American-Eurasian J. Agric. & Environ. Sci., 1 (2): 127-132, 2006
ISSN 1818-6769

Morphogenesis of Two *Candida albicans* Strains as Influenced by Growth Media, pH Value and Incubation Temperature

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Abstract: The pattern of growth and mycelial formation of two strains M (oral isolate) and G (genitourinary tract isolate) of *Candida albicans*, in horse-serum, MSB and RPMI 1640 media, indicated that the first medium promotes filamentation in both strains. While, MSB intiates mediun mycelial formation for M strain and RPMI stimulates the yeast growth and low filamentation. However, RPMI promotes moderate filamentation and growth values for *C. albicans* G. The peak of mycelium production appeared between 1.5 and 4.5 h after inoculation of *C. albicans* M and between 1.5 to 6.0 and 7.5 h for *C. albicans* G. The pH value of 7.4 appeared to be optimal for filamentation on MSB at 37°C for *C. albicans*, M (oral pH slightly alkaline), While, pH of 4.4 was responsible for highest mycelial formation of *C. albicans* G, (vaginal pH around 4.5) under same cultural conditions. Incubation temperature of 37°C was concomitant with the highest germ-tube formation by *C. albicans* M, on MSB at pH 7.4. While 34°C was optimum for filamentation by *C. albicans* G, on MSB at pH 4.4. These results indicated that the pattern of filamentation by *C. albicans* depends mainly on the yeast strain and the nutritional and cultural conditions control the route of yeast dimorphism. The two *C. albicans* strains (M and G) showed the same phenotypic switching when grown in solid media of MSB, RPMI, horse serum and blood agar base, in presence of 7% CO_2 (anaerobic) and in absence of 7% CO_2 (aerobic) conditions). However, *C. albicans* G showed phenotypic switching on chocolate agar (feet appendages in presence of 7% CO_2 and normal growth in absence of CO_2), but *C. albicans* M showed no switching under the same conditions.

Key words: Morphogenesis · germ-tube · yeast · hyphal form · *Candida albicans* · phenotypic

INTRODUCTION

Candida albicans is the most frequent opportunistic fungal infection of man. Although antifungal resistance in *C. albicans* is less frequent than in other species, an increasing number of resistant strains are emerging [1, 2]. The major virulence factors of *C. albicans* are proteinase secretion, hyphal formation, adhesion and phenotypic switching [3]. *C. albicans* a dimorphic fungus is able to grow in yeast and hyphal forms depending on environmental conditions [4]. A wide range of environmental factors have been shown to be important in selectively favoring yeast or hyphal forms, the most important being the growth medium, temperature of incubation and external pH value [5-9]. *C. albicans* is a normal inhabitant of the oral cavity and the gastrointestinal and genitourinary tracts, where it persists in equilibrium with the host's microflora [4, 10].

In a previous work the induction of myeclial development in *C. albicans*, (ATTC 10231) as influenced by some environmental and nutritional shifts and also its phenotypic switching were studied [11]. The present work aimed to compare between two *Candida albicans* strains (one was isolated from the oral cavity of infant and the other from genitourinary tract of pregnant women) and their mycelial formation as influenced by growth media, incubation temperature and external pH value. Also, to characterize their phenotypic switching under 7% CO_2 or in aerobic conditions.

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MATERIALS AND METHODS

Organisms: *Candida albicans* isolates used in this work were obtained from the mouth of hospitalized children (M) and the other from genitourinary tract of pregnant women (G) during routine preoperative screening. None of the patients had overt candidiasis nor had they antifungal therapy. The two isolates were kindly provided by the Hospital of King Abdulaziz University, Jeddah, Saudi Arabia. The yeasts were kept on Sabouraud's glucose agar slopes at 4°C. Inoculates were prepared from cultures on Sabouraud's agar slopes incubated at 37°C for 16-18 h. The yeast cells were washed in water, centrifuged and resuspended in water (under aseptic conditions). The number of blastospores/ml of suspension were determined by haemocytometer counting and a suitable volume of suspension was added to 250 ml Erlenmeyer flasks containing 100 ml of broth to yield an initial concentration of 10^6 blastospores/ml [5].

Growth media and culture conditions: The three media tested for their ability to stimulate filamentation in *C. albicans* strains (M and G), were modified Sabouraud's glucose broth (MSB), comprising 1% mycological peptone (Oxoid) and 0.2% glucose, adjusted to pH 7.4 [12], horse serum medium (Oxioid, SR0035C), pH 6.8 and RPMI 1640 medium (Sigma, MF0009), pH 7.4.

Media were warmed to their incubation temperature before inoculation. The flasks of media were then agitated on a rotary shaker (150 rpm) for 12 h. The growth of the culture was recorded together with the ability to form germ-tubes and pseudomycelium (mycelium or filamentation) during the incubation period.

