Research Article

Effect of Tyrosol and Farnesol on Virulence and Antibiotic Resistance of Clinical Isolates of Pseudomonas aeruginosa

Shaymaa Hassan Abdel-Rhman,1 Areej Mostafa El-Mahdy,1,2 and Mohammed El-Mowafy1

1Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
2Department of Pharmaceutical Sciences, Faculty of Pharmacy, Princess Norah Bint Abdulrahman University, Riyadh 11671, Saudi Arabia

Correspondence should be addressed to Mohammed El-Mowafy; mohammedasaad7@gmail.com

Received 11 September 2015; Revised 31 October 2015; Accepted 14 December 2015

1. Introduction

Quorum sensing (QS) compounds are signaling molecules that are released by the cells in a manner depending on their population density. In bacteria, the concentration of these compounds is elevated as the number of bacteria increases. Such autostimulatory compounds induce the population to cooperate in diverse behaviors such as bioluminescence, antibiotic production, virulence, biofilm formation, and sporulation [1]. In Gram-negative bacteria, these compounds are usually acyl homoserine lactones, whereas in Gram-positive bacteria, they are often modified peptides [2]. In Candida albicans, farnesol (sesquiterpene derivative) [3] and tyrosol (tyrosine derivative) [4] were identified as the main QS compounds in this pathogenic yeast.

Farnesol is a metabolic product of mevalonate/sterol synthetic pathway in eukaryotes [3]. Weber et al., 2008, have shown that farnesol produced in situ by planktonic C. albicans cultures inhibits biofilm formation [5]. The accumulation of farnesol was found to block the morphological shift from yeast to hyphae form at high cell densities [3], suggesting that Candida pathogenesis may be reduced by farnesol [6]. Contrary to farnesol, tyrosol was found to accelerate the morphological transition from yeasts to hyphae [7].

Quorum sensing signals were found not only to modulate single species bacterial and fungal communications, but also to mediate crosstalk between bacteria and the fungus C. albicans [8]. Mixed bacterial-C. albicans coinfections can be associated with an increase in the severity of the disease. For example, coinoculation of C. albicans and Staphylococcus aureus in mice was associated with high mortality in comparison with sole inoculation of S. aureus [9, 10]. The bacterial-C. albicans interactions were not restricted to enhancement of virulence of bacterial species but they were also extended to affect the bacterial resistance to antimicrobial agents. For instance, it was demonstrated that the human pathogen S. aureus forms larger biofilms with increased resistance to vancomycin when it is cocultured with C. albicans [11]. Biofilms of oral streptococci and C. albicans are similarly
more resistant to antibiotics than their single species counterparts [8, 10, 12].

A variety of populations of bacteria and fungi colonizes humans. Interactions between these microbial populations can be useful or detrimental to the host either in healthy state or in the settings of disease [8, 10]. A significant proportion of human microbial infections are biofilm associated, wherein the formation of mixed-species biofilms could create a protected environment that allows for survival to external assaults and facilitates different bacterial-fungal interactions [8].

The coexistence of *Pseudomonas aeruginosa* and *C. albicans* is an example of a pathogenic community. *P. aeruginosa* is extremely difficult to treat due to its high intrinsic and adaptive antibiotic resistance, ability to form biofilms in chronic infections, and broad arsenal of virulence factors [13]. Several reports indicate that *P. aeruginosa* and *C. albicans* can coexist in different opportunistic infections [14–18], and a variety of different molecular interactions between these two organisms have been investigated [18–22]. In the last years, both pathogenic organisms have been shown to be involved in device-associated nosocomial infections for almost all types of indwelling devices [23].

Therefore in this study we were interested in investigating the possible effect of *C. albicans* QS compounds, tyrosol, on the antibiotic resistance and some of the virulence factors of Egyptian isolates of *P. aeruginosa*.

### 2. Methods

#### 2.1. Bacterial Strains and Reagents

The strains of *P. aeruginosa* used in this study were clinical isolates collected from Mansoura University Hospital and obtained from different sources: burn, wound, urine, and pus. All the clinical isolates were collected after approval from the administrative authorities (Research Ethics Committee) in the Faculty of Pharmacy, Mansoura University, Egypt, on 13/10/2014, with the code number 214–70. The isolates were stored at −70°C till use. Farnesol (Sigma number 277541) and tyrosol (Sigma number 79058) were stored at −20°C before preparation of 500 mM stock solution in DMSO or water for farnesol and tyrosol, respectively. Farnesol stock solution was prepared immediately prior to each experiment and it was added to the final concentration just at the time of inoculation, where control cultures received an equivalent amount of DMSO [24]. Muller-Hinton (MH) broth, MH agar, and antibiotic sensitivity disks were obtained from Oxoid.

