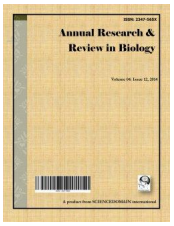

Annual Research & Review in Biology

24(6): 1-8, 2018; Article no.ARRB.40071
ISSN: 2347-565X, NLM ID: 101632869

Growth Kinetic Profile of *Candida albicans* Under Varying Environmental Conditions

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/ARRB/2018/40071
Editor(s):
(1) Ibrahim Farah, Professor, Jackson State University, Mississippi, USA.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Maria Luisa Carrillo Inungaray, Universidad Autónoma de San Luis Potosí, Mexico.
(2) Bryan Larsen, Marian University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/23538

Original Research Article

Received 19th November 2017
Accepted 28th February 2018
Published 8th March 2018

ABSTRACT

Several environmental conditions were identified to influence the growth and morphogenesis of *Candida albicans*. The present study quantitatively examined the growth rate and described the kinetic profile of *C. albicans* under different pH, temperature, and culture medium. When *C. albicans* was monitored over the 12-h period under different culture media, temperature values, and pH levels, the growth kinetic profile of the organism behaved in accordance with the first-order rate equation. The organism exhibited a relatively faster growth rate when incubated at 37ºC in modified Sabouraud glucose broth medium with pH 7.4. Moreover, the growth profile exhibited a linear pattern between 1.5 h and 6 h after inoculation of *C. albicans* culture which coincides with the mycelium production, and subsequently shifted to an exponential increase beyond 6 h. Given the environmental conditions selectively supporting the growth and morphogenesis of *C. albicans*, quantitative descriptions of the rate kinetic profile of *C. albicans* population offer an objective approach in comparing environmental conditions with varying physicochemical characteristics and biochemical compositions.

Keywords: Candida albicans; growth rate; kinetic models; first-order rate equation.

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1. INTRODUCTION

Candida albicans, a relatively harmless commensal organism, normally thrives in the oral cavity, skin, and genitourinary and gastrointestinal tracts of healthy individuals [1]. However, disturbances in the normal microbiota resulting from surgical procedures, antibiotic therapy, and prolonged use of medical devices can lead to *C. albicans* infection [2], the most prevalent opportunistic fungal infection in humans [3]. During fungal infections, host microenvironments of varying physicochemical and biochemical conditions can significantly influence the growth, survival, and pathogenicity of the organism [4-6]. Moreover, there were several known factors influencing the morphogenesis [7] and filamentation [4] of *C. albicans* such as biochemical and nutritional compositions of the medium, CO_2 and O_2 concentrations, pH, temperature, and cell density, which apparently suggest an array of signals [4] recognized by the organism.

Candida albicans is capable of reversible morphogenetic transitions between yeast and filamentous growth [3,7,8], and these morphological structures differ in population dynamics, architectural dimensions, molecular chemistry, genetic expression, virulence, and pathogenicity [9]. Considering that *C. albicans* survives in different host niches with varying available nutrients, pH levels, O_2 and CO_2 concentrations [6,10], the present study quantitatively described the growth kinetic profile of *C. albicans* under environmental conditions of varying temperature, pH, and culture medium. Although yeast growth is favored in low pH and temperatures, enriched media, and high cell density and osmolarity [7], no study has been done to quantify growth rates in order to describe the kinetic profile of *C. albicans* population under specified conditions. The quantitative descriptions on the rate kinetic profile of *C. albicans* population offer an objective criterion in comparing population dynamics of organisms under environmental conditions with varying physicochemical characteristics and biochemical compositions.

