Impact of a Cross-Kingdom Signaling Molecule of *Candida albicans* on *Acinetobacter baumannii* Physiology

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Multidrug-resistant (MDR) *Acinetobacter baumannii* is an opportunistic human pathogen that has become highly problematic in the clinical environment. Novel therapies are desperately required. To assist in identifying new therapeutic targets, the antagonistic interactions between *A. baumannii* and the most common human fungal pathogen, *Candida albicans*, were studied. We have observed that the *C. albicans* quorum-sensing molecule, farnesol, has cross-kingdom interactions, affecting the viability of *A. baumannii*. To gain an understanding of its mechanism, the transcriptional profile of *A. baumannii* exposed to farnesol was examined. Farnesol caused dysregulation of a large number of genes involved in cell membrane biogenesis, multidrug efflux pumps (AcrAB-like and AdeIJK-like), and *A. baumannii* virulence traits such as biofilm formation (*csuA*, *csuB*, and *ompA*) and motility (*pilZ* and *pilH*). We also observed a strong induction in genes involved in cell division (*minD*, *minE*, *ftsK*, *ftsB*, and *ftsL*). These transcriptional data were supported by functional assays showing that farnesol disrupts *A. baumannii* cell membrane integrity, alters cell morphology, and impairs virulence characteristics such as biofilm formation and twitching motility. Moreover, we showed that *A. baumannii* uses efflux pumps as a defense mechanism against this eukaryotic signaling molecule. Owing to its effects on membrane integrity, farnesol was tested to see if it potentiated the activity of the membrane-acting polymyxin antibiotic colistin. When coadministered, farnesol increased sensitivity to colistin for otherwise resistant strains. These data provide mechanistic understanding of the antagonistic interactions between diverse pathogens and may provide important insights into novel therapeutic strategies.

Acinetobacter baumannii is a Gram-negative coccobacillus that has emerged as a clinically important pathogen worldwide (1). Its success in the hospital environment can be attributed to its ability to survive for extended periods on abiotic hospital surfaces (2, 3) and its ability to rapidly acquire antibiotic resistance (4, 5). In fact, human infections with *A. baumannii* isolates that are resistant to all known antibiotics have now been reported (6). The range of infections caused by *A. baumannii* is extensive and includes bacteremia, pneumonia, urinary tract infection, skin and wound infections, meningitis, and endocarditis (7). With the decline in the discovery and development of new antimicrobials, novel therapeutic approaches for organisms such as *A. baumannii* are desperately required.

Within the hospital setting, *A. baumannii* often shares an ecological niche with the yeast *Candida albicans*, especially in intensive care units (6, 8, 9). We and others have previously described an antagonistic interaction between these two organisms, whereby *A. baumannii* can kill *C. albicans* and inhibit its ability to form filaments *in vivo* (10). However, when *C. albicans* was allowed to form a quorum in a biofilm environment, *A. baumannii* survival was significantly decreased (10). This counteroffensive by *C. albicans* was shown to be mediated by farnesol (10).

Farnesol was first identified as an extracellular sesquiterpene that was responsible for mediating quorum sensing in *C. albicans* (11). Quorum sensing is a method of cell-cell communication used by microorganisms to coordinate their gene expression in response to population density (12, 13). Farnesol is produced continuously during *C. albicans* growth and has been shown to prevent yeast-to-filament conversion (11). Farnesol has also been reported to affect the viability and virulence of several bacterial species (14–18).

In this study, we investigated the molecular mechanisms of reduced *A. baumannii* survival in the presence of farnesol. By using transcriptome-wide profiling and functional studies, we showed that farnesol interferes with *A. baumannii* membrane integrity and critical cell division machinery and inhibits key virulence characteristics such as biofilm formation and motility. Applying this mechanistic understanding, we showed that farnesol synergizes with the membrane-acting antibiotic colistin in killing multidrug-resistant *A. baumannii*, providing greater insights into potential future therapeutics.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study are shown in Table 1. Cultures were grown in heart infusion (HI) broth at 37°C with vigorous shaking. Farnesol (Sigma-Aldrich) was prepared as a 50 mM stock solution in methanol and added to the concen-

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TABLE 1 *Acinetobacter baumannii* strains used in this study

Strain	Details	Source or reference(s)
ATCC 19606	Type strain; clinical isolate (urine)	American Type Culture Collection
ATCC 17978	Clinical isolate (meningitis); colistin MIC of 2 µg/ml	American Type Culture Collection
AB307	Clinical isolate (blood); antibiotic susceptible	45, 46
AB0059	Clinical isolate (urine); multidrug resistant; colistin MIC of 1 µg/ml	23
A9844	Clinical isolate; multidrug resistant	10, 24

tration desired. Control cultures received an equimolar concentration of methanol. After 18 h of incubation, bacterial growth was determined by viable counts.

