables 2 and 3). As tryptic peptide profile was different for individual proteins among protein homolog pairs of these two species, the ProteinPilot 3.0 software could not cluster most of protein orthologs as "pairs". In this study, it clustered only 24 protein orthologs among the 500 identified proteins belonging to both species. Thus, quantitation data determined by the ProteinPilot 3.0 software were biologically relevant for only these 24 ortholog proteins. Therefore, we performed in-house bioinformatics analysis to determine protein pairs and their relative expression levels based on the relative expression profile of common tryptic peptides as follows. For each protein identified, we determined its counterpart in other species under investigation using a reciprocal BLAST analysis. We identified another set of 35 ortholog proteins in addition to 24 ortholog proteins clustered by ProteinPilot 3.0. At least one common tryptic peptide was observed for these 59 ortholog pairs identified in this study. We calculated weighted average ratio for relative expression level of peptides for these 59 protein pairs using the formula used by ProteinPilot 3.0 software. The values of reporter ion ratio 115:114 for all of these protein pairs were found to be near 1 as expected with an average of 1.05±

Table 2 A partial list of proteins with higher levels of expression in *Candida glabrata*

	Gene IDs	Protein name	Relative expression (*C. glabrata*/*C. albicans*)	Common tryptic peptides (≤95% confidence)	Common tryptic peptides (≥95% confidence)
1	3640000	Likely cobalamin-independent methionine synthase Met6 [*C. albicans*]	1.51	ALDADVVSIEFSK, FWVNPDCGLK	–
	2889085	Hypothetical protein CAGL0I04994g [*C. glabrata*]			
2	3641547	Likely adenylylsulfate kinase Met14 [*C. albicans*]	1.53	DLGFSEADRNENIR	–
	2890901	Hypothetical protein CAGL0L02321g [*C. glabrata*]			
3	3640659	Adenosine triphosphatase Pma1 [*C. albicans*]	1.54	GYLVAMTGDGVNDAPSLK, SAADIVFLAPGLSAIIDALK, GAPLFVLK, KQAIVQK	KGLDAIDK
	2886465	Hypothetical protein CAGL0A00495g [*C. glabrata*]			
4	3642461	Likely 26 S proteasome regulatory particle ATPase Rpt1 [*C. albicans*]	1.57	KVEFSLPDLEGR	VIGSELVQKYVGEGAR, YVGEGARMVRELFEMAR
	2887298	Hypothetical protein CAGL0E06490g [*C. glabrata*]			
5	3644290	Mitochondrial ketol-acid reductoi somerase Ilv5 [*C. albicans*]	1.60	TLYFSHGFSPVFK, DNGLNVIIGVR, YGMDYMYDACSTTAR	–
	2886604	Hypothetical protein CAGL0B03047g [*C. glabrata*]			
6	3647367	Histone H3 Hht2 [*C. albicans*]	1.72	KSAPSTGGVK, YKPGTVALR	QTARKSTGGK, KSTGGK, STGGKAPR, KQLASK, KLPFQR
	2886846	Hypothetical protein CAGL0M06655g [*C. glabrata*]			
7	3637507	Heat shock protein Hsp90 [*C. albicans*]	2.10	GVVDSEDLPLNLSR, SPFLDALK	EDQLEYLEEK, TKPLWTR, KNNIK
	2891108	Hypothetical protein CAGL0L00495g [*C. glabrata*]			
8	3644934	Cytosolic NADP-specific isocitrate dehydrogenase Idp2 [*C. albicans*]	2.24	NILGGTVFR, CATITPDEAR, LIDDMVAQMLK	–
	2886496	Hypothetical protein CAGL0B04917g [*C. glabrata*]			
9	3647488	Mitochondrial NADP-specific isocitrate dehydrogenase Idp1 [*C. albicans*]	2.36	CATITPDEAR, NILGGTVFR	–
	2887212	Hypothetical protein CAGL0D00770g [*C. glabrata*]			
10	3639407	Beta-tubulin Tub2 [*C. albicans*]	2.44	KLAVNLVPFPR, LAVNLVPFPR	–
	2890421	Hypothetical protein CAGL0K12650g [*C. glabrata*]			

0.08. Also, values of (116:114)/(117:114) were also expected to be near 1 and found with an average of 0.93±0.1. MS/MS spectra of representative peptides belonging to four differentially expressed protein pairs are given in Fig. 2. The relative intensities of reporter ions from these spectra are shown in the insets. As shown in the Fig. 2, reporter ion intensities of individual common peptides belonging to same ortholog protein pairs were found to be in agreement with each other. Intensities of these reporter ions among technical replicates were also found to be in the same range. The mass spectrometry data generated in this study has been submitted to *Peptidome* resource (http://www.ncbi.nlm.nih.gov/pepdome).

