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Effect of Farnesol on *Staphylococcus aureus* Biofilm Formation and Antimicrobial Susceptibility

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Staphylococcus aureus **is among the leading pathogens causing bloodstream infections able to form biofilms on host tissue and indwelling medical devices and to persist and cause disease. Infections caused by *S. aureus* are becoming more difficult to treat because of increasing resistance to antibiotics. In a biofilm environment particularly, microbes exhibit enhanced resistance to antimicrobial agents. Recently, farnesol was described as a quorum-sensing molecule with possible antimicrobial properties. In this study, the effect of farnesol on methicillin-resistant and -susceptible strains of *S. aureus* was investigated. With viability assays, biofilm formation assessment, and ethidium bromide uptake testing, farnesol was shown to inhibit biofilm formation and compromise cell membrane integrity. The ability of farnesol to sensitize *S. aureus* to antimicrobials was assessed by agar disk diffusion and broth microdilution methods. For both strains of staphylococci, farnesol was only able to reverse resistance at a high concentration (150 μM). However, it was very successful at enhancing the antimicrobial efficacy of all of the antibiotics to which the strains were somewhat susceptible. Therefore, synergy testing of farnesol and gentamicin was performed with static biofilms exposed to various concentrations of both agents. Plate counts of harvested biofilm cells at 0, 4, and 24 h posttreatment indicated that the combined effect of gentamicin at 2.5 times the MIC and farnesol at 100 μM (22 μg/ml) was able to reduce bacterial populations by more than 2 log units, demonstrating synergy between the two antimicrobial agents. This observed sensitization of resistant strains to antimicrobials and the observed synergistic effect with gentamicin indicate a potential application for farnesol as an adjuvant therapeutic agent for the prevention of biofilm-related infections and promotion of drug resistance reversal.**

Staphylococcus aureus is a leading cause of nosocomial infections and the etiologic agent of a wide range of diseases associated with significant morbidity and mortality. Some of the diseases mediated by this species include endocarditis, osteomyelitis, toxic shock syndrome, food poisoning, and skin infections (1, 34). Those diseases that are due to the colonization of implanted medical devices, however, are particularly problematic since they provide a route past the body's barrier defenses and a surface for cell growth (3, 11, 13, 27, 43, 25). This ability of *Staphylococcus* spp. to adhere to and form multilayered biofilms on host tissue and other surfaces is one of the important mechanisms by which they are able to persist in these diseases (3, 6, 27).

Biofilms are communities of surface-associated microorganisms embedded in a self-produced extracellular polymeric matrix that are notoriously difficult to eradicate and are a source of many recalcitrant infections (8, 11, 14, 37, 1, 35). This sessile mode of life provides biofilm-embedded microbes with ample environmental nutrients and protection from host phagocytic clearance, greatly limiting the ability of the host to adequately deal with the infection (14, 18). A more important consequence of biofilm growth, however, with profound clinical implications is the markedly enhanced resistance to antimicrobial agents where biofilm-associated microorganisms are estimated to be 50 to 500 times more resistant than their planktonic counterparts (1, 4, 5, 29, 31, 37, 39).

The first mechanism by which inherent resistance to antimicrobial factors is mediated is through very low metabolic levels and drastically down-regulated rates of cell division of the deeply embedded microbes (8). Therefore, clearance strategies that depend upon robust and actively dividing microbes (such as the β-lactam antibiotic family) are often ineffective (40). In addition, the polymeric matrix that forms the majority of biofilms was shown to retard the inward diffusion of a number of antimicrobials (1, 38). The reactive oxidants produced by the host immune response or reactive chlorine species (such as hypochlorite, chloramines, or chlorine dioxide) in a number of antimicrobial and/or antifouling agents may also be deactivated in the outer layers of the biofilm faster than they can diffuse into the lower layers (16, 33, 36). A number of studies have shown that the gene expression within biofilms is altered due to the physical action of attachment (15). Although this change in gene expression is a biologically programmed response to attachment and nutrient deprivation, the link between antimicrobial resistance and this altered gene expression is currently being elucidated. The ability of biofilm-embedded *S. aureus* to resist clearance by antimicrobial agents points to the importance of a continuous search for novel agents that are effective against bacteria in this mode of growth or work in synergy with the currently available myriad of antimicrobial agents (31).

