Mycoscience (2011) 52:356–360
DOI 10.1007/s10267-011-0110-y

NOTE

Acetaldehyde inhibits the yeast-to-hypha conversion and biofilm formation in *Candida albicans*

Nitin M. Chauhan · Jayant S. Raut · S. Mohan Karuppayil

Received: 3 April 2010 / Accepted: 3 February 2011 / Published online: 1 March 2011

Abstract In *Candida albicans*, alcohol metabolism is implicated in biofilm formation. The alcohol dehydrogenase gene (*ADH1*) is involved in the conversion of acetaldehyde to ethanol and reported to be downregulated during biofilm formation. *C. albicans* produces acetaldehyde under both in vivo and in vitro conditions. Mutations in *ADH* genes result in increased acetaldehyde production in vitro, but studies are lacking on the morphogenetic role(s) of acetaldehyde in *C. albicans*. We report here that acetaldehyde at a concentration of 7 mM was able to inhibit the conversion from yeast to hyphal forms induced by four standard inducers at 37°C. The hyphal inhibitory concentrations did not adversely affect the growth and viability of *C. albicans* cells. The same concentration of acetaldehyde also significantly inhibited biofilm development, and only adhered yeast cells were found. We hypothesize that acetaldehyde produced by *C. albicans* may exert a morphogenetic regulatory role influencing yeast-to-hypha conversion, biofilm formation, dissemination and establishment of infection.

Keywords Alcohol dehydrogenase · *Candida* biofilm · Dimorphism

N. M. Chauhan · J. S. Raut · S. M. Karuppayil (✉)
School of Life Sciences, SRTM University,
Nanded 431-606, MS, India
e-mail: karuppayil@yahoo.com

N. M. Chauhan
e-mail: nitinchauhan84@yahoomail.com

J. S. Raut
e-mail: rautjayant@yahoo.com

Yeast–hypha morphogenesis is considered to be a virulence factor in the human fungal pathogen *Candida albicans* (Cutler 1991; Odds 1994; Gow 1997). Hyphal mutants of *C. albicans* are reported to be avirulent in animal models (Lo et al. 1997). Various metabolites produced by yeasts, such as *Saccharomyces cerevisiae* and *C. albicans*, in planktonic as well as biofilm cells are known to influence morphogenesis (Hogan 2006; Nickerson et al. 2006; Martins et al. 2007; Dickinson 2008). Interestingly, the effect of such molecules, including farnesol, tryptophol, phenyl ethyl alcohol, tyrosol, isoamyl alcohol, among others, on morphogenesis are not the same in these yeasts, making comparisons hard (Chen and Fink 2006). The mechanisms of action of these molecules in *C. albicans* are of interest because of its clinical relevance.

The alcohol dehydrogenase gene (*ADH1*), which is involved in the conversion of acetaldehyde to ethanol in *C. albicans*, is downregulated during biofilm formation. Mukherjee et al. (2006) reported that deletion of *ADH1* results in enhanced biofilm formation, indicating the involvement of an ethanol-dependent mechanism during biofilm development. Preliminary studies carried out by our group revealed that ethyl alcohol could be a potential morphogenetic regulatory molecule in *C. albicans* (Chauhan N., unpublished). However, the roles of the various metabolites involved in ethanol metabolism in *C. albicans* have not yet been clearly defined. Acetaldehyde, the first metabolite of ethanol oxidation, is reported to be produced in the human body through the action of liver ADH (Jokelainen et al. 1994). The ADH of both bacteria and *C. albicans* also contributes to the production of acetaldehyde in the body (Tillonen et al. 1999; Poschl and Seitz 2004). Previous studies have shown that sulphur amino acid metabolism and the polyamine transporter gene are induced in *S. cerevisiae* cells exposed to acetaldehyde

(Aranda and del Olmo 2004). Walker-Caprioglio and Parks (1987) reported that treatment of *S. cerevisiae* with 0.01% of acetaldehyde mitigated growth inhibition by ethanol, and Tillonen et al. (1999) found a significantly higher rate of yeast colonization, mainly by *C. albicans*, in individuals with high acetaldehyde-containing saliva than those with saliva containing low levels of acetaldehyde. However, studies are lacking on the morphological roles of acetaldehyde in *C. albicans*. Here, we report on the effects of acetaldehyde on yeast-to-hypha morphogenesis and biofilm formation in two standard strains of *C. albicans*.

