JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2000, p. 902–904 Vol. 38, No. 2
0095-1137/00/$04.00+0

Growth Competition between *Candida dubliniensis* and *Candida albicans* under Broth and Biofilm Growing Conditions

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Received 17 August 1999/Returned for modification 28 September 1999/Accepted 15 November 1999

Seven isolates each of *Candida albicans* and *Candida dubliniensis* were paired (11 pairs) and examined for competitive interaction. Equal numbers of CFU of each competitor were inoculated into Sabouraud dextrose broth and incubated at 37°C with vigorous shaking under conditions favorable to either broth or biofilm growth. Surviving proportions of each competitor were calculated from the broth culture at 24 and 96 h and the biofilm culture at 96 h, with species differentiation done on CHROMagar Candida medium. *C. albicans* had a competitive advantage over *C. dubliniensis* in broth culture and under biofilm growing conditions; however, with the presence of a supporting structure for biofilm formation, *C. dubliniensis* was able to better withstand the competitive pressures from *C. albicans*.

Candida dubliniensis is an opportunistic yeast with remarkable phenotypic similarity to *Candida albicans* (13, 16). *C. dubliniensis* has been implicated in oropharyngeal candidiasis (OPC) in human immunodeficiency virus (HIV)-infected individuals and has been recovered from the oral cavities of HIV-negative individuals (13, 27, 28). While *C. albicans* is generally considered to be the most pathogenic of the *Candida* species, a variety of other members of this genus, notably *C. krusei*, *C. glabrata*, *C. tropicalis*, and *C. dubliniensis*, have been cited as the causative agents of an increasing number of infections (10, 16, 26). Causes for this recent epidemiological deviation toward less pathogenic yeasts in oral infections remain unclear (29). Some evidence suggests that the increased use of fluconazole or other antifungal agents may be responsible for the emergence of yeasts with decreased antifungal susceptibility, and this may, in turn, alter the epidemiology of OPC (16, 17). A number of other risk factors, such as the use of broad-spectrum antibacterial antibiotics, surgical procedures, and immunosuppression related to organ transplant, have also been implicated in this epidemiological shift (1).

Oropharyngeal candidiasis is a notable cause of morbidity among HIV-infected and AIDS patients (24). Other forms of mucosal candidiasis are frequently found in other patient groups, such as denture wearers, infants, the elderly, and recent recipients of antibiotic therapy (6). One of the first steps in the development of infection is the adherence of yeasts to host tissues or prosthetic devices (20, 25). Several *Candida* species, including *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis*, have demonstrated the potential to colonize plastic surfaces (19, 20, 25, 30). Factors known to have an effect on the retention or distribution of oral *Candida* species are many. Acidic pH, sucrose- or glucose-rich diets, and *Candida* cell surface mannoprotein and cell surface hydrophobicity all tend to enhance adhesion (14, 15, 30), yet the complex subject of candidal adhesion and colonization remains poorly understood (5, 19, 30).

The mouth is diverse as a microbial habitat. It contains soft mucosal tissues as well as hard tissues (teeth) and junctures between them; each of these, being warm, moist, and bathed in nutrients, provides an environment suitable for the proliferation of mixed-species colonization (9, 15). A variety of factors, such as changes in pH, nutrient availability, and temperature, may lead to changes in the microbial balance (4). In bacteria, one means of survival available to many oral species is the ability to form biofilms, which confers, in some measure, resistance to antibiotics and host defenses (1, 9). In this regard, adherent (sessile) cells may display properties different from those of their free-living (planktonic) counterparts (7, 11, 12). In nature, most microorganisms do not exist as pure cultures of free-living cells but are associated with surfaces and multispecies consortia (7). In suspension cultures of bacteria, where the constituent microorganisms all experience exposure to a common environment, a single phenotype tends to predominate (9). In biofilms, the situation is more diverse. The complex structure of the biofilm allows stratification into spatially organized populations of mixed-species communities, where some degree of interspecies cooperation develops (4, 7, 9).

While previous studies of biofilm development and species interaction have focused largely on bacterial species, the present experiments highlight interactions between two similar yeasts, *C. dubliniensis* and *C. albicans*. Competition growth assays were performed using several pairs of these two yeasts, grown under conditions favoring the growth of either free-living cells or biofilms.

