Rapid Induction of High-frequency *MTL* Homozygosis and Microbiological Polymorphism in *Candida albicans* by Fluconazole

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Candida albicans is most common fungal pathogen of opportunistic infections. *C. albicans* was previously noted as a diploid, apparently asexual fungus. Morphological and genetic studies had found mating type-like locus (*MTL*a/α) and demonstrated its relations to morphological switching, virulence, and fluconazole-resistance. A parasexual cycle was found between opposite *MTL* homozygotes. However, *C. albicans* was found with *MTL* homozygous genotype in only around 3.2 % of clinical strains, and MTL heterozygotes had a low frequency (about 10^{-4}) of white-opaque (W/O) switch to be *MTL* homozygotes in nature. In the present study, reference *C. albicans* strain SC5314 was used for fluconazole-inducing assay to harvest 35 first-generation survival daughter strains from 70 colonies picked from fluconazole inhibitory zone. Further separation with spreading culture and micromanipulator methods was further employed to gain second- and third-generation strains with accordance to first-generation daughter strains. PCR analysis based on *MTL* a1, α1 and α2 was employed to demonstrate *MTL* genotypes of these isolates. Microscopic observation and flowcytometry assay were employed for cell/colony morphology and DNA content analysis on progeny SC5314 and its daughter strains. High frequency of *MTL* gene loss (24 of 35, 68.57 %) and homozygotes (16 of 35, 45.71 %) were found in fluconazole-inducing survival daughter strains with accordance to first-generation daughter strains. Polymorphism of cell/colony morphology and decreasing DNA content were also found in these fluconazole-inducing daughter strains. Fluconazole treatment not only inhibited the growth of *C. albicans* but also altered phenotypic characteristics in cell/colony morphology as well as induced rapid high-frequency *MTL* gene loss and homozygosity in fluconazole inhibition zone. The DNA content of these fluconazole-inducing daughter strains were also obviously reduced with comparison to their progeny SC5314 suggesting a possibility of chromosome loss and DNA rearrangement during the fluconazole treatment to *C. albicans*.

(292 words)

Keywords: *Candida albicans*, fluconazole, mating type like gene, homozygosis

Rapid Induction of High-frequency *MTL* Homozygosis, Microbiological Polymorphism and Changing Antifungal Susceptibility in *Candida albicans* by Fluconazole

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Keywords: *Candida albicans*, fluconazole, mating type like gene, homozygosis

- Background. *Candida albicans* is most common fungal pathogen of opportunistic infections. *C. albicans* was previously noted as a diploid, apparently asexual fungus. Morphological and genetic studies had found mating type-like locus (*MTLa*/ α) and demonstrated its relations to morphological switching, virulence, and fluconazole-resistance. A parasexual cycle was found between opposite MTL homozygotes. However, *C. albicans* was found with *MTL* homozygous genotype in only around 3.2 % of clinical strains, and MTL heterozygotes had a low frequency (about 10^{-4}) of white-opaque (W/O) switch to be *MTL* homozygotes in nature.
- Methods. In the present study, reference *C. albicans* strain SC5314 was used for fluconazole-inducing assay to harvest 35 first-generation survival daughter strains from 70 colonies picked from fluconazole inhibitory zone. Further separation with spreading culture and micromanipulator methods was further employed to gain second- and third-generation strains with accordance to first-generation daughter strains. PCR analysis based on *MTL* a1, α 1 and α 2 was employed to demonstrate *MTL* genotypes of these isolates. Microscopic observation and flowcytometry assay were employed for cell/colony morphology and DNA content analysis on progeny SC5314 and its daughter strains.
- Results. High frequency of *MTL* gene loss (24 of 35, 68.57 %) and homozygotes (16 of 35, 45.71 %) were found in fluconazole-inducing survival daughter strains with accordance to first-generation daughter strains. Polymorphism of cell/colony morphology and decreasing DNA content were also found in these fluconazole-inducing daughter strains.
- Conclusions. Fluconazole treatment not only inhibited the growth of *C. albicans* but also altered phenotypic characteristics in cell/colony morphology as well as induced rapid high-frequency *MTL* gene loss and homozygosity in fluconazole inhibition zone. The DNA content of these fluconazole-inducing daughter strains were also obviously reduced with comparison to their progeny SC5314 suggesting a possibility of chromosome loss and DNA rearrangement during the fluconazole treatment to *C. albicans*.

