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cDNA Microarray Analysis of Differential Gene Expression in *Candida albicans* Biofilm Exposed to Farnesol

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Candida albicans biofilms are structured microbial communities with high levels of drug resistance. Farnesol, a quorum-sensing molecule that inhibits hyphal formation in _C. albicans_, has been found to prevent biofilm formation by _C. albicans_. There is limited information, however, about the molecular mechanism of farnesol against biofilm formation. We used cDNA microarray analysis to identify the changes in the gene expression profile of a _C. albicans_ biofilm inhibited by farnesol. Confocal scanning laser microscopy was used to visualize and confirm normal and farnesol-inhibited biofilms. A total of 274 genes were identified as responsive, with 104 genes up-regulated and 170 genes down-regulated. Independent reverse transcription-PCR analysis was used to confirm the important changes detected by microarray analysis. In addition to hyphal formation-associated genes (e.g., _TUP1_, _CRK1_, and _PDE2_), a number of other genes with roles related to drug resistance (e.g., _FCR1_ and _PDR16_), cell wall maintenance (e.g., _CHT2_ and _CHT3_), and iron transport (e.g., _FTR2_) were responsive, as were several genes encoding heat shock proteins (e.g., _HSP70_, _HSP90_, _HSP104_, _CaMSI3_, and _SSA2_). Further study of these differentially regulated genes is warranted to evaluate how they may be involved in _C. albicans_ biofilm formation. Consistent with the down-regulation of the cell surface hydrophobicity-associated gene (_CSH1_), the water-hydrocarbon two-phase assay showed a decrease in cell surface hydrophobicity in the farnesol-treated group compared to that in the control group. Our data provide new insight into the molecular mechanism of farnesol against _C. albicans_ biofilm formation.

Candida albicans is a pleiomorphic fungus that can exist as either a commensal or an opportunistic pathogen and is capable of causing superficial to life-threatening infections. Predisposing factors for *C. albicans* infections include immunosuppressive therapy, antibiotic therapy, human immunodeficiency virus infection, diabetes, and old age. In addition, structured microbial communities attached to surfaces, commonly referred to as biofilms (29), have increasingly been found to be the sources of *C. albicans* infections. Biomaterials such as stents, shunts, prostheses (voice, heart valve, and knee prostheses), implants (lens and breast implants and dentures), endotracheal tubes, pacemakers, and various types of catheters have all been shown to facilitate *C. albicans* colonization and biofilm formation (1, 20, 27, 28). It is estimated that biofilms might be involved in 65% of infections (30).

Biofilms are spatially organized heterogeneous communities of cells embedded within an extrapolymeric matrix. In comparison with planktonic cells, biofilm cells display unique phenotypic traits (10, 11), the most outstanding of which is that they are notoriously resistant to both antimicrobial agents and host immune factors. Biofilm-associated infections are therefore difficult to treat because of their decreased susceptibilities to antimicrobial therapy. It is reported that *C. albicans* biofilms are resistant to a variety of clinical antifungal agents, including amphotericin B and fluconazole (7, 31).

Cell-cell signaling, particularly quorum sensing, has been one of the focuses of microbiological research over the past decade. It has been demonstrated that quorum-sensing molecules are essential for bacterial biofilm formation and that a threshold concentration triggers biofilm formation (25, 35). It was reported (16) that *C. albicans* produces farnesol as an extracellular quorum-sensing molecule, and farnesol is the first quorum-sensing molecule to be identified in a eukaryotic organism. When farnesol accumulates above a threshold level, it can prevent *C. albicans* from converting from the yeast form to the mycelium form and from forming biofilms (33). It is thought to be a novel target for the development of drugs intended to prevent biofilm production by *C. albicans*. However, the mechanism by which farnesol prevents *C. albicans* from forming biofilms is unknown.

In the present study, we used cDNA microarray analysis to identify differentially expressed genes associated with biofilm formation by comparing the transcriptional profile of a farnesol-inhibited *C. albicans* biofilm to that of a matched normal *C. albicans* biofilm. A number of genes were found to be differentially expressed between these two groups, including several genes involved in hyphal formation, cell surface hydrophobicity (CSH), and drug resistance.

