Expression of Efflux Pumps and Fatty Acid Activator One Genes in Azole Resistant *Candida Glabrata* Isolated From Immunocompromised Patients

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Abstract- Acquired azole resistance in opportunistic fungi causes severe clinical problems in immunosuppressed individuals. This study investigated the molecular mechanisms of azole resistance in clinical isolates of Candida glabrata. Six unmatched strains were obtained from an epidemiological survey of candidiasis in immunocompromised hosts that included azole and amphotericin B susceptible and azole resistant clinical isolates. Candida glabrata CBS 138 was used as reference strain. Antifungal susceptibility testing of clinical isolates was evaluated using Clinical and Laboratory Standards Institute (CLSI) methods. Complementary DNA-Amplified Fragment Length Polymorphism (cDNA-AFLP) technology, semiquantitative RT-PCR, and sequencing were employed for identification of potential genes involved in azole resistance. Candida glabrata Candida drug resistance 1 (CgCDR1) and Candida glabrata Candida drug resistance 2 (C_gCDR2) genes, which encode for multidrug transporters, were found to be upregulated in azole-resistant isolates (\geq 2-fold). Fatty acid activator 1 (FAA1) gene, belonging to Acyl-CoA synthetases, showed expression in resistant isolates 22-fold that of the susceptible isolates and the reference strain. This study revealed overexpression of the CgCDR1, CgCDR2, and FAA1 genes affecting biological pathways, small hydrophobic compounds transport, and lipid metabolism in the resistant clinical C.glabrata isolates. © 2016 Tehran University of Medical Sciences. All rights reserved. Acta Med Iran, 2016;54(7):458-464.

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Introduction

Candida glabrata, formerly rarely seen in fungal infections, has become an important cause of serious systemic infections in immunosuppressed hosts (1-3). *Candida glabrata* is an agent of bloodstream infection (4-6). Most clinical isolates of this organism show low susceptibility to azole antifungal compounds, and treatment of its infection is problematical. The basis of azole resistance in *Candida* species may be the upregulation or mutation of the *ERG11* gene that encodes for the azole target enzyme, cytochrome p-450

lanosterol 14-alpha demethylase (6-9). Another important mechanism of resistance to azole may be overexpression of the efflux pump genes *Candida glabrata Candida* drug resistance 1 and 2 (*CgCDR1* and *CgCDR2*) belonging to the ATP-binding cassette (ABC) transporter family (10-12). The major facilitator superfamily is another class of drug transporters involved in drug efflux pumps (12). In addition, the *C.glabrata* sensitivity to 4 Nitroquinoline N-oxide gene (*CgSNQ2*), encodes a protein belonging to the ABC transporter superfamily and is controlled by the *C.glabrata* pleiotropic drug response1 gene (*CgPDR1*).

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The overexpression of CgPDR1could is associated with the development of azole resistance in C.glabrata (13). Several studies have demonstrated the central role of the CgPDR1 gene in acquired azole resistance (14-18). CgPDR1 acquired gain-of-function (GOF) mutations are responsible for upregulation of many genes, especially ABC transporters, lipid biological pathway genes, and the aldo-ketoreductase gene (14,17,19,20). Since the molecular pathways in the resistant Iranian strains of C.glabrata are unclear, the present study investigated the transcript profile of the genes involved in antifungal resistance mechanisms using cDNA-AFLP method. This technology is a PCRbased genome-wide expression analysis method (21). cDNA-AFLP has been successfully applied to assess expression of several genes in various organisms (22, 23).

Yeast isolates and mycological criteria

Six unmatched Candida glabrata isolates used in this study were obtained from the culture collection of an candidiasis epidemiological survey of in immunosuppressed patients at the Department of Medical Mycology and Parasitology in Tehran University of Medical Sciences (Table 1) (20). The C.glabrata strain CBS 138 was used as a reference. All clinical isolates were subcultured on CHROMagar Candida medium. The strains obtained were confirmed by standard biological criteria containing the assimilation patterns using the API 20C AUX system (bioMérieux, France). The isolates for chlamydospores and pseudohyphal production were subcultured on commeal agar with 1% Tween 80. All isolates and the reference strain were grown on yeast extract, peptone, glucose (YEPD) agar plates and incubated for 48 h at 37°C.