RNA extraction and transcriptome analysis. An overnight culture of *A. baumannii* ATCC 17978 was diluted 1:100 in 100 ml of fresh HI broth and grown to an optical density at 600 nm (OD₆₀₀) of 1.5 at 37°C with vigorous shaking. The culture was then divided into two 40-ml cultures in 250-ml glass flasks and exposed to either 0 mM or 0.25 mM farnesol for 1 h at 37°C with shaking. RNA extraction and transcriptome analysis were performed as previously described (19). The ratio of normalized reads between *A. baumannii* without and with farnesol exposure was calculated, and genes with a ≥2-fold change and a false-discovery rate of <0.01 were considered to be significant.

Quantitative real-time PCR. Quantitative real-time PCR was used to verify the transcriptional changes of a subset of differentially regulated genes ($n = 6$). Reverse transcription was performed as previously described (19). Triplicate quantitative real-time PCRs were conducted using a Mastercycler Ep Realplex4 (Eppendorf). A *t* test was used to determine statistical significance between samples (data not shown).

Ethidium bromide uptake. Ethidium bromide uptake was used to measure cell membrane permeability. One-milliliter volumes of 1×10^8 CFU of bacteria were suspended in phosphate-buffered saline (PBS) with or without 0.5 mM farnesol and then incubated at 37°C with shaking for 2 h. Cell suspensions were exposed to 100 µM ethidium bromide (Promega) and incubated at room temperature for 15 min. Cells were washed and resuspended in PBS. Ethidium bromide uptake was recorded by measuring the fluorescence intensity (λ_{excite} , 510 nm; λ_{emit} , 590 nm) using an Infinite M200 plate reader (Tecan). The experiment was performed four times.

Assessment of resistance to oxidative stress. Bacterial resistance to oxidative stress was assessed by serial microdilution of oxidizing agents (hydrogen peroxide or cumene hydroperoxide) in HI medium in the presence or absence of farnesol. The MIC was determined after 16 h of incubation at 37°C.

SEM. *A. baumannii* ATCC 17978 was grown overnight in the presence of 0.5 mM farnesol. Cells were collected by centrifugation at $4,000 \times g$ for 5 min, resuspended in 2.5% glutaraldehyde, and fixed onto 13-mm plastic coverslips (Thermanox) for 2 h. Coverslips were prepared for scanning electron microscopy (SEM) as described by Uwamahoro et al. (47). The samples were viewed on a Hitachi S570 scanning electron microscope, and images were captured with a Gatan model 791 digital camera.

Twitching motility assay. Twitching motility assays were conducted as described previously (20). Briefly, a 1-µl drop of an *A. baumannii* ATCC 17978 overnight culture was placed onto the center of a 0.25% modified LB agar plate containing 0 mM, 0.25 mM, or 0.5 mM farnesol. Plates were incubated at 37°C for up to 5 days, and three or more independent experiments were performed.

Biofilm formation. Biofilms were assessed using 96-well polystyrene microtiter plates (Falcon). Dilutions (100-fold) of *A. baumannii* were grown statically at 37°C for 24 h in 200 µl of HI broth supplemented with 0 mM, 0.25 mM, 0.5 mM, 1 mM, or 2 mM farnesol. Each experiment consisted of 20 replicates at each concentration. Cell biomass was estimated by measuring the OD₆₀₀ of the culture. Biofilms were quantified by staining with crystal violet as described previously (21). The OD₅₉₀/OD₆₀₀ ratio was used to normalize the amount of biofilm formed to the total cell

content in each well and was represented as an average of 20 technical replicates. The biofilm assay was performed four times. *A. baumannii* biofilm formation was also assessed on glass. Cultures were grown as described for the transcriptome analysis. The OD₆₀₀ was measured after 1 h of exposure to 0 mM or 0.25 mM farnesol, and biofilms were quantified as described above. The biofilm assay was performed four times.