Sabouraud liquid broth modified antibiotic medium 13 (SDB) (BBL, Cockeysville, Md.) was prepared from a powdered medium according to the manufacturer's instructions. The solution was dissolved, dispensed in 4-ml volumes into 18- by 150-mm culture tubes, and autoclaved. An additional set of tubes was prepared as described above, but each contained a single 1-cm piece of polyvinyl chloride (PVC) "feeding tube and urethral catheter" (Monoject, St. Louis, Mo.). The tubes of medium were cooled, stored at 4°C, and used within 1 week.

Clinical samples were obtained from HIV-infected patients enrolled in a longitudinal study of OPC at the University of Texas Health Science Center at San Antonio and the South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio (23, 24). These patients had advanced AIDS with mean CD4 cell counts of $<50/mm^3$. Samples were obtained weekly during therapy and quarterly as surveillance

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TABLE 1. Yeast isolate pairings and retention of *C. dubliniensis* after 24 and 96 h of growth competition

Yeast isolate pairing (MIC[a])		Avg % *C. dubliniensis* at:		
C. albicans	*C. dubliniensis*	24 h (broth)	96 h (broth)	96 h (catheter)
412 (0.5)	903 (0.25)	25	0	0
1002 (0.25)	1680 (0.25)	8	0	52
1649 (0.25)	1770 (0.25)	32	42	11
1490 (0.5)	2696 (0.5)	0	0	1
580 (4)	1154 (4)	9	0	0
2307 (>64)	2419 (64)	24	0	0
2823 (>64)	4712 (64)	0	0	0
1490 (0.5)	2419 (64)	12	0	0
1649 (0.25)	4712 (64)	16	37	29
2307 (>64)	1770 (0.25)	35	0	2
2823 (>64)	2696 (0.5)	8	0	0

[a] 48-h broth macrodilution fluconazole MIC.

cultures by having patients swish and spit 10 ml of normal saline to be used for culturing (21, 24). One hundred microliters of the swish solution was plated on media with and without fluconazole at concentrations of 8 and 16 μg/ml and incubated at 30°C for 48 h before growth was assessed. CHROMagar Candida (CHROMagar Company, Paris, France) with fluconazole was used to improve detection of non-*C. albicans* species and resistant isolates (21). Growth assessment was recorded and three to five yeast colonies from each culture were stored on Sabouraud dextrose slants (BBL) at −70°C and in sterile deionized H_2O at room temperature.

Clinical samples were submitted to the Fungus Testing Lab (the University of Texas Health Science Center) for MIC determination by both the National Committee for Clinical Laboratory Standards broth macrodilution procedure (18) and broth microdilution adaptation (2, 8, 18).

Competition growth assays were performed in duplicate with pairs of clinical isolates of *C. albicans* and *C. dubliniensis*. Seven clinical samples each of *C. albicans* and *C. dubliniensis* were selected to represent both fluconazole susceptibility and resistance and have previously been described in more detail (13, 14). Yeast isolates, stored at room temperature in sterile deionized H_2O, were subcultured onto Sabouraud dextrose agar to ensure purity and viability (3) and then subcultured again to select for isolated colonies. Each competitor of the pair was inoculated at 5×10^5 CFU (total of 10^6 CFU) into 4 ml of SDB alone and into 4 ml of SDB containing a single 1-cm piece of PVC feeding tube and urethral catheter and incubated at 37°C with vigorous shaking. Daily transfers of 100 μl of resultant broth culture or gently washed catheter section into fresh medium were performed. Surviving proportions of each competitor were calculated from the broth culture at 24 and 96 h with species differentiation done on CHROMagar Candida medium. Proportions of each competitor were obtained from the piece of PVC at 96 h, after the PVC piece had been washed and then vigorously vortexed and sonicated for 10 min at 26°C in 4 ml of PBS using a Branson model 2510 sonicator (Branson Ultrasonics Corporation, Danbury, Conn.), the water having been previously degassed in the bath for 5 min.

Two sets of experiments were performed, each in duplicate, differing in growth conditions by the presence or absence of a piece of PVC catheter and means of subsequent inoculation. As shown in Table 1, at 24 h, in broth culture, both *C. albicans* and *C. dubliniensis* were detected in 9 of 11 (82%) (range of *C. dubliniensis* detected, 8 to 35%) of the cultures and *C. albicans* alone was detected in 2 of 11 (18%). At 96 h, in broth culture, both species were detected in only 2 of 11 (18%) (range of *C. dubliniensis* detected, 37 to 42%) of the cultures and *C. albicans* alone was detected in 9 of 11 (82%). In both instances in which mixed culture was maintained, the same *C. albicans* isolate (1649) was used in the competition pairing. At 96 h, both species were detected in 5 of 11 (45%) (range of *C. dubliniensis* detected, 1 to 52%) of the cultures from the PVC pieces, and *C. albicans* alone was detected in 6 of 11 (55%).