Quorum sensing has been the focus of much research, and quorum-sensing molecules have been demonstrated to be essential for biofilm formation (12, 21, 31, 32). Quorum sensing

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is a strategy of cell-cell communication benefiting the biofilm community by controlling unnecessary overpopulation and competition for nutrients with important implications for the infectious process (14, 19). Recently, quorum-sensing activities have been described for the sesquiterpenoid farnesol. In this role, farnesol produced extracellularly prevented the transition from yeast to hyphal growth in *Candida albicans* and greatly compromised biofilm formation by this fungus (10, 12, 20, 21, 32).

In mammalian cells, farnesol as an isoprenoid is a key intermediate in de novo synthesis of cholesterol and is metabolically derived from farnesyl pyrophosphate. Inhibition of the biosynthesis of farnesyl pyrophosphate results in blocking of DNA synthesis and arrest of cell growth (12, 21, 30, 32). Although the effect of farnesol on *S. aureus* has not been fully investigated, some studies have indicated a possible interaction of farnesol with cell membranes of certain bacterial species, including *Streptococcus mutans* (2, 7, 22, 24, 30). In *S. mutans*, for example, brief exposure to farnesol affected growth and metabolism by disrupting the bacterial membrane, as well as the accumulation and polysaccharide content of biofilms of the streptococci (24). Investigations into the mode of action of terpene alcohols on *S. aureus* suggested that damage to cell membranes might be one of the major antibacterial mechanisms. By monitoring changes in the amount of K^+ ions in bacterial suspensions in the presence of these alcohols, the rate of leakage of K^+ ions from the bacterial cells was found to increase with increased concentrations of the compounds added (22).

In this study, methicillin-susceptible and methicillin-resistant strains were used to investigate the effect of farnesol on *S. aureus*. The growth inhibition effect of farnesol on *S. aureus* was assessed with Alamar blue, an indicator of metabolic activity. The activity of farnesol on *S. aureus* biofilm formation was evaluated microscopically by confocal scanning laser microscopy (CSLM) of biofilms grown in the absence and presence of farnesol. In order to test whether the mechanism of farnesol-mediated antimicrobial activity was due to a disruptive effect on the integrity of the bacterial cell membrane, ethidium bromide uptake testing was performed. In addition, the ability of farnesol to sensitize both strains of *S. aureus* to a variety of antimicrobials with different modes of action was assessed by agar disk diffusion and broth microdilution methods. Lastly, in order to test the ability of farnesol to act as a synergistic agent with gentamicin, static biofilms were treated with various concentrations of the antibiotic in the absence and presence of increasing concentrations of farnesol. Survival was determined by counting CFU in bacterial biofilm populations.

MATERIALS AND METHODS

Bacterial strains and reagents. The strains of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) used in these studies were clinical isolates obtained from patients with osteomyelitis who were undergoing treatment at the University of Texas Medical Branch, Galveston. The strains were stored at −70°C in defibrinated sheep blood and used as needed. Farnesol was obtained from Sigma Chemical Co. (St. Louis, MO), and Alamar blue was obtained from Alamar Biosciences, Inc. (Sacramento, CA). Tryptic soy broth (TSB), tryptic soy agar, cation-adjusted Mueller-Hinton broth (CAMHB), and BBL Antibiotic Sensi-disks were obtained from Becton Dickinson & Co. (Cockeysville, MD). Fluorescent stains and ethidium bromide were obtained from Molecular Probes, Inc. (Eugene, OR), and Promega (Madison, WI), respectively.

Effect of farnesol on metabolic activity. In order to evaluate the effect of farnesol on the vitality of the MSSA and MRSA strains, 25 μl of Alamar blue, an oxidation-reduction indicator, was added to 5×10^5 CFU/ml *S. aureus* cell suspensions (100 μl) in the wells of microtiter plates in the presence of farnesol (0, 30, 100, 125, 150, 200, 250, and 300 μM; 0 to 66 μg/ml) and the suspensions were incubated at 37°C for 24 h. Growth was indicated by a change in color from dark blue to red or pink. The tolerance threshold was defined as the lowest concentration of farnesol that prevented the development of a red color.