Table 1. Characteristics of *Candida albicans* strains series in present study

Strains series[a]	Characteristics	*MTL* genotypes[b]	number of strains	Loss of *MTL* locus[c]	*MTL* homozygosis[c]
SC5314 (n=1)	progeny	a1/α1/α2	1		
FI-FGDS (n=35) (First generation)	Derivate from SC5314	a1/α1/α2	31	4/35 (11.4%)[c]	4/35 (11.4%)[c]
		a1	2		
		α1/α2	2		
		bizarre[b]	0		
		died	35		
FI-SGDS (n=87) (Second generation)	From FI-FGDS	a1/α1/α2	71	14/35 (40.0%)[c]	10/35 (28.6%)[c]
		a1	5		
		α1/α2	7		
		bizarre[b]	4		
		died	0		
FI-TGDS (n=141) (Third generation)	From FI-SGDS	a1/α1/α2	84	24/35 (68.6%)[c]	16/35 (45.7%)[c]
		a1	22		
		α1/α2	17		
		bizarre[b]	18		
		died	0		

NOTE. *Candida albicans* strain SC5314 and its derivates daughter strains.
MTL, matting type like; FI-FGDS, fluconazole-inducing first-generation daughter strains; FI-SGDS, fluconazole-inducing second-generation daughter strains; FI-TGDS, fluconazole-inducing third-generation daughter strains.
[a] SC5314 is the reference strain purchased from ATCC. The other strains were renamed in present study.
[b] *MTL* genotypes of a1/α1, a1/α2, α1, or α2 were defined as bizarre.
[c] The ratios were defined with accordance to first-generation FI-FGDS.

Table 2. Frequencies of *Candida albicans* with *MTL* homozygosis in different literatures

Strains features	Frequencies of *MTL* homozygotes	Literatures
Nature happening	$1.4x10^{-4}$	[Slutsky et al. 1987]
Strain WO-1[a]	100%	[Lockhart et al, 2002]
Clinical isolates (220 strains)	$3.2x10^{-2}$	[Lockhart et al, 2002]
Fluconazole-inducing assay on strain SC5314	$4.57x10^{-1}$	Present study

NOTE. Literatures about frequencies of *MTL* homozygotes for comparing with the results in this study.
MTL, matting type like.

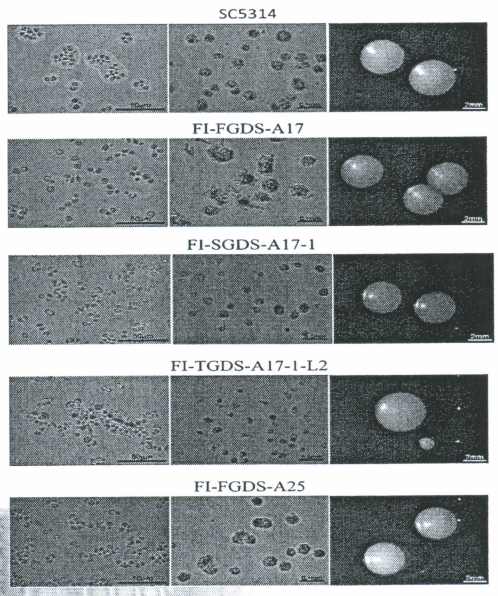

Figure 1. Polymorphism of cell/colony morphologies were found in initial-stage colonies of daughter strains, compared to progeny SC5314. Cell, initial-stage colony, and 5-day colony morphologies of strains SC5314, FI-FGDS-A17, FI-SGDS-A17-1, FI-TGDS-A17-1-L2, and FI-FGDS-A25. Cell (left column) morphologies were shown after growth on YPD agar for 12 hours at 30 ℃ and visualized by light microscopy and photographed at 400x objectvie. Initial-stage colony (middle column) morphologies were shown after growth on YPD agar for 12 hours at 30 ℃ and visualized by light microscopy and photographed at 100x objectvie. 5-day colony (right column) morphologies were shown after growth on YPD agar for 120 hours at 30 ℃ and visualized by light microscopy and photographed at 10x objectvie. White/opaque cells were seen in Lee's medium series daughter strains. Filamentous cells were frequently seen in fluconazole-inducing daughter strains.