Growth and mycelial formation: At intervals (1.5 h) during growth, the cell concentration was determined as single cell (blastospores) by direct count in a Burke haemocytometer. Germ-tube and pseudomycelium (mycelium or filamentation) formation were measured by counting the number of individual cells showing a definite out growing tube and expressing this as percentage of the total cell population.

* Total cell population = Number of blastospores + Germ-tube forming cells

Effect of pH value: The influence of different starting pH values (3.4-9.4) of modified Sabouraud's glucose broth (MSB), the cheapest and lead to high induction of mycelial formation in the tested *Candida albicans* strains (M and G), was tested.

Effect of incubation temperature: *C. albicans*, strains were allowed to grow under the best pH value (7.4 for M and 8.4 for G) that induces mycelial formation and at incubation temperatures of 34, 37, 40 and 45°C.

Phenotypic switching of *C. albicans* strains (M and G): *C. albicans* strains (M and G) were allowed to grow in solid media of MSB, RPMI 1640 (Sigma, MF0009), horse serum (Oxoid, SR0035C), blood agar base (HIMEDIA, M089) and chocolate agar (HIMEDIA) under 7% CO_2 (anaerobically) and aerobically for 48 h at 37°C. Thereafter, the colony growth was described, either go as normal yeast growth or forming feet appendages at the colony edges [2].

RESULTS AND DISCUSSION

Effect of growth media: In a preliminary experiment the two *C. albicans* strains (M and G) failed to form germ-tubes when grown in both Lee's medium [13], amino acid synthetic medium and Winge medium (glucose 2% and 0.4 yeast extract [14].

The pattern of growth and mycelial formation of *C. albicans* strains (M and G) in horse-serum, MSB and RPMI 1640 media (Table 1) indicated that horse - serum medium noticeably promotes filamentation in both yeast strains (about 97% for M and 96% for G). While MSB resulted in only 52% germ- tube forming cells in M strain. However, RPMI constituents were not in harmony with mycelial formation by the same strain (about 11% filamentation). On the other hand, the last medium was more conducive for mycelial formation (about 45%) than MSB (about 16%) medium for *C. albicans* G. These findings indicated that filamentation of *C. albicans* depends mainly on the yeast strain and to a lesser extent on the growth medium formulation. However, horse-serum natural ingredients of hemin, hormones and other blood serum constituents appeared to be necessary for germ-tube formation and hence increased pathogenicity for most *C. albicans* strains [7, 15, 16]. Media containing high levels of glucose (Winge, Lee's and RPMI, have 2, 1.25 and 0.45% glucose, respectively), high phosphate (RPMI and Lee's, 6, 2.5 g/l, respectively), amino acids and biotin (RPMI and Lee's) reduce the ability of *C. albicans* M to form germ tube. In accordance with

Table 1: Effect of different growth media in germ-tube production in *Candida albicans* strains (M and G) incubated at 37°C for 12 h

		Horse serum medium		MSB medium		RPMI 1640 medium	
Yeast strain	Incubation period (h)	Yeast count/ ml × 10^4	% of cells forming germ - tube	Yeast count/ ml × 10^4	% of cells forming germ - tube	Yeast count/ ml × 10^4	% of cells forming germ - tube
M	1.5	141	96.92	120	15.00	146	0.0
	3.0	152	94.23	135	52.27	162	0.28
	4.5	194	81.00	179	39.44	218	3.07
	6.0	260	73.89	240	29.87	278	10.67
	7.5	480	71.70	430	17.80	491	9.49
	9.0	680	56.88	615	12.52	688	6.82
	10.5	915	42.86	880	8.86	993	4.97
	12.0	1200	37.16	1105	7.19	1270	3.97
G	1.5	120	95.60	108	11.43	120	16.58
	3.0	149	95.12	140	15.71	141	37.76
	4.5	175	92.68	164	14.02	205	45.45
	6.0	238	85.71	218	11.00	270	35.55
	7.5	360	77.78	316	8.23	435	23.16
	9.0	440	64.29	460	6.09	642	18.31
	10.5	526	54.29	689	4.21	904	13.67
	12.0	720	40.28	913	3.28	1187	10.64

this finding that *C. albicans* strain could respond for glucose, phosphate, amino acids and biotin starvation by activating hyphal development [6, 17, 18]. On the other hand, *C. albicans* strain G appeared to be in need to complex media for filamentation where horse-serum was the best tested media and RPMI medium with complex components of amino acids, vitamins, hormones etc. gave detectable filamentation. The influence of amino acids and vitamins for initiation of germ-tube formation and pathogenicity of some *C. albicans* strains were reported [7, 8, 13, 19].