#### 2.2. *P. aeruginosa* Growth Assay upon Farnesol or Tyrosol Treatment

The growth of representative eight Egyptian isolates of *P. aeruginosa* was tested in the presence of different concentrations of tyrosol and farnesol according to [25] with little modification. Briefly, $2 \times 10^5$ cells/mL of *P. aeruginosa* were inoculated in MH broth supplemented with different concentrations of farnesol (10, 50, 100, and 200 μM) or tyrosol (1.2, 2.5, 5, 10, 20, 40, and 80 μM), employing 96-well plates in a final volume of 200 μL. In case of farnesol containing wells, the final volume of DMSO was 1% the total volume in each well. Farnesol and tyrosol free controls were supplemented with 1% DMSO and water, respectively. Cultures were allowed to grow at 37°C for 16 hours. The growth of the cells was determined by measuring the optical density (OD) at 600 nm using the absorbance microplate reader, BioTek ELx800. All experiments were carried out in triplicate.

The effect of farnesol and tyrosol on cell viability was also estimated by colony counting. The cell densities, incubation conditions, and the concentrations of tyrosol and farnesol were followed as previously mentioned in the measurement of OD _600 nm_ in the 96-well plates except that the culture volume was adjusted to 2 mL shaking at 150 rpm. After incubation, the cells were harvested by centrifugation at 4000 rpm for 5 min and washed with MH broth before being plated on MH agar for at least 24 h at 37°C to determine their viability. The survival rate of these cultures, expressed as colony forming units (CFU), was compared to farnesol and tyrosol free cultures.

#### 2.3. Disk Diffusion Assay

The susceptibility of *P. aeruginosa* strains to a number of clinically important antibiotics was investigated in the presence of farnesol or tyrosol. Cultures were grown for overnight at 37°C with shaking in MH broth. Aliquots (100 μL) were streaked onto MH agar plates with farnesol final concentration (10 μM) or tyrosol final concentration of (1.2 μM) followed by application of the following antibiotic-impregnated disks (Oxoid Ltd.). Nucleic acid inhibitors (ciprofloxacin [5 μg] and norfloxacin [10 μg]), cell wall synthesis inhibitors (ceftriaxone [30 μg] and cefepime [30 μg]), intermediary metabolism inhibitors (trimethoprim and sulfamethoxazole [25 μg]), and protein synthesis inhibitors (erythromycin [15 μg] and gentamicin [10 μg]) were applied to pseudomonas-seeded agar plates at specified concentration of farnesol or tyrosol. Plates were incubated at 37°C for 18 h, and the zones of inhibition surrounding the antibiotic disks were measured and recorded [26].

#### 2.4. Effect of Farnesol and Tyrosol on *P. aeruginosa* Total Proteases and Hemolysin

The effect of farnesol and tyrosol on the production of total protease and hemolysin enzymes of *P. aeruginosa* isolates was performed using previously prepared culture supernatant. Total protease activity was measured using a casein substrate as previously described [27].

The hemolysis test was carried out by incubating 0.6 mL of fresh sheep red blood cells (2%) with 0.6 mL of bacterial supernatant at 37°C for 2 h. Hemoglobin release was measured at OD _540 nm_. The test was also performed in absence of the bacterial supernatant (negative control) or in presence of SDS (0.2%) instead of the bacterial supernatant (positive control) [28].

#### 2.5. Statistical Analysis

Statistical analysis was performed using Student’s t-test for paired data. Significance was set at 0.1% using Microsoft Excel.

### 3. Results

#### 3.1. Farnesol and Tyrosol Inhibit the Growth and Viability of *P. aeruginosa*

In this work we evaluated the influence
of the quorum sensing compounds of \textit{C. albicans} (farnesol and tyrosol) on the growth of representative eight Egyptian isolates of \textit{P. aeruginosa}. These results were identical in the eight tested isolates of \textit{P. aeruginosa}. A representative data for the effect of farnesol and tyrosol on one of these isolates (isolate 1) is shown in Figures 1 and 2, respectively. The growth was inhibited after 16 h by 30% or more than 50% in the presence of 200 $\mu$M farnesol or 10 $\mu$M tyrosol, respectively. On the other side, 1.2 $\mu$M tyrosol and 50 $\mu$M farnesol either had no effect on growth or inhibited it by 10%, respectively.