Efflux pump inhibition. To determine whether multidrug efflux pumps play a role in farnesol resistance, *A. baumannii* ATCC 17978 was cultured overnight with or without 0.5 mM farnesol in the presence of the efflux pump inhibitor phenyl-arginine-β-naphthylamide dihydrochloride (PAβN) at 100 mg/liter (Sigma-Aldrich).

Time-kill assays. The killing kinetics of farnesol (0.5 mM) in combination with colistin (1 µg/ml) or imipenem (1 µg/ml) was assessed against the farnesol-resistant strains ATCC 17978 and AB0059. This colistin concentration was used because it is an achievable concentration in human serum and was at or below the MIC for each strain. Time-kill assays were performed in triplicate in cation-adjusted Mueller-Hinton II broth as described previously (22).

RESULTS AND DISCUSSION

The *Candida albicans* quorum-sensing molecule, farnesol, affects the viability of *A. baumannii*. In the hospital environment, particularly in intensive care units and in patients with burn wounds, *C. albicans* cells often interact with hospital-acquired bacterial pathogens, such as *A. baumannii*. It was found that the *C. albicans* quorum-sensing molecule, farnesol, has inhibitory effects on *A. baumannii* (10).

The effect of farnesol on the growth of *A. baumannii* ATCC 19606 was further analyzed, and it was found that growth was significantly inhibited by farnesol, by ~2 log CFU/ml, at a concentration of 0.5 mM (Fig. 1). Growth inhibition was also seen with up to 2 mM farnesol (data not shown). The inhibitory effects of farnesol were tested against other clinical *A. baumannii* isolates

FIG 1 Inhibitory effects of farnesol on the growth of various *A. baumannii* strains. Bacteria were grown for 18 h in HI medium with or without 0.5 mM farnesol, and the growth was assessed by viable counts. Error bars correspond to standard errors. *, $P < 0.05$ (Student's *t* test).

FIG 2 Graphical representation of differential *A. baumannii* gene regulation in the presence of farnesol. Genes with a significant difference in regulation in the presence of farnesol were clustered according to reported function, cluster of orthologous group (COG), and gene ontology (GO) group. The dashed lines represent the 2-fold up- and downregulation threshold.

with different degrees of antibiotic resistance (Fig. 1). Farnesol significantly inhibited the growth of an antibiotic-susceptible isolate (AB307) but not multidrug-resistant clinical isolates AB0059 (23) and A9844 (24) (Fig. 1).

Farnesol impacts *A. baumannii* gene expression relating to membrane integrity, structure, and function. To determine possible mechanisms by which farnesol impairs the viability of *A. baumannii*, RNA sequencing was performed on exponentially growing *A. baumannii* ATCC 17978 exposed to farnesol for 1 h. A subinhibitory concentration of farnesol was used (0.25 mM) to minimize transcriptional changes due to stress; this concentration of farnesol had no appreciable impact on the growth rate of ATCC 17978 (data not shown). *A. baumannii* ATCC 17978 was selected for analysis due to the availability of a fully sequenced genome and the ability to align reads accurately. Farnesol exposure had a dramatic effect on the global transcriptome of *A. baumannii*, with 382 genes significantly upregulated and 618 genes downregulated (see Tables S1 and S2 in the supplemental material). Differentially expressed genes were categorized based on cluster of orthologous groups (COG) and gene ontology (GO) assignment. Interestingly, one of the most highly represented categories was related to membrane structure, with 89 differentially regulated genes classified as GO 0031224 (“intrinsic to membrane”) (51 down and 38 up) and 76 genes related to lipid metabolism and membrane biogenesis (33 down and 43 up) (Fig. 2). This suggests that farnesol may interfere with membrane structure, triggering a compensatory response. Also highly represented were GO categories relating to DNA binding and transcription (105 genes), suggesting that the response to farnesol exposure may be mediated by numerous transcription factors. Of interest, the virulence factor *ompA*

FIG 3 Impact of farnesol on various phenotypes of *A. baumannii* ATCC 17978. (A) Cellular morphology in the presence of farnesol. Overnight cultures of bacteria grown in the presence or absence of farnesol were fixed and viewed by scanning electron microscopy. (B) Motility in the presence of farnesol. A bacterial culture (1 μ l) was dropped onto the center of a modified LB plate (solidified with 0.25% agar) and incubated for 5 days. (C and D) Biofilm formation on a polystyrene surface after 24 h of incubation at 37°C with various concentrations of farnesol (C) or a glass surface after incubation for 1 h with 0.25 mM farnesol (D). Biofilm density was quantified by crystal violet staining (OD_{590}) and adjusted by the concentration of planktonic cells in broth (OD_{600}). Error bars correspond to one standard error. *, $P < 0.05$ (Student's *t* test from three to five independent experiments).