Materials and Methods

fluco	fluconazole, itraconazole, and amphotericin B susceptibility				
Identification	Age of the	MIC ^b (µg/ml)			
No. ^a	patient (years)	Fluconazole	Itraconazole	Amphotericin B	
94	62	0.25	0.5	0.25	
45	59	0.5	0.25	0.25	
51	53	64	2	0.25	
153	35	64	4	0.25	
137	35	64	2	0.25	
219	38	64	2	0.25	

 Table 1. List of clinical C.glabrata isolates used in this study with their fluconazole, itraconazole, and amphotericin B susceptibility

a: All strains have been isolated from the oropharynx b: MIC, Minimum Inhibitory Concentration

Antifungal susceptibility assays

The susceptibility of *C.glabrata* isolates to fluconazole, itraconazole, and amphotericin B was tested by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards, M27-A3 broth microdilution method (24). Briefly, final inocula of 0.5×103 to 2.5×103 organisms per ml were distributed in wells of a microtiter plate in RPMI 1640 medium buffered to pH 7.0 with 0.165M morpholinepropanesulfonic acid and incubated at 37°C. The MICs were determined as the lowest azole concentration that reduced yeast growth by 50% compared to that of a non-treated control.

RNA extraction

Total RNA extraction was prepared from logarithmic growth phase cultures in YEPD broth with an RNeasy protect mini kit (Qiagen, Germany). For mechanical disruption, the yeast cell pellet was sonicated with 600 μ l volume of acid-washed glass beads (0.45-0.52 mm

diameter). Total RNA was treated through an RNasefree DNase treatment step (Qiagen, Germany). The purity of RNA template was determined with the NanoDrop 1000 spectrophotometer (Thermo scientific, USA).

Complementary DNA-amplified fragment length polymorphism (cDNA-AFLP)

cDNA-AFLP method was conducted as described previously with minor modifications (20). For complementary DNA (cDNA) synthesis, an equivalent amount (6 μ g) of total RNA from each sample was incubated at 65°C for 10 min followed by cooling on ice. The master mixture included a 5x reverse transcriptase (RT) buffer (Fermentas, Canada), 1 μ l of Oligo d (T) (20 pmol/ μ l), 2 μ l of dNTPs (10 mM), 2 μ l of Ribolock (20 U) (Fermentas, Canada), and DEPC treated water. Two hundred units of Moloney Murine Leukemia Virus (M-MuLV) RT enzyme (Fermentas, Germany) were added. cDNA was controlled with the reference gene *URA3* (orotidine-5'-phosphate decarboxylase). Primers were designed by using GenBank sequences of *C.glabrata* genes (as templates)

with the Primer3 program (http://primer3.wi.mit.edu/) (Table 2).

			0	
Gene	Primer	Sequence	Gene location (5 ⁻ -3 ⁻)	Product length
IIDA2	URA3 F	GGGCTCTTTAGCTCATGGTG	432-451	173hn
UKAS	URA3 R	CAAGTGCATCGCCTTTATCA	604-585	1730p
	CgCDR1 F	AAGTTGGTTTCCCCTCGTCT	3518-3537	109hm
CgCDKI	CgCDR1R	CTGCTGTAGCAATGGGTTGA	3715-3696	1980p
C-CDD1	CgCDR2 F	CACATCGCTAAGCAATCGAA	467-486	220 h-
CgCDK2	CgCDR2 R	AAGAACATGGCTGCACCTCT	705-686	239 bp
EAAI	FAA1 F	TCGTTCCTAACCACACACCA	1793-1812	106hm
ГААІ	FAA1 R	TCGTCATCGAAGAACACAGC	1988-1969	1960p

Table 2. Primers used for semi-quantitative RT-PCR and internal control

The second strand was synthesized using DNA polymerase I (Fermentas, Germany) at 16°C for 3 h and precipitated with ethanol. The integrity of dscDNA was confirmed with the Nanodrop 1000 spectrophotometer. Two micrograms of dscDNA were digested with *MboI* restriction enzyme (25) (Fermentas) for 4 h at 37°C, and the enzyme was inactivated at 80°C for 20 min. Eight μ g of ADMboI and 4 μ g of abMboI, AFLP adaptors (Table 3) (20), were ligated to digested dscDNA fragments by T4 DNA Ligase (Takara Bio Inc. Japan).The following protocol was used: 1 min at 65°C, decreasing to 10°C over 1 h. T4 DNA Ligase was added to the mixture incubated in advance at 16°C for 16 h. The pre-

amplification was conducted with the PreAmp adaptor as primer with the touchdown PCR program: 5 min at 94°C; 20 cycles of 30 s at 94°C, 45 s initially at 65°C reduced 0.5°C per cycle; 20 cycles of 30 s at 94°C, 45 s at 55°C, and 2 min 72°C; and final extension 5 min at 72°C. In the sensitive amplification steps, ten PCRs were conducted, including all sensitive adaptors combinations. The PCR products were visualized on 8% non-denaturated polyacrylamide gel electrophoresis (PAGE), and using silver nitrate staining. Differentiated transcription-derived fragments (TDFs) were generated. The cDNA AFLP gels were covered with a plastic seal and scanned.