Both the percent of growth (expressed as OD$_{600\text{ nm}}$) and the number of viable bacterial cells decreased proportionally with farnesol and tyrosol concentrations, suggesting that both compounds have an antibacterial activity against \textit{P. aeruginosa}.

3.2. Influence of Farnesol and Tyrosol on the Antibiotic Sensitivity of \textit{P. aeruginosa}. Disk diffusion assay showed no detectable difference in the inhibition zones obtained by the discs of tested antibiotics in presence or absence of either of the two investigated QS compounds. These results were further confirmed by data obtained from broth microdilution experiments (data not shown). These experiments were performed using twofold serial dilutions of three of the most widely administrated broad spectrum antibiotics: ceftriaxone, ciprofloxacin, and gentamicin at concentrations of 64000-0.12, 4000-0.004, and 2000-0.002 $\mu$g/mL, respectively. In both disk diffusion and broth microdilution experiments, the effect of tyrosol and farnesol was tested at concentrations 1.2 and 10 that affect the growth yield by not more than 10%.

3.3. Effect of Farnesol and Tyrosol on Total Proteases and Hemolysin Production by \textit{P. aeruginosa}. We next examined the effect of farnesol and tyrosol on the production of total proteases and hemolysin. Treating \textit{P. aeruginosa} isolates with farnesol (10 $\mu$M) or tyrosol (1.2 $\mu$M) caused reduction in the production level of hemolysin (Table 1) and total proteases (Table 2).

Tyrosol significantly inhibited the production of hemolysin ($p < 0.001$) and total proteases ($p < 0.001$) in the investigated isolates. Additionally, farnesol significantly inhibited the production hemolysin ($p < 0.001$) without signification inhibition of total proteases.

4. Discussion

The interaction between \textit{C. albicans} and \textit{P. aeruginosa} in mixed communities was reported in many studies. From the side of \textit{P. aeruginosa}, the effect of either the bacterial pathogen itself or its metabolites on \textit{C. albicans} was extensively investigated in numerous studies [11, 18, 29, 30]. Few reports have investigated the effect of coculturing of \textit{C. albicans} or incubation with farnesol on \textit{P. aeruginosa} regarding growth, virulence, and antibiotic resistance of this bacterial pathogen. For example, \textit{C. albicans} has been shown to enhance the production of \textit{P. aeruginosa} phenazine toxins in cocultured...
Table 1: Effect of farnesol and tyrosol on hemolysin production in Egyptian isolates of *P. aeruginosa*.

| Isolate number | Control Hemolysis percent caused by the isolate (SD=30.6) | Tyrosol effect Before normalization | After normalization | Farnesol effect Before normalization | After normalization |
|---------------|-----------------------------------------------------------|-----------------------------------|---------------------|-------------------------------------|---------------------|
| 1             | 23.5                                                      | 4.8175                            | −79.5               | 6.4625                              | −72.5               |
| 2             | 12.3                                                      | 1.1931                            | −90.3               | 2.46                                | −80                 |
| 3             | 27.4                                                      | 22.8242                           | −16.7               | 24.4956                             | −10.6               |
| 4             | 60.7                                                      | 11.6544                           | −80.8               | 33.8099                             | −44.3               |
| 5             | 59.8                                                      | 3.887                             | −93.5               | 17.94                               | −70                 |
| 6             | 34.1                                                      | 22.744                            | −33.3               | 30.008                              | −12                 |
| 7             | 72.3                                                      | 19.521                            | −73                 | 7.5915                              | −89.5               |
| 8             | 38.8                                                      | 30.458                            | −21.5               | 29.4104                             | −24.2               |
| 9             | 89.9                                                      | 36.4095                           | −59.5               | 21.576                              | 76                  |
| 10            | 100                                                       | 1                                 | −99                 | 25                                  | −75                 |
| 11            | 10                                                        | 9                                 | −10                 | 9                                   | −10                 |
| 12            | 79.4                                                      | 6.352                             | −92                 | 29.2192                             | −63.2               |
| 13            | 71                                                        | 20.519                            | −71.1               | 47.357                              | −33.3               |
| 14            | 22.3                                                      | 15.2978                           | −31.4               | 12.9117                             | −42.1               |
| 15            | 50.5                                                      | 13.5845                           | −73.1               | 14.7965                             | −70.7               |
| 16            | 81.1                                                      | 7.3801                            | −90.9               | 20.275                              | −75                 |
| 17            | 7.6                                                       | 6.802                             | −10.5               | 7.448                               | −2                  |
| 18            | 92.3                                                      | 27.69                             | −70                 | 50.765                              | −45                 |
| 19            | 90                                                        | 21.51                             | −76.1               | 34.65                               | −61.5               |
| 20            | 31.7                                                      | 21.4609                           | −32.3               | 10.1123                             | −68.1               |

