Original Article

White-opaque Switching in Different Mating Type-like Locus Gene Types of Clinical *Candida albicans* Isolates

Hou-Min Li[1], Yumi Shimizu-Imanishi[2], Reiko Tanaka[2], Ruo-Yu Li[3], Takashi Yaguchi[2]

[1]Department of Dermatology, Peking University People's Hospital, Beijing 100044, China
[2]Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan
[3]Department of Dermatology, Peking University First Hospital, Beijing 100034, China

Abstract

Background: *Candida albicans* (*C. albicans*) can become a pathogen causing superficial as well as life-threatening systemic infections, especially in immunocompromised patients. Many phenotypic attributes contribute to its capacity to colonize human organs. In our study, 93 *C. albicans* isolates from patients of various candidiasis in a hospital of China were surveyed. We aimed to investigate the white-opaque (WO) switching competence, drug sensitivity, and virulence of mating type-like (*MTL*) a/α isolates.

Methods: Internal transcribed spacer (*ITS*) gene and the *MTL* configuration were detected in all the isolates by reverse transcription-polymerase chain reaction. White/opaque phenotype and doubling time of cell growth were determined. The minimum inhibitory concentrations of antifungal agent were measured using broth microdilution method.

Results: Sixty-four isolates (69.6%) were classified to serotype A, 19 (20.6%) to serotype B, and 9 (9.8%) to serotype C. Moreover, phylogenetic analysis showed that these isolates were divided into four different subgroups of *ITS* genotypes. Most of our clinical isolates were *MTL*a/α type, while 6.8% remained *MTL*a or *MTL*α type. The frequency of opaque phenotype was 71.0% (66 isolates). Following the guidelines of Clinical and Laboratory Standards Institute M27-A3, all isolates were susceptible to caspofungin and a few (0.6–3.2%) of them showed resistance against amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole.

Conclusions: From these analyses, there were comparatively more *C. albicans* strains classified into serotype B, and the frequency of opaque phase strains was significant in the clinical isolates from China. Genetic, phenotypic, or drug susceptibility patterns were not significantly different from previous studies. *MTL*a/α isolates could also undergo WO switching which facilitates their survival.

Key words: *Candida albicans*; Drug Susceptibility; Genotype; Mating Type-like; White-opaque

Introduction

Candida albicans (*C. albicans*) is a commensal microorganism living on the gastrointestinal and urogenital mucosa in healthy individuals.[1] However, it can become a pathogen causing superficial as well as life-threatening systemic infections, especially in immunocompromised patients.[1] Many phenotypic attributes, such as the yeast and filamentous forms, contribute to its capacity to colonize all body organs virtually.[2,3] *C. albicans* is once considered to be an imperfectible fungal species, lacking a sexual cycle. However, this paradigm was challenged when mating type-like (*MTL*) loci, *MTL*a and *MTL*α, were identified, which is orthologous of *MAT*a and *MAT*α in *Saccharomyces cerevisiae*.[4]

In 1987, a spontaneously and reversibly switch termed white-opaque (WO) switching from the normal, round-to-oval yeast form to an elongate cell form was found in *C. albicans* WO regulator-1 strain.[5] All strains undergoing WO switching were considered to be homozygous at the *MTL* locus.[6] The homeodomain protein *MTL*a1-α2 complex represses WO switching in *MTL*a/α cells.[7,8] This is why only about 3% of naturally occurring strains are homozygous at the *MTL* locus, and most clinical isolates produced white colonies.[6] Recent studies also found that these two cell

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.193442

Address for correspondence: Prof. Takashi Yaguchi, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan
E-Mail: t-yaguchi@faculty.chiba-u.jp

Received: 21-07-2016 **Edited by:** Qiang Shi
How to cite this article: Li HM, Shimizu-Imanishi Y, Tanaka R, Li RY, Yaguchi T. White-opaque Switching in Different Mating Type-like Locus Gene Types of Clinical *Candida albicans* Isolates. Chin Med J 2016;129:2725-32.

types had different pathogenic traits.[9] In 2013, Xie *et al*.[10] discovered that a number of natural *MTL*a/α strains were capable of WO switching under condition mimicking aspects of the host environment.

In this context, we surveyed the microbiological characteristics including genetic, phenotypic, and drug susceptibility patterns of 93 *C. albicans* isolates from patients in a hospital of China and found some intriguing phenomenon of natural strains.

