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ORIGINAL ARTICLE

Proteomic Analysis of Intracellular and Membrane Proteins From Voriconazole-Resistant *Candida glabrata*

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Abstract
Objectives: The proteomic analysis of voriconazole resistant *Candida glabrata* strain has not yet been investigated. In this study, differentially expressed proteins of intracellular and membrane fraction from voriconazole-susceptible, susceptible dose-dependent (S-DD), resistant *C. glabrata* strains were compared with each other and several proteins were identified.
Methods: The proteins of intracellular and membrane were isolated by disrupting cells with glass bead and centrifugation from voriconazole susceptible, S-DD, and resistant *C. glabrata* strains. The abundance of expressed proteins was compared using two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis and proteins showing continuous twofold or more increase or reduction of expression in resistant strains compared to susceptible and S-DD strain were analyzed by liquid chromatography/mass spectrometry-mass spectrometry method.
Results: Of 34 intracellular proteins, 15 proteins showed expression increase or reduction (twofold or more). The identified proteins included regulation, energy production, carbohydrate transport, amino acid transport, and various metabolism related proteins. The increase of expression of heat shock protein 70 was found. Among membrane proteins, 12, 31 proteins showed expression increase or decrease in the order of susceptible, S-DD, and resistant strains. This expression included carbohydrate metabolism, amino acid synthesis, and response to stress-related proteins. In membrane fractions, the change of expression of 10 heat shock proteins was observed, and 9 heat shock protein 70 (Hsp70) showed the reduction of expression.
Conclusion: The expression of Hsp70 protein in membrane fraction is related to voriconazole resistant *C. glabrata* strains.

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1. Introduction

Fungal infection in humans is increasing; *Candida* species are the most frequently reported organisms. Approximately 95% of all invasive *Candida* infections are caused by five species: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis,* and *Candida krusei* [1]. Among the *Candida* species, *C. albicans* is the most prevalent in both healthy patients and those with infection [2,3]. Recently, the four non-*C. albicans* species were found to be more frequently isolated in humans than *C. albicans* [4]. *C. glabrata* was the second most common non-*C. albicans* species in fungemia in the United States and also most commonly recovered from the oral cavities of patients with human immunodeficiency virus [5]. The increase in the number of *C. glabrata* systemic infections is cause for concern because the high mortality rate associated with *C. glabrata* fungemia [6]. Because fungal infections are increasing, the use of antifungal agents has correspondingly increased. In particular, fluconazole is a highly effective antifungal agent used for the treatment of candidiasis. Voriconazole is a triazole derivative of fluconazole, and the activity for *Candida* may be better than that of fluconazole. However, the widespread and prolonged use of fluconazole in recent years has led to the development of drug resistance in *Candida* species [7,8]. In addition, the resistance of *Candida* to fluconazole is highly predictive of resistance to voriconazole agent. The observation of cross-resistance in *C. glabrata* strains receiving fluconazole and voriconazole therapy of *C. glabrata* in patients with candidemia was reported [9]. The resistant mechanisms to azole antifungal agents have been studied in *C. albicans* [10−12]. However, *C. glabrata* has an intrinsic resistant tendency to fluconazole, and the molecular basis for the intrinsically low susceptibility of *C. glabrata* remains unclear. Several mechanisms of acquired resistance to the azole antifungal agents have been described in *C. glabrata*. These include upregulation of genes encoding adenosine triphosphate (ATP) binding cassette (ABC) transporters encoded by *CDR1* and *CDR2* [13]. Overexpression of *ERG11*, the gene encoding the target of the azole antifungal agents, has also been associated with acquired azole resistance [14]. Recently, proteomic analysis of azole-susceptible and -resistant *Candida* isolates was accomplished to understand the mechanisms underlying azole antifungal resistance [12,15]. Proteomic analysis has also been used to study the adaptive response of *C. albicans* to fluconazole and itraconazole [16]. Currently, no proteomic analysis exists for voriconazole resistant *C. glabrata* strain. So, we analyzed the expression of proteins of voriconazole-susceptible, susceptible dose-dependent (S-DD), and resistant strains to investigate proteins associated with voriconazole resistance.