C. albicans strain M showed higher growth values than that of G strain, on the tested media under the cultural condition. The peak of mycelium production appeared between 1.5 and 4.5 h after inoculation of *C. albicans* M in horse-serum and MSB media and between 3.0 and 7.5 h in RPMI for the same strain. As for *C. albicans* G, the peak of mycelial formation in horse-serum and RPMI media appeared between 1.5 and 7.5 h for the first medium and 1.5 and 6.0 h for RPMI, whereas for MSB medium it was between 1.5 and 6.0 h. The fore mentioned results indicated that *C. albicans* filamentation and its peak depends mainly on the yeast strain and in the nutritional factors. The importance of nutritional factors that induces germ-tube formation by *C. albicans* strains was reported [6, 7, 9, 14].

Effect of pH value: The influence of starting pH value of MSB medium on mycelial formation by *C. albicans* strains (M and G) (Table 2), indicated that *C. albicans* M failed to form germ-tube at the acidic pH 3.4 and as the pH value shifted toward neutrality (pH 7.4) filamentation percentages increased at the different incubation periods. On the other hand, as the pH's tends to alkalinity mycelial formation decreased. A pH range of 3.4 to 7.4 favored the growth of yeast (M) than the alkaline pH's. As for *C. albicans* G, the shift of pH from neutrality to acidity favored yeast growth and germ-tube formation and pH 4.4 proved to be optimum for yeast strain G filamentation and growth, under the tested conditions. As mentioned before *C. albicans* M was of oral origin, pH slightly alkaline, so the optimum pH for mycelial formation by the strain was at neutral pH value (7.4). But *C. albicans* (G) was isolated from genitourinary tract of acidic pH (4.5). So, pH value of (4.4) of the test medium favored the best yeast (G) growth and filamentation.

The peak of mycelium production appeared between 1.5 and 6.0 h after inoculation *C. albicans* M at pH range (4.4-4.7), while it was between 3 and 9 h for pH 8.4 and at pH value of 9.4 (highly alkaline) the peak appeared between 6 and 12 h of incubation. These indicated retardation of the time of mycelium formation as

Table 2: Influence of pH value in germ-tube production in *Candida albicans* strains (M and G) cultivated in modified Sabouroud's broth (MSB) medium at 37°C for 12 h

		pH value													
		3.4		4.4		5.4		6.4		7.4 (basal)		8.4		9.4	
Yeast strain	Incubation period (h)	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT
M	1.5	120	0.0	122	2.25	130	6.62	122	11.48	120	15.00	108	0.0	106	0.0
	3.0	162	0.0	168	3.52	180	8.71	160	13.54	135	52.27	128	5.68	120	0.0
	4.5	209	0.0	228	4.48	320	5.94	220	9.96	179	39.44	158	10.23	146	0.0
	6.0	301	0.0	335	4.63	467	4.71	285	7.69	240	29.87	210	15.94	200	6.06
	7.5	428	0.0	449	3.61	648	3.73	460	5.93	430	17.80	388	13.41	332	11.90
	9.0	580	0.0	640	3.15	686	3.64	680	5.22	615	12.52	450	11.67	400	14.00
	10.5	880	0.0	890	2.58	899	3.00	886	3.27	880	8.86	790	6.71	696	15.78
	12.0	1115	0.0	1186	2.11	1219	2.31	1221	2.63	1105	7.19	980	5.46	848	14.03
G	1.5	128	3.85	130	13.91	124	13.32	120	12.11	108	11.43	107	9.37	106	0.85
	3.0	175	2.91	182	18.43	168	17.83	154	16.61	140	15.71	129	11.68	123	3.95
	4.5	251	2.19	268	18.65	239	17.88	195	16.83	164	14.02	146	10.96	138	10.64
	6.0	352	1.98	384	15.10	326	14.12	266	13.16	218	11.00	192	8.85	188	8.50
	7.5	506	1.45	579	10.36	441	10.91	410	9.02	316	8.23	262	6.87	240	7.09
	9.0	680	1.09	745	8.32	615	8.15	560	6.89	460	6.09	396	4.79	370	4.73
	10.5	930	0.81	946	6.76	812	6.40	786	5.10	689	4.21	580	3.45	527	3.42
	12.0	1210	0.62	1228	5.37	1106	4.88	1084	3.89	913	3.28	866	2.37	810	2.28

CFGT = Cell forming germ - tube

Table 3: Influence of incubation temperature on germ- tube production in *Candida albicans* strains (M and G) cultivated in modified Sabouroud's broth (MSB) medium at pH 7.4 for (M) and pH 4.4 for (G), for 12 h of incubation