MATERIALS AND METHODS

Organism and growth medium. *C. albicans* collection strain SC5314 was kindly provided by William A. Fonzi (Department of Microbiology and Immunology, Georgetown University, Washington, D.C.). Cells were propagated in yeast peptone dextrose (YPD) medium (1% [wt/vol] yeast extract, 2% [wt/vol] peptone, 2% [wt/vol] dextrose). Batches of medium (20 ml) were inoculated from YPD agar plates containing freshly grown *C. albicans* and incubated overnight in an orbital shaker at 30°C. *C. albicans* grew in the budding-yeast phase under these conditions. Cells were harvested and washed in sterile phosphate-buffered saline

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(PBS; 10 mM phosphate buffer, 2.7 mM potassium chloride, 137 mM sodium chloride [pH 7.4]). The cells were then suspended in RPMI 1640 medium supplemented with L-glutamine and buffered with morpholinepropanesulfonic acid (MOPS), counted in a hemocytometer, and adjusted to the desired cell density (1.0×10^6 cells/ml). Farnesol (mixed isomers; lot 082K2520; Sigma Chemical Co.) was obtained as a 4 M stock solution and then diluted in 100% (vol/vol) methanol to obtain a 40 mM working stock solution. The working concentration of farnesol was 40 μM when it was prepared in RPMI 1640 medium.

Biofilm formation and treatment. Standardized *C. albicans* cells (1.0×10^6 cells/ml in RPMI 1640 medium) were prepared and added to 25-ml portions of RPMI 1640 medium in 75-cm^2 tissue culture flasks with vented caps. The flasks were incubated statically for 1 h to allow initial adherence of the cells, after which the medium was decanted and replaced with 50-ml portions of prewarmed (37°C) RPMI 1640 medium containing 40 μM farnesol. This farnesol concentration was chosen because it still maintained an inhibitory effect on biofilm formation, while it allowed the recovery of a sufficient cellular mass for RNA extraction. The flasks were then gently rocked (50 rpm) at 37°C for 24 h to promote biofilm formation (33). A farnesol-free control was also included.

CSH. The CSH of *C. albicans* was measured by the water-hydrocarbon two-phase assay, as described previously (18). Briefly, *C. albicans* cultures to which different concentrations (0, 1, 10, and 100 μM) of farnesol were added were cultured as described above to form biofilms, and the cells of the biofilm were removed from the flask surfaces with a sterile scraper to prepare a cell suspension (optical density at 600 nm [OD_{600}], 1.0 in YPD medium). A total of 1.2 ml of a suspension from each group was drawn into a clean glass tube and overlaid with 0.3 ml of octane. The mixture was vortexed for 3 min for phase separation. Soon after the two phases had separated, the OD_{600} of the aqueous phase was determined. The OD_{600} for the group without the octane overlay in YPD medium was used as the negative control. Three repeats were performed for each group. The relative hydrophobicity was obtained as [(OD_{600} of the control − OD_{600} after octane overlay)/OD_{600} of the control] × 100.

Confocal scanning laser microscopy. Confocal scanning laser microscopy was performed as described in the literature (8) to demonstrate the inhibitory effect of farnesol on biofilm formation. The formation of *C. albicans* strain SC5314 biofilms was achieved by adding 4 ml of a standardized cell suspension onto six-well plates containing plastic disks. Following farnesol exposure and biofilm formation, the disks were removed and transferred to new six-well culture plates and incubated for 45 min at 37°C in 4 ml of PBS containing the fluorescent stains FUN-1 (10 μM; Molecular Probes, Eugene, Oreg.) and concanavalin A–Alexa Fluor 488 conjugate (ConA; 25 μg/ml; Molecular Probes). FUN-1 (excitation wavelength, 543 nm; emission wavelength, 560 nm; long-pass filter) is converted to an orange-red cylindrical intravacuolar structure by metabolically active cells, while ConA (excitation wavelength, 488 nm; emission wavelength, 505 nm; long-pass filter) binds to the glucose and mannose residues of cell wall polysaccharides and emits a green fluorescence. After incubation with the dyes, the disks were flipped and the stained biofilms were observed with a Leica TCS sp2 confocal scanning laser microscope equipped with argon and HeNe lasers.