Biofilm formation. Overnight cultures of MSSA and MRSA were diluted 1:100 in fresh TSB and grown with shaking for 2 h at 37°C. This logarithmic-stage culture was used to inoculate TSB to a final density of 1.0×10^6 CFU/ml. CSLM was performed on biofilms formed on plastic coverslips by dispensing 4 ml of cell suspensions into the wells of six-well microtiter plates with farnesol to produce final concentrations ranging from 0 to 300 μM (0 to 66 μg/ml). Plates were incubated for 24 h at 37°C. Coverslips with cells grown in farnesol-free medium were included. Following incubation, the coverslips were washed with phosphate-buffered saline (PBS) and stained with the FUN-1 fluorescent cell stain (Molecular Probes, Eugene, OR) for 30 min at 37°C in the dark. Stained coverslips were washed with sterile PBS and observed with a confocal scanning laser microscope (Eclipse TE2000-S; Nikon) and a 488-nm argon ion laser.

Ethidium bromide uptake. The disruptive effect of farnesol on MSSA and MRSA cell membranes was assessed by using farnesol-mediated ethidium bromide uptake. One-milliliter volumes of 1.0×10^6 CFU/ml cell suspensions of *S. aureus* in PBS were incubated with 10, 50, 100, 200, and 300 μM (0 to 66 μg/ml) farnesol at 37°C for 2 h with shaking. Ethidium bromide (100 μM of a 1 mg/ml solution) was added to all concentrations, and cells were incubated at room temperature for 15 min. Cells with ethidium bromide and no farnesol served as negative controls. Cells were washed and resuspended in PBS, and a drop of each suspension was examined with a confocal scanning laser microscope for red fluorescence.

Disk diffusion assays. The susceptibility of *S. aureus* strains to a number of clinically important antibiotics was investigated in the presence of farnesol. MSSA and MRSA cultures were grown overnight at 37°C with shaking in TSB. Aliquots (100 μl) were streaked onto tryptic soy agar plates with increasing concentrations of farnesol (0 to 300 μM; 0 to 66 μg/ml). Antibiotic-impregnated disks (BBL Antibiotic Sensi-disks) that included nucleic acid inhibitors (ciprofloxacin [5 μg] and rifampin [15 μg]), cell wall synthesis inhibitors (penicillin [10 μg], nafcillin [1 μg], cefazolin [30 μg], carbenicillin [100 μg], and vancomycin [30 μg]), intermediary metabolism inhibitors (trimethoprim [1.25 μg] and sulfamethoxazole [23.75 μg]), and protein synthesis inhibitors (chloramphenicol [30 μg], erythromycin [15 μg], tetracycline [30 μg], streptomycin [10 μg], and gentamicin [10 μg]) were applied to staphylococcus-seeded agar plates at each concentration of farnesol. Plates were incubated at 37°C for 18 h, at which time the zones of inhibition surrounding the antibiotic disks were measured and recorded.

MIC determination. Standardized planktonic antibiotic MICs were determined by the broth microdilution method outlined by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) (28). The antibiotic susceptibilities of the *S. aureus* strains were measured for gentamicin with and without increasing concentrations of farnesol in CAMHB. In each of the studies, the MICs and minimum bactericidal concentrations of the test antibiotics were determined. Briefly, the test antibiotic was serially diluted twofold in tubes containing 5 ml of CAMHB. The *S. aureus* inoculum for the series of tubes was 100 μl of a 5.0×10^6 CFU/ml dilution in CAMHB of an overnight CAMHB culture. The MIC was the lowest concentration of antibiotic that prevented turbidity after 20 h of incubation at 37°C.

Static-biofilms and synergism studies. Static biofilms were grown for 7 days as previously described (25). Briefly, MSSA and MRSA cultures were grown overnight at 37°C with shaking in TSB. The cultures were diluted 1:1,000 in fresh prewarmed (37°C) TSB. An aliquot (2.5 ml) of each diluted culture was added to each well of a six-well sterile polystyrene plate and incubated without shaking overnight at 37°C. Following incubation, plates were placed on an orbital shaker run at 150 rpm for 10 min and spent medium and planktonic cells were then removed and fresh TSB was added. This was repeated daily for 7 days. At the end of the 7 days of biofilm growth, the spent medium and the planktonic cells were removed as described above and fresh TSB was added again. However, the TSB contained farnesol at 0, 25, 50, or 100 μM (0, 5.5, 11, and 22 μg/ml, respectively) and gentamicin (0, 1, or 10 μg/ml). Biofilm samples were harvested at 0, 4, and 24 h posttreatment by removing excess medium from the wells, adding 1 ml of PBS, and scraping the biofilm. Harvested biofilm was homogenized, serially diluted, and plated on Trypticase soy agar with 5% defibrinated sheep blood to determine the numbers of viable cells remaining.