 Table 3. cDNA-AFLP adaptors used in current

	study
Adaptors	Sequence (5´- 3´)
ADMbo1	AGCACTCTCCAGCCTCTCACCGCA
adMBO1	GATCTGCGGTGA
Pre Amp	AGCACTCTCCAGCCTCTCACCGCAGATC
S1Mbo1	AGCACTCTCCAGCCTCTCACCGCAGATCC
S2Mbo1	AGCACTCTCCAGCCTCTCACCGCAGATCG
S3Mbo1	AGCACTCTCCAGCCTCTCACCGCAGATCA
S4Mbo1	AGCACTCTCCAGCCTCTCACCGCAGATCT

cDNA-AFLP fragments

The TDFs displaying differential expression were extracted from silver stained PAGE and re-amplified using the suitable sensitive adaptors (Table 3). The amplified fragments were cloned using a TA-cloning kit (Invitrogen-USA), and the recombinant plasmids were confirmed by using M13 universal primers with the following PCR conditions: 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min with extension of the final cycle at 72°C for 7 min. PCR products were visualized by 2% agarose gel electrophoresis. The recombinant plasmids including unknown DNA were

sequenced using M13 forward (-20) and M13 reverse universal primers. Some TDFs were determined by direct sequencing (Bioneer, Korea). Sequence data were verified in non-redundant nucleic and protein databases BLAST (http://www.ncbi.nim.nih.gov/BLAST/).

Semi-quantitative RT-PCR technique

Semi-quantitative RT-PCR analysis was used to confirm the mRNA specific expression pattern visualized in the cDNA-AFLP profile (23). An equivalent amount (6 μ g) of total RNA from clinical isolates and CBS standard strain was conducted for the first strand cDNA synthesis as described above, and the evaluation pattern in cDNA-AFLP was performed using specific primers (Table 2) on RNA of clinical isolates and the reference strain. The *URA3* gene was applied as a reference gene and internal control. The PAGE image was captured digitally with a Sony XC-ST50CE camera (Sony, Japan). The intensity of band was analyzed with gel analysis software UVI (Roche, Germany).

Sequencing

For reconfirming the genes which analyzed in semiquantitative RT-PCR technique were sequenced with the same primers (Bioneer, Korea). Sequence results were analyzed using the BLAST program.

Results

Standard mycological study

The clinical isolates and reference strain produced pinkpurple colonies, in contrast to the white colonies exhibited by *C.bracarensis* and *C.nivariensis*, on CHROMagar *Candida* medium (26). The isolates could not produce chlamydospore and pseudohyphae on cornmeal agar with 1% Tween 80. The isolates of *Candida glabrata* were able to assimilate glucose and trehalose and could not assimilate L-lysine and glycerol (27).

Antifungal sensitivity

Susceptibility to fluconazole, itraconazole, and amphotericin B was examined by the CLSI method (document M27-A3). MICs for fluconazole, itraconazole, and amphotericin B are shown in Table 1.

cDNA-AFLP profile

The TDFs of cDNA-AFLP were separated on 8% non-

denaturing PAGE by silver staining (Figure 1). Several TDFs were visualized using ten primer combinations. The TDFs that showed differential expression were selected from the silver stained PAGE and identified using cloning and DNA sequencing with the sequences analyzed by BLAST (Table 4). Three differentially expressed TDFs were produced at approximately 200-600 bp length when using S2Mbo1, S4Mbo1 and S1Mbo1, S3Mbo1 and S3Mbo1, and S4Mbo1 as sensitive primers (Figure 1).



Figure 1. cDNA-AFLP expression profile on PAGE with silver nitrate staining. Sensitive amplification of cDNA-AFLP using four sensitive adaptor combinations, S1Mbo1, S2Mbo1, S3Mbo1, and S4Mbo1, as primers. The lane numbers correspond to the clinical isolates presented in Table 1. The arrows show differentially expressed TDFs. M=Marker 100 bp.

The sequencing results showed matches the TDFs with CgCDR1, CgCDR2, and Fatty acid activator 1 (*FAA1*) genes. An additional clone contained an unnamed protein sequence (Table 4).