2. Materials and methods

2.1. *C. glabrata* strains and growth conditions

A total of 56 *C. glabrata* strains collected from tertiary and nontertiary hospitals were used in this study. We previously reported the results of an antifungal susceptibility test [17]. We selected three *C. glabrata* strains according to voriconazole susceptibility for a comparative proteomic study. All strains were stored at −80 °C, and prior to the experiment each strain was subcultured twice on sabouraud dextrose agar to ensure viability and purity. For the proteomic experiment, an aliquot of glycerol stock from each strain was diluted in yeast peptone dextrose (YPD; 1% yeast extract, 2% peptone, 1% dextrose) and grown overnight at 30 °C in a shaking incubator. The cultures were diluted to an optical density 0.2 at OD_{600} in 0.5 L of YPD and grown to the exponential phase of growth.

2.2. Cellular protein extraction

To isolate the cellular proteins, *C. glabrata* cells were cultured in YPD broth at 30 °C to the exponential phase of growth. Cells were harvested in centrifugation 4000 rpm for 15 minutes. The pellet cells were pooled and washed twice using 50 mM Tris-HCl pH 7.6 buffer solution. The cells were disrupted using 0.45-μm glass beads (Sigma, St. Louis, MO, USA) on ice. After homogenization, the solution was centrifuged twice at 14,000 rpm for 20 minutes. The supernatant was harvested carefully without contaminant similar to a lipid component, and it was freeze dried for further experiment.

2.3. Membrane protein extraction

After an exponential phase of growth, cells were harvested, washed with distilled water, and resuspended in homogenizing buffer (50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1 mM phenylmethylsulfonylfluoride). After disruption of the cell using the glass bead, cell debris and unbroken cells were removed by centrifugation at 5000 *g* for 10 minutes. A crude membrane fraction was isolated from the cell-free supernatant by second centrifugation at 30,000 *g* for 30 minutes. The pellet was washed in GTE buffer (10 mM Tris-HCl, pH 7.0, 0.5 mM EDTA, 20% glucose), resuspended in GTE buffer, and stored at −80 °C. The protein concentration was determined by a micro-Bradford assay using a protein assay kit II (Bio-Rad, Hercules, CA, USA).

2.4. Sample preparation and 2-Dimentional Gel Electrophoresis

The harvested samples were suspended in 0.5 mL of 50 mM Tris buffer containing 7 M urea, 2 M thiourea, 4% [weight/volume (w/v)] CHAPS, and 16 μL protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN, USA). The lysates were homogenized

and centrifuged at 12,000 × *g* for 15 minutes. Fifty units of Benzonase (250 units/μL; Sigma) was added to the mixture and suitably stored at −80 °C until use after quantitation by the Bradford method. For 2-DE analysis, pH 3−10 immobilized pH gradient (IPG) strips (Amersham Biosciences, UK, Ltd) were rehydrated in swelling buffer containing 7 M urea, 2 M thiourea, 0.4% (w/v) Dithiothreitol, and 4% (w/v) CHAPS. The protein lysates (500 μg) were cup-loaded into the rehydrated IPG strips using a Multiphor II apparatus (Amersham Biosciences, UK, Ltd) for a total of 57 kVh. The two-dimensional separation was performed on 8−16% (v/v) linear gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels. Following fixation of the gels for 1 hour in a solution of 40% (v/v) methanol containing 5% (v/v) phosphoric acid, the gels were stained with Colloidal Coomassie Blue G-250 solution for 5 hours. The gels were destained in 1% (v/v) acetic acid for 4 hours and then imaged using a GS-710 imaging calibrated densitometer (Bio-Rad).

Protein spot detection and two-dimensional pattern matching were carried out using ImageMasterTM 2D Platinum software (Amersham Biosciences, UK, Ltd). For comparison of protein spot densities between control and treated samples, more than 20 spots throughout all gels were correspondingly landmarked and normalized. The quantified spots of candidate proteins were compared with the aid of histograms. For ensuring the reproducibility of 2DE experiments, each sample was analyzed in duplicate.