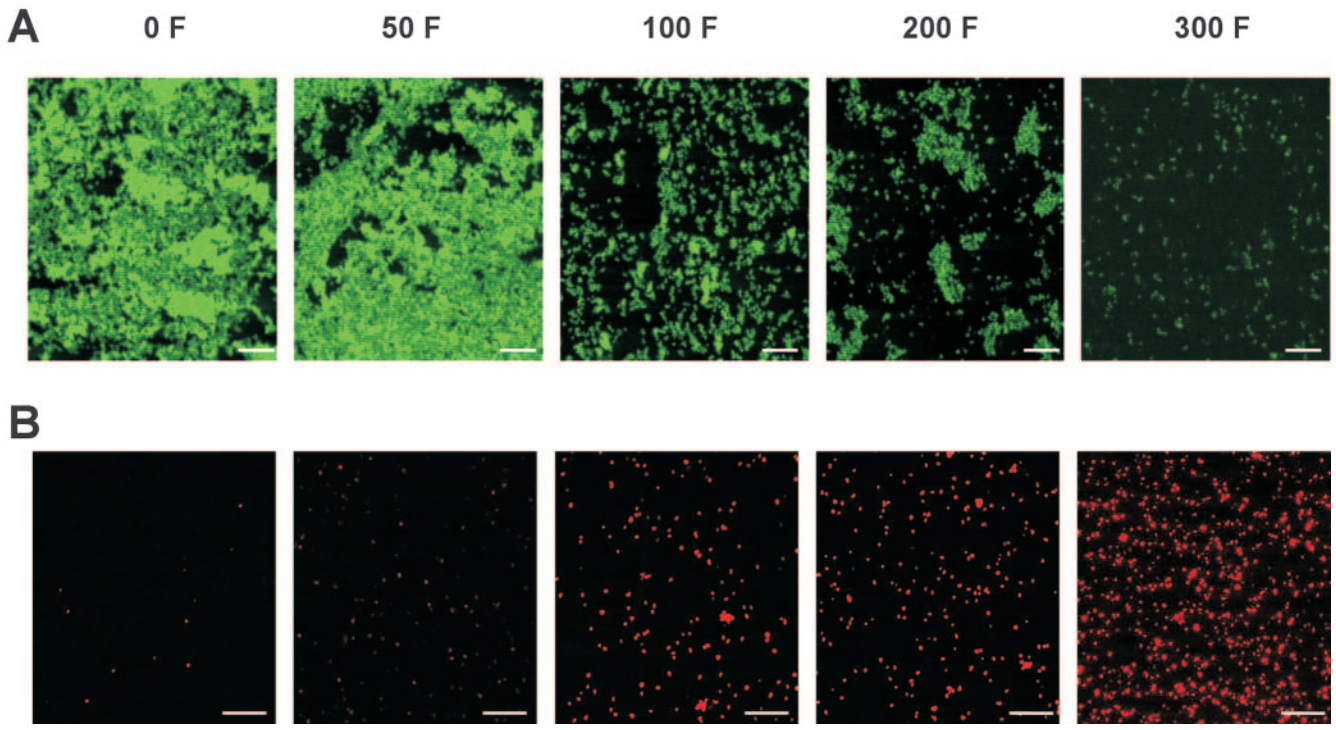

FIG. 1. (A) Biofilm formation by MSSA in the absence and presence of 300 μM farnesol (F). (B) Ethidium bromide uptake following exposure to 0 to 300 μM farnesol as assessed by CSLM. Bars, 10 μm.

Statistical evaluation. Unless otherwise mentioned, all experiments were performed in triplicate and comparisons between group values were performed by analysis of variance with a P value of <0.05 required for statistical significance.

RESULTS

Effect of farnesol on metabolic activity. An oxidation-reduction indicator (Alamar blue) was added to suspensions in the presence of farnesol. The viability assay showed that incubation of cells in the presence of 200 μM (44 μg/ml) farnesol resulted in no change in color from blue to pink, indicating that this concentration inhibited oxidation-reduction reactions in the suspensions.

Biofilm formation and CSLM. Microscopic images showed that both *S. aureus* strains formed a homogeneous biofilm when grown in the absence of farnesol, as well as in the presence of 0 to 50 μM concentrations; however, 100 μM resulted in minimal biofilm formation and higher concentrations completely inhibited biofilm formation (Fig. 1A).

