Antimicrobial Agents and Chemotherapy, May 2008, p. 1859–1861 Vol. 52, No. 5
0066-4804/08/$08.00+0 doi:10.1128/AAC.01646-07
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Secretion of *E*,*E*-Farnesol and Biofilm Formation in Eight Different *Candida* Species[▽]

K. Weber,[1] R. Sohr,[2] B. Schulz,[1] M. Fleischhacker,[1] and M. Ruhnke[1]*

Division of Oncology and Hematology, Department of Medicine,[1] and Institute of Pharmacology,[2] Charité—Universitätsmedizin Berlin, Berlin, Germany

Received 21 December 2007/Returned for modification 20 January 2008/Accepted 2 March 2008

Production of *E*,*E*-farnesol (FOH) and biofilm formation were studied under various conditions in 56 strains of eight *Candida* spp. FOH production differed significantly not only between *Candida* spp. but within *Candida albicans* strains as well. FOH concentrations and biofilm formation were the highest for *C albicans*.

Candida albicans is a major human fungal pathogen, causing both superficial and invasive tissue infections. The ability of *C. albicans* to form biofilms on medical devices has a profound impact on its capacity to cause human disease. *C. albicans* and other members of the genus *Candida* are able to grow in different forms as budding yeast, pseudohyphae, and true hyphae, which is called dimorphism (1, 8). This transition from yeast to hyphal growth can be induced by various conditions (2, 25). Progression to a mature biofilm is dependent on cell adhesion, extracellular matrix production, and the yeast-to-hypha transition in *C. albicans* (2, 3). However, biofilm development in non-*C. albicans Candida* spp. (NCAC) is not well understood.

Suppression of biofilm formation in *C. albicans* may be achieved by quorum-sensing molecules (5). *E*,*E*-Farnesol (FOH) has been reported to inhibit the induction of hyphal growth and biofilm formation in *C. albicans* (10, 11). In this study, FOH secretion by *C. albicans* and eight NCAC was examined under various culture conditions. In addition, the development of biofilms was studied. Finally, the correlation between FOH secretion and biofilm formation was analyzed for all of the isolates studied.

We studied 56 strains of eight *Candida* species (Table 1). All isolates were cultivated in RPMI 1640 medium with or without the addition of 10% fetal calf serum (FCS) at 37°C for 24 h under continuous rotation at 125 rpm. Two milliliters of sterile filtered (0.45 μm) culture supernatant was extracted with 5 ml *n*-hexane–ethanol (90:10, vol/vol) and derivatized with 9-anthroylnitrile as previously described (13). Quantification was done with *n*-butanol as an internal standard (50 ng added to each sample). Reverse-phase high-performance liquid chromatography was done with a YMC Hydrosphere C_{18} column (5 μm, 150 by 2.1 mm [inside diameter]). A linear gradient of acetonitrile-water (85% to 100% over 20 min) was used as the mobile phase. Standard concentrations ranged from 0.004 μM to 40 μM FOH. Detection, determination of recovery, and calculations were performed as previously described (4, 13). The development of biofilms by all of the *Candida* spp. was studied according to Krom et al. (6), with minor differences such as using 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt instead of 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide. In addition to visual reading at 450 nm, optical density was measured at 405 nm for data validation (15). All tests were done twice and analyzed with the two-tailed paired Student *t* test comparing 0 and 10% FCS cultivations (significance was set at a *P* value of ≤0.05).

The quantification and standardization of FOH showed good linearity with $R^2 = 0.99$. The recovery rate was determined as approximately 95%. The FOH concentrations for all 56 *Candida* strains are shown in Table 1. Under FCS-free conditions, the highest concentrations of FOH were measured for *C. albicans* isolates, with a mean of 35.6 μM (range, 13.7 to 58.5 μM). The quantity was up to 35 times higher than for NCAC, except for *C. dubliniensis*. The mean FOH concentration for *C. dubliniensis* was 8.3 μM (range, 6.0 to 17.5 μM). Individual *C. albicans* isolates varied remarkably in their ability to produce FOH. FOH concentrations were almost four times as high as for *C. dubliniensis*. All other NCAC showed relatively low concentrations of FOH (mean, 0.6 ± 0.2 μM), independently of whether the *Candida* strains were cultivated with or without supplementation with 10% FCS.