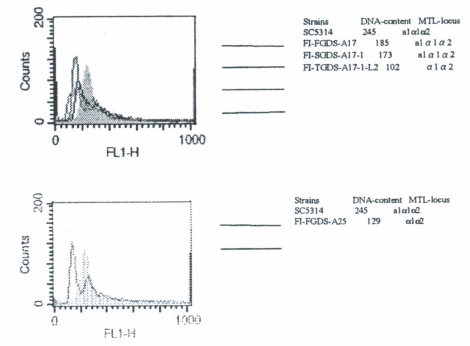

Figure 2. Decreasing DNA content in daughter strains. DNA contents of strain SC5314, FI-FGDS-A17, FI-SGDS-A17-1, FI-TGDS-A17-1-L2, and FI-FGDS-A25. DNA contents were evaluated by Sytox Green dye stained flowcytometric analysis for the indicated strains. With accordance to indicated first-generation daughter strains, analyses on each FI-FGDS associated strains were merged for comparison to their progeny strain SC5314. (A) FI-FGDS-A17. (B) FI-FGDS-A25.

References.

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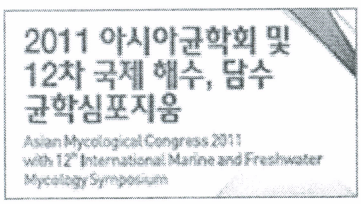

Scientific Program

№ 2011078

Program at a Glance

Date	Time	AMC2011 Hall 12-101	AMC2011 Hall 12-103
08. 07	13:00-14:00		Registration
	14:00-14:30		Opening Ceremony (Art Hall)
	14:30-15:20		Plenary Lecture 1 (Art Hall)
	15:20-15:40		Coffee Break
	15:40-17:40	Symposium 1-1 Fungal Systematics: Basidiomycota and Ascomycota I	Symposium 3 Mycorrhizae and Endophytic Fungi
	18:00-20:00		Welcome Reception
08. 08	08:30-09:30		Registration
	09:30-10:20		Plenary Lecture 2 (Art Hall)
	10:20-11:00		Photo Session/Coffee Break
	11:00-13:00	Symposium 1-2 Fungal Systematics: Ascomycota II	Symposium 5 Mushroom Biology and Cultivation Technology Ta
	13:00-14:00		Lunch
	14:00-14:50		Plenary Lecture 3 (Hall 12-103)
	14:50-16:00		Coffee Break/Poster Session
	16:00-18:00	Symposium 2-1 Fungal Systematics: Biodiversity	Symposium 6 Genetics and Molecular Cell Biology Bio
08. 09	09:30-10:20		Plenary Lecture 4 (Art Hall)
	10:20-11:00		Coffee Break/Poster Session
	11:00-13:00	Workshop "Outcomes-based Teaching and Learning in Mycology - Enhancement of Learning through Assessment"	Symposium 7 Physiology and Biochemistry Bioc
	13:00-14:00	Lunch	
	14:00-14:50	Plenary Lecture 5 (Hall 12-103)	
	14:50-15:20	Coffee Break/Poster Session	
	15:20-17:20	Symposium 8 Fungal Plant Pathology	Symposium 9 Fungal Ecology and Community I Bior an comp aq

	09:30-11:30	Symposium 10 Molecular Plant-Fungal Interactions	Symposium 11 Medicinal and Food Mycology
	11:30-12:00		Coffee Break/Poster Session
	12:00-13:00	Offered paper session 1	Offered paper session 2
	13:00-14:00		Lunch
08. 10	14:00-16:00	Symposium 13 Fungal Bioremediation	Symposium 14 Inventory and Databases of Fungi (MSJ & KSM)
	16:00-16:10		Coffee Break/Poster Session
	16:10-18:10	Symposium 16 Quarantine and Diagnosis	Offered paper session 4
	18:10-19:20		Congress Dinner
	19:30-20:20		Art performance (Art Hall)
	20:20-20:50		Closing (ART Hall)
08. 11	09:00		Tour

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