(A1S_2840) was upregulated in the presence of farnesol (3.2-fold). *A. baumannii* OmpA has been shown to facilitate the adhesion to, and killing of, *C. albicans* (25), and it is therefore possible that farnesol exposure causes *A. baumannii* cells to equip themselves for defense against this fungal pathogen.

Farnesol impacts *A. baumannii* cell division machinery, predisposing it to reduced viability. Apart from changes in expression of genes involved in membrane biogenesis, many genes responsible for cell division also had altered expression in farnesol-exposed *A. baumannii*, including *minD* (A1S_0880; 2.4-fold up), *minE* (A1S_0879; 2-fold up), *ftsK* (A1S_0876; 2.1-fold up), a *ftsB*-like gene (A1S_1896; 2.4-fold down), and *ftsL* (A1S_3205; 2.6-fold down) (26). To determine the impact of farnesol exposure on cell structure, the morphology of farnesol-treated cells was examined by scanning electron microscopy (Fig. 3A). Untreated *A. baumannii* cells exhibited a normal, coccobacillus shape. In

contrast, farnesol-treated cells were abnormally shaped, with many losing their coccoid form and becoming long, nonseptate filaments. Furthermore, the farnesol-treated cells had rougher surfaces with visible pits that were rarely observed in control cells (Fig. 3A), suggesting compromised membrane integrity.

The defects in cell division in farnesol-treated bacteria can be explained through previous studies. In farnesol-exposed cells, *minD* and *minE*, which are required for the correct positioning of the Z-ring at the midcell (27), were upregulated. MinD, along with MinC, forms a nonspecific septation inhibitor that prevents the assembly of the Z-ring (28). MinE shields the midcell site from MinCD in order to allow Z-ring assembly at this site (29). Overexpression of *minD*, as seen in farnesol-exposed *A. baumannii*, has been shown to inhibit Z-ring formation throughout the cell, causing filamentation and cell death (30). We also observed overexpression of *ftsK*, which can similarly inhibit Z-ring formation and cell division (31). FtsB and FtsL, which were downregulated in farnesol-exposed *A. baumannii*, are small membrane proteins that form a complex with FtsQ (32). In *E. coli*, deletion of *ftsL* led to impaired cell division, with mutants forming long, nonseptate filaments that eventually ceased to grow and underwent lysis (33). Together, these data explain the observed defects in cell division in farnesol-exposed *A. baumannii* and provide a potential reason for the reduced viability of *A. baumannii* in the presence of this eukaryotic quorum-sensing molecule.

Farnesol inhibits *A. baumannii* twitching motility. *A. baumannii* is capable of flagellum-independent movement on agar plates, either on the surface or at the plate-agar interface. This motility may be twitching motility mediated by the extension and retraction of type IV pili (20), and it may be important for host colonization and biofilm formation (21, 34). The genes encoding numerous pilus biogenesis or motility-associated proteins were downregulated in the presence of farnesol, including the genes for PilH (2.6-fold), PilI (2.2-fold), PilS (2.5-fold), PilV (6.7-fold), PilW (2.3-fold), and PilZ (4.9-fold), the A1S_1507 gene (3.3-fold), and the A1S_1508 gene (3.2-fold). Motility assays confirmed the functional impact of farnesol, with motility of ATCC 17978 abolished in the presence of the molecule at 0.25 mM and 0.5 mM. An impact upon motility was observed after 24 h of incubation, and plates were monitored for up to 5 days (Fig. 3B). The motility of strain AB307 was also ablated by farnesol, while no motility was observed for A9844 (data not shown). Transmission electron microscopy (TEM) analysis of control and 0.5 mM farnesol-treated ATCC 17978 cells found no difference in pilus formation between the two groups (data not shown), suggesting that the motility deficiency may lie in the pilus structure or extension and retraction.