Significant decreases in the secretion of FOH were observed for *C. albicans* ($P = 0.001$), *C. dubliniensis* ($P = 0.0007$), and *C. guilliermondii* ($P = 0.01$) under FCS-supplemented culture conditions. The concentration of FOH for *C. albicans* decreased to 2.0 ± 0.9 μM (mean). The comparison of the FCS-free and 10% FCS cultivations indicated a significant disparity ($P = 0.003$).

The investigation of biofilm formation showed comparable results for both techniques (Fig. 1). An optical density cutoff of ≥0.2 (>35% transmission blocked) was used to discriminate biofilm producers according to recent suggestions (14, 15). With both media, *C. albicans* and *C. dubliniensis* formed good biofilms. *C. dubliniensis* isolates produced 48% more biofilm under FCS-free conditions than did *C. albicans* (no statistically significant difference). Addition of 10% FCS to the growth medium induced significantly better biofilm formation in five of the eight *Candida* species tested (Fig. 1; $P < 0.05$). With *C. albicans*, increased biofilm formation (by 76%) was observed with 10% FCS in the medium. Increased biofilm formation was

* Corresponding author. Mailing address: Charité—Universitätsmedizin Berlin, Department of Medicine, Division of Oncology and Hematology, Charitéplatz 1, 10117 Berlin, Germany. Phone: 49 (30) 450 51 30 36. Fax: 49 (30) 450 51 39 37. E-mail: markus.ruhnke@charite.de.
▽ Published ahead of print on 10 March 2008.

TABLE 1. *Candida* spp. used in this study and concentrations of secreted FOH

Strain[a]	FOH concn [μM]		Strain[a]	FOH concn [μM]	
	0% FCS	10% FCS		0% FCS	10% FCS
C. albicans strains			*C. kefyr* strains		
ATCC 90028	13.7	1.7	Y0601[b]	0.8	0.7
ATCC 24433	32.0	2.4	RKI 01-0709	0.7	0.1
ATCC 36801	34.6	0.5	RKI 95-0329.02	0.2	1.1
ATCC 44373	16.9	0.9	RKI 95-1915.02	0.5	2.8
ATCC 44374	51.2	3.0	RKI 95-2066	0.1	2.6
ATCC 76615	58.5	2.4	RKI 97-0656	0.2	5.6
Y0119[b]	42.2	2.3	RKI 97-0768.02	0.5	0.2
Mean ± SD	35.6 ± 16.5	2.0 ± 0.9	Mean ± SD	0.5 ± 0.3	1.9 ± 1.8
C. glabrata strains			*C. krusei* strains		
ATCC 90030	0.8	0.5	MB16[b]	0.5	0.4
Y3390[b]	0.5	0.4	ATCC 6258	0.7	0.8
RKI 04-0388	0.2	0.2	ATCC 90878	0.8	0.6
RKI 05-0284.01	0.4	0.3	RKI 03-450.02	0.5	2.5
RKI 05-0445.01	0.1	0.2	RKI 04-0167.04	0.5	0.9
RKI 05-0559.02	0.4	0.1	RKI 05-0126	0.6	0.9
RKI 06-0367	0.3	0.2	RKI 06-0365	0.6	0.6
Mean ± SD	0.4 ± 0.2	0.3 ± 0.1	Mean ± SD	0.6 ± 0.1	1.0 ± 0.6
C. parapsilosis strains			*C. guilliermondii* strains		
Y0501[b]	0.3	0.4	ATCC 90877	1.3	0.7
ATCC 90018	1.2	0.8	RKI 01-0546	0.8	0.6
ATCC 22019	0.8	0.9	RKI 02-0043	0.4	0.3
RKI 00-0438	0.5	0.4	RKI 04-0347	0.9	0.6
RKI 02-0579	0.5	0.5	RKI 05-0091	0.4	0.4
RKI 04-0241	0.4	0.6	RKI 95-1889	0.5	0.3
RKI 06-0220	0.2	0.1	RKI 95-2865	0.7	0.1
Mean ± SD	0.6 ± 0.3	0.6 ± 0.3	Mean ± SD	0.8 ± 0.3	0.5 ± 0.2
C. tropicalis strains			*C. dubliniensis* strains		
ATCC 750	1.2	0.4	CBS 8500	17.5	2.1
ATCC 90874	2.2	0.4	CBS 8501	7.8	2.9
RKI 03-0108	0.3	0.1	RKI 01-0170.01	6.0	0.1
RKI 04-0326	0.4	0.8	RKI 01-0265	12.5	2.5
RKI 05-559.01	0.6	0.6	RKI 01-0268.02	10.2	1.7
RKI 98-0463	0.5	0.4	RKI 05-0037	6.7	0.4
RKI 99-0499.02	1.2	0.4	RKI 06-0019	9.2	1.4
Mean ± SD	1.0 ± 0.7	0.5 ± 0.02	Mean ± SD	8.7 ± 3.8	1.5 ± 1.0