2.5. In-gel protein digestion

Protein bands of interest were excised and digested in-gel with sequencing grade, modified trypsin (Promega, Madison, WI, USA). In brief, each protein spot was excised from the gel, placed in a polypropylene tube, and washed four to five times with 150 μL of 1:1 acetonitrile/25 mM ammonium bicarbonate, pH 7.8. The gel was dried in a Speedvac concentrator, and then rehydrated in 30 μL of 25 mM ammonium bicarbonate, pH 7.8, containing 20 ng of trypsin. After incubation at 37 °C for 20 hours, the liquid was transferred to a new tube. Tryptic peptides remaining in the gel matrix were extracted for 40 min at 30 °C with 20 μL of 50% (v/v) aqueous acetonitrile containing 0.1% (v/v) formic acid. The combined supernatants were evaporated in a Speedvac concentrator and dissolved in 8 μL of 5% (v/v) aqueous acetonitrile solution containing 0.1% (v/v) formic acid for mass spectrometric analysis.

2.6. Identification of proteins by liquid chromatograph/tandem mass spectrometry

The resulting tryptic peptides were separated and analyzed using reversed phase capillary high-performance liquid chromatography (HPLC) directly coupled to a Finnigan LCQ ion trap mass spectrometer [liquid chromatography-tandem mass spectrometry (LC-MS/MS)]. A 0.1 × 20 mm trapping and a 0.075 × 130 mm resolving column were packed with Vydac 218 MS low trifluoroacetic acid C18 beads (5 μm in size, 300 Å in pore

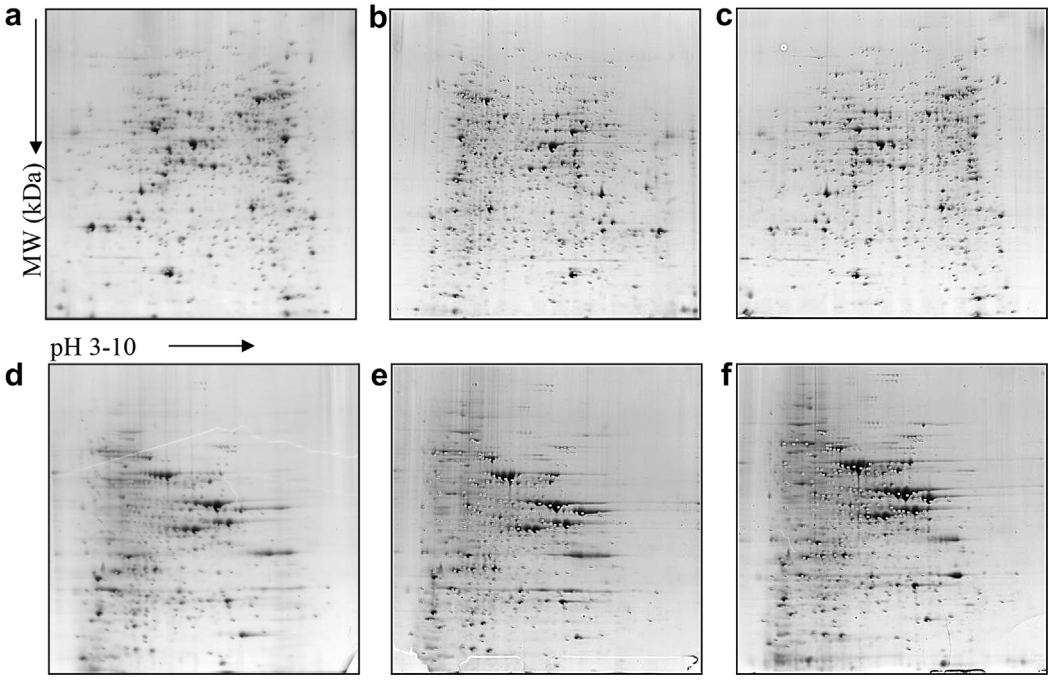

Figure 1. Cellular and membrane protein spot of *C. glabrata* strains resolved by 2D gel electrophoresis. Spots representing differentially expressed proteins that were identified by LC-Ms/Ms peptide mass fingerprinting. (a) cellular protein spot of voriconazole susceptible strain, (b) cellular protein spot of voriconazole SDD strain, (c) cellular protein spot of fluconazole susceptible strain, (d) membrane protein spot of voriconazole susceptible strain, (e) membrane protein spot of voriconazole SDD strain, (f) membrane protein spot of voriconazole resistant strain.