The two standard strains of *C. albicans* used in this study were ATCC 90028 (MTCC 3017) and ATCC 10231 (MTCC 227). Methodology for the growth and storage of *C. albicans* were as described by Devkatte et al. (2005). Prior to use, cells were washed three times in phosphate buffer saline (PBS) (10 mM phosphate buffer, 2.7 mM potassium chloride, and 137 mM sodium chloride, pH 7.4). The various inducers used for germ-tube formation were (1) 20% horse serum in deionized distilled water, (2) yeast nitrogen broth without amino acid but supplemented with 2.5 mM *N*-acetylglucosamine, (3) 10 mM proline prepared in PBS, (d) RPMI-1640 medium (Mosel et al. 2005).

Filamentation assays were performed using a microplate-based assay in 96-well microtiter plates (Hudson et al. 2004). Briefly, cell samples at a density of 1×10^6 cells/ml were inoculated into medium supplemented with an inducer. Various concentrations of acetaldehyde (Ranbaxy Fine Chemicals Limited, New Delhi, India), ranging from 0.875 to 14 mM, were added to the wells. The final volume of assay solution in each well was 200 µl. The plates were incubated at 37°C on an orbital shaker (200 rpm) for 2–3 h (2 h for serum and 3 h for *N*-acetylglucosamine, proline and RPMI-1640 medium). Following the incubation, the cells were observed microscopically and the percentage of germ-tube formation in each well calculated relative to that of control using the following formula: percentage of germ-tube formation = (number of germ tubes in treatment/number of germ tubes in control) × 100. All experiments were performed in triplicate, and differences were considered to be statistically significant at $P < 0.05$. Figures 1 and 2 show that acetaldehyde effectively prevented germ-tube formation induced by the four standard inducers in both strains of *C. albicans*. This inhibition of hyphal formation was concentration-dependent. At 7 mM acetaldehyde, there was complete inhibition of germ-tube formation in both strains in serum, RPMI-1640 medium, and proline, and an 85–90% inhibition of hyphal formation in *N*-acetylglucosamine treatment. At 3.5 mM of acetylglucosamine, a significant (around 50%) reduction of hyphal forms was observed (Figs. 1, 2).

Minimum inhibitory concentrations of acetaldehyde were determined using CLSI M27—a microbroth dilution

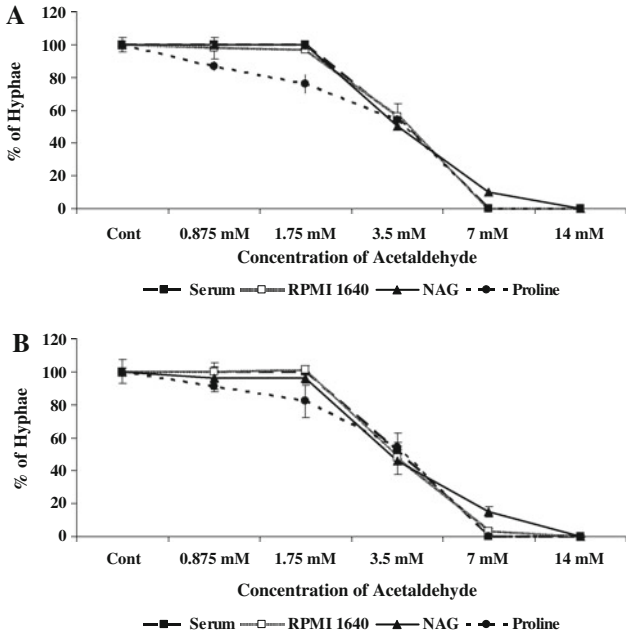

Fig. 1 Inhibition of hyphae formation by acetaldehyde in two strains of *Candida albicans.* Cells were incubated at 37°C for 2–3 h in the respective medium together with various concentrations of acetaldehyde, following which the morphology of the cell cultures was assessed. *Cont* Control (wells without acetaldehyde), *NAG N*-acetylglucosamine. **a** *C. albicans* ATCC 90028, **b** *C. albicans* 10231