Farnesol-mediated ethidium bromide uptake. As assessed by the emission of red fluorescence and CSLM, non-farnesol-treated *S. aureus* cells stained with ethidium bromide showed no fluorescence except for very few isolated cells. However, farnesol-treated cells exhibited increased uptake of ethidium bromide proportional to the increase in the farnesol concentration, with 300 μM (66 μg/ml) resulting in complete and homogeneous staining of cells (Fig. 1B).

Disk diffusion assays. When increasing concentrations of farnesol were added to the agar plates, the zones of inhibition surrounding the antibiotic-impregnated disks (i.e., the antibiotic efficacy) increased for all classes of antibiotics against MSSA cultures ($P < 0.02$), indicating a steady increase in sensitivity in this strain that was proportional to the concentrations of farnesol (Fig. 2). In addition, the antibiotic efficacy was increased only for those antibiotics that the MRSA strain was already susceptible to, namely, tetracycline, ciprofloxacin, and vancomycin (data not shown). For the antibiotics to which the strains were initially resistant, the increasing concentrations of farnesol had no effect upon the size of the zone of inhibition until it reached the bacteriostatic concentration (200 μM or 44 μg/ml) in this assay.

MIC determination. The antibiotic broth microdilution sensitivities of the *S. aureus* strains were measured for gentamicin with and without increasing concentrations of farnesol. Addition of farnesol even at low concentrations was able to greatly reduce the MIC for MSSA by increasing the permeability of staphylococci to gentamicin (Fig. 3A) ($P < 0.001$). However, this reduction was not seen in MRSA since this strain is inherently resistant to gentamicin (Fig. 3B). Therefore, while gentamicin may have increased access to the antimicrobial targets (i.e., the S12 and L6 proteins of the 30S and 50S bacterial ribosomal subunits, respectively), the resistance to the antibiotic is still present. As a result, the only reduction in the MIC was due to the antibacterial properties of farnesol itself.

Static biofilms and synergism studies. Static MSSA biofilms were grown and then treated with various concentrations of gentamicin (0, 1, and 10 μg/ml) in the presence of farnesol at 0, 25, 50, 100, and 200 μM (0, 5.5, 11, 22, and 44 μg/ml, respectively). Following a 4-h treatment, a statistically significant ($P < 0.03$) reduction was only noted in biofilms that were treated with a gentamicin concentration 2.5 times higher than

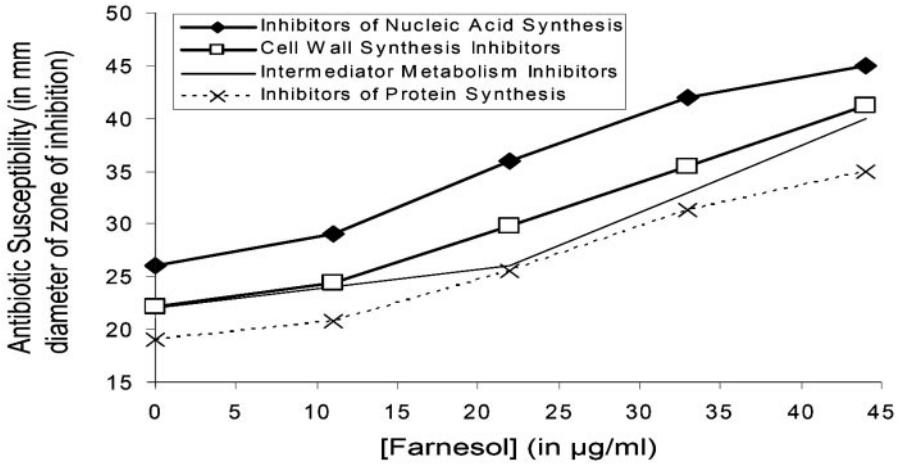

FIG. 2. Antibiotic susceptibility, determined by disk diffusion assay, of MSSA in the presence of farnesol at 0 to 44 μg/ml. Data are means of three independent experiments.