Table 1. Differentially expressed intracellular proteins (by twofold or more), as identified by liquid chromatography-tandem mass spectrometry between voriconazole susceptible, susceptible dose-dependent, and voriconazole resistant strains

Spot	Protein	Molecular mass (Dalton)	pI	Fold change (R/S)[a]
25	C1-tetrahydrofolate synthase	102,203	5.98	3.09
27	Formyltetrahydrofolate synthetase (FTHFS)	102,203	5.98	2.06
79	ACO1 aconitate hydratase aconitase	85,429	6.78	2.53
90	Potassium efflux protein KefA	73,694	5.41	2.05
115	Sphingolipid long-chain base sensory protein	40,387	5.54	2.27
116	TKL1 transketolase	73,704	6.01	2.50
127	Heat shock protein 70	112,540	7.87	3.5
189				2.15
540				2.13
154	Acetolactate synthase	73,300	8.55	2.41
202	LEU4 2-isopropylmalalate synthase	67,290	5.52	2.51
218	Acetyl-CoA hydrolase/transferase N-terminal domain	58,541	6.16	2.38
228	Phosphoribosylaminoimidazole carboxylase	62,672	6.95	4.42
238	Pyruvate kinase	55,563	6.25	2.66
263	Pyruvate decarboxylase and related thiamine pyrophosphate-requiring enzymes	61,726	5.59	3.94
295	Aldehyde dehydrogenase family	55,937	5.09	2.03
304	SES1 seryl-transcription RNA synthetase	52,775	5.8	2.19
319	Iinosine monophosphate dehydrogenase	56,969	6.69	2.86
396	Serine hydroxymethyltransferase	52,271	6.74	3.1
397				3.6
411	GDP dissociation inhibitor	50,582	5.66	3.02
476	Protein with specific affinity for G4 quadruplex nucleic acids	42,134	8.61	2.33
504	Isocitrate dehydrogenase	46,728	5.23	2.52
505	spP36046 *Saccharomyces cerevisiae* YKL195w	44,592	4.45	2.78
507	Chromosome segregation adenosine triphosphatases (ATPases)	55,271	8.81	14.1
535	Malate dehydrogenases (MDH) glycosomal and mitochondrial	39,024	6.15	2.42
550	Cyclophilin_ABH_like	41,620	5.61	2.2
560	Aspartate/tyrosine/aromatic aminotransferase	45,608	7.2	2.73
576	Quinone reductase and related Zn-dependent oxidoreductases	40,823	6.01	2.24
603	Branched-chain aminotransferase	41,550	5.82	2.58
619	Highly similar to *S. cerevisiae* YBR249c ARO4	38,617	6.51	2.24
636	RPC40 DNA-directed RNA polymerase I	37,577	5.22	2.05
645	*S. cerevisiae* YGR080w	36,175	5.02	3.49
777	Peptidase_S8 (serine proteinase)	50,008	5.75	2.25
107	Glycerol-3-phosphate dehydrogenase	43,961	5.85	−2.82
142	TKL1 transketolase	73,704	6.01	−3.57
321	F0F1 ATP synthase	58,485	8.99	−2.46
454	Effector domain of the CAP family of transcription factors	44,936	5.92	−2.14
570	Acetyl-CoA hydrolase			−2.53
609	Oxidoreductases	46,710	5.76	−2.4
613				−3.75
615	Malate dehydrogenases glycosomal and mitochondrial	40,487	9.18	−2.14
625	Phosphoglycerate kinase	44,590	6.37	−8.90
628	Arginase	35,061	5.27	−3.13
633	Highly similar to spP53252 *S. cerevisiae* YGR086c	35,129	4.68	−3.03
723	Uncharacterized enzymes related to aldose 1-epimerase	33,397	5.06	−3.15
774				−2.07
909	Hypothetical protein CAGL0I00616g	2,183	5.37	−9.09
912	ATP synthase D chain, mitochondrial (ATP5H)	19,918	6.64	−2.73