Methods

Fungal isolates

Ninety-three clinical *C. albicans* strains isolated from candidiasis patients in Peking University First Hospital were used in this study. Isolates were collected from vagina (67, 72.0%), glans penis (16, 17.2%), skin (5, 5.4%), and oral cavity (5, 5.4%). All the isolates were from immunocompetent patients and were sampled by swap. These clinical isolates were purified to single colony on CHROMagar Candida™ (Kanto Chemical Co. Inc., Japan) and deposited to Medical Mycology Research Center, Chiba University, Japan (as IFM 61638–61730). When these clinical isolates were purified, we isolated two colonies LH770-1 and LH770-2 which have different colony sizes compared with the LH770 strain.

The standard strain *Candida parapsilosis* (American Type Culture Collection [ATCC] 22019), *Candida krusei* (ATCC 6258), and *C. albicans* (ATCC 90028) were included in antifungal susceptibility assays as quality control. The isolates were streaked onto potato dextrose agar (PDA, Becton, Dickinson and Company, USA) slants and incubated at 25°C overnight. The experiments were done in Medical Mycology Research Center, Chiba University, Japan.

Genotyping

Primers CA-INT-L and CA-INT-R were used for serotype determination of *C. albicans* on the basis of 25S rDNA.[11] Internal transcribed spacer (*ITS*) gene was detected in these isolates.[12] *ITS*5 (forward) and *ITS*4 (reverse) primers were used to amplify the *ITS*1, 5.8S, and *ITS*2 regions according to the results of reverse transcription-polymerase chain reaction (RT-PCR).[12]

Analysis of the mating type-like configuration

The *MTL* configuration (heterozygous or homozygous) was determined by RT-PCR using primers specific for *MTL*a and *MTL*α.[6] RT-PCR reactions were carried out on Bio-Rad T100 thermocycler (Bio-Rad, USA) using a standard program: 94°C incubation 5 min, and then 35 cycles of 94°C for 60 s, 55°C for 60 s, 72°C for 60 s, followed by a final extension step at 72°C for 10 min. Primer sequences were as follows: *MTL*a1 forward, 5'-TTGAAGCGTGAGAGGCAGGAG-3' and *MTL*a1 reverse, 5'-GATTAGGCTGTTTGTTCTTCTCG-3'; and *MTL*α2 forward, 5'-CATGAATTCACGTCTGGAGGCAC-3' and *MTL*α2 reverse, 5'-AAGCAGCCAACTCAGGTCAC-3'.

Determination of white/opaque phenotype

Yeast peptone dextrose agar (YPD, 1% yeast extract, 2% peptone, 2% dextrose, and 2% agar) supplemented with 50 μg/ml phloxine B (Wako Pure Chemical Industries, Ltd., Japan) was used for white/opaque phenotype detection. Cells were streaked onto YPD-phloxine B plates and incubated at room temperature for 2 weeks. The phenotype of colonies was observed under a stereoscopic dissecting microscope (Leica M125, Leica, Germany), and the phenotype of cells was observed under a scanning electron microscope (JSM-7200F, JEOL, Japan).

Determination of doubling time of cell growth

Cells were inoculated into liquid YPD medium and incubated overnight at 25°C at 20 ×*g*. Culture was diluted by 200 folds to 5 ml of fresh liquid YPD medium, and growth at 25°C at 20 ×*g* was automatically recorded as A_{600nm} using the TVS062CA Bio-photorecorder (Advantec, Tokyo, Japan).

Fluorescence-activated cell sorting analysis

Strain was precultured in liquid YPD medium at 25°C, and a stationary phase culture was diluted 100 folds to 10 ml of a new YPD. Cell suspension was diluted 10 folds to normal saline buffer containing 10 μg/ml of propidium iodide (Wako Pure Chemical Industries, Japan) and 1 mg/ml of RNase (Wako Pure Chemical Industries, Japan) and incubated at 37°C for 2 h. Stained cells were diluted 10 folds to sterilized distilled water and applied to a flow cytometry, On-chip Sort (FISHMAN, On-Chip Biotechnologies, Japan) according to the procedure manuals.

Antifungal susceptibility

The minimum inhibitory concentrations (MICs) of antifungal agents; amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and caspofungin against the tested strains were measured using broth microdilution methods following the guidelines of the Clinical and Laboratory Standards Institute M27-A3. Microtiter plates (Dry Plate; Eiken Chemical Co., Ltd., Japan) were used in the assay. *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258), and *C. albicans* (ATCC 90028) were used as controls.

Statistical analysis

Statistical analysis was performed using Excel 2010 software (Microsoft Corporation, USA). Student's *t*-test was applied in the analysis of the correlation between WO switching and cellular growth rates. A value of $P < 0.05$ was considered statistically significant.