method (Hazma et al. 2008). Briefly, various concentrations (range 0.875–14 mM) were added to RPMI 1640 medium in 96-well microtiter plates. Each well was inoculated with a cell density of 1×10^3 cells/ml, and the plates were incubated at 35°C on a shaker for various time intervals (2, 4, 6, 8, 12 and 24 h), following which absorbance was measured using a 96-well microplate reader (Multiscan-Ex; Thermo Fisher Scientific, Waltham, MA) at a wavelength of 620 nm. The viability assay consisted of a viability plate count (Westwater et al. 2005). Cells from the respective wells were diluted to obtain countable colonies, and an aliquot of each sample was spread on YPD agar plates. The plates were incubated at 30°C for 48 h and the colonies counted. The effect of acetaldehyde on the growth and viability of *C. albicans* was studied up to 14 mM of acetaldehyde. Growth, as determined by absorbance at 620 nm, was unaffected at 7 mM of acetaldehyde, which is a germ-tube inhibitory concentration. At 14 mM of acetaldehyde, 80–100% of growth was observed relative to that of the control. Table 1 shows the viability of *C. albicans* cells in the presence of acetaldehyde. The viability plate count revealed that both standard strains had a viability of around 70–80% at 7 mM of acetaldehyde; however, at 14 mM of acetaldehyde, viability fell to 50–60% of that of the control (i.e. without acetaldehyde) (Table 1).

To study the effect of acetaldehyde on biofilm development, we developed *Candida* biofilms using standard