[a]*Expression ration of voriconazole-resistant (R) over voriconazole-susceptible (S) strains. The minus sign (−) indicates decreased protein expression of voriconazole-resistant strains in comparison with voriconazole-susceptible strains.*

size; Vydac, Hesperia, CA, USA) and placed in-line. Next, the peptides were bound to the trapping column for 10 minutes with 5% (v/v) aqueous acetonitrile containing 0.1% (v/v) formic acid, then the bound peptides were eluted with a 50-minute gradient of 5-80% (v/v) acetonitrile containing 0.1% (v/v) formic acid at a flow rate of 0.2 μL/min. For tandem mass spectrometry, a full mass scan range mode was m/z = 450−2000 Da. After determination of the charge states of an ion on zoom scans, product ion spectra were acquired in MS/MS mode with relative collision energy of 55%. The individual spectra from MS/MS were processed using the TurboSEQUEST software (Thermo Quest, San Jose, CA). The generated peak list files were used to query either the MSDB database or National Center for Biotechnology Information (NCBI) using the MASCOT program (http://www.matrixscience.com). Modifications of methionine and cysteine, peptide mass tolerance at 2 Da, MS/MS ion mass tolerance at 0.8 Da, allowance of missed cleavage at 2, and charge states (+1, +2, and +3) were taken into account. Only significant hits as defined by MASCOT probability analysis were initially considered.

3. Results

3.1. Strains

Among the *C. glabrata* strains, voriconazole susceptible strain [*C. glabrata* I-49, minimum inhibitory concentration (MIC) 0.5 μg/mL], S-DD strain (*C. glabrata* D-54, MIC 2 μg/mL) and resistant strain (*C. glabrata* D-91, MIC 4 μg/mL) were selected. All strains were isolated from blood specimen of patients.

3.2. Expression of intracellular proteins and identification

The two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (2D-SDS PAGE) gels are shown in Figure 1. The profiling of 459 intracellular proteins was detected in three strains. Of the total proteins, 38 proteins having abundance ratios of twofold or more showed continuous increase of expression from susceptible and S-DD to resistant strain. In addition, 34 proteins were identified by LC-MS/MS (Table 1). The 15 proteins showing decrease of expression from susceptible and S-DD to resistant strain were also identified. Among the identified proteins, aldehyde dehydrogenase family, serine hydroxymethyltransferase, acetolactate synthase, heat shock protein, pyruvate kinase, potassium efflux protein, isocitrate dehydrogenase, and other proteins showed increased expression. Expression was decreased in proteins such as glycerol-3-phosphate dehydrogenase, ATP synthase, acetyl-coA hydrolase, oxidoreductase, and malate dehydrogenases (Table 1). Among the proteins for which expression was decreased, phosphoglycerate kinase protein showed the largest decreased expression, at 9.09 times reduction of expression. The identified proteins, classified according to their function, are summarized in Table 2. The

Table 2. Functional classification of identified intracellular proteins from voriconazole susceptible, susceptible dose-dependent, and resistant strains

Protein	Function
Cell regulation	
Similar with bacterial potassium efflux protein KefA	Regulate iron balance
Sphingolipid long chain base sensory protein	Cell wall, antifungal protection
Heat shock protein 70, 90, 60	Stress, protein folding
SES1 seryl-transcription RNA (tRNA) synthetase	Catalyze the formation of aminoacyl-tRNA
GDP dissociation inhibitor	GTP binding protein regulator
similar to *Saccharomyces cerevisiae* YKL195w	Promotes retention of newly imported proteins
Chromosome segregation adenosine triphosphatases (ATPases)	Cell division
Highly similar to *S. cerevisiae* YGR086c	Unknown function that are induced on cell stress
CAP family of transcription factors	Control transcription of genes
Carbohydrate transport and metabolism	
Pyruvate decarboxylase	Related thiamine pyrophosphate-requiring enzymes
Hexokinase	Phosphorylates a six-carbon sugar, a hexose to a hexose phosphate
Amino acid transport and metabolism	
SAM1 S-adenosyl methionine synthetase	Catalyzes transfer of the adenosyl group of ATP to the sulfur atom of methionine
Energy production and conversion	
F1 ATP synthase	Catalyze the ATP synthesis
Phosphoglycerate kinase	Catalyzes the transfer of the high-energy phosphate group of 1,3-biphosphoglycerate to adenosine diphosphate

Table 3. Differentially expressed membrane proteins (by twofold or more), as identified by liquid chromatography-tandem mass spectrometry between voriconazole susceptible, susceptible dose-dependent, and voriconazole resistant strains