Results

Genotyping

In 93 isolates, 64 isolates (68.8%) were classified into serotype A, 19 (20.4%) to serotype B, and 10 (10.8%) to serotype C [Tables 1 and 2]. This result showed no significant difference in the genotyping with previous studies with the other sources of *C. albicans* isolates.[13] It was also showed that 70.0% of isolates from oral cavity and 50.0% of isolates from sputum were serotype B [Table 2], which were higher than

Table 1: Summarized information of the source, *MTL* type, ABC serotype, and phenotype of *Candida albicans* isolates

Strain	No.	Source	*MTL* type	ABC genotype	*ITS* phylogenetic analysis	White/opaque phenotype
LH495	61638	Glans penis discharge	a/α	C	3	100% pink
LH496	61639	Vaginal discharge	a/α	A	1	98% pink
LH498	61640	Vaginal discharge	a/α	A	1	84% pink
LH502	61641	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH521	61642	Vaginal discharge	a/α	A	1	White
LH527	61643	Vaginal discharge	a/α	A	1	Almost 100% pink
LH529	61644	Vaginal discharge	a/α	A	1	Almost 100% pink
LH532	61645	Vaginal discharge	a/a	A	1	Almost 100% pink
LH533	61646	Vaginal discharge	a/α	A	1	White
LH534	61647	Sputum	a/α	A	3	Pink
LH537	61648	Sputum	a/α	B	–	Pink
LH538	61649	Sputum	a/α	B	2	Few pink, under 50%
LH544	61650	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH565	61651	Sputum	a/α	B	1	Pink
LH566	61652	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH567	61653	Vaginal discharge	a/α	B	3	Few pink, under 50%
LH568	61654	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH569	61655	Vaginal discharge	a/α	A	1	21.6% pink
LH570	61656	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH573	61657	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH574	61658	Vaginal discharge	a/α	A	1	White
LH575	61659	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH576	61660	Vaginal discharge	a/α	B	3	Few pink, under 50%
LH577	61661	Vaginal discharge	a/α	C	1	White
LH602	61662	Vaginal discharge	a/α	A	1	78% pink
LH603	61663	Vaginal discharge	a/α	A	1	White
LH605	61664	Vaginal discharge	a/α	A	1	White
LH606	61665	Vaginal discharge	a/α	A	1	White
LH607	61666	Vaginal discharge	α	A	1	46.8% pink
LH613	61667	Unknown	a/α	A	1	White
LH623	61668	Sputum	a/α	A	1	13.7% pink
LH685	61669	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH729	61670	Vaginal discharge	a/α	B	3	White
LH730	61671	Vaginal discharge	a/α	A	1	White
LH731	61672	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH732	61673	Oral cavity	a/α	B	1	White
LH735	61674	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH737	61675	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH739	61676	Unknown	a/α	A	3	White
LH740	61677	Oral cavity	a/α	B	1	Few pink, under 50%
LH742	61678	Glans penis discharge	α	B	4	Few pink, under 50%
LH743	61679	Unknown	a/α	A	2	55.8% pink
LH744	61680	Unknown	a/α	C	3	Few pink, under 50%
LH549	61681	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH610	61682	Vaginal discharge	a/α	A	1	White
LH734	61683	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH738	61684	Vaginal discharge	a/α	A	3	White
LH745	61685	Unknown	a/α	A	1	White
LH746	61686	Onychomycosis	a/α	A	1	Few pink, under 50%
LH747	61687	Unknown	a/α	C	3	White
LH748	61688	Sputum	a/α	C	3	White
LH750	61689	Tinea corporis	a/α	A	1	45.4% pink
LH751	61690	Unknown	a/α	A	3	White

Contd...

Table 1: Contd...