The values in the control column represent the levels of hemolysin production by the isolates (SD = 30.6), indicated by hemolysis percent, without addition of farnesol and tyrosol. The values in the columns of tyrosol and farnesol stand for either nonnormalized values (SD = 10.2 and 13.5 for tyrosol and farnesol, resp.) or normalized values. The latter values indicate the percent of either decrease (negative values) or increase (positive values) in the production of hemolysin after normalization to untreated samples (control). All values represent the mean of three independent experiments.

colony biofilms [18, 31]. Moreover, the quorum sensing-regulated phenazines in *P. aeruginosa* were stimulated when cultured with *C. albicans* [31]. Farnesol was shown to inhibit the swarming motility in *P. aeruginosa* [32].

In this work we were interested to determine how the *C. albicans* QS compounds (tyrosol and farnesol) might affect the antibiotic sensitivity and production of the virulence factors, hemolysin and total proteases in Egyptian isolates of *P. aeruginosa*. To our knowledge, the effect of tyrosol on *P. aeruginosa* is not yet reported. Care was taken to perform firstly growth studies of a concentration-dependent manner for farnesol and tyrosol. Our results showed that the growth of *P. aeruginosa* was inhibited by more than 50% in the presence of 10 µM tyrosol, while 1.2 µM was the highest concentration that did not exhibit any effect on the growth of *P. aeruginosa*. On the other side, farnesol inhibited the growth of *P. aeruginosa* by 10 and 30% at concentrations of 50 and 200 µM, respectively. Previous report showed that farnesol did not affect the growth of *P. aeruginosa* strain PA14 at a concentration of 250 µM [24]. It is important to mention that the corresponding physiological relevant concentration of tyrosol and farnesol produced by stationary-phase *C. albicans* cultures is about 350–400 µM [4] and 50 µM [5, 33], respectively. This indicates that the *C. albicans* QS molecule, tyrosol, would certainly affect the growth of *P. aeruginosa* in mixed biofilms. Our results of the inhibitory effects of farnesol are consistent with the observed antibacterial effect of farnesol against other bacterial species such as *Staphylococcus aureus* [26], *Streptococcus mutans* [34], and *Staphylococcus epidermidis* [35].

For further investigation of the effect of tyrosol and farnesol on antibiotic sensitivity and the production of hemolysin and total proteases in the isolates of *P. aeruginosa*, subinhibitory concentrations that were not exceeding the corresponding physiological relevant concentration were further used in this study (1.2 and 10 µM for tyrosol and farnesol, resp.).

Jabra-Rizk et al., 2006, have shown that the antibiotic sensitivity in *S. aureus* was increased in the presence of farnesol [26]; they related this synergistic effect to the possible membrane permeabilizing action of farnesol that subsequently increases the sensitivity of *S. aureus* to antibiotics. Indeed, many compounds that are able to increase the sensitivity to antibiotics might have outer membrane permeabilizing action [36]. Our results showed that neither tyrosol nor farnesol, at their subinhibitory concentrations, was found to have any effect on the antibacterial activity of different antibiotics on *P. aeruginosa*. We might also conclude that farnesol and tyrosol do not have membrane permeabilizing action on *P. aeruginosa*, which is in contrast to the membrane permeabilizing action on *P. aeruginosa* strain PA14.
Table 2: Effect of farnesol and tyrosol on total protease production in Egyptian isolates of P. aeruginosa.