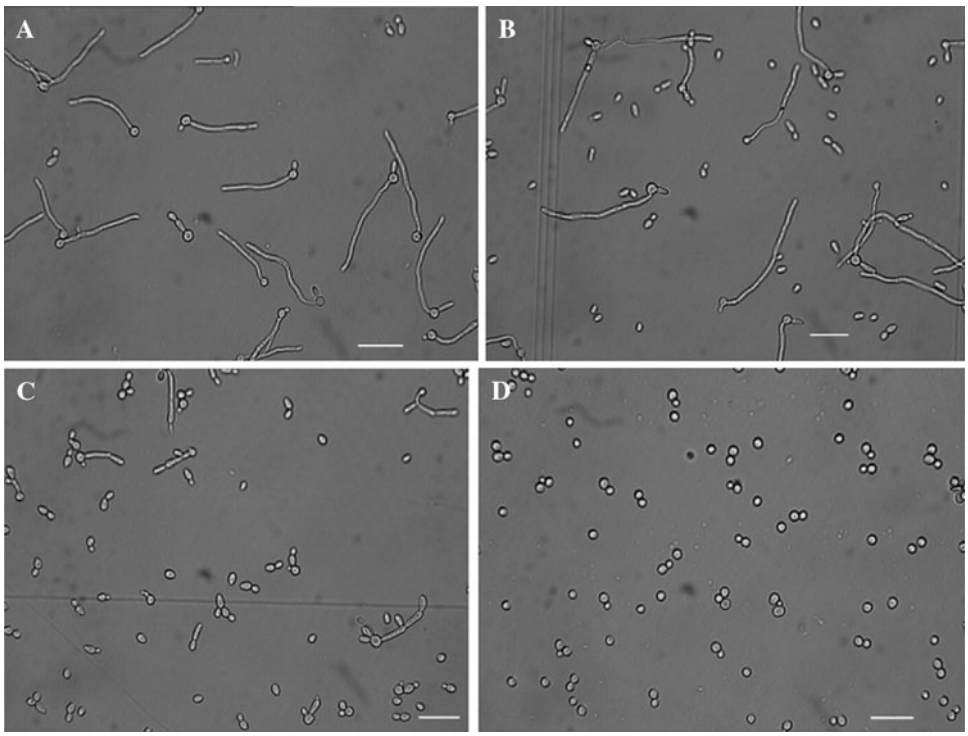

Fig. 2 Effect of acetaldehyde on RPMI-1640-induced germ-tube formation in *C. albicans* ATCC 90028. **a** Control (no acetaldehyde). **b–d** Germ-tube germination in acetaldelyde-supplemented RPMI-1640 medium: **b** 1.75 mM, **c** 3.5 mM, **d** 7 mM. Cells were incubated for 2 h at 37°C, and morphology was assessed by Labomed (Korntal, Germany) microphotography system. *Bar*: 50 μm (×400)

Table 1 Viability of *Candida albicans* cells in the presence of acetaldehyde

Concentrations of acetaldehyde (mM)	Percentage viability						Percentage viability					
	ATCC 90028						ATCC 10231					
	2 h	4 h	6 h	8 h	12 h	24 h	2 h	4 h	6 h	8 h	12 h	24 h
0.00	100 ± 5	100 ± 6	100 ± 2	100 ± 6	100 ± 12	100 ± 8	100 ± 1	100 ± 8	100 ± 4	100 ± 7	100 ± 8	100 ± 6
0.87	101 ± 8	91 ± 5	99 ± 2	94 ± 6	98 ± 5	95 ± 15	96 ± 2	95 ± 2	98 ± 7	100 ± 7	94 ± 12	92 ± 6
1.75	84 ± 1	81 ± 6	92 ± 2	98 ± 7	91 ± 6	86 ± 5	91 ± 5	84 ± 9	85 ± 5	92 ± 3	95 ± 12	87 ± 10
3.50	85 ± 5	80 ± 7	77 ± 8	86 ± 4	86 ± 15	85 ± 3	87 ± 1	83 ± 3	80 ± 5	82 ± 1	78 ± 11	77 ± 7
7.00	77 ± 2	80 ± 5	72 ± 2	76 ± 3	80 ± 6	74 ± 2	80 ± 5	80 ± 6	77 ± 9	71 ± 6	79 ± 6	78 ± 6
14.0	65 ± 3	51 ± 9	64 ± 5	50 ± 6	58 ± 8	62 ± 7	64 ± 8	62 ± 1	52 ± 7	53 ± 9	54 ± 6	53 ± 6

Values are given as the mean of triplicate viable counts ± standard deviation

Cells were incubated at 35°C at various time intervals on an orbital shaking incubator in RPMI-1640 medium containing various concentrations of acetaldehyde. Viability count was done after a 48-h incubation

protocols with minor modifications, i.e., a polystyrene surface was used in place of a silicone disc (Chandra et al. 2001). A cell suspension of 1×10^7 cells/ml was prepared in PBS, and 100-μl aliquots were inoculated into each well of a microtiter plate. The plate was incubated at 37°C at 50 rpm for 90 min for adhesion of the *Candida* cells to the polystyrene surface. After 90 min of incubation, non-adhered cells were removed by washing the wells with PBS. RPMI-1640 medium containing various concentrations of acetaldehyde (range 0.875–14 mM and one control (RPMI-1640 medium without acetaldehyde) were added to the microplate well. The plates were incubated at 37°C for 24 and 48 h, respectively, to allow biofilm formation. After biofilm formation, the wells were washed to remove any planktonic cells and the biofilms were observed under an inverted light microscope (Metzer, India). Biofilm quantification was performed using the crystal violet assay (Peeters et al. 2008). As shown in Fig. 3, acetaldehyde at 7 and 14 mM concentration, inhibited hyphal forms in biofilms formed by both strains of *C. albicans* included in this study and only adhered yeast cells were observed. In contrast, biofilms in the control consisted of both yeast and hyphal forms (Fig. 3). Analysis of cell biomass by the crystal violet assay showed that biofilm formation decreased with increasing concentrations of acetaldehyde in both *C. albicans* ATCC 90028 ($P = 0.005$) and ATCC 10231