the MIC but only if the farnesol concentration was at least 22 μg/ml (100 μM) (Fig. 4A). However, the effect of farnesol on gentamicin efficacy was much more pronounced with a more lengthy treatment regimen (i.e., 24 h) (Fig. 4B). Treatment with gentamicin alone at 10 μg/ml (i.e., 2.5 times higher than the MIC for this MSSA strain) was only able to reduce bacterial biofilm populations from 1.46×10^9 CFU/cm^2 to 1.04×10^9 CFU/cm^2, a nearly 0.5 log reduction. Farnesol alone was somewhat more effective but was still only able to reduce bacterial numbers by more than 0.5 log unit, from 1.46×10^9 CFU/cm^2 to 4.37×10^8 CFU/cm^2, at a concentration of 200 μM (44 μg/ml) in samples without gentamicin. However, the combined effect of gentamicin at 2.5 times the MIC and 200 μM (44 μg/ml) farnesol was able to reduce bacterial populations by more than 2 log units, demonstrating synergy between the two antimicrobial agents ($P < 0.02$). In fact, even at a low concentration of farnesol (i.e., 25 μM or 5.5 μg/ml), the gentamicin was able to significantly ($P < 0.02$) reduce bacterial biofilm populations at concentrations below the MIC for staphylococci.

DISCUSSION

Due to the increasing involvement of *S. aureus* in foreign-body-related infections, the rapid development and exhibition of multiple antibiotic resistance, as well as its great propensity to lead to persistent, chronic, and recurrent infections, this pathogen has continued to receive significant attention (1, 3, 11, 13, 27, 35, 42, 43). These types of infections are mediated by the ability of *S. aureus* to form biofilms. Bacteria that grow as a biofilm encased in a self-produced matrix are protected from host defenses and often exhibit reduced antibiotic susceptibility, contributing to the persistence of biofilm infections (1, 3, 6, 14, 17, 27). Adherence of organisms to surfaces is a prerequisite to the formation of a biofilm where attachment of cells is followed by proliferation and biofilm formation (42, 43).

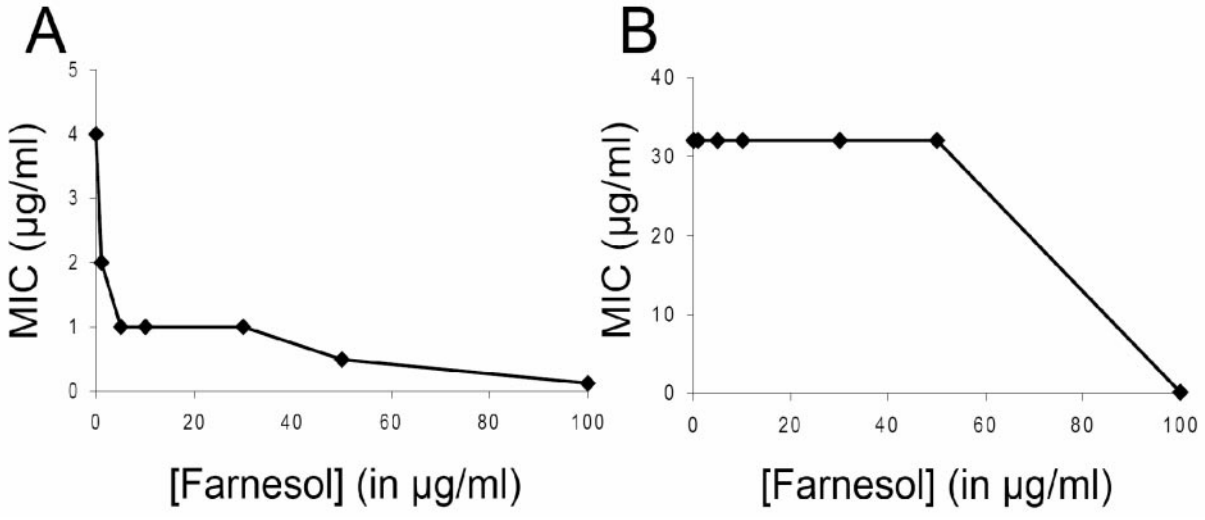

FIG. 3. MICs (micrograms per milliliter) of farnesol at 0 to 100 μg/ml for MSSA (A) and MRSA (B). Data are means of three independent experiments.