RNA isolation. Cells were washed in ice-cold sterile PBS in the flasks and then removed from the flask surfaces with a sterile scraper. Following collection of the cells by centrifugation, the cells were resuspended in 12 ml of AE buffer (50 mM sodium acetate [pH 5.2], 10 mM EDTA) at room temperature, after which 800 μl of 25% sodium dodecyl sulfate (SDS) and 12 ml of acid phenol were added. The cell lysate was then incubated for 10 min at 65°C with vortexing each minute, cooled on ice for 5 min, and subjected to centrifugation at 12,000 × *g* for 15 min. The supernatants were transferred to new tubes containing 15 ml of chloroform, mixed, and subjected to centrifugation at 200 × *g* for 10 min. RNA was precipitated from the resulting aqueous layer by transferring that portion to new tubes containing 1 volume of isopropanol and 0.1 volume of 2 M sodium acetate (pH 5.0) and mixed well. The mixture was centrifuged at 18,000 × *g* for 35 min at 4°C. The supernatants were removed. The pellet was resuspended in 10 ml of 70% ethanol and centrifuged at 18,000 × *g* for 20 min at 4°C to collect the RNA. The supernatants were removed again, and the RNA was resuspended in diethyl pyrocarbonate-treated water. The OD_{260} and OD_{280} were measured, and the integrity of the RNA was visualized by subjecting 2 to 5 μl of the sample to electrophoresis through a 1% agarose-MOPS gel. Poly(A) mRNA was extracted by using an Oligotex mRNA kit (Qiagen, Hilden, Germany) and was quantitated by using a RiboGreen RNA quantitation kit (Molecular Probes).

Microarray preparation. The microarray (3132 chip; United Gene Holdings, Ltd., Shanghai, People's Republic of China) used in our study consisted of 3,132 spots (3,102 sequences), including full-length and partial cDNA sequences representing the sequences of novel, known, and control genes of *C. albicans*. Most of the genes were obtained by sequencing the clones isolated from a library of *C. albicans* with full-length cDNA at a high ratio by using the switching mechanism at the 5′ end of the RNA transcript. Sequencing of the clones was carried out at United Gene Holdings. Other known genes involved in drug resistance and biofilm formation were selected from the National Center for Biotechnology Information Unigene set and were cloned into plasmid vectors. The control spots included human glycerol-3-phosphate dehydrogenase (8 spots), actin genes (8 spots), and spotting solution alone without DNA (16 spots). The 3132 chip was constructed by our previously described method (22). In brief, the cDNA inserts were amplified by PCR with universal primers specific for the plasmid vector sequences and then purified by isopropanol precipitation. All PCR products were examined by agarose gel electrophoresis to ensure the quality and the identity of the amplified clones, which were as expected. The amplified PCR products were dissolved in a buffer containing 3× SSC solution (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). These solutions were spotted onto sialylated slides (CEL Associates, Houston, Tex.) with a Cartesian PixSys 7500 motion-control robot (Cartesian Technologies, Irvine, Calif.) fitted with ChipMaker Micro-Spotting technology (TeleChem International, Sunnyvale, Calif.). The glass slides spotted with cDNA were then hydrated for 2 h in an atmosphere with 70% humidity, dried for 0.5 h at room temperature, and UV cross-linked (65 mJ/cm^2). They were further processed at room temperature by soaking them in 0.2% SDS for 10 min, distilled water for 10 min, and 0.2% sodium borohydride for 10 min. The slides were dried again, at which time they were ready for use.