Strain	No.	Source	*MTL* type	ABC genotype	*ITS* phylogenetic analysis	White/opaque phenotype
LH752	61691	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH753	61692	Oral cavity	a/α	A	3	White
LH756	61693	Vaginal discharge	a/α	A	1	14.5%
LH759	61694	Unknown	a/α	A	1	Few pink, under 50%
LH760	61695	Onychomycosis	a/α	A	1	98% pink
LH761	61696	Vaginal discharge	a/α	A	1	99% pink
LH766	61697	Vaginal discharge	a/α	A	1	White
LH767	61698	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH768	61699	Vaginal discharge	a/α	A	1	All white
LH769	61700	Glans penis discharge	a/α	C	3	All white
LH770-1	61701	Vaginal discharge	a/α	C	–	100% pink
LH770-2	61702	vaginal discharge	α	C	–	Few pink, under 50%
LH771	61703	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH772	61704	Glans penis discharge	a/α	A	3	few pink, under 50%
LH773	61705	Vaginal discharge	a	A	1	Few pink, under 50%
LH775	61706	Vaginal discharge	a/α	B	1	White
LH776	61707	Vaginal discharge	a/α	A	2	Almost 100% pink
LH777	61708	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH778	61709	Unknown	a	A	1	38.8% pink
LH791	61710	Vaginal discharge	a/α	A	3	White
LH795	61711	Vaginal discharge	a/α	A	3	White
LH804	61712	Vaginal discharge	a/α	A	1	White
LH805	61713	Vaginal discharge	a/α	B	1	Almost 100% pink
LH806	61714	Vaginal discharge	a/α	A	1	Few pink, under 50%
LH807	61715	Vaginal discharge	a/α	A	4	Few pink, under 50%
LH808	61716	Vaginal discharge	a/α	B	1	White
LH852	61717	Sputum	a/α	A	1	Few pink, under 50%
LH855	61718	Sputum	a/α	B	3	Few pink, under 50%
LH856	61719	Sputum	a/α	B	3	White
LH857	61720	Oral cavity (AIDS)	a/α	B	3	White
LH858	61721	Sputum	a/α	A	1	White
LH864	61722	Glans penis discharge	a/α	A	1	Few pink, under 50%
LH865	61723	Oral cavity (AIDS)	a/α	B	4	Few pink, under 50%
LH866	61724	Oral cavity (AIDS)	a/α	B	3	Few pink, under 50%
LH867	61725	Oral cavity (AIDS)	a/α	C	1	Almost 100% pink
LH868	61726	Blood culture	α	A	1	Almost 100% pink
LH869	61727	Oral cavity (AIDS)	a/α	B	1	All white
LH871	61728	Urine culture	a/α	B	2	All white
LH874	61729	Oral cavity	a/α	B	3	Almost 100% pink
LH875	61730	Oral cavity (AIDS)	a/α	C	3	All white

–: Not applicable; *MTL*: Mating type-like; *ITS*: Internal transcribed spacer; AIDS: Acquired immune deficiency syndrome.

11.1% of isolates from vaginal discharge. Phylogenetic analyses divided the 91 clinical isolates from China into four different subgroups. In 52 isolates from vaginal discharge, most were subgroup I (86.5%), seven isolates (13.5%) were subgroup III, and no strain was subgroup II or IV [Table 3]. From other sources, strains of subgroups I, II, III, and IV could be isolated although there was no strain of subgroup III from oral cavity.

Analysis of the mating type-like configuration

MTL analysis showed that 86 strains (92.5%) were a/α type, while only seven strains (6.5%) were considered to be aa or αα type [Table 1]. We discovered that minimal a/a or α/α single colonies can be isolated from a/α isolates. As shown in Figure 1, from LH770 a/α isolates, we isolated single colonies LH770-1 and LH770-2. The LH770-1 remained a/α mating type, but LH770-2 was α/α mating type. This demonstrates that a/α clinical isolate may transform to a/a or α/α strain in host as well as in nature.

Determination of white/opaque phenotype

WO phenotype in these isolates was also observed by phloxine B staining. Cells from white isolates were smaller in size and had a smooth surface and while cells from pink isolates were larger in size and had a rough surface [Figure 2], which

we identified as opaque phase. In 93 isolates, about 29.0% isolates (27 strains) showed all white colonies on the solid medium and the remaining isolates (71.0%, 66 isolates) showed some pink colonies on the solid medium [Tables 1 and 4]. In these 66 strains producing opaque colony, 59 strains were heterozygous at the mating-type locus. We determined *MTL*a DNA sequences of 10 *MTL*a/α and WO switchable strains and found that the three strains (498, 874, and 805 strains) had a point mutagenesis in their *MTL*a1 sequence. DNA sequence changed thymine to cytosine at the 139 position and amino acid sequence changed serine to lysine [Figure 3].