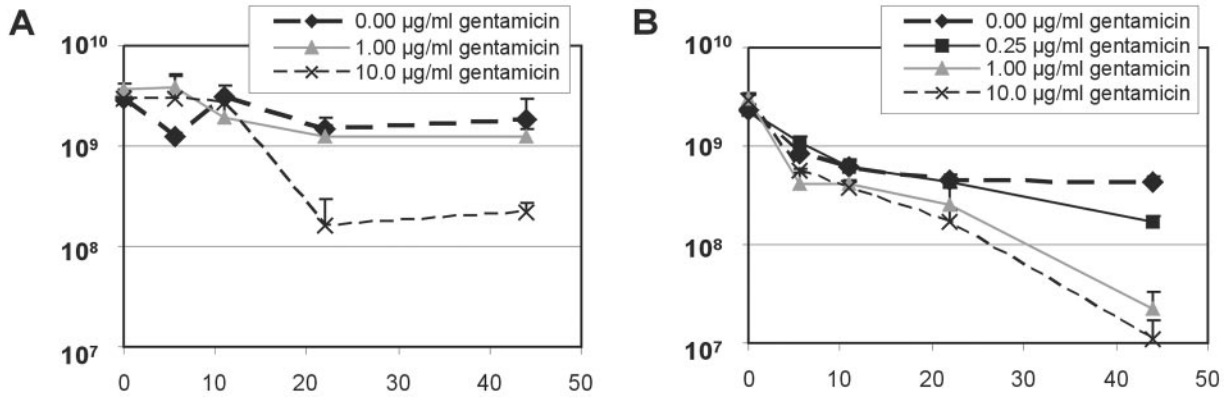

FIG. 4. Synergy testing of gentamicin (0 to 10 μg/ml) and farnesol (0 to 44 μg/ml) against MSSA biofilms following 4 h (A) and 24 h (B) of treatment. Data are means of three independent experiments. Bars represent the standard error of the mean.

Among the recently identified quorum-sensing molecules is the sesquiterpene alcohol farnesol, which has been shown to affect the morphogenesis and biofilm formation of the yeast *C. albicans* (21, 32). Farnesol is naturally found in essential oils of citrus fruits and was shown to be devoid of toxic effects and nonmutagenic in vitro and in vivo (9, 24, 26). Farnesol exposure significantly affected the rate of glucan synthesis in *S. mutans*, the main polysaccharide in the biofilm matrix, and consequently reduced the accumulation and biomass of the biofilms (24). Polysaccharides provide organisms with a unique microenvironment for their growth, metabolism, and survival (24). Therefore, inhibition of formation and accumulation of biofilm communities by affecting the synthesis of polysaccharides is an attractive route for preventing biofilm-related infections. Agents that disrupt the properties of cell membranes may also affect glucan synthesis and consequently reduce the accumulation and biomass of biofilms (24). The effect of farnesol on *S. aureus* polysaccharide production, however, was not investigated (24).

In this study, we investigated the effect of farnesol on the viability of *S. aureus* biofilm formation, as well as its interaction with a variety of antibiotics and the impact it has on antimicrobial resistance. Data from this investigation indicated that modest concentrations of farnesol (22 μg/ml) were sufficient to exhibit an antibacterial effect and significantly inhibit biofilm formation, as was shown by viability assays and fluorescence microscopy for both of the MRSA and MSSA strains tested.

The hydrophobic nature of farnesol favors its accumulation in the membrane, possibly causing membrane leakage. In fact, exposure to terpene alcohols has been recently shown to affect the cell membranes of *S. aureus*, *Escherichia coli*, and *Listeria monocytogenes*, resulting in leakage of K^+ ions from cells (2, 7, 22, 24). However, the farnesol levels used in these experiments (>1 mM) were found to be inhibitory to *S. aureus* and therefore the effect of farnesol on *S. aureus* was not evaluated or investigated further at lower concentrations. Ethidium bromide is a membrane-impermeant drug which enters the cell when membranes are compromised and intercalates within DNA to become highly fluorescent. These properties allow it to be used as a reporter of membrane integrity (7). Therefore, experiments were designed to assess farnesol-mediated uptake of ethidium bromide in cells exposed to farnesol as a measurement of cell integrity. Microscopic images revealed an enhanced uptake of ethidium bromide by farnesol-exposed cells that was proportional to the farnesol concentration, supporting the hypothesis that farnesol causes disruption of the cytoplasmic membrane. The rapid increase in the penetration of cells by ethidium bromide just minutes after the addition of farnesol indicated that this compound was able to quickly damage the bacterial cell membrane.