Probe labeling and hybridization. The labeling procedures were conducted as follows: the fluorescent cDNA probes were synthesized from purified mRNA with Cy3- or Cy5-dUTP (Amersham Pharmacia Biotech, Piscataway, N.J.) by oligo(dT)-primed polymerization with Superscript II reverse transcriptase (Invitrogen). The reaction buffer mixture contained deoxynucleoside triphosphates (200 μmol of dATP, dCTP, and dGTP per liter, 60 μmol of dTTP per liter, and 60 μmol of Cy3- or Cy5-dUTP per liter), 2 μl of Superscript II reverse transcriptase, and 1× reaction buffer. The reactions were carried out at 42°C for 2 h. Then the RNA was hydrolyzed by the addition of 4 μl of 2.5 mol of NaOH per liter and incubation at 65°C for 10 min, and then the RNA was neutralized with 4 μl of 2.5 mol of HCl per liter. Dye swap was used to avoid dye-associated effects on cDNA synthesis. That is, three independent hybridization experiments were performed, with RNA from the farnesol-treated group labeled with Cy5-dUTP two times and with Cy3-dUTP one time. The two color probes were then mixed and diluted to 500 μl with TE (Tris-EDTA), concentrated to 10 μl with a Microcon YM-30 filter (Millipore, Bedford, Mass.), and vacuum dried.

The probes were dissolved in 20 μl of hybridization solution (5× SSC [0.75 mol of NaCl per liter and 0.075 mol of sodium citrate per liter], 0.4% SDS, 50% formamide). The microarrays were prehybridized with hybridization solution containing 0.5 mg of denatured salmon sperm DNA per ml at 42°C for 6 h. The fluorescent probe mixtures were denatured at 95°C for 5 min and were then applied onto the prehybridized chip under a cover glass. The chip was hybridized in a homemade chamber at 42°C for 15 to 17 h. The hybridized chip was then washed at 60°C in solutions of 2× SSC–0.2% SDS, 0.1× SSC–0.2% SDS, and 0.1× SSC for 10 min in each solution and then dried at room temperature.

Detection and analysis. The chips were scanned with a ScanArray 3000 apparatus (GSI Lumonics, Bellerica, Mass.) at two wavelengths to detect the emissions from both Cy3 and Cy5. The acquired images were analyzed with ImaGene (version 3.0) software (BioDiscovery, Los Angeles, Calif.). The intensities of each spot at the two wavelengths represent the quantities of Cy3-dUTP and Cy5-dUTP, respectively, that hybridized to each spot. The ratios of Cy5 to Cy3 were calculated for each location on each microarray. To minimize artifacts that arise from low expression values, only genes with raw intensity values of >800 counts for both Cy3 and Cy5 were chosen for differential analysis. Genes were identified as differentially expressed if the absolute value of the natural logarithm of the ratios was >0.69 (22).

RESULTS AND DISCUSSION

Experimental design and global findings. Farnesol acts as a naturally occurring quorum-sensing molecule that inhibits filamentation in *C. albicans* and that was also reported to inhibit *C. albicans* biofilm formation (16, 33). In the present study, we compared *C. albicans* biofilms cultured for 24 h in the presence of either the control solution or farnesol (40 μmol/liter). The 24-h time point was chosen since it was reported that the biofilm is mature at 24 h (33) and drug resistance appears at this time point (8, 32). Farnesol was used at a concentration of 40 μmol/liter and was added after the original incubation