[a] Shown are the strains used and the concentrations of FOH secreted by them when they were cultivated in RPMI 1640 medium with 10% FCS and without FCS ($n = 2$; agreement of ≤25%) and the mean ± SD of each *Candida* species, respectively. Strains were obtained from the Centraal Bureau voor Schimmelcultures (CBS), Baarn, The Netherlands; the American Type Culture Collection (ATCC), Manassas, VA; and the Robert Koch Institute (RKI), Berlin, Germany.

[b] *C. albicans* Y0119, *C. glabrata* Y3390, *C. parapsilosis* Y0501, *C. kefyr* Y0601, and *C. krusei* MB16 were supplied by Pfizer Laboratories, Illertissen, Germany.

also obtained for *C. dubliniensis* (34%), *C. tropicalis* (34%), and *C. krusei* (61%).

Quorum-sensing molecules, and FOH in particular, are considered to play an important role in the development of biofilms by *C. albicans* on the surfaces of medical devices (10–12). In this study, we found that *C. dubliniensis* can produce significant amounts of FOH but still less than *C. albicans*. In contrast, biofilm formation was more pronounced in *C. dubliniensis* than in any of the other *Candida* spp. tested. It is unclear whether this is related to the phylogenetic relationship between the two species or to other conditions (7). *C. dubliniensis* is a rare human pathogen and causes much less common invasive *Candida* infections in humans than do *C. albicans* and other NCAC. Furthermore, we have observed that several other NCAC do not produce any notable FOH but may form a biofilm (e.g., *C. tropicalis*, *C. parapsilosis*). The mechanism of reduced FOH production after supplementation with 10% FCS is unclear, but it is known that culture conditions may affect the global transcriptional response of *Candida* spp. (*C. albicans*) (9). It is assumed that the ability to form a biofilm is linked to FOH secretion as the major quorum-sensing molecule in *C. albicans* and *C. dubliniensis*, as shown in this study. We found that other *Candida* spp. can form biofilms without high levels of FOH, and it may be concluded that these NCAC