Correlation between white-opaque switching and cellular growth rates or chromosomal ploidy

WO switching has been previously described to be controlled by mating-type locus homeodomain proteins,[8] to be induced by *N*-acetyl glucosamine, 5% CO_2,[10] or to be related with cell growth.[14] In the 59 WO switchable and a/α strains, the switching was neither rigidly related with *MTL* homozygotes nor induced by *N*-acetyl glucosamine and CO_2. Growth

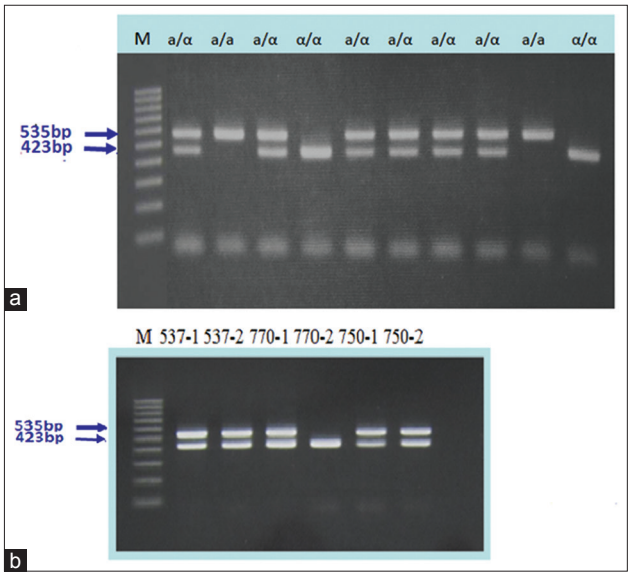

Figure 1: Mating type-like locus gene type of *Candida albicans*: (a) Type (heterozygous or homozygous) was determined by reverse transcription-polymerase chain reaction using primers specific for *MTL*a and *MTL*α. (b) We isolated different single colonies from LH770 a/α isolate. LH770-1 remained a/α mating type and LH770-2 was α/α mating type. M: Molecular marker. *MTL*: Mating type-like.

Table 2: Relation between the ABC genotypes and sources of *Candida albicans* isolates

Source of isolates	ABC genotypes, *n* (%)			Total, *n*
	A	B	C	
All isolates	64 (68.8)	19 (20.4)	10 (10.8)	93
Oral cavity	1	7	2	10
Sputum	4	5	1	10
Vaginal discharge	45 (83.3)	6 (11.1)	3 (5.6)	54
Others	14	1	4	19

Table 3: Distribution of *Candida albicans* isolates in four phylogenetic groups

Source of isolates	*ITS* Subgroups, *n* (%)				Total, *n*
	I	II	III	IV	
All isolates	61 (67.8)	4 (4.4)	22 (24.4)	3 (3.3)	90
Oral cavity	4	0	5	1	10
Sputum	3	1	4	1	9
Vaginal discharge	45 (86.5)	0	7 (13.5)	0	52
Others	9	3	6	1	19

ITS: Internal transcribed spacer.

Table 4: White-opaque phenotype in *Candida albicans* isolates from different sources

Source of isolates	Phenotypes, *n* (%)		
	Only white	White-opaque	Total
All isolates	27 (29.0)	66 (71.0)	93
Oral cavity	6	4	10
Sputum	3	7	10
Vaginal discharge	11 (32.1)	43 (67.9)	54
Others	7	12	19

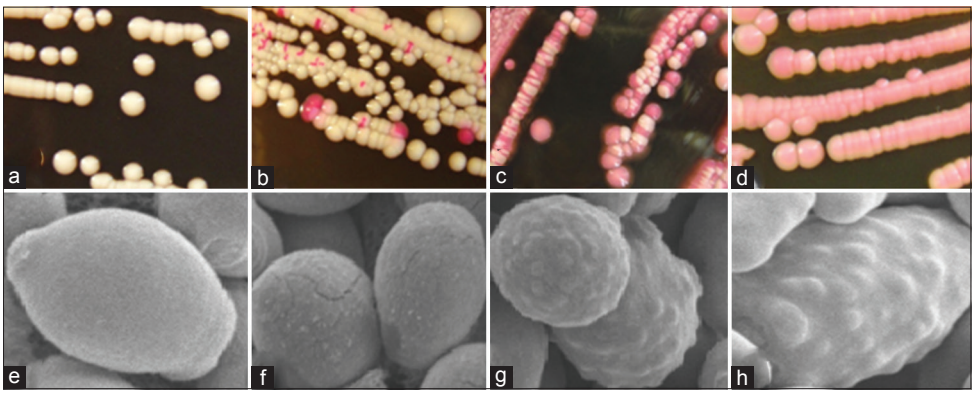

Figure 2: Determination of white/opaque phenotype of *Candida albicans*. Phenotypes were stained with phloxine B. (a) Stereomicroscope image showed all white phenotypes. (b and c) Stereomicroscope images showed white-opaque phenotype. (d) Stereomicroscope image showed all opaque phenotypes. (e and f) Scanning electron microscope images showed that white cells have smaller size and smooth surface. (g and h) Scanning electron microscope images showed that opaque cells have larger size and rough surface. Original magnification: a–d, ×8; e–h, ×20,000.

rates of strains indicated white phase and WO transition, 1.08 h and 1.30 h, respectively. We observed 8 white cells and 17 opaque cells. The doubling time for white and opaque cells was 1.08 (standard deviation [SD] = 0.16) and 1.30 (SD = 0.16), respectively, and there was a significant difference ($P = 0.004$).