		34°C		37°C (basal)		40°C		43°C	
Yeast strain	Incubation period (h)	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT	Yeast count/ ml × 10^4	% of CFGT
M	1.5	145	0.00	120	15.00	118	12.06	104	4.870
	3.0	176	7.39	135	52.27	129	11.62	114	5.090
	4.5	234	15.06	179	39.44	147	10.27	139	5.370
	6.0	323	11.14	240	29.87	207	7.34	196	4.080
	7.5	460	7.85	430	17.80	366	4.23	297	2.860
	9.0	667	5.54	615	12.52	580	2.74	489	1.840
	10.5	915	4.06	880	8.86	793	2.02	698	1.029
	12.0	1310	2.86	1105	7.19	964	1.72	880	1.090
G	1.5	143	15.49	130	13.91	121	9.84	114	3.600
	3.0	196	22.21	182	18.43	169	14.92	147	12.050
	4.5	287	24.68	268	18.85	248	10.88	214	9.340
	6.0	396	18.18	384	15.10	314	9.55	289	7.960
	7.5	499	15.03	579	10.36	530	6.03	500	5.200
	9.0	773	9.96	745	8.32	706	4.86	685	4.240
	10.5	982	7.95	946	6.76	911	4.06	879	3.750
	12.0	1300	6.23	1228	5.37	1200	3.38	1099	3.180

CFGT = Cell forming germ tube

alkalinity increases. However, the peak of filamentation by C. *albicans* G appeared between 1.5 and 4.5 h after inoculation, at the different tested pH's. It was reported that the extracellular pH is one of the environmental factors that modified the physiology of the cell [20]. It was also reported that the gene of cell wall protein is expressed at a pH ≥ 5.5 and is required for Systemic candidiasis (blood pH is near neutrality), whereas its paralogue gene is expressed only at acidic pH (pH ≤ 5.5) and is required for vaginal candidiasis (vaginal pH is around 4.5) [21, 22].

Effect of incubation temperature: The influence of temperature range (34°C-43°C) on mycelial formation (Table 3) by *C. albicans* strains (M and G) revealed as 37°C was optimal for filamentation percentages by the strain M, 34°C was so for G strain. 34°C proved to be suitable for growth of both strains, while higher temperatures resulted in a gradual decrease in yeasts growth.

The peak of mycelium formation by *C. albicans* (M) appeared between 1.5 and 4.5 h of inoculation. But for G strain it appeared between 1.5 and 6.0 h of incubation. It was reported that germ-tube induction in *C. albicans* strains may involve different signaling pathways triggered by distinct environmental factors which regulate different or overlapped subsets of gene systems controlling dimorphism. The functionally of these path ways may depend in both culture conditions and the growth phase [7]. The influence of incubation temperature in dimorphism of *C. albicans* was reported by many workers [18, 23-26].

The above mentioned results indicated that the pattern of filamentation by *C. albicans* depends mainly on the yeast strain, genetically controlled and the nutritional and cultural conditions control the route of yeast dimorphism.

Phenotypic switching of *C. albicans* strains M and G on solid media: The phenotypic of *C. albicans* strains M and G growth on solid media of MSB, RPMI, horse-serum, blood agar base and chocolate agar under 7% CO_2 (anaerobically) and aerobically for 48 h at 37°C, indicated that the two strains showed the same phenotypic switching on the first four media. However, on chocolate agar medium feet appendages emerged under the tested conditions (absence and presence of 7% CO_2) for *C. albicans* M, while strain G showed no feet appendages in absence of CO_2 and normal yeast growth was estimated. The two strains (M and G) showed no phenotypic switching when growth on MSB agar medium, either in 7% CO_2 or in aerobic conditions. So, normal yeast growth was shown. They also showed feet appendages (no phenotypic switching) when grown in both RPMI and horse-serum media, (under presence or absence 7% CO_2). i.e.: the last two media stimulated formation of feet appendages. However, *C. albicans* M and G showed phenotypic switching as grown on blood agar base, where they showed feet appendages in presence of 7% CO_2 and normal growth in absence of CO_2. The above results indicated that the two strains have the same phenotypic growth in the tested media, but differ only when grown on chocolate agar medium where strain G showed phenotypic switching (feet appendages in 7% CO_2 and normal growth in absence of CO_2) and strain M showed no switching.

The phenotypic switching in colonies form of *C. albicans* in aerobic (absence of 7% CO_2) and anaerobic conditions (7% CO_2) used as indication for its virulence and pathogenecity, as well as, resistance to fungal antibiotics. The strains isolated from candidiasis ills have the ability for phenotypic switching, while that inhabit healthy persons lack this phenomenon [2, 27]. Phenotypic switching is one of the major virulence factors and has been shown to be effective in defense of the immune system, increase in adherence, increase in enzyme secretion and decrease in susceptibility to antifungal [1-3].

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