Since the principal interaction of farnesol appears to be with the cytoplasmic membrane, it is likely that farnesol can nonspecifically enhance the permeability of bacterial cells to certain exogenous chemical compounds, including antimicrobials. In order to test this hypothesis, disk diffusion and broth microdilution methods were used to determine the ability of farnesol to sensitize *S. aureus* to a variety of antibiotics with different modes of action (28). For both strains of staphylococci, farnesol was only able to reverse resistance at a high concentration (150 μM). However, it was very successful at enhancing the antimicrobial efficacy of all of the antibiotics to which the strains were somewhat susceptible. Therefore, the generalized stress upon the bacterial cells caused by this membrane-permeabilizing agent might increase the ease with which antimicrobial agents can eliminate *S. aureus* populations. This ability of farnesol to sensitize *S. aureus* to such a heterogeneous group of antibiotics underlines the nonspecific nature of this enhancing activity. Since the farnesol cell-cell signaling molecule and the entire quorum-sensing system of *Candida* species are reasonably different from the autoinducing peptides and accessory gene regulator system of *S. aureus*, it is highly doubtful that the antimicrobial effect of farnesol has anything to do with quorum-sensing cross talk. In addition, the farnesol-dependent disruption of membrane integrity adequately accounts for the results presented in this study.

We hypothesized that farnesol, while nonspecifically enhancing the efficacy of a wide variety of antimicrobial agents, may act synergistically with certain groups of antibiotics, in particular, aminoglycosides (e.g., gentamicin). This class of antibiotics cannot readily penetrate intact bacterial cells to affect ribosomal function and requires ATP-dependent transport to enter the cells. Therefore, one may conclude that this class of antimicrobials would not be very effective against the anaerobic and nutrient-limited regions of the staphylococcal

biofilm, where cells are intact but ATP is in short supply, as was shown in a number of studies (4, 5, 38, 41). Therefore, we tested the ability of farnesol to act in synergy with gentamicin by disrupting the membrane of biofilm-embedded bacterial cells, thereby facilitating gentamicin entry and aiding in the clearance of staphylococcal biofilms.

The synergy testing of farnesol and gentamicin was performed with static staphylococcal biofilms exposed to various concentrations of both agents. Colony counts of harvested biofilm cells at 0, 4, and 24 h posttreatment indicated that the combined effect of gentamicin at 2.5 times the MIC and farnesol at 100 μM (22 μg/ml) was able to reduce bacterial populations by more than 2 log units, demonstrating synergy between the two antimicrobial agents. This observed sensitization of resistant strains to antimicrobials and the observed synergistic effect with gentamicin indicate a potential application for farnesol as an adjuvant therapeutic agent. While addition of farnesol to gentamicin in the treatment of *S. aureus* biofilms was able to reduce the bacterial concentrations by more than 2 log units, the resulting 10^7 CFU/cm^2 was still a significant number of bacteria. However, these studies were performed with a single farnesol-and-gentamicin dose applied to the biofilms for 24 h. Considering that the usual treatment for most biofilm infections like osteomyelitis or prosthetic implant infections is removal of the nidus of infection, followed by 4 to 6 weeks of continuous-dose intravenous antibiotic administration, farnesol may demonstrate even higher efficacy in vivo.

Because of the increasing resistance of *S. aureus* to various antibiotics, much effort is being exerted to identify novel compounds with antibacterial activity and to analyze their mechanisms of action. Specifically, there is a critical need for identifying therapeutic strategies that are directed toward the inhibition of biofilm formation and effective treatment of biofilms once they have formed. Although the mechanism of killing is not fully understood, the data from this investigation indicate a potential application for farnesol as an adjuvant therapeutic agent for the treatment or prevention of biofilm-related infections and for promoting antimicrobial resistance reversal.

Biofilms more often consist of mixed microbial species (1). Staphylococci and *Candida* are the most commonly isolated bloodstream pathogens, the result of indwelling medical devices often infected by both species at the same time (1, 23). Their coisolation from septicemic patients is usually an indication of an adverse outcome. Immunocompromised individuals in particular are the most susceptible. The potency of farnesol against bacteria and fungi indicate a potential for application in polymicrobial biofilms, where these species often coexist and are difficult to treat.

There is a critical need for the development of novel antimicrobial compounds to treat the growing number of infections where antibiotic resistance is a serious threat, especially in situations where biofilms are involved. Investigations into the molecular mechanisms involved in the inhibition of biofilm by farnesol and its involvement in the complex quorum response in *S. aureus* are therefore warranted and are currently being pursued in our laboratory.

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