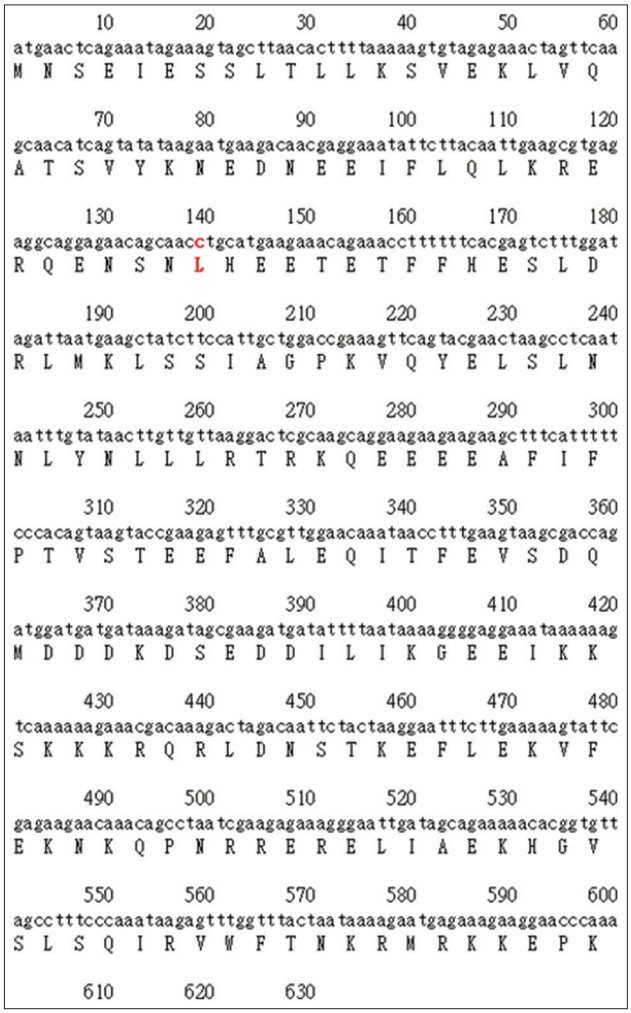

Figure 3: DNA sequence and amino acid sequence of *MTLa1* in LH498, LH874, and LH805 strains. The position of point mutation was indicated in red. *MTL*: Mating type-like.

We discovered that the LH770 (a/α) strain produced the colony *MTL*α/α (770-2). This led to the hypothesis that some WO switchable and a/α strains occurred parasexual reproduction and changed from diploid to tetraploid. Fluorescence-activated cell sorting analysis using DNA propidium iodide staining was conducted for a sample of 45 WO transition strains and 9 white phase strains picked out in a random manner. From the pattern of fluorescence intensity, we classified three groups; pattern I, pattern II, and pattern III [Figure 4]. Strains of the pattern I containing fifty strains were diploid. In the pattern II or pattern III, fluorescence intensity was lower or higher than that of the Pattern I, and strains classified into these patterns seemed to be an irregular ploidy.

Antifungal susceptibility and resistance

The 93 isolates were tested for antifungal susceptibilities [Table 5]. All were susceptible to micafungin with MIC between 0.015–0.250 μg/ml. The MIC of amphotericin B against *C. albicans* was 0.250–4.000 mg/ml. Most of the isolates were susceptible with only four isolates (4.3%) resistant to this medicine. The MIC of flucytosine was between 0.125 and 64.000 mg/ml. Two isolates were resistance to this antifungal drug and three isolates showed intermediate with MIC, higher than susceptible and lower than resistance. In our study, the MICs of miconazole, fluconazole, itraconazole, and voriconazole were between 0.030–4.000, 0.125–64.000, 0.015–4.000, and 0.015–0.500 mg/ml, respectively. Most of the isolates showed high susceptibility to these drugs. In the commonly used azole drugs such as fluconazole, itraconazole, and voriconazole, two isolates and one isolate showed resistance to fluconazole and itraconazole, respectively, but all isolates were susceptible to voriconazole.