Substantial differences exist between the genomes and proteomes of *C. albicans* and *C. glabrata*, which influences protein identification in these two species. *C. albicans* is diploid with considerable heterozygosity, whereas *C. glabrata* thrives as a simple haploid. *C. albicans* has 12,015 genes encoded by its heterozygous diploid genome including alleles as described by genome sequencing consortium [26, 27], whereas *C. glabrata* has only over 5,000 genes as described by Dujon et al. [28]. It was also observed that the proteome diversity of *C. albicans* is further enhanced by ambiguous usage of CUG codon to randomly incorporate either amino acid leucine or serine in the proteome, whereas such codon alternation is not reported in *C. glabrata* [55–57].

This protein pair analysis identified several proteins which are expressed only in either *C. albicans* or *C. glabrata*. MS/MS spectra of these peptides showed only reporter ions belonging to either *C. albicans* or *C. glabrata* (Fig. 3). Proteins which are found to be expressed only in *C. albicans* included 3-phosphoglycerate kinase (Pgk1), pyruvate decarboxylase (CaO19.2877), immunogenic alcohol dehydrogenase (Adh1), and heat shock protein 60 (Hsp60). It is interesting to note that several proteins involved in glucose metabolism including gluconeogenesis are highly expressed in *C. albicans*. There is also a marked difference in the expression of serine-, lysyl-, leucyl-, and valyl-tRNA synthetases and several ribosomal subunits, which is significant in the context of differential usage of specific codons in *C. albicans* or *C. glabrata* [53]. Proteins expressed unique to *C. glabrata* included hypothetical proteins CAGL0I06600g (similar to ubiquitin-specific proteinase Ubp3), CAGL0I01496g (similar to heat shock protein Ssc1), CAGL0E03916g (similar to glycerol kinase Gut1), and CAGL0L12364g (similar to acetyl-CoA C-acetyltransferaseYPL028w).

Analysis of expression levels of orthologous proteins showed that 19 proteins of *C. albicans* were found to be higher (>1.5-fold) in abundance when compared to their corresponding orthologs in *C. glabrata* (Table 1). Conversely, expression levels of 12 *C. glabrata* proteins were found to be higher when compared to their counterparts in *C. albicans* (Table 2). The relative protein expression ratios (i.e., 116:114 or 117:114) for the remaining 28 protein pairs ranged from 0.75 to 1.5 (Fig. 4). Proteins with higher expression levels in *C. albicans* included enolase (Eno1), fructose-bisphosphate aldolase (Fba1), dihydroxyacid dehydratase (Ilv3), glutathione oxidoreductase (Glr1), ubiquitin-activating enzyme (Uba1), and chaperonin CCT ring complex subunit (Cct2). Interestingly, fructose-bisphosphate aldolase has already been investigated as a target in *C. albicans* using a methionine/cysteine-conditional mutant [58]. Proteins expressed higher in *C. glabrata* include several hypothetical proteins CAGL0F06941g (similar to pyruvate carboxylase fragment Pyc2), CAGL0L00495g (similar to heat shock protein Hsp90), CAGL0A00495g (similar to adenosine triphosphatase Pma1), CAGL0L02321g (likely adenylylsulfate kinase Met14), CAGL0B04917g (similar to cytosolic NADP-specific isoci-