| Isolate number | Control Total protease production by the isolate (U/mL) | Control Total protease production by the isolate (U/mL) | Tyrosol effect | Tyrosol effect | Farnesol effect | Farnesol effect |
|---------------|------------------------------------------------|------------------------------------------------|--------------|--------------|----------------|----------------|
|               | Before normalization | After normalization | Before normalization | After normalization | Before normalization | After normalization |
| 1             | 107 | 75.6 | -29.3 | 89 | -16.8 |
| 2             | 95 | 61 | -35.8 | 57.5 | 39.5 |
| 3             | 100 | 88.5 | -11.5 | 80 | -20 |
| 4             | 60.8 | 57.3 | -5.6 | 10.6 | 82.5 |
| 5             | 198.8 | 154.6 | -22.2 | 102 | -48.7 |
| 6             | 22 | 21 | -4.5 | 22 | -0.1 |
| 7             | 76 | 60.8 | -20 | 64 | -15.8 |
| 8             | 102.8 | 85.9 | -16.4 | 102 | -0.8 |
| 9             | 128.9 | 118.2 | -8.3 | 96 | -25.5 |
| 10            | 147.9 | 115.4 | -22 | 121.8 | -17.6 |
| 11            | 65.5 | 54.2 | -17.3 | 65 | 0.8 |
| 12            | 127 | 76.4 | -39.8 | 165.4 | -30.2 |
| 13            | 33.5 | 46.4 | 38.5 | 32 | -4.5 |
| 14            | 94.5 | 78.4 | -17 | 75.5 | 20.1 |
| 15            | 160 | 91.3 | -42.9 | 143 | -10.6 |
| 16            | 63.7 | 61.8 | -3 | 62 | -2.7 |
| 17            | 90 | 70.7 | -21.4 | 101.7 | 13 |
| 18            | 109 | 100.8 | -7.5 | 99 | -9.2 |
| 19            | 130.7 | 123.8 | -5.3 | 82 | -37.3 |
| 20            | 163 | 101.5 | -37.7 | 150 | -8 |

The values in the control column represent the levels of total protease production by the isolates (SD = 44.3), as determined in [27], without addition of farnesol and tyrosol. The values in the columns of tyrosol and farnesol stand for either nonnormalized values (SD = 40 and 40.5 for tyrosol and farnesol, resp.) or normalized values. The latter values indicate the percent of either decrease (negative values) or increase (positive values) in the production of total protease after normalization to untreated samples (control). All values represent the mean of three independent experiments.

P. aeruginosa was repressed in the presence of tyrosol; however no significant decrease was observed in case of farnesol. Together with our novel observation of the antibacterial activity of tyrosol against P. aeruginosa, a suggestion for effective antimicrobial strategy may be broadly applicable to other bacterial species. Therefore, our research highlights the importance of studying effect of C. albicans QS compounds on P. aeruginosa as an example for bacterial pathogen that can coexist in mixed-species of microbial communities and might play an important role in vivo. Further studies are now in progress to identify the mechanism of inhibition of these virulence factors by tyrosol particularly the expression of hemolysin gene.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. B. Miller and B. L. Bassler, “Quorum sensing in bacteria,” Annual Review of Microbiology, vol. 55, pp. 165–199, 2001.
[2] B. L. Bassler, “Small talk. Cell-to-cell communication in bacteria,” Cell, vol. 109, no. 4, pp. 421–424, 2002.
[3] J. M. Hornby, E. C. Jensen, A. D. Lise et al., “Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol,” Applied and Environmental Microbiology, vol. 67, no. 7, pp. 2982–2992, 2001.

[4] H. Chen, M. Fujita, Q. Feng, J. Clardy, and G. R. Fink, “Tyrosol is a quorum-sensing molecule in Candida albicans,” Proceedings of the National Academy of Sciences of the United States of America, vol. 101, no. 14, pp. 5048–5052, 2004.

[5] K. Weber, R. Sohr, B. Schulz, M. Fleischhacker, and M. Ruhne, “Secretion of E, E-farnesol and biofilm formation in eight different Candida species,” Antimicrob Agents Chemother, vol. 52, no. 5, pp. 1859–1861, 2008.

[6] N. Décanis, N. Tazi, A. Correia, M. Vilanova, and M. Rouabha, “Farnesol, a fungal quorum-sensing molecule triggers Candida Albicans morphological changes by downregulating the expression of different secreted aspartyl proteinase genes,” Open Microbiology Journal, vol. 5, pp. 119–126, 2011.

[7] M. A. S. Alem, M. D. Y. Oteef, T. H. Flowers, and L. J. Douglas, “Production of tyrosol by Candida albicans biofilms and its role in quorum sensing and biofilm development,” Eukaryotic Cell, vol. 5, no. 10, pp. 1770–1779, 2006.