Discussion

In recent years, the sexual mating and WO switching of *C. albicans* have aroused a great interest.[2,3,6-10,14] In this context, we compared the genetic and phenotypic heterogeneities of clinical strains isolated from China and previously described articles. It is previously considered

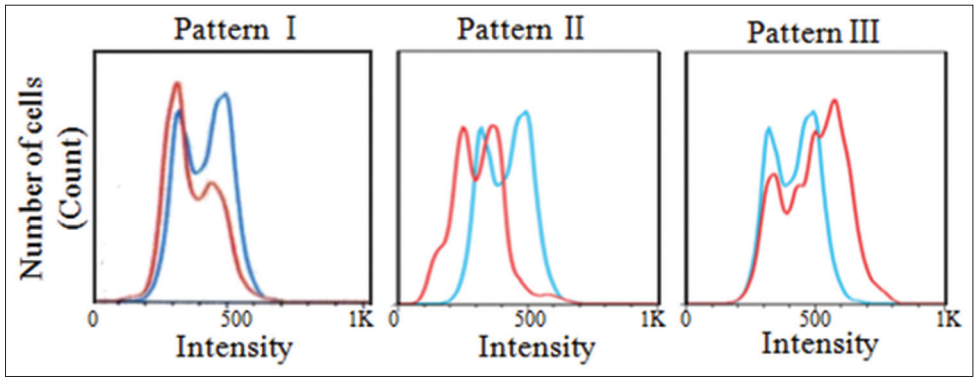

Figure 4: Three patterns of diagram of fluorescence intensity and distribution of *Candida albicans* isolates determined by fluorescence-activated cell sorting analysis. Red lines indicate the diagram of LH729 (left), LH607 (center), and LH722 strains (right), respectively, and blue lines indicate the diagram of LH740 strain.

Table 5: Antifungal susceptibility of *Candida albicans* isolates, *n* = 93

Antifungals	MIC (mg/ml)	Susceptible, *n* (%)	Susceptible-dose dependent, *n* (%)	Intermediate, *n* (%)	Resistant, *n* (%)	Nonsusceptible, *n* (%)
Amphotericin B	0.250–4.000	89 (95.7)	–	–	4 (4.3)	–
Flucytosine	0.125–64.000	88 (94.6)	–	3 (3.2)	2 (2.2)	0
Micafungin	0.015–0.250	93 (100)	–	–	2 (2.2)	–
Fluconazole	0.125–64.000	90 (96.7)	1 (1.1)	–	1 (1.1)	–
Itraconazole	0.015–4.000	89 (95.7)	3 (3.2)	–	0	–
Voriconazole	0.015–0.500	93 (100)	0	–	–	–

–: Not applicable; MIC: Minimum inhibitory concentration.

that before mating, *C. albicans* strains must first undergo homozygosis in *MTL* allele, and then switch from white to opaque. White cells are round or oval which form smooth colonies that hardly be dyed by phloxine B, while opaque cells are long shaped which form rough colonies that can be dyed into pink by phloxine B. In our research, we found a higher frequency of a/α opaque phenotype [71.0%, 66 isolates, Table 4]. Therefore, we hypothesize that even a/α isolates can also switch to opaque phenotype and mate *in vivo* in host environment. Of course, in natural a/α strains, they have been induced to WO switching by using *N*-acetyl glucosamine as the sole carbon source and incubation in 5% CO_2. However, in our experiments, these isolates could have WO transition when they grew on YPD solid medium at room temperature. Pendrak *et al*.[15] found that decreased expression of hemoglobin response gene 1 (*HBR*1) has been shown to alter the expression of the *MTL*. Our finding suggested that a/α cells may mate *in vivo* which coincides with the hypothesis of Pendrak *et al*.[15] Moreover, we found a point mutagenesis in *MTL*a1 DNA sequences of *MTL*a/α WO-switchable strains. Some mutations like this mutation might have effect on WO transition.

Currently, the most effective drugs for *C. albicans* include azoles and echinocandins.[12] However, repeated exposure to triazole drugs is a major risk factor for drug resistance, and *in vitro* resistance to fluconazole and itraconazole in *C. albicans* had been reported. Opaque cells reported to be more resistance to amphotericin B, nystatin, 5-fluorocytosine, and miconazole nitrate than white cells.[16] In our results, fluconazole and itraconazole resistance ratio was under 3%. No voriconazole resistance was seen. This may be related to the duration the antifungal drug has been available clinically. We detected the susceptibility of micafungin and found no resistance. Moreover, most of the clinical isolates were sensitive to amphotericin B with 4.3% resistance ratio. Although an appearance of WO switching was remarkable in the clinical isolates, we could not find a correlation between WO switching and resistance to amphotericin B, 5-fluorocytosine, and miconazole nitrate.