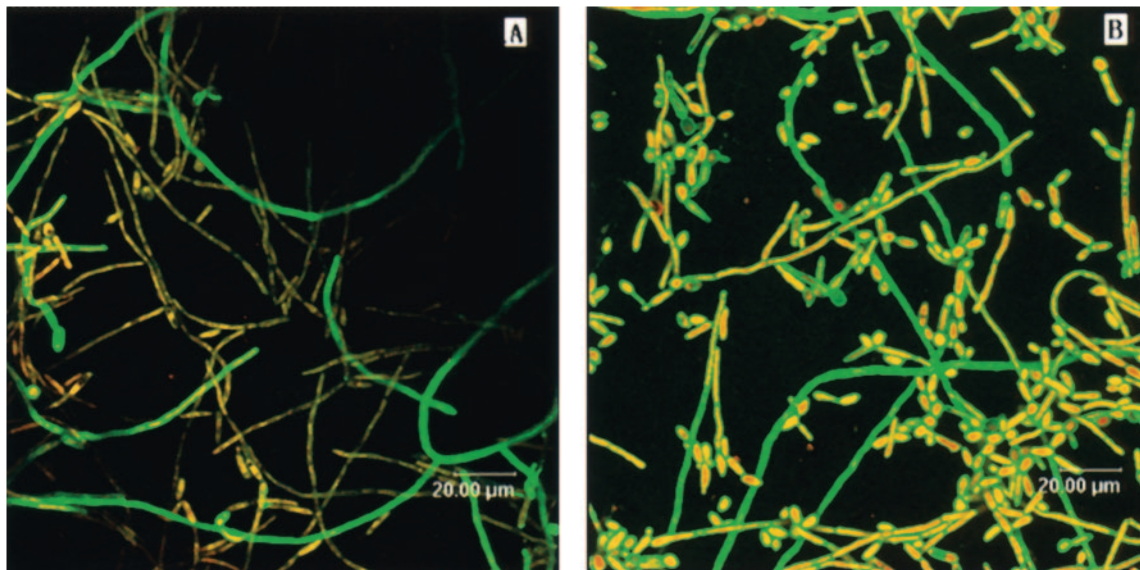

FIG. 1. Confocal scanning laser microscopy showing the effect of farnesol on *C. albicans* biofilm (24 h) formation. ConA (green) and FUN-1 (red) staining were used to generate the images. Metabolically active cells are shown in red, and cell wall polysaccharides are shown in green. Cells treated with 40 μM farnesol (B) show poor biofilm architectures, with the biofilm predominantly composed of yeast cells and pseudohyphae. True hyphae were present in farnesol-treated cells at levels much less than those in the farnesol-free control (A).

of 1 h to suppress biofilm formation while enough cells were retrieved for RNA extraction and hybridization (Fig. 1). After farnesol treatment, 274 genes (mainly identified at http://genolist.pasteur.fr/CandidaDB/) altered differentially with a threshold of a 2.0-fold change in the expression level (see the supplementary table available at http://www.chinagenenet.com/ca_microarray). Differential expression for these genes was then confirmed independently by reverse transcription-PCR (RT-PCR). The important genes selected from the differentially expressed genes are shown in Table 1, and the RT-PCR results for some of these genes are shown in Fig. 2.

Roles of genes involved in hyphal formation. Consistent with previous findings that farnesol prevents hyphal development in a dose-dependent manner (33), we found that several hyphal formation-associated genes were differentially expressed. *TUP1* encodes a global transcriptional corepressor. Deletion of the *TUP1* gene causes hyperfilamentation under conditions favorable for growth of the yeast form (3, 4). Up-regulation of *TUP1* may therefore contribute to the inhibition of hyphal formation. However, we did not find that the *TUP1*-repressed genes, which are associated with morphology, as reported previously (5), were differentially expressed.

CRK1 is a new member of the Cdc2 kinase subfamily (9). Deletion of *CRK1* dramatically impaired hyphal formation under various hypha-inducing conditions, whereas the ectopic expression of its catalytic domain promoted hyphal colony formation, even under conditions favorable for growth of the yeast form (44). Down-regulation of the *CRK1* gene may account for the yeast phenotype.

The cAMP-dependent pathway is one of the pathways that regulate yeast-to-hypha morphogenesis in *C. albicans*, which is controlled by changes in cyclic AMP (cAMP) levels determined by the processes of synthesis and hydrolysis. *CaPDE2* encodes the high-affinity cAMP phosphodiesterase. Deletion of *CaPDE2* causes elevated cAMP levels, elevated responsiveness to exogenous cAMP, and highly reduced levels of transcription of *EFG1*, which is an important hypha-associated gene. In vitro in hypha-inducing liquid medium, *CaPDE2* deletion prohibits normal hyphal development (17). Thus, down-regulation of *PDE2* may affect morphogenesis.