[8] D. K. Morales and D. A. Hogan, “Candida albicans interactions with bacteria in the context of human health and disease,” PLoS Pathogens, vol. 6, no. 4, Article ID e1000886, 2010.

[9] E. Carlson, “Effect of strain of Staphylococcus aureus on synergism with Candida albicans resulting in mouse mortality and morbidity,” Infection and Immunity, vol. 42, no. 1, pp. 285–292, 1983.

[10] M. E. Shirliff, B. M. Peters, and M. A. Jabra-Rizk, “Cross-kingdom interactions: Candida albicans and bacteria,” FEMS Microbiology Letters, vol. 299, no. 1, pp. 1–8, 2009.

[11] L. J. Holcombe, G. McAlester, C. A. Munro et al., “Pseudomonas aeruginosa secreted factors impair biofilm development in Candida albicans,” Microbiology, vol. 156, no. 5, pp. 1476–1485, 2010.

[12] L. J. Douglas, “Candida biofilms and their role in infection,” Trends in Microbiology, vol. 11, no. 1, pp. 30–36, 2003.

[13] A. Neidig, A. T. Yeung, T. Rosay et al., “TypA is involved in virulence, antimicrobial resistance and biofilm formation in Pseudomonas aeruginosa,” BMC Microbiology, vol. 13, no. 1, article 77, 2013.

[14] A. Bauernfeind, G. Hörl, R. Jungwirth et al., “Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis,” Infection, vol. 15, no. 4, pp. 270–277, 1987.

[15] C. Hermann, J. Hermann, U. Munzel, and R. Rüschel, “Bacterial flora accompanying Candida yeasts in clinical specimens,” Mycoses, vol. 42, no. 11-12, pp. 619–627, 1999.

[16] M. A. El-Azizi, S. E. Starks, and N. Khardori, “Interactions of Candida albicans with other Candida spp. and bacteria in the biofilms,” Journal of Applied Microbiology, vol. 96, no. 5, pp. 1067–1073, 2004.

[17] S. Nseir, E. Jozefowicz, B. Cavestri et al., “Impact of antifungal treatment on Candida-Pseudomonas interaction: a preliminary retrospective case-control study,” Intensive Care Medicine, vol. 33, no. 1, pp. 137–142, 2007.

[18] J. Gibson, A. Sood, and D. A. Hogan, “Pseudomonas aeruginosa-Candida albicans interactions: localization and fungal toxicity of a phenazine derivative,” Applied and Environmental Microbiology, vol. 75, no. 2, pp. 504–513, 2009.

[19] J. R. Kerr, “Suppression of fungal growth exhibited by Pseudomonas aeruginosa,” Journal of Clinical Microbiology, vol. 32, no. 2, pp. 525–527, 1994.

[20] J. R. Kerr, G. W. Taylor, A. Rutman, N. Hoiby, P. J. Cole, and R. Wilson, “Pseudomonas aeruginosa pyocyanin and 1-hydroxyphenazine inhibit fungal growth,” Journal of Clinical Pathology, vol. 52, no. 5, pp. 385–387, 1999.

[21] D. A. Hogan and R. Kolter, “Pseudomonas-Candida interactions: an ecological role for virulence factors,” Science, vol. 296, no. 5576, pp. 2229–2232, 2002.

[22] D. A. Hogan, A. Vik, and R. Kolter, “A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology,” Molecular Microbiology, vol. 54, no. 5, pp. 1212–1223, 2004.

[23] G. E. Pierce, “Pseudomonas aeruginosa, Candida albicans, and device-related nosocomial infections: implications, trends, and potential approaches for control,” Journal of Industrial Microbiology and Biotechnology, vol. 32, no. 7, pp. 309–318, 2005.

[24] C. Cugini, M. W. Calfee, J. M. Farrow III, D. K. Morales, E. C. Pesci, and D. A. Hogan, “Farnesol, a common sesquiterpene, inhibits PQS production in Pseudomonas aeruginosa,” Molecular Microbiology, vol. 65, no. 4, pp. 896–906, 2007.

[25] L. S. Derengowski, C. De-Souza-Silva, S. V. Braz et al., “Antimicrobial effect of farnesol, a Candida albicans quorum sensing molecule, on Paracoccidioides brasiliensis growth and morphogenesis,” Annals of Clinical Microbiology and Antimicrobials, vol. 8, article 13, 2009.