2. MATERIALS AND METHODS

2.1 The Data Set [11]

In the present study, information regarding *C. albicans* population was obtained from literature [11], where yeast to hyphal transition of *Candida albicans* was investigated under different growth media, pH, and temperature. Briefly, the morphogenesis of *C. albicans* was studied using three different growth media, namely Horse serum medium (HSM) at pH 6.8, RPMI-1640 at pH 7.4, and modified Sabouraud glucose broth (MSGB) at pH 7.4 [11], which were all incubated at 37°C for 12 h. Moreover, the compositions of the three media were described as follows [11]: HSM contained yeast extract peptone dextrose (YEPD) broth and serum; RPMI-1640 medium contained distilled water, inorganic salts, amino acids, vitamins, glucose, glutathione, and phenol red; and MSGB medium contained distilled water, peptone, and glucose. The three media were warmed to their incubation temperature prior to *C. albicans* inoculation. For the inoculum preparation, *C. albicans* culture was maintained on SDA at 4ºC, subsequently inoculated in YEPD broth and incubated at 37ºC for 24 h. The final suspensions were prepared by adjusting the centrifuged washed yeast cells with sterile water to 0.5 Mc Farland solutions. The effect of temperature on the morphogenesis of *C. albicans* was investigated using MSGB medium at pH 7.4 under varying temperature values (34ºC, 37ºC, and 40ºC). Assessment on the effect of pH (5.4, 6.4, and 7.4) was done using MSGB medium incubated at 37ºC for 12 h. In all experimental setups, the growth of *C. albicans* was monitored at 1.5-h interval using hemocytometer to measure cell concentrations.

Table 1. Nonlinear and linear forms of the different growth models [12-14]

Kinetic model	Nonlinear model	Linear form
Zero-order	$\frac{dP_t}{dt} = k_o$	$P_t = k_0 t + P_o$
First-order	$\frac{dP_t}{dt} = k_1 P_t$	$\log P_t = \log P_o + \frac{k_1}{2.303} t$
Second-order	$\frac{dP_t}{dt} = k_2 {P_t}^2$	$\frac{-1}{P_t} = k_2 t + \left(\frac{-1}{P_0}\right)$

P_t (count/ml): yeast population at time t (h); P_0 (count/ml): initial yeast population; k_o (count/ml/h): zero-order rate constant; k_1 (count/ml/h): first-order rate constant; k_2 (ml/count/h): second-order rate constant

2.2 The Kinetic Models

In this study, nonlinear curves were generated to describe the relationship between *C. albicans* population (count x 10^6 per ml) and time (h). Several kinetic models [12-14] including zero-order, first-order, and second-order rate equations were utilized to elucidate the rate mechanisms and to describe the growth profile of *C. albicans* under specified conditions. The model parameters were estimated employing the linearized forms of the kinetic equations (Table 1) using linear regression analysis. All statistical and numerical analyses were performed using Microsoft Excel® at 5% level of significance.

3. RESULTS AND DISCUSSION

When the growth of *C. albicans* was monitored over the 12-h period under different culture media, the kinetic profile of the organism behaved in accordance with the first-order rate equation (Table 2). The organism exhibited a relatively faster growth rate in MSGB medium compared to HSM and RPMI-1640. However, it was reported that MSGB medium promoted moderately low filamentation while HSM had noticeable filamentation [11]. Moreover, as early as 1.5 h at 40ºC, the germ tube of *C. albicans* developed, but with highest germ tube formation at 37ºC [11]. In the present study, *C. albicans* population had a relatively faster growth rate (2.3 x 10^5 yeast count per ml per h) when incubated at 37ºC in MSGB with pH 7.4 (Table 2). Under varying pH levels, the growth profile of the organism behaved under the first-order kinetic equation and there is a direct relation between the pH level of the culture medium and the growth rate of the organism (Table 2). This was a similar phenomenon exhibited between pH levels and the induction of filamentation of *C. albicans*, higher pH favored filamentation, with pH 7.4 identified as best suited for germ tube formation [11]. Similar to the different culture media and temperature values, the growth profile of *C. albicans* under varying pH values likewise obeyed the first-order kinetic equation.