AFLP profiles					
Accession No.	Product length (bp)	Explanation			
XM_447677.1	375	Fatty acid activator			
AF109723	330	Candia glabrata candida drug resistance 1			
XM_446088	330	Candia glabrata candida drug resistance 2			
XM_445802.1	300	unnamed protein			

Semi-quantitative RT-PCR assay

In order to verify the genes found in the cDNA-AFLP profile, a semi-quantitative RT-PCR assay was carried out using cDNAs from resistant and sensitive isolates and the CBS 138 reference strain. Figure 2 shows the upregulation of *CgCDR1*, *CgCDR2*, and

FAA1 mRNA expression in resistant isolates. The semiquantitative RT-PCR showed CgCDR1 mRNA expression levels to be \geq 2-fold those of the sensitive isolates (Figures 2,3). In addition, CgCDR2 was upregulated in four resistant isolates to levels 2-3-fold those of the susceptible isolates and the CBS 138 reference strain (Figures 2, 3).



Figure 2. Semi-quantitative RT-PCR assay. Comparison of expression patterns of *CgCDR1*, *CgCDR2*, and *FAA1* genes in azole resistant and azole susceptible *C.glabrata* isolates. *URA3* gene was used as internal control. The lane numbers correspond to the clinical isolates presented in Table 1.



Figure 3. Expression levels of *CgCDR1*, *CgCDR2*, and *FAA1* genes in azole resistant *C.glabrata* isolates compared to azole sensitive isolates. Expression levels are shown after normalization with the *URA3* gene. The sample numbers correspond to the clinical isolates presented in Table 1.

The semi-quantitative RT-PCR results of resistant strains showed that mRNA expression levels of the *FAA1* gene, which encodes Faa1p, a principal long chain acyl-CoA synthetase (ACSL), were ≥ 2 fold those observed in the sensitive isolates and reference strain (Figures 2,3). The *URA3* gene was used as internal control (Figures 2,3).

Sequencing results

The sequencing results showed highly matched with *CgCDR1*, *CgCDR2*, and Fatty acid activator 1 (*FAA1*) genes of *C.glabrata* CBS 138 genome.

Discussion

Among non-*C.albicans* species, *C.glabrata*, has emerged as an important cause of mucosal and systemic candidiasis in immunocompromised patients. Due to the extensive use of antimycotic drugs in the prophylaxis of candidiasis, azole resistant clinical isolates of *C.glabrata* have been increasingly reported (28).

In the present study, we used the cDNA-AFLP method to investigate the genes involved the resistance phenotypes of clinical *C.glabrata* isolates. The cDNA-AFLP method is a PCR-based technique widely used to assess gene expression levels and the effects of agents controlling up- and down-regulation of genes (21,23).

Previous studies have indicated that ABC-transporter genes CgCDR1, CgCDR2, and CgSNQ2 are the most important biological pathway in azole resistance of C.glabrata, eventually leading to broad cross-resistance to antifungal triazoles (29,30). In this study, differential expression of TDFs of cDNA-AFLP was determined by DNA sequencing. DNA sequencing showed a match with the CgCDR1 and CgCDR2 ABC transporter, which are involved in protecting organisms against cytotoxins and xenobiotics. Semi-quantitative analysis showed increased mRNA expression levels of CgCDR1 and CgCDR2 genes in resistant clinical C.glabrata isolates, whereas these genes were normally regulated in susceptible isolates as well as CBS reference strain.

Upregulation and mutation of genes involved in sterol biosynthesis are important in azole resistance of Candida sp. Molecular pathways of azole resistance in Candida sp. are also associated with enhanced relative mRNA expression levels or mutation in the ERG11 gene, which encodes cytochrome p-450 lanosterol $14-\alpha$ demethylase, the principle step in ergosterol biosynthesis (31-33). The results of cDNA-AFLP profile and semi-quantitative RT-PCR showed FAA1 gene mRNA expression levels in resistant isolates ≥ 2 -fold that of sensitive isolates (Figures 2,3). As expected, results revealed that the regulation patterns of FAA1 in susceptible isolates were normal. In Saccharomyces cerevisiae, four ACSL genes, FAA1 to FAA4, have been characterized (34,35). This family of enzymes plays important roles in lipid metabolism, fatty acid transport, energy production, and cell wall synthesis (36). Faa1p, which constitutes the major cellular activity located in the endoplasmic reticulum, plasma membrane and vesicles catalyzes acyl-CoA. A study of a fluconazoleresistant mutant of C.glabrata reported overexpression of multiple genes that were found to be responsible for small molecules transport and cell wall function, as well