Log-phase doubling times were determined for each isolate of *C. albicans* and *C. dubliniensis* used in the growth assays. Yeast isolates were purified and subcultured as described above. Three to five colonies were then suspended in 5.0 ml of sterile deionized H_2O and mixed thoroughly on a vortex mixer. The suspension was adjusted to a 0.5 McFarland turbidity standard (10^6 CFU/ml) using a spectrophotometer (22). Each isolate was inoculated at 10^6 CFU into 4 ml of SDB and incubated at 37°C with vigorous shaking. Absorbance readings (600 nm) were obtained at 2-h intervals for 8 h. Growth curves were generated using CA-CricketGraph III (Computer Associates, Islandia, N.Y.). Doubling times were obtained from portions of the curves corresponding to the logarithmic growth phase of each yeast.

Log-phase cell doubling times (± standard errors) for pure cultures of *C. albicans* averaged 2.0 ± 0.11 h (range, 1.7 to 2.6 h), while those for *C. dubliniensis* were 2.7 ± 0.16 h (range, 2.2 to 3.3 h) ($P < 0.005$). *C. dubliniensis* had a higher log-phase doubling rate in only one pair of isolates examined, isolate 1770 (*C. dubliniensis*) and 1649 (*C. albicans*). In all other combinations, the *C. albicans* isolate had the higher doubling rate.

Results of the planktonic growth competition between the two species in SDB showed that *C. albicans* had a competitive advantage, unrelated to MIC, over *C. dubliniensis* was undetectable in two sets of assays, while in the other nine sets of tubes, *C. dubliniensis* ranged from 8 to 35% of broth composition. At 96 h, the results were even more dramatic, with only 2 of 11 sets showing retention of *C. dubliniensis*. *C. albicans* had a higher log-phase cell doubling rate than did *C. dubliniensis* in 10 of 11 pairings. With the exception of one pairing involving *C. albicans* 1649, at 96 h, the slower-growing isolate was overwhelmed by the faster-growing isolate, as might be expected. These results support observations from bacterial studies that microorganisms in a well-mixed planktonic culture tend to display a single phenotype (9); thus, competitive pressures within suspension cultures may drive the development of a monoculture, allowing the retention of low levels of competitors. Under such uniform conditions, all cells have equal access to nutrients, dispersion of metabolic waste, temperature, and pH; therefore, those phenotypes or organisms with superior growth rates would be expected to dominate over time, as seen here at both 24 and 96 h.

Growth competition between the two species under conditions supportive of biofilm growth revealed that *C. albicans* again had a competitive advantage over *C. dubliniensis* at 96 h, apparently unrelated to MIC. *C. dubliniensis* was undetectable in 6 of 11 sets of assays yet was present at a concentration of 1 to 52% in the other 5 sets. Population variations due to differences in growth rate were less apparent under the biofilm growing conditions. Growth within a biofilm community tends to vary according to cellular spatial organization. Organisms near the nutrient source exhibit a different phenotype from that of organisms deep within the biofilm substratum (9). Such heterogeneity within the community may induce metabolic cooperation between its members (7), enhancing the survival of the biofilm and thereby encouraging the persistence of yeasts that, under planktonic conditions, might succumb to competitive pressure.

Little is known about interspecies interactions between *C. albicans* and *C. dubliniensis*, yet these species have been isolated concomitantly from the oral cavities of immunocompromised patients. In this series of experiments, equal numbers of CFU of *C. albicans* and *C. dubliniensis* were coinoculated into SDB and incubated under similar growing conditions, differing chiefly by the presence of a PVC substrate. Results of these growth competition studies showed that *C. albicans* had a distinct competitive advantage over *C. dubliniensis*. Under planktonic growth conditions, *C. albicans* demonstrated superior growth characteristics over *C. dubliniensis*; however, under biofilm growing conditions, with supporting structure afforded by PVC, *C. dubliniensis* was able to better withstand the rigorous competitive pressures from *C. albicans*.

This work was supported by a grant from Pfizer Inc. and by Public Health Service grants 1R29 AI42401 (to J.L.L.-R.), 5 RO1 DE11381 (to T.F.P.), and M01-RR-01346 (for the Frederic C. Bartter General Clinical Research Center).

Chromogenic medium was provided by CHROMagar Company. We thank the Fungus Testing Laboratory at UTHSCSA for performing antifungal susceptibility testing.

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