CSH affects biofilm formation. A typical laboratory model of fungal biofilm formation involves three steps: adhesion, biofilm growth, and maturation (2, 8). Using different species of *Candida*, previous researchers observed that fungal adherence to plastic surfaces is correlated with CSH (14, 18, 34, 38, 39). Recently, it was reported (21) that CSH plays a major role in biofilm formation in *C. albicans*. A positive correlation between biofilm formation abilities and CSH has been found. The *CSH1* gene, which codes for the CSH-associated protein, is the first candidate gene that has been demonstrated to play a role in affecting the CSH phenotype in *C. albicans*. Knockout of this gene resulted in a decrease in measurable CSH and a decrease in the level of adhesion of *C. albicans* to fibronectin (40). We found that *CSH1* was down-regulated about threefold in this experiment. Since initial adhesion is important in terms of the ability of farnesol to inhibit biofilm formation, there may be a relationship between CSH and the farnesol concentration. To confirm this, we determined the CSH of biofilms treated with different concentrations of farnesol. We found a negative correlation between the farnesol concentrations and CSH (Fig. 3). It is therefore possible that a decrease in CSH may contribute to the inhibition of biofilm formation.

Genes related to drug resistance. *C. albicans* biofilms are highly resistant to the actions of clinically important antifungal agents, especially fluconazole (8, 13). *CDR1*, *CDR2*, and *MDR1*, which encode major multidrug efflux pumps in *C. albicans* and which play important roles in the drug resistance of planktonic cells, cannot be used to explain why mature biofilms show high-level drug resistance (26, 32). The antifungal resistance of biofilms involves multiple factors and phase-specific

TABLE 1. Selected important genes differentially expressed in farnesol-treated *C. albicans* biofilm

Gene group and CandidaDB entry no.	Function (gene)	Gene regulation[a]		
		Test vs control group (A)	Test vs control group (B)	Control vs test group
Hyphal development associated				
CA3450	Nucleotide phosphodiesterase (*PDE2*)	0.24	0.27	4.05
CA0861	Protein kinase (*CRK1*)	0.47	0.41	2.42
CA3852	General transcription repressor (*TUP1*)	6.58	6.39	0.30
CSH, AY330272[b]	CSH-associated protein (*CSH1*)	0.30	0.32	3.27
Drug resistance				
CA5890	Zinc cluster transcription factor (*FCR1*)	2.64	2.92	0.37
396066A06.sl.seq[c]	Drug resistance (*PDR16*)	0.17	0.18	6.21
Heat shock proteins				
CA1230	70-kDa heat shock protein (*HSP70*)	0.25	0.26	4.15
CA4959	90-kDa heat shock protein (*HSP90*)	0.38	0.35	2.65
CA6306	104-kDa heat shock protein (*HSP104*)	0.28	0.23	4.10
CA2857	Heat shock protein of HSP70 family (*SSA1*)	0.32	0.25	4.70
AB061274[b]	Chaperone protein (*CaMSI3*)	0.34	0.33	3.15
Cell wall maintenance				
CA1051	Chitinase (*CHT2*)	3.69	2.36	0.29
CA5987	Chitinase (*CHT3*)	5.56	5.70	0.13
Cell cycling and DNA processing				
CA2479	G_1 cyclin (*CCN1*)	9.38	11.31	0.14
CA2950	G_1/S-specific cyclin (*CLN2*)	4.54	4.39	0.23
CA5315	B-type cyclin (*CYB2*)	2.20	2.35	0.44
CA2805	Ribonucleotide reductase large subunit (*RNR1*)	4.45	4.05	0.24
CA5495	DNA helicase (*SGS1*)	3.93	2.56	0.28
CA2471	DNA topoisomerase II (*TOP2*)	3.21	2.82	0.32
Small molecule and iron transport				
CA5354	High-affinity iron permease (*FTR2*)	4.21	3.71	0.24
CA0357	Putative purine-cytosine permease (*FCY22*)	4.16	4.75	0.22
Other functions				
CA5986	Inositol-1-phosphate synthase (*IN01*)	0.05	0.05	15.39
CA4700	Secreted aspartic proteinases (*SAP9*)	0.44	0.34	2.56
CA2660	Secreted aspartic proteinases (*SAP1*)	0.38	0.41	2.46
CA4040	Galactokinase (*GAL1*)	0.44	0.47	2.23
CA5152	Carnitine *o*-acetyltransferase (*CAT2*)	3.03	3.28	0.32
CA4857	pH-responsive protein (*PHR1*)	2.73	3.15	0.34
CA5546	Alpha-tubulin (*TUB1*)	2.67	2.38	0.40
CA0895	Serine hydroxymethyltransferase II (*SHMII*)	2.15	2.60	0.42
CA0972	Peptidyl-propyl *cis-trans* isomerase (*CYP1*)	0.28	0.28	3.47
CA5932	polyubiquitin (*UB14*)	0.26	0.32	3.45
CA2189	Alternative oxidase (*AOX2*)	0.22	0.23	4.79