as lipid and fatty acid metabolism (17). In addition, mutation of the PDR1 gene was found to control the expression of many genes, such as those involved in sterol biological pathways (14,17). Ferrari et al., showed that 626 genes were regulated by at least one GOF mutation in CgPDR1. Analysis of the differentially regulated genes showed them to be involved in specific biological pathways. The POX1 gene CAGL0A03740g (fatty acyl-coenzyme A oxidase), which is involved in the fatty acid beta-oxidation pathway (highly similar to sp P13711 Saccharomyces cerevisiae), showed upregulation in C.glabrata. Also, the FAS1 gene CAGL0D00528g (Beta unit of fatty acid synthetase), which catalyzes the synthesis of long-chain saturated fatty acids, was over-expressed in resistant C.glabrata. The studies showed fatty acid metabolism to be essential for cellular life (34-36), and upregulation of genes belonging to this pathway in resistant C.glabrata provides a protective effect. The results of the present study underscore overexpression of the FAA1 gene as an important factor in lipid metabolism and suggest that upregulation of the ABC efflux pump genes is a principal mechanism of azole resistance. Many investigations indicate that several molecular pathways are involved in the development of azole resistance in C.glabrata.

Candida glabrata often exhibits low susceptibility to fluconazole, and the incidence of all clinical forms of candidiasis caused by this microorganism is on the rise. The results of the cDNA-AFLP assay revealed that overexpression of the ABC efflux pump genes (CgCDR1, CgCDR2) and FAA1 (the principal ACSL gene) is associated with azole resistance in clinical isolates. In addition, Faa1p, which is encoded by the FAA1 gene, is necessary during the exponential growth-phase for fatty acid transport, metabolism, and energy production. This study reports upregulation of FAA1 in azole resistant clinical C.glabrata isolates. Further studies are needed to clarify the role of FAA1 gene.

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References

- Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 2007;20:133-63.
- Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis 2009;48:1695-703.
- Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 2013;62:10-24.
- Ruhnke M. Epidemiology of Candida albicans infections and role of non-Candida-albicans yeasts. Curr Drug Targets 2006;7:495-504.
- Bassetti M, Merelli M, Righi E, Diaz-Martin A, Rosello EM, Luzzati R, et al. Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol 2013;51:4167-72.
- Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev 2012;36:288-305.
- Marichal P, Vanden Bossche H, Odds FC, Nobels G, Warnock DW, Timmerman V, et al. Molecular biological characterization of an azole-resistant Candida glabrata isolate. Antimicrob Agents Chemother 1997;41:2229-37.
- Espinel-Ingroff A. Mechanisms of resistance to antifungal agents: yeasts and filamentous fungi. Rev Iberoam Micol 2008;25:101-6.
- Kanafani ZA, Perfect JR. Antimicrobial resistance: resistance to antifungal agents: mechanisms and clinical impact. Clin Infect Dis 2008;46:120-8.
- Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of Candida glabrata collected during a hospital survey of antifungal resistance. Antimicrob Agents Chemother 2005;49:668-79.
- Sanglard D, Ischer F, Bille J. Role of ATP-bindingcassette transporter genes in high-frequency acquisition of resistance to azole antifungals in Candida glabrata. Antimicrob Agents Chemother 2001;45:1174-83.
- Prasad R, Gaur NA, Gaur M, Komath SS. Efflux pumps in drug resistance of Candida. Infect Disord Drug Targets 2006;6:69-83.
- 13. Torelli R, Posteraro B, Ferrari S, La Sorda M, Fadda G,

Sanglard D, Sanguinetti M. The ATP-binding cassette transporter-encoding gene CgSNQ2 is contributing to the CgPDR1-dependent azole resistance of Candida glabrata. Mol Microbiol 2008;68:186-201.