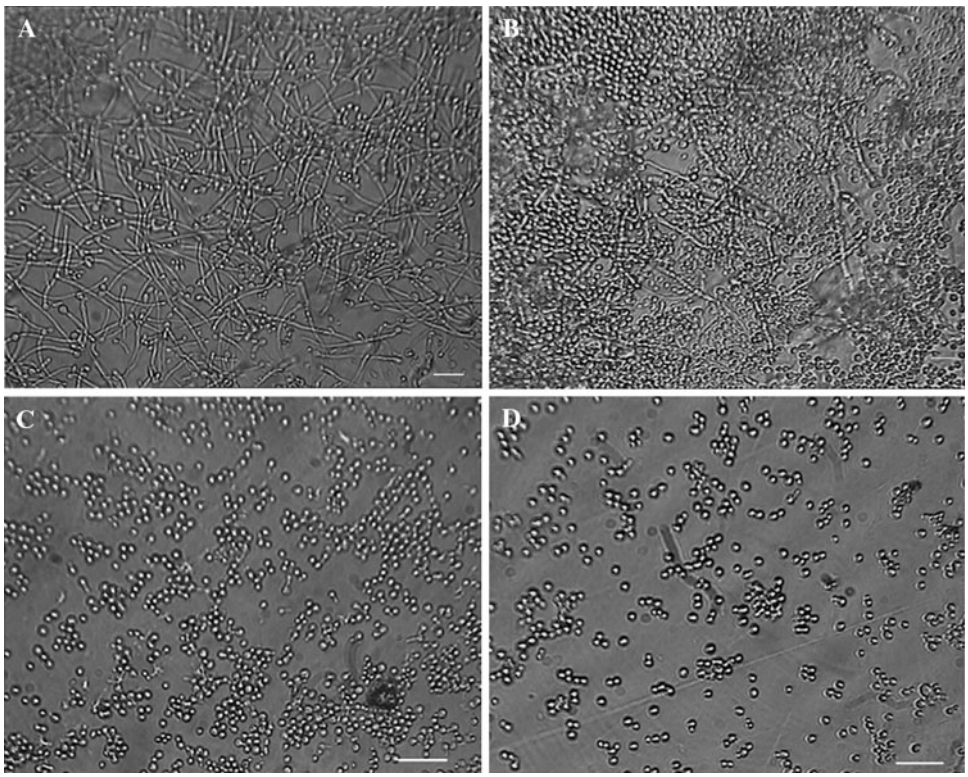

Fig. 3 Effect of acetaldehyde on biofilm development in *C. albicans* ATCC 90028. *Candida* biofilms were developed on a polystyrene surface. **a** Control, 24 h; **b** 7 mM acetaldehyde, 24 h; **c** control, 48 h; **d** 7 mM acetaldehyde, 48 h. The various concentrations of acetaldehyde were added after the adhesion phase. The plate was incubated at 37°C for 24 and 48 h, respectively. *Bar*: 60 μm (×200)

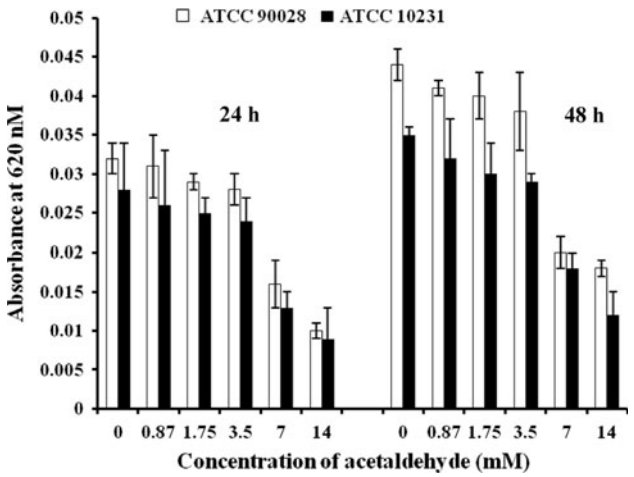

Fig. 4 Effect of acetaldehyde on biofilm formation in two strains of *C. albicans*. In vitro biofilm formation in the presence of acetaldehyde was quantified using the crystal violet assay. RPMI-1640 medium containing various concentrations of acetaldehyde and one control solution (RPMI-1640 medium without acetaldehyde) were incubated at 37°C for 24 and 48 h, respectively

($P = 0.002$), with the reduction (less than 50%) being significant ($P < 0.05$) at 7 and 14 mM acetaldehyde compared to the control. At concentrations <7 mM of acetaldehyde, biofilm formation was 80–100% of that of the control (Fig. 4).