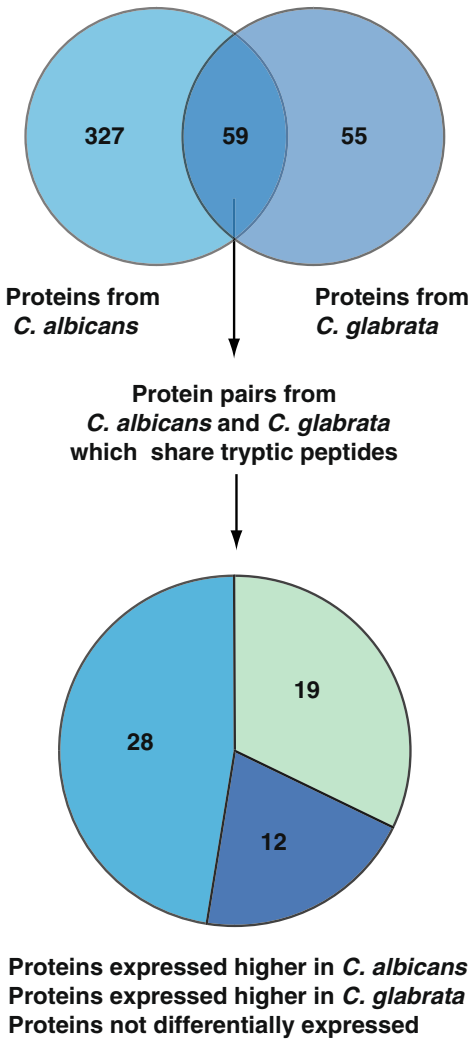


Fig. 4 Proteins identified in *C. albicans* and *C. glabrata*. (*Venn diagram*) Out of 500 proteins identified, shared tryptic peptides were obtained for 59 protein orthologs. (*Pie diagram*) Based on relative intensities of these shared peptides, abundances of these proteins between two organisms were calculated. Out of 59 ortholog proteins, 19 were more abundant in *C. albicans* and 12 in *C. glabrata*

trate dehydrogenase Idp2), and CAGL0D00770g (similar to mitochondrial NADP-specific isocitrate dehydrogenase Idp1). Overexpression of Hsp90 in *C. glabrata* is interesting in the light of its established role in the emergence of azole drug resistance. It was shown that loss of Hsp90 function in yeast and *C. albicans* resulted in the decreased resistance to antifungal drugs including echinocandins [59, 60].

The peptides/proteins which are unique to one organism and/or differentially expressed between two organisms should be attractive candidates as potential biomarkers for the diagnosis and monitoring of treatment. Development of similar rapid diagnostic methods have been investigated in order to identify infections by *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, and *Plasmodium malariae* using antibodies generated against merozoite surface protein 1, which are highly expressed among these pathogens [61, 62]. Similarly, histidine-rich protein-2 (HRP-2) of *P. falciparum* and lactate dehydrogenase (Pv-pLDH) of *P. vivax* have been targeted in the FK80 kit (Standard Diagnostics, Korea) to differentially diagnose malaria infection caused by *P. falciparum* and *P. vivax* [63]. Another successful attempt has been the development of ELISA-based antigen capture assays targeting non-structural protein (NS1) for laboratory detection of acute primary and secondary dengue [61, 64, 65]. Our study provides a list of proteins which are uniquely or differentially expressed in *C. albicans* and *C. glabrata* which can be used in the development of such diagnostic tests. This study also demonstrates a comparative proteomics platform which will facilitate the discovery of several such candidate target molecules with diagnostic potential in many other human diseases with multiple etiologies.

Conclusions

Generally, quantitative proteomic studies are carried out to compare the proteomes of the same species under more than one condition. Often, there is a need to compare two distinct but related proteomes. The orthology-based approach has been used to profile proteomes of organisms with unsequenced genomes [66], but not for differential profiling of proteomes between two closely related organisms. Our study outlines a strategy to investigate protein expression patterns across closely related organisms, by combining orthology information with quantitative proteomics. Such studies should help identify mechanisms responsible for distinct biological features of clinical importance among closely related organisms as exemplified in *C. albicans* and *C. glabrata*. As discussed previously, although *C. albicans* and *C. glabrata* cause similar infections, they exhibit distinct biological features. As most proteins from *C. albicans* and *C. glabrata* are yet to be investigated in detail for their role in various biological processes, our study provides the basis to guide such investigations in the future.

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