In the present study, *C. albicans* exhibited a remarkably heterogeneous growth pattern when monitored under varying environmental conditions suggesting variability in the kinetic growth profile of the organism which can likewise influence the structural architecture, adhesion potential, and biofilm formation. *C. albicans* hyphae developed synchronously in methionine assay medium, Eagle's minimal essential medium, serum, and buffered *N*-acetylglucosamine, but hyphal evaginations in modified Sabouraud broth and amino acids salts medium emerged over a relatively extended period [15]. In an in vitro study, when *C. albicans* was incubated at 37ºC in phosphate buffer saline adjusted at pH 5, maximum adherence to the vaginal epithelium was identified for a logarithmic phase-culture grown at 25ºC [16], and potent in initiating and forming in vitro biofilms in RPMI-1640 medium [17]. The formation of these fungal biofilms was dictated not only by the morphogenetic state and structural diversity of *C. albicans* [18-22], but also by substrate biomaterial and contact surface type [18,19,22], and other environmental parameters [23] including growth medium [24], carbohydrate source and concentration [25], oxygen availability [26], and pH [27,28].

The morphological development of *C. albicans* is influenced by environmental pH [29], similar to any other nutritional and stress signals, pH is important in the regulation of morphogenesis and pathogenesis of the organism [4]. In a hypoxic environment, secondary to tissue necrosis resulting from pathogen invasion and influx of immune cells at the infection site, *C. albicans* can adapt to oxygen limitation [30]. However, proteolytic activity is critically regulated by pH, relatively small changes in pH can significantly modify extracellular proteolytic activity [31], and proteolytic action on the fungal cell surface can alter hydrophobicity and subsequent adherence to the host-cell surface [32].

Furthermore, at room temperature, *C. albicans* cells produced abundant germ tubes which escaped phagocytosis, and were more virulent [33], since morphogenesis of *C. albicans* from yeast to hypha is one of the virulence factors promoting its pathogenicity [1,34]. This suggests that differences in growth temperature can influence yeast cell surface hydrophobicity [35,36], since *C. albicans* cells grown at 25ºC and 37ºC were hydrophobic and hydrophilic, respectively [36,37]. Moreover, *C. albicans* yeast cells can synthesize heat-shock proteins at elevated temperatures, with 45ºC optimum temperature for the heat-shock response [38].

In the present study, during 1.5 h to 6 h, the yeast count increased linearly, however, the increase shifted exponentially beyond 6 h (Figs. 1-3). The peak of mycelium production appeared between 1.5 h and 6 h after inoculation of *C. albicans* culture [11]. The mycelia of the

Table 2. Parameter estimates of the different kinetic models describing growth of *Candida albicans* under varying conditions

Kinetic model	Parameter	Medium[+]			Temperature[#]			pH*		
		HSM	RPMI-1640	MSGB	34ºC	37ºC	40ºC	5.4	6.4	7.4
Zero-order	k_0	0.5397	1.1706	1.0159	0.7222	0.4167	0.3810	1.1556	0.9357	1.1310
	P_o	-0.5179	-2.3393	-1.2321	-0.6250	0.5000	0.6786	-0.9250	-1.7536	-1.7964
	R^2	0.9370	0.7087	0.9716	0.9213	0.8883	0.9458	0.9059	0.8412	0.9043
	SSE	1.8512	53.2173	2.8512	4.2083	2.0625	0.7857	13.1083	15.6182	12.7882
	p	8.01e-5	8.75e-3	7.24e-6	1.57e-4	4.55e-4	5.08e-5	2.70e-4	1.33e-3	2.84e-4
First-order	k_1	0.1914	0.2177	0.2316	0.1965	0.1282	0.1246	0.1903	0.2146	0.2180
	P_o	0.6972	0.9392	0.8892	0.9123	1.2601	1.2778	1.5488	0.8109	1.0280
	R^2	0.9704	0.9078	0.9326	0.9516	0.9592	0.9535	0.9307	0.9845	0.9838
	SSE	0.0199	0.0857	0.0691	0.0350	0.0125	0.0135	0.0481	0.0129	0.0139
	p	8.16e-6	2.54e-4	9.84e-5	3.61e-5	2.16e-5	3.20e-5	1.07e-4	1.16e-6	1.33e-6
Second-order	k_2	0.0868	0.0666	2.0757	0.0735	0.0450	0.0451	0.0438	0.0680	0.0592
	P_o	0.9298	1.2823	1.1807	1.1486	1.4878	1.4798	1.9081	1.2047	1.4406
	R^2	0.8774	0.7022	0.77111	0.7818	0.9291	0.9210	0.7053	0.9481	0.8530
	SSE	0.0993	0.1780	0.1608	0.1426	0.0146	0.0165	0.0758	0.0239	0.0570
	p	6.04e-4	9.38e-3	4.12e-3	3.56e-3	1.15e-4	1.60e-4	9.08e-3	4.45e-5	1.05e-3