Acetaldehyde is known to be formed in the human body by ADH in the liver. To a lesser extent, ethanol is oxidized to acetaldehyde by other tissues, such as kidneys, respiratory tract, intestine, and bone marrow (Jokelainen et al. 1994). In vivo colonic bacteria can significantly contribute to the production of acetaldehyde from alcohol (Jokelainen et al. 1994). Acetaldehyde is also reported to be produced in saliva through the metabolic activity of oral microorganisms (Tillonen et al. 1999). In alcoholics, acetaldehyde is believed to be produced from ethanol, predisposing them to cancer (Poschl and Seitz 2004). However, the potential effect of acetaldehyde on resident flora of the human body (like *C. albicans*) has not been investigated. *C. albicans* is also known to produce acetaldehyde in culture supernatants (Mukherjee et al. 2006). Mukherjee et al. (2006) reported the production of 0.01 mg/ml acetaldehyde in wild-type *C. albicans*, whereas the *ADH1* mutant produced up to 0.14 mg/ml of acetaldehyde. Interestingly, *C. albicans* isolated from high-acetaldehyde-producing saliva produced more acetaldehyde from ethanol (Tillonen et al. 1999). Acetaldehyde was found to act as a substitute for the xanthine oxidase–myeloperoxidase halide system that caused damage to *C. albicans* hyphae (Diamond et al. 1980). The effects of acetaldehyde on the physiology and, in particular, morphogenesis of *C. albicans* are unclear.

Our results are the first to show that acetaldehyde has morphogenetic regulatory properties in *C. albicans*. It inhibited yeast-to-hyphal dimorphism induced by four standard inducers at 37°C—in a concentration-dependent manner—in two strains of *C. albicans* (Figs. 1, 2).

Acetaldehyde at a concentration of 7 mM caused an 80–100% of inhibition of morphogenesis (Fig. 1) but did not have much effect on the growth and viability of the *C. albicans* cells (Table 1). However, above this effective concentration, viability did decrease. Interestingly, 7 and 14 mM of acetaldehyde prevented the formation of hypha in biofilms of *C. albicans* (Fig. 4) and also significantly inhibited (<50%) biofilm development, with only adherent yeast cells present (Fig. 3). The mechanisms behind these effects are unclear. Aranda and del Olmo (2004) reported that the exposure of *S. cerevisiae* cells to 1 mg/ml concentration of acetaldehyde had an adverse effect on cell cycle progression, DNA replication and protein biosynthesis, without any significant effects on viability. They also found that exposure of *S. cerevisiae* cells to acetaldehye caused an induction of the *MET* genes involved in sulfur metabolism and the repression of genes involved in growth and maintenance of cell polarity (Aranda and del Olmo 2004). Similar responses may occur in *C. albicans*.

In vivo, at locations where *C. albicans* predominates, the accumulation of acetaldehyde may inhibit hyphal forms to favor the formation of yeast-type cells. This may facilitate the dissemination of *C. albicans* inside the host. Acetaldehyde-mediated prevention of hyphal forms may inhibit typical biofilms but favor easily detachable 'yeast-only' biofilms. We propose that acetaldehyde has morphogenesis inhibitory properties in planktonic as well as biofilm forms of *C. albicans*. How acetaldehyde influences *C. albicans* morphogenesis and which mechanisms are involved are currently under investigation in our laboratory.