Spot	Protein	Molecular mass (Dalton)	pI	Fold change (R/S)[a]
12, 314	Enolase	46,710	5.76	2.69, 2.56
132, 169	Hsp70 protein	6,635	5.32	2.18, 2.72
195, 379	Pyruvate kinase (PK)	54,572	8.26	2.13, 2.21
244	Cysteine synthase	55,388	5.51	2.34
255, 291	Pyruvate decarboxylase	61,726	5.59	2.12, 2.31
276	Pyrophosphate-requiring enzymes	46,993	4.46	3.66
284	WD40 domain adaptor/regulatory modules in signal transduction	46,504	4.44	2.34
457	Phosphoglycerate kinase (PGK)	44,590	6.37	2.77
50	Heat shock protein	80,983	4.82	−2.43
119, 149	Hsp70 protein	69,469	4.96	−2.43, −4.09
153, 226				−2.18, −2.74
357, 552				−2.54, −3.88
138, 174				−28.0, −2.37
172				−3.35
175	*Saccharomyces cerevisiae* YLR259c Heat shock protein	60,351	5.14	−4.77
229	Hexokinase	53,772	5.23	−2.39
260	Aldehyde dehydrogenase family	56,131	6.07	−2.11
292	F1 adenosine triphosphate (ATP) synthase beta subunit, nucleotide-binding domain	54,176	5.14	−2.68
298	Nicotinamide adenine dinucleotide phosphate -glutamate dehydrogenase	49,711	5.58	−3.17
360	SAM1 S-adenosylmethionine synthetase	41,700	5.10	−2.19
398	ATPase alpha2,Na/K	116,305	5.41	−2.56
462	N terminal of the Stm1 protein	29,791	9.65	−6.48
465	Adenosine kinase (AK)	36,250	5.23	−2.05
548	Exo-beta-1,3-glucanase	33,667	4.41	−2.50
557	Elongation factor 1 beta (EF1B) guanine nucleotide exchange domain	22,903	4.33	−3.57
560	Predicted epimerase, PhzC/PhzF homolog	32,286	4.98	−2.35
578	Phosphoglycerate mutase 1	27,468	5.48	−11.1
597	Phosphoglycerate kinase	18,458	7.85	−2.51
602	Mitochondrial ribosomal protein MRP8	24,160	4.73	−2.12
616	Ribosome antiassociation factor IF6	26,367	4.52	−2.62
629	TrpR binding protein WrbA	29,728	6.54	−2.08
632	Alcohol dehydrogenase GroES-like domain	36,721	6.21	−2.25
645	Type 1 glutamine amidotransferase (GATase1)	25,479	5.16	−4.45
658	Phosphoglycerate kinase (PGK)	44,590	6.37	−2,15
715	Chain A, yeast Cu, Zn enzyme superoxide dismutase	15,714	5.63	−7.19

[a]*Expression ration of voriconazole-resistant (R) over voriconazole-susceptible (S) strains. The minus sign (−) indicates decreased protein expression of voriconazole-resistant strains in comparison with voriconazole-susceptible strains.*

functional category showed that the identified proteins were cell regulation, energy production, carbohydrate transport, amino acid transport, and various metabolism-related proteins.

3.3. Expression of membrane proteins and identification

A total of 329 membrane proteins were resolved by 2D gel electrophoresis. Of the 17 spots (differential ratio twofold or more) for which expression was increased, 12 proteins were identified. The identified proteins showed enolase, heat shock protein 70, pyruvate kinase, cysteine synthase, pyruvate decarboxylase, pyrophosphate requiring enzyme, regulatory modules in signal transduction, and phosphoglycerate kinase (Table 3). Among the identified proteins, phosphate requiring enzymes showed the most increased expression (3.66 times). Enolase and phosphoglycerate kinase proteins also showed 2.69 and 2.77 times increased

Table 4. Functional classification of identified membrane proteins from voriconazole susceptible, susceptible dose-dependent, and resistant strains