k_0 (count/ml/h): zero-order rate constant; k_1 (count/ml/h): first-order rate constant; k_2 (ml/count/h): second-order rate constant;
P_0 (count/ml): initial yeast population; R^2: coefficient of determination; SSE: sum of squares of the error; p: p-value;
HSM: Horse serum medium; MSGB: modified Sabouraud glucose broth;
[+]Horse serum medium (pH 6.8), RPMI-1640 (pH 7.4), and MSGB (pH 7.4) incubated at 37°C for 12 h;
*[#]MSGB medium with pH 7.4; *MSGB medium incubated at 37°C*

dimorphic fungus *C. albicans* in serum-containing medium, early colony formation displayed unusual characteristics of linear germ tube extension with prolonged delay between septation and the onset of branch formation [39]. The lag phases of *C. albicans* hyphae development in serum, methionine assay medium, modified Sabouraud broth, Eagle's minimal essential medium, amino acids salts medium, and buffered *N*-acetylglucosamine were never more than 1 h with *C. albicans* hyphae extending at a linear rate and with parent yeast cells having constant volume throughout the first 3 h of growth [15]. Despite of the variations in the physicochemical characteristics and biochemical compositions of the environmental conditions influencing the morphogenesis of *C. albicans*, the organism can sense its surroundings, adapt to evolving microenvironments for survival, and eventually still cause opportunistic infections [4].

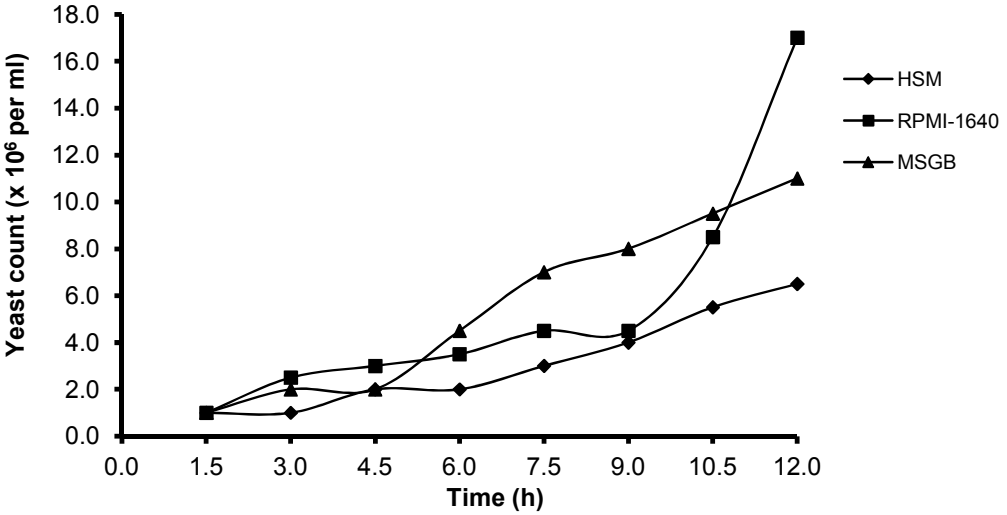

Fig. 1. Population kinetic profile of *Candida albicans* incubated at 37°C for 12 h under different growth media (HSM: Horse serum medium; MSGB: modified Sabouraud glucose broth)