References

Aranda A, del Olmo ML (2004) Exposure of *Saccharomyces cerevisiae* to acetaldehyde induces sulfur amino acid metabolism and polyamine transporter genes, which depend on Met4p and Haa1p transcription factors, respectively. Appl Environ Microbiol 70:1913–1922

Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA (2001) Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol 183:5385–5394

Chen H, Fink GR (2006) Feedback control of morphogenesis in fungi by aromatic alcohol. Genes Dev 20:1150–1161

Cutler JE (1991) Putative virulence factors of *Candida albicans*. Annu Rev Microbiol 45:187–218

Devkatte AN, Zore GB, Karuppayil SM (2005) Potential of plant oils as inhibitors of *Candida albicans* growth. FEMS Yeast Res 5:867–873

Diamond RD, Clarke RA, Haudenschild CC (1980) Damage to *Candida albicans* hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of neutrophil metabolism in vitro. J Clin Invest 66:908–917

Dickinson JR (2008) Filament formation in *Saccharomyces cerevisiae*—a review. Folia Microbiol 1:3–14

Gow NA (1997) Germ tube growth of *Candida albicans*. Curr Top Med Mycol 8:43–55

Hazma OJ, Matee MI, Moshi MJ, Simon EN, Mugusi F, Miks FH, Helderman WH, Rijs AJ, van der Ven AJ, Verweij PE (2008) Species distribution and in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol 8:135–143

Hogan DA (2006) Talking to themselves: autoregulation and quorum sensing in fungi. Eukaryot Cell 5:613–619

Hudson DA, Sciascia QL, Sanders RJ, Norris GE, Edwards PJ, Sullivan PA, Farley PC (2004) Identification of the dialyzable serum inducer of germ-tube formation in *Candida albicans*. Microbiology 150:3041–3049

Jokelainen K, Roine RP, Vaananen H, Farkkila M, Salaspuro M (1994) In vitro acetaldehyde formation by human colonic bacteria. Gut 35:1271–1274

Lo HJ, Köhler JR, DiDomenico B, Loebenberg D, Cacciapuoti A, Fink GR (1997) Nonfilamentous *C. albicans* mutants are avirulent. Cell 90:939–949

Martins M, Henriques M, Azeredo J, Rocha SM, Coimbra MA, Oliveria R (2007) Morphogenesis control in *Candida albicans* and *Candida dubliniensis* through signaling molecules produced by planktonic and biofilm cells. Eukaryot Cell 6:2429–2436

Mosel DD, Dumitru R, Hornby JM, Autkin AL, Nickerson KW (2005) Farnesol concentrations required to block germ tube formation in the presence and absence of serum. Appl Environ Microbiol 71:4938–4940

Mukherjee PK, Mohammed S, Chandra J, Kuhn D, Liu S, Antar OS, Munyon R, Mitchell AP, Andes D, Chance MR, Rouabhia M, Ghannoum MA (2006) Alcohol dehydrogenase restricts the ability of pathogen *Candida albicans* to form a biofilm on catheter surfaces through an ethanol-based mechanism. Infect Immun 74:3804–3816

Nickerson KW, Atkin AL, Hornby JM (2006) Quorum sensing in dimorphic fungi: farnesol and beyond. Appl Environ Microbiol 72:3805–3813

Odds FC (1994) *Candida* species and virulence. ASM News 60:313–318

Peeters E, Nelis HJ, Coenye T (2008) Comparision of multiple methods for quantification of microbial biofilms grown in microtitre plates. J Microb Methods 72:157–165

Poschl G, Seitz HK (2004) Alcohol and cancer. Alcohol Alcohol 39:155–165

Tillonen J, Homann N, Rautio M, Jousimies-Somer H, Salaspuro M (1999) Role of yeasts in the salivary acetaldehyde production from ethanol among risk groups for ethanol-associated oral cavity cancer. Alcohol Clin Exp Res 23:1409–1415

Walker-Caprioglio HM, Parks LW (1987) Autoconditioning factor relieves ethanol-induced growth inhibition of *Saccharomyces cerevisiae*. Appl Environ Microbiol 53:33–35

Westwater C, Balish E, Schofield DA (2005) *Candida albicans*-conditioned medium protects yeast cells from oxidative stress: a possible link between quorum sensing and oxidative stress resistance. Eukaryot Cell 10:1654–1661