[26] M. A. Jabra-Rizk, T. F. Meiller, C. E. James, and M. E. Shirliff, “Effect of farnesol on Staphylococcus aureus biofilm formation and antimicrobial susceptibility,” Antimicrobial Agents and Chemotherapy, vol. 50, no. 4, pp. 1463–1469, 2006.

[27] S. H. M. Abdel-Rhma, A. M. El-Mahdy, and E. S. A. Abdelmegeed, “Optimization of protease production by Pseudomonas PAO1 and Physico-chemical characterization of the enzyme,” Journal of American Science, vol. 10, no. 7, pp. 62–72, 2014.

[28] G. Rossignol, A. Merieau, J. Guerillon et al., “Involvement of a phospholipase C in the hemolytic activity of a clinical strain of Pseudomonas fluorescens,” BMC Microbiology, vol. 8, article 189, 2008.

[29] D. K. Morales, N. J. Jacobs, S. Rajamani, M. Krishnamurthy, J. R. Cubillos-Ruiz, and D. A. Hogan, “Antifungal mechanisms by which a novel Pseudomonas aeruginosa phenazine toxin kills Candida albicans in biofilms,” Molecular Microbiology, vol. 78, no. 6, pp. 1379–1392, 2010.

[30] D. K. Morales, N. Grahl, C. Okegbe, L. E. P. Dietrich, N. J. Jacobs, and D. A. Hogan, “Control of Candida albicans metabolism and biofilm formation by Pseudomonas aeruginosa phenazines,” mbio, vol. 4, no. 1, Article ID e00526-12, 2013.

[31] C. Cugini, D. K. Morales, and D. A. Hogan, “Candida albicans-produced farnesol stimulates Pseudomonas quinolone signal production in LasR-defective Pseudomonas aeruginosa strains,” Microbiology, vol. 156, no. 10, pp. 3096–3107, 2010.

[32] G. McAlester, F. O’Gara, and J. P. Morrisey, “Signal-mediated interactions between Pseudomonas aeruginosa and Candida albicans,” Journal of Medical Microbiology, vol. 57, no. 5, pp. 563–569, 2008.

[33] M. L. Langford, A. L. Atkin, and K. W. Nickerson, “Cellular interactions of farnesol, a quorum-sensing molecule produced by Candida albicans,” Future Microbiology, vol. 4, no. 10, pp. 1353–1362, 2009.

[34] H. Koo, P. L. Rosalen, J. A. Cury, Y. K. Park, and W. H. Bowen, “Effects of compounds found in propolis on Streptococcus mutans growth and on glucosyltransferase activity,”
[35] F. I. A. Gomes, P. Teixeira, J. Azeredo, and R. Oliveira, “Effect of farnesol on planktonic and biofilm cells of *Staphylococcus epidermidis,*” *Current Microbiology,* vol. 59, no. 2, pp. 118–122, 2009.

[36] H.-L. Alakomi, A. Paananen, M.-L. Suihko, I. M. Helander, and M. Saarela, “Weaken effect of cell permeabilizers on gram-negative bacteria causing biodeterioration,” *Applied and Environmental Microbiology,* vol. 72, no. 7, pp. 4695–4703, 2006.

[37] D. E. Woods, M. S. Schaffer, H. R. Rabin, G. D. Campbell, and P. A. Sokol, “Phenotypic comparison of *Pseudomonas aeruginosa* strains isolated from a variety of clinical sites,” *Journal of Clinical Microbiology,* vol. 24, no. 2, pp. 260–264, 1986.

[38] V. Majtán, A. Hostacká, A. Kosiarová, L. Majtánová, and S. Kohútová, “On the toxinogenicity of *Pseudomonas aeruginosa* strains,” *Journal of Hygiene, Epidemiology, Microbiology, and Immunology,* vol. 35, no. 2, pp. 217–224, 1991.

[39] J. L. Kadurugamuwa and T. J. Beveridge, “Virulence factors are released from *Pseudomonas aeruginosa* in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion,” *Journal of Bacteriology,* vol. 177, no. 14, pp. 3998–4008, 1995.

[40] M. Kuroda, S. Nagasaki, R. Ito, and T. Ohta, “Sesquiterpene farnesol as a competitive inhibitor of lipase activity of *Staphylococcus aureus,*” *FEBS Microbiology Letters,* vol. 273, no. 1, pp. 28–34, 2007.