- Ferrari S, Sanguinetti M, Torelli R, Posteraro B, Sanglard D. Contribution of CgPDR1-regulated genes in enhanced virulence of azole-resistant Candida glabrata. PLoS One 2011;6:e17589.
- 15. Tsai HF, Krol AA, Sarti KE, Bennett JE. Candida glabrata PDR1, a transcriptional regulator of a pleiotropic drug resistance network, mediates azole resistance in clinical isolates and petite mutants. Antimicrob Agents Chemother 2006;50:1384-92.
- Berila N, Subik J. Molecular analysis of Candida glabrata clinical isolates. Mycopathologia 2010;170:99-105.
- 17. Vermitsky JP, Earhart KD, Smith WL, Homayouni R, Edlind TD, Rogers PD. Pdr1 regulates multidrug resistance in Candida glabrata: gene disruption and genome-wide expression studies. Mol Microbiol 2006;61:704-22.
- Caudle KE, Barker KS, Wiederhold NP, Xu L, Homayouni R, Rogers PD. Genomewide expression profile analysis of the Candida glabrata Pdr1 regulon. Eukaryot Cell 2011;10:373-83.
- Berila N, Borecka S, Dzugasova V, Bojnansky J, Subik J. Mutations in the CgPDR1 and CgERG11 genes in azoleresistant Candida glabrata clinical isolates from Slovakia. Int J Antimicrob Agents 2009;33:574-8.
- 20. Farahyar S, Zaini F, Kordbacheh P, Rezaie S, Safara M, Raoofian R, et al. Overexpression of aldo-keto-reductase in azole-resistant clinical isolates of Candida glabrata determined by cDNA-AFLP. Daru 2013;21:1.
- 21. Reijans M, Lascaris R, Groeneger AO, Wittenberg A, Wesselink E, van Oeveren J, et al. Quantitative comparison of cDNA-AFLP, microarrays, and GeneChip expression data in Saccharomyces cerevisiae. Genomics 2003;82:606-18.
- 22. Jayaraman A, Puranik S, Rai NK, Vidapu S, Sahu PP, Lata C, et al. cDNA-AFLP analysis reveals differential gene expression in response to salt stress in foxtail millet (Setaria italica L.). Mol Biotechnol 2008;40:241-51.
- Saffari M, Dinehkabodi OS, Ghaffari SH, Modarressi MH, Mansouri F, Heidari M. Identification of novel p53 target genes by cDNA AFLP in glioblastoma cells. Cancer Lett 2009;273:316-22.
- 24. CLSI. Reference Method for Broth Dilution Antifungal

Susceptibility Testing of Yeast; Approved Standard. 3rd ed. CLSI document M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2008:9-16.

- 25. Levterova V, Panaiotov S, Brankova N, Tankova K. Typing of genetic markers involved in stress response by fluorescent cDNA-amplified fragment length polymorphism technique. Molecular biotechnology 2010;45:34-8.
- 26. Lockhart SR, Messer SA, Gherna M, Bishop JA, Merz WG, Pfaller MA, et al. Identification of Candida nivariensis and Candida bracarensis in a large global collection of Candida glabrata isolates: comparison to the literature. J Clin Microbiol 2009;47:1216-7.
- Warren TA, McTaggart L, Richardson SE, Zhang SX. Candida bracarensis bloodstream infection in an immunocompromised patient. J Clin Microbiol;48:4677-9.
- Richardson M, Lass-Florl C. Changing epidemiology of systemic fungal infections. Clin Microbiol Infect 2008;14:5-24.
- 29. Coleman JJ, Mylonakis E. Efflux in fungi: la piece de resistance. PLoS Pathog 2009;5:e1000486.
- 30. Morschhauser J. Regulation of multidrug resistance in pathogenic fungi. Fungal Genet Biol 2010;47:94-106.
- 31. Lamping E, Ranchod A, Nakamura K, Tyndall JD, Niimi K, Holmes AR, et al. Abc1p is a multidrug efflux transporter that tips the balance in favor of innate azole resistance in Candida krusei. Antimicrob Agents Chemother 2009;53:354-69.
- Akins RA. An update on antifungal targets and mechanisms of resistance in Candida albicans. Med Mycol 2005;43:285-318.
- Tavakoli M, Zaini F, Kordbacheh M, Safara M, Raoofian R, Heidari M. Upregulation of the ERG11 gene in Candida krusei by azoles. Daru 2010;18:276-80.
- 34. Li H, Melton EM, Quackenbush S, DiRusso CC, Black PN. Mechanistic studies of the long chain acyl-CoA synthetase Faa1p from Saccharomyces cerevisiae. Biochim Biophys Acta 2007;1771:1246-53.
- Black PN, DiRusso CC. Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. Biochim Biophys Acta 2007;1771:286-98.
- 36. Scharnewski M, Pongdontri P, Mora G, Hoppert M, Fulda M. Mutants of Saccharomyces cerevisiae deficient in acyl-CoA synthetases secrete fatty acids due to interrupted fatty acid recycling. FEBS J 2008;275:2765-78.