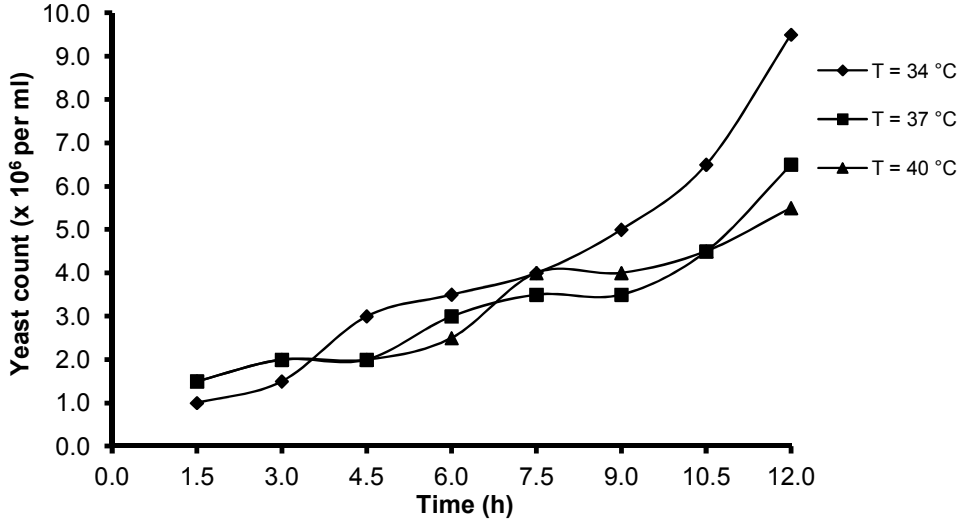

Fig. 2. Population kinetic profile of *Candida albicans* inoculated in modified Sabouraud glucose broth medium under pH 7.4 incubated for 12 h at different temperature values

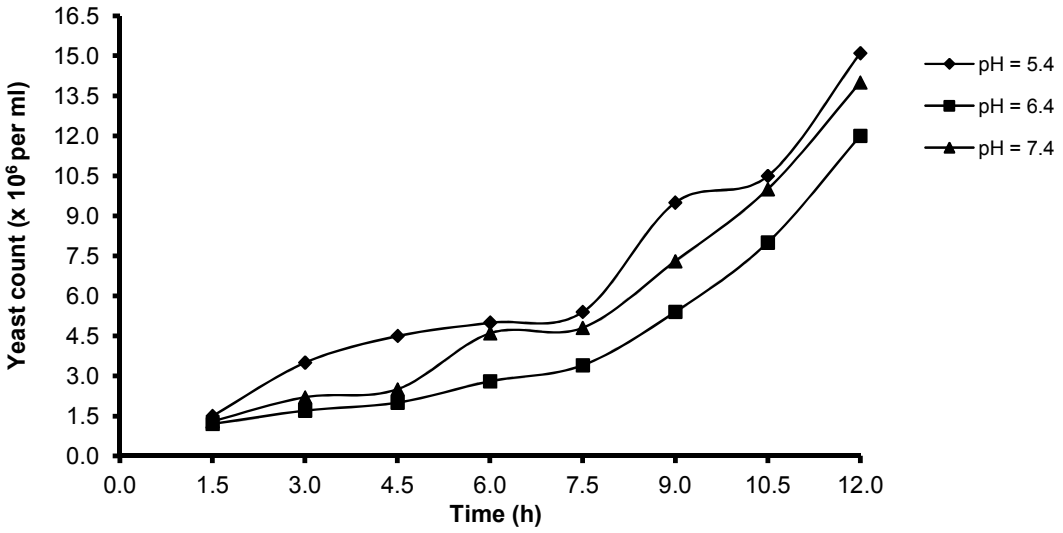

Fig. 3. Population kinetic profile of *Candida albicans* inoculated in modified Sabouraud glucose broth medium incubated at 37ºC for 12 h under different pH values

4. CONCLUSION

The growth kinetic profile of *Candida albicans* under different culture media, temperature values, and pH levels obeyed the first-order rate equation with relatively faster growth rate in modified Sabouraud glucose broth medium, pH 7.4, and 37°C incubation temperature. Between 1.5 h and 6 h after inoculation, the growth profile of *C. albicans* culture exhibited a linear pattern which coincides with the mycelium production, and subsequently shifted to an exponential increase beyond 6 h. These quantitative descriptions of the rate kinetic profile of *C. albicans* population offer an objective criterion in comparing population dynamics of organisms under environmental conditions with varying physicochemical characteristics and biochemical compositions.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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