***Candida*-derived farnesol antagonizes the key virulence attribute of *A. baumannii*: biofilm formation.** In the hospital environment, *Acinetobacter* species are frequently isolated from abiotic hospital surfaces and medical devices, such as ventilator tubing and catheters (1). Survival of *Acinetobacter* on surfaces relies on the ability to form biofilms, and pili encoded by the *csuA/BABCDE* chaperone-usher secretion system are essential for this process (35, 36). Farnesol exposure led to significant downregulation of components of this pilus assembly system, *csuA* (A1S_2217; 6.9-fold) and *csuB* (A1S_2216; 4.8-fold). We therefore hypothesized that farnesol may impact *A. baumannii* biofilms. A significant reduction in biofilm formation was observed with farnesol-exposed *A. baumannii* ATCC 17978 on plastic and

FIG 4 Drug efflux pumps are required for farnesol resistance in *A. baumannii* ATCC 17978. Bacteria were cultured overnight in either HI medium containing either farnesol (0.5 mM), the efflux pump inhibitor phenyl-arginine- β -naphthylamide dihydrochloride (PA β N; 100 mg/liter), or a combination of both reagents. Bacterial growth was determined by viable count. Error bars correspond to one standard error. ****, $P < 0.0001$ (Student's *t* test from three independent experiments).

glass, consistent with the findings of the transcriptomic analysis (Fig. 3C and D). Farnesol exposure caused a significant decrease in biofilm formation on plastic for AB0059, while a slight increase in biofilm formation was observed for strain A9844 (data not shown). These effects may confer a direct competitive advantage to *C. albicans* in the clinical setting through the perturbation of normal *A. baumannii* biofilm formation. Furthermore, these data provide important insights into new therapeutics targeting *A. baumannii* biofilms.

Efflux pumps are important for farnesol resistance. Two clusters representing RND efflux pumps were found to be upregulated in the farnesol-exposed cells. The genes A1S_3445/3446 (a single gene in most annotations of *A. baumannii*, suggesting an error in the sequence of ATCC 17978) and A1S_3447 (encoding a putative AcrAB multidrug efflux pump) were 7- to 10-fold upregulated in the presence of farnesol. The A1S_2735, A1S_2736, and A1S_2737 products represent an AdeIJK multidrug efflux pump, and the genes encoding these proteins were 3- to 4-fold upregulated in the presence of farnesol. Both AcrAB and AdeIJK efflux pumps provide resistance to multiple antibiotics, including tetracycline, β -lactams, chloramphenicol, fluoroquinolones, novobiocin, erythromycin, fusidic acid, and rifampin (37, 38). To determine the importance of drug efflux pumps in farnesol resistance, multidrug efflux in *A. baumannii* strain ATCC 17978 was inhibited by the broad-spectrum pump inhibitor PA β N. PA β N alone and farnesol alone had no significant effect on *A. baumannii* growth, with bacterial growth similar to that of the control. However, when *A. baumannii* was cultured in the presence of both PA β N and farnesol, a significant (5-log) reduction in CFU per milliliter was observed ($P < 0.001$) (Fig. 4). This suggests that efflux-based mechanisms are important for *A. baumannii* resistance to farnesol and that increasing the expression of these pumps in the presence of farnesol may be a defense mechanism. In addition to drug export, RND efflux pumps have also been shown to accommodate the export of the homoserine lactones involved in quorum sensing (39). As farnesol shares structural homology with the *A. baumannii* homoserine lactone, it is plausible that its efflux from bacterial cells would be mediated via the same pumps.

Farnesol affects *A. baumannii* membrane permeability. A large proportion of genes differentially regulated in the presence of farnesol were related to membrane components or membrane synthesis. Furthermore, scanning electron microscopy identified

abnormal surface appearance indicative of membrane abnormalities (Fig. 3B). The membrane integrity of farnesol-exposed *A. baumannii* was analyzed using an ethidium bromide exclusion assay; ethidium bromide is membrane impermeable and is excluded from intact cells, but it becomes highly fluorescent when it enters permeabilized cells and binds to DNA (14, 15). After 15 min of exposure to ethidium bromide, farnesol-treated cells were 7-fold more fluorescent than untreated cells (12,439 luminescent units versus 1,746 units; $P < 0.0001$). This result confirms that farnesol disrupts *A. baumannii* membrane integrity.