[a] Values >2 mean that the genes were up-regulated in test cells versus the regulation in control cells; values <0.5 mean that the genes were down-regulated in test cells versus the regulation in control cells.
[b] Genes were identified at http://www.ncbi.nih.gov/BLAST/.
[c] *PDR16* was identified at http://www-sequence.stanford.edu:8080/bin/blastnComb.

mechanisms. In the present study, *FCR1* and *PDR16*, which are associated with drug resistance, were found to be differentially expressed.

FCR1 codes for a transcription factor of the C_6 zinc cluster family homologous to *Saccharomyces cerevisiae* Pdr1p. It behaves as a negative regulator of drug resistance in *C. albicans*. The *fcr1*/*fcr1* mutant displays hyperresistance to fluconazole and other antifungal drugs (42). Up-regulation of *FCR1* in the farnesol-treated group suggests that the level of fluconazole resistance decreased in inhibited biofilms.

PDR16 is another differentially expressed gene involved in drug resistance and belongs to the ABC family of transporters. De Deken and Raymond (12) recently reported that *C. albicans* azole-resistant clinical isolates overexpress the *PDR16* gene. In *S. cerevisiae*, deletion of PDR16 led to strongly increased sensitivity to azole antifungals (43). Furthermore, experiments showed that this deletion affects the phospholipid and sterol compositions of the plasma membrane and that the changes in phospholipid and sterol compositions result in changes in the total yeast lipid composition. It was reported

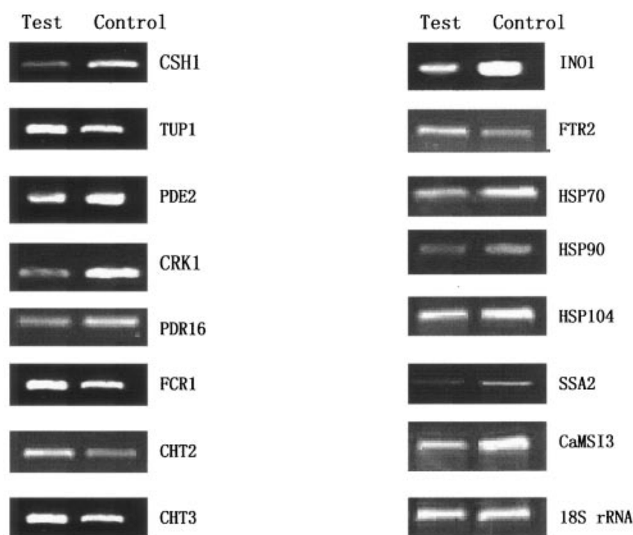

FIG. 2. Confirmation by RT-PCR of differential expression of selected important *C. albicans* genes found to be differentially expressed by cDNA microarray analysis. 18S rRNA was tested as a control.

(19) that alteration of the phospholipid content in *C. albicans* is associated with drug resistance. Consistent with this, we observed *INO1*, which encodes inositol-1-phosphate synthase, a key enzyme in the synthesis of inositol for phosphotidylinositol synthesis (41), was down-regulated dramatically in the farnesol-treated group. Of note, *INO1* was reported to be up-regulated in drug-resistant isolates (37). Down-regulation of *PDR16* and *INO1* thus suggests that altered phospholipid metabolism is associated with azole resistance.

Other responsive genes related to heat shock protein, cell wall maintenance, and iron transport genes. Farnesol exposure resulted in the down-regulation of several genes encoding heat shock proteins (e.g., *HSP70*, *HSP90*, *HSP104*, *CaMSI3*, and *SSA2*). Presumably, the production of heat shock proteins contributes to the protection of cells from damage and repair of cell damage following stress, which may occur in biofilms. Besides, heat shock proteins play important roles in major growth-related processes, such as cell division, DNA synthesis, transcription, translation, protein folding and transport, and membrane translocation (23). Recently, a *C. albicans* biofilm microarray experiment revealed the overexpression of genes involved in protein synthesis (15), which indicated that, because of their role in helping with protein synthesis, up-regulation of heat shock proteins could also occur in normal biofilms, while down-regulation of these proteins could occur in impaired biofilms.