Protein	Function
Cell regulation	
Similar with bacterial potassium efflux protein KefA	Regulate iron balance
Sphingolipid long-chain base sensory protein	Cell wall, antifungal protection
Heat shock protein 70, 90, 60	Stress, protein folding
SES1 seryl-transcription RNA (tRNA) synthetase	Catalyze the formation of aminoacyl-tRNA
GDP dissociation inhibitor	GTP binding protein regulator
similar to *Saccharomyces cerevisiae* YKL195w	promotes retention of newly imported proteins
Chromosome segregation adenosine triphosphatases (ATPases)	Cell division
highly similar to *Saccharomyces cerevisiae*YGR086c	Unknown function that are induced on cell stress
Cu, Zn enzyme superoxide dismutase	Catalyse the conversion of superoxide radicals to oxygen
CAP family of transcription factors	Control transcription of genes
Molecular chaperone DnaK	Posttranslational modification, protein turnover, chaperones
Carbohydrate transport and metabolism	
Pyruvate decarboxylase	Related thiamine pyrophosphate-requiring enzymes
Hexokinase	Phosphorylates a six-carbon sugar, a hexose to a hexose phosphate
Amino acid transport and metabolism	
SAM1 S-adenosylmethionine synthetase	Catalyzes transfer of the adenosyl group of ATP to the sulfur atom of methionine
Elongation factor 1 beta (EF1B)	catalyzes the exchange of GDP bound to the G-protein, EF1A, for GTP
Energy production and conversion	
F1 ATP synthase	Catalyze the ATP synthesis
Phosphoglycerate kinase	catalyzes the transfer of the high-energy phosphate group of 1,3-biphosphoglycerate to adenosine diphosphate

expression, respectively. Thirty-seven spots showed decreased expression in the order of susceptible, S-DD, and resistant strains. Among the 37 spots, 31 proteins were identified. The identified membrane proteins included heat shock protein 70, aldehyde dehydrogenase, nicotinamide adenine dinucleotide phosphate-glutamate dehydrogenase, phosphoglycerate mutase I, glutamine aminotransferase, superoxide dismutase, Stm1 protein, phosphoglycerate kinase, and others. A total of 12 heat shock proteins were observed and heat shock protein 70 was 11. In addition, 9 heat shock protein 70 showed the deceased expression in resistant strain compared to susceptible and S-DD strain. The identified membrane proteins were classified into carbohydrate metabolism, amino acid synthesis, and response to stress-related proteins (Table 4).

4. Discussion

C. glabrata is a major opportunistic fungal pathogen of humans and also part of the gastrointestinal microflora in many healthy human beings [1]. The most effective classes of antifungal agents used to treat *C. glabrata* infections are the azoles agents, specifically fluconazole and voriconazole [9]. However, the occurrence of azole-resistant strains resulted in a difficulty of treatment. Currently, the available information of voriconazole resistance in protein levels is sparse. In this study, we compared the expression changes of proteins using the voriconazole susceptible, S-DD, and resistant strains. The results of proteomic analysis showed the tendency of expression increase (38 proteins) was observed in intracellular fractions of resistant strain compared to membrane fraction of susceptible and S-DD strain (17 proteins). The membrane fraction of resistant strain had the tendency of expression decrease (37 proteins) compared to intracellular fraction of susceptible and S-DD strains (18 proteins). The results indicated that the metabolism process is continuously increased from voriconazole susceptible to S-DD, resistant strain but the biochemical reaction may be decreased in membrane fraction to endure the antifungal stress environment. Among the identified proteins, heat shock protein was observed in various spots of intracellular and membrane fractions. Usually, heat shock protein is known as a stress and response related protein. In this study, the expression increase of heat shock protein in intracellular proteins of voriconazole resistant strain was observed in three spots, but 9 heat shock 70

protein showed decreased expression in membrane proteins. This finding indicated that heat shock protein 70 is related to voriconazole resistance. Among the *C. albicans* triazole resistance mechanisms, the molecular chaperone Hsp90 is known to share a correlation. The Hsp90 protein stabilizes calcineurin, thereby enabling calcineurin-dependent stress responses that are required for triazole tolerance of *Candida* strains [18]. In this study, the heat shock protein identified most often was Hsp70 protein, and 9 Hsp70 proteins showed a decrease of expression in membrane fraction, but the exact mechanism with voriconazole resistance needs more investigation. Among the identified membrane proteins, expression of DnaK and Stml protein was reduced in voriconazole resistant strains compared with S-DD and susceptible strains. These proteins are related to protein posttranslation modification and apoptosis, respectively. There has been little information of voriconazole resistance in *C. glabrata* strain, so the proteomic investigation can be useful information for further study.

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