There was no significant difference between *ITS* sequence and ABC typing in previous studies as well as the other sources of *C. albicans* isolates.[6,13] However, variation of genotype was significantly observed in isolates from oral cavity or sputum against isolates from vaginal discharge in our results [Tables 2 and 3]. High frequency of a/α opaque phenotype was not associated with the variation of genotypes. We will clarify why the isolates from China occurred significantly WO transition. Furthermore, the mechanism of WO switching in *MTL*a/α isolates has not been clearly explained yet, although stress-activated protein kinase pathway and *WOR*1 gene have been reported to play roles in the process of switching,[17] further investigations are still needed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lim CS, Rosli R, Seow HF, Chong PP. *Candida* and invasive candidiasis: Back to basics. Eur J Clin Microbiol Infect Dis 2012;31:21-31. doi: 10.1007/s10096-011-1273-3.
2. Odds FC. *Candida* and Candidiasis: A review and Bibliography. 2nd ed. London, UK: Bailliere Tindall; 1988.
3. Lengeler KB, Davidson RC, D'souza C, Harashima T, Shen WC, Wang P, *et al.* Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 2000;64:746-85. doi: 10.1128/MMBR.64.4.746-785.2000.
4. Hull CM, Johnson AD. Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans*. Science 1999;285:1271-5. doi: 10.1126/science.285.5431.1271.
5. Slutsky B, Staebell M, Anderson J, Risen L, Pfaller M, Soll DR. White-opaque transition: A second high-frequency switching system in *Candida albicans*. J Bacteriol 1987;169:189-97.
6. Lockhart SR, Pujol C, Daniels KJ, Miller MG, Johnson AD, Pfaller MA, *et al.* In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics 2002;162:737-45.
7. Morschhäuser J. Regulation of white-opaque switching in *Candida albicans*. Med Microbiol Immunol 2010;199:165-72. doi: 10.1007/s00430-010-0147-0.
8. Miller MG, Johnson AD. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell 2002;110:293-302. doi: http://dx.doi.org/10.1016/S0092-8674(02)00837-1.
9. Geiger J, Wessels D, Lockhart SR, Soll DR. Release of a potent polymorphonuclear leukocyte chemoattractant is regulated by white-opaque switching in *Candida albicans*. Infect Immun 2004;72:667-77. doi: 10.1128/IAI.72.2.667-677.2004.
10. Xie J, Tao L, Nobile CJ, Tong Y, Guan G, Sun Y, *et al.* White-opaque switching in natural MTLa/α isolates of *Candida albicans*: Evolutionary implications for roles in host adaptation, pathogenesis, and sex. PLoS Biol 2013;11:e1001525. doi: 10.1371/journal.pbio.
11. Liu Y, Kang Y, Yokoyama K, Gonoi T, Mikami Y. Molecular differentiation and antifungal susceptibility of *Candida albicans* isolated from patients with respiratory infections in Guiyang Medical College Hospital, China. Nihon Ishinkin Gakkai Zasshi 2009;50:175-8.

12. Li F, Wu L, Cao B, Zhang Y, Li X, Liu Y. Surveillance of the prevalence, antibiotic susceptibility, and genotypic characterization of invasive candidiasis in a teaching hospital in China between 2006 to 2011. BMC Infect Dis 2013;13:353. doi: 10.1186/1471-2334-13-353.
13. McCullough MJ, Clemons KV, Stevens DA. Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. J Clin Microbiol 1999;37:417-21.
14. Alby K, Bennett RJ. Stress-induced phenotypic switching in *Candida albicans*. Mol Biol Cell 2009;20:3178-91. doi: 10.1091/mbc.E09-01-0040.
15. Pendrak ML, Yan SS, Roberts DD. Hemoglobin regulates expression of an activator of mating-type locus alpha genes in *Candida albicans*. Eukaryot Cell 2004;3:764-75.doi: 10.1128/EC.3.3.764-775.2004.
16. Ghannoum MA, Swairjo I, Soll DR. Variation in lipid and sterol contents in *Candida albicans* white and opaque phenotypes. J Med Vet Mycol 1990;28:103-15.
17. Liang SH, Cheng JH, Deng FS, Tsai PA, Lin CH. A novel function for Hog1 stress-activated protein kinase in controlling white-opaque switching and mating in *Candida albicans*. Eukaryot Cell 2014;13:1557-66. doi: 10.1128/EC.00235-14.