Chitinase is an essential component in the maintenance of cell wall plasticity during fungal growth and proliferation. *C. albicans* contains three chitinase genes, *CHT1*, *CHT2*, and *CHT3*. Previous reports (6, 24) showed that *CHT2* and *CHT3* are preferentially expressed in the yeast phase. The up-regulation of these two genes in our study is consistent with the yeast phenotype in the farnesol-treated group.

We found that the high-affinity iron permease gene *FTR2* is up-regulated more than fourfold. Previous studies have indicated a relationship between iron accessibility and azole resistance in *C. albicans*. Moreover, *FTR2* is down-regulated in fluconazole-resistant *C. albicans* isolates compared to its regulation in fluconazole-susceptible isolates (36). The up-regulation of *FTR2* may therefore contribute to the decrease in the level of azole resistance.

Conclusions. To our knowledge, this study is the first to use cDNA microarray analysis to characterize changes in gene expression between normal biofilms and biofilms inhibited by farnesol, a quorum-sensing molecule in *C. albicans*. The ability of cDNA microarray analysis to examine thousands of genes simultaneously allows not only the identification of the differential expression of genes involved in hypha1 formation, which is the primary function of farnesol, but also that of several genes associated with CSH and drug resistance. This supports the interpretation of *C. albicans* biofilms. That is, the higher the level of CSH is, the easier it is for a biofilm to form; and when a biofilm has formed, high levels of drug resistance occur. In addition, genes functionally related to cell wall maintenance

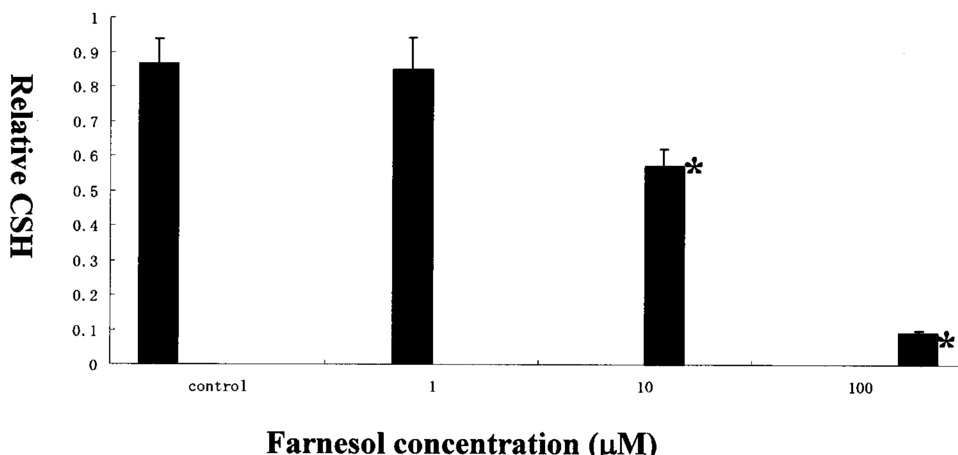

FIG. 3. Effect of farnesol on CSH of *C. albicans* biofilms. Different farnesol concentrations (0, 1, 10, and 100 μM) were added to *C. albicans* cells 1 h after attachment, and the cells were incubated under conditions favorable for biofilm growth. CSH was estimated by the water-hydrocarbon two-phase assay. Mean relative CSH values and standard deviations for three separate experiments are shown. Statistically significant differences between farnesol-treated biofilms and biofilms formed in the absence of farnesol were determined by Student's t test (*, $P < 0.01$).

or iron transport as well as genes encoding heat shock proteins were found to be differentially expressed for the first time. The present study identifies new potential targets in the development of pharmacological approaches to circumventing biofilm formation and the antifungal resistance derived from biofilm formation.

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