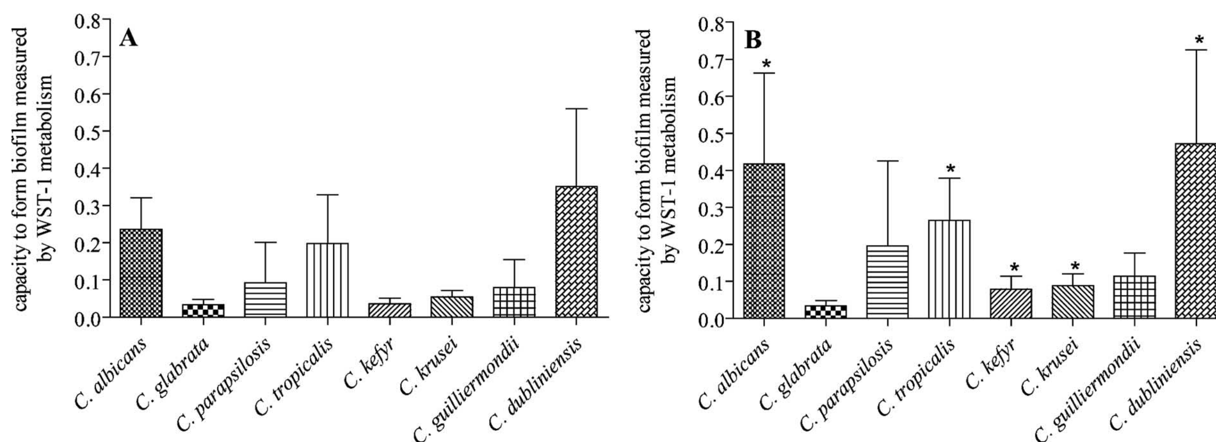

FIG. 1. Biofilm formation by *Candida* species. Fifty-six *Candida* spp. were incubated for 24 h in RPMI 1640 medium containing 0% (A) or 10% (B) FCS. Biofilm formation was measured as described in the text. The average for all of the isolates of a species in Table 1 is shown as the result of 14 values calculated for each column. *, Significantly increased biofilm ($P < 0.05$) when cultured in medium with 10% FCS. WST-1, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt.

use other pathways or quorum-sensing molecules for biofilm formation without FOH secretion.

Biofilm formation by NCAC is not well understood and requires further exploration with other models (e.g., gene regulation, detection of regulatory pathways).

REFERENCES

1. **Calderone, R. A.** 2002. Taxonomy and biology of *Candida*, p. 15–28. *In* R. A. Calderone (ed.), *Candida* and candidiasis. ASM Press, Washington, DC.
2. **Calderone, R. A., and W. A. Fonzi.** 2001. Virulence factors of *Candida albicans*. Trends Microbiol. **9:**327–335.
3. **Douglas, L. J.** 2003. *Candida* biofilms and their role in infection. Trends Microbiol. **11:**30–36.
4. **Glowka, F. K., M. Karazniewicz, and E. Lipnicka.** 2006. RP-HPLC method with fluorescence detection for determination of small quantities of triamcinolone in plasma in presence of endogenous steroids after derivatization with 9-anthroyl nitrile; pharmacokinetic studies. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **839:**54–61.
5. **Hornby, J. M., and E. C. Jensen.** 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl. Environ. Microbiol. **67:**2982–2992.
6. **Krom, B. P., J. B. Cohen, G. E. Elhaney Feser, and R. L. Cihlar.** 2007. Optimized candidal biofilm microtiter assay. J. Microbiol. Methods **68:**421–423.
7. **Mähnss, B., F. Stehr, W. Schafer, and K. Neuber.** 2005. Comparison of standard phenotypic assays with a PCR method to discriminate Candida albicans and C. dubliniensis. Mycoses **48:**55–61.
8. **Mitchell, A. P.** 1998. Dimorphism and virulence in *Candida albicans*. Curr. Opin. Microbiol. **1:**687–692.
9. **Murillo, L. A., G. Newport, C. Y. Lan, S. Habelitz, J. Dungan, and N. M. Agabian.** 2005. Genome-wide transcription profiling of the early phase of biofilm formation by *Candida albicans*. Eukaryot. Cell **4:**1562–1573.
10. **Nickerson, K. W., A. L. Atkin, and J. M. Hornby.** 2006. Quorum sensing in dimorphic fungi: farnesol and beyond. Appl. Environ. Microbiol. **72:**3805–3813.
11. **Ramage, G., S. P. Saville, B. L. Wickes, and J. L. Lopez-Ribot.** 2002. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl. Environ. Microbiol. **68:**5459–5463.
12. **Ramage, G., K. VandeWalle, B. L. Wickes, and J. L. López-Ribot.** 2001. Characteristics of biofilm formation by *Candida albicans*. Rev. Iberoam. Micol. **18:**163–170.
13. **Saisho, Y., A. Morimoto, and T. Umeda.** 1997. Determination of farnesyl pyrophosphate in dog and human plasma by high-performance liquid chromatography with fluorescence detection. Anal. Biochem. **252:**89–95.
14. **Shin, J. H., S. J. Kee, M. G. Shin, S. H. Kim, D. H. Shin, S. K. Lee, S. P. Suh, and D. W. Ryang.** 2002. Biofilm production by isolates of *Candida* species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. J. Clin. Microbiol. **40:**1244–1248.
15. **Tumbarello, M., B. Posteraro, E. M. Trecarichi, B. Fiori, M. Rossi, R. Porta, K. de Gaetano Donati, M. La Sorda, T. Spanu, G. Fadda, R. Cauda, and M. Sanguinetti.** 2007. Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J. Clin. Microbiol. **45:**1843–1850.