Studies have shown that farnesol affects the cell membrane integrity of *Staphylococcus aureus*, *Lactococcus fermentum*, and *Streptococcus mutans* (14–16). The lipophilic nature of farnesol suggests that it may accumulate in bacterial membranes, where it may cause perturbations in membrane permeability and fluidity (11), thereby inducing a transcriptional response to repair the damage. Indeed, the hydrophobic autoinducer of the *Pseudomonas* quinolone signal (PQS) system of *P. aeruginosa* is delivered to recipient cells through perturbations of outer membrane structure; PQS inserts into the host cell membrane, altering membrane fluidity and inducing vesicle formation. The resulting vesicles then act as a vehicle to deliver signals to recipient cells (40). It is possible that the hydrophobic molecule farnesol has similarly disruptive effects on Gram-negative bacterial membranes.

Perturbation of the outer membrane in other Gram-negative bacteria, such as *Vibrio cholerae* and *E. coli*, has been shown to escalate internal oxidative stress (41). We observed that a number of oxidative stress response genes were upregulated in farnesol-exposed *A. baumannii*, including catalases, superoxide dismutases, and glutathione peroxidase (see Table S1 in the supplemental material), which may have occurred as a consequence of membrane damage. Despite the apparent response to oxidative stress, exposure to farnesol did not enhance the susceptibility of *A. baumannii* to oxidative stress induced by hydrogen peroxide or cumene hydroperoxide (data not shown).

Farnesol sensitizes *A. baumannii* to the killing effects of the antibiotic colistin. The outer membrane of bacteria is an essential permeability barrier to antibiotics and other environmental stresses. The disruption of membrane integrity by farnesol raised the possibility that farnesol may sensitize *A. baumannii* to antibiotics. To test this theory, a sensitive (ATCC 17978) and a multi-drug-resistant (AB0059) *A. baumannii* strain were exposed to the polymyxin antibiotic colistin, either alone or in combination with 0.5 mM farnesol. Both strains were initially inhibited by colistin but recovered over the duration of the experiment (Fig. 5). In contrast, when exposed to farnesol and colistin in combination, both strains were significantly inhibited. Neither strain was inhibited by farnesol alone. In contrast, no synergy was observed between farnesol and the β -lactam antibiotic imipenem (data not shown).

Polymyxins are amphipathic molecules that initially undergo a charge-based interaction with the anionic lipopolysaccharide (LPS) of the bacterial outer membrane (42); accordingly, mutations that reduce the net charge of the outer membrane confer colistin resistance (43). Subsequently, polymyxin molecules undergo hydrophobic interaction with the outer membrane, resulting in the release of LPS into the extracellular milieu (42). Farnesol may increase the activity of colistin by disrupting the outer membrane, thereby increasing the ability of colistin to bind and solubilize membrane lipids. Various sesquiterpenoids have previously

FIG 5 Impact of farnesol on susceptibility to the antibiotic colistin. *A. baumannii* strains ATCC 17978 (A) and AB0059 (B) were grown in HI medium alone (control) or HI medium containing either a subinhibitory concentration of farnesol (0.5 mM), colistin (1 µg/ml), or a combination of the two reagents. Viable counts were determined over a 48-h period. Results for representative experiment from triplicate assays are presented. The limit of detection was 10 CFU.

been shown to sensitize *S. aureus* and *E. coli* to the action of different antibiotics, most likely through disruption of the permeability barrier function of cell membranes (15).

Conclusions. *A. baumannii* has been described by the Infectious Diseases Society of America as one of the greatest threats to our current antibiotic armamentarium (44). The development of novel antimicrobial therapies for the treatment of antibiotic-resistant infections is critical. Our investigation has shown that farnesol affects the viability of different *A. baumannii* strains and is also capable of disrupting bacterial cell membranes. This study found that farnesol inhibits processes important for host infection, such as twitching motility and biofilm formation, and identified a role for RND efflux pumps in the resistance of *A. baumannii* to farnesol. Furthermore, farnesol resulted in increased sensitivity to colistin, most likely through perturbations in outer membrane structure. The findings of this study highlight the value of investigating bacterial-fungal interactions for the purpose of understanding the mechanisms by which competing organisms antagonize each other. This could potentially lead to the identification of targets for novel antimicrobials for the treatment of problematic human pathogens.

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