Trop J Nat Prod Res, May 2024; 8(5):7259-7263 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Biofilm Formation by Polymicrobial Interactions between *Candida albicans* with *Staphylococcus aureus* and *Enterococcus faecalis*

Fransisca Puspitasari[1], Agung D.W. Widodo[2]*, Marijam Purwanta[3]

[1]*Master Program of Basic Medical Science, Faculty of Medicine, Airlangga University, Prof. DR. Moestopo Street, Surabaya, Indonesia*
[2]*Department of Clinical Microbiology, Faculty of Medicine, Airlangga University, Prof. DR. Moestopo Street, Surabaya, Indonesia*
[3]*Department of Clinical Microbiology, Faculty of Medicine, Airlangga University, Prof. DR. Moestopo Street, Surabaya, Indonesia*

ARTICLE INFO

Article history:
Received 12 February 2024
Revised 13 May 2024
Accepted 13 May 2024
Published online 01 June 2024

ABSTRACT

Biofilm polymicrobial infections have gained international attention, especially cases involving fungi and bacteria. Such infections require patients to undergo costly, extended hospital stays and have caused a considerable amount of morbidity and mortality. Polymicrobial biofilms also have greater mortality rates than monomicrobial biofilms. This study aims to evaluate the overall biomass of biofilms produced by *Candida albicans* interacting with two Gram-positive bacteria: *E. faecalis* and *S. aureus*. Tryptic Soy Broth (TSB) medium were used for the bacterial and fungal suspensions. Three 96-well microplates were used for the biofilm cultivation. The microplates were then incubated for 24, 48, and 72 hours at 37˚C. Next, after conducting the Crystal Violet test, the biofilms' Optical Density (OD) values were measured by an ELISA reader. The mixed species treatment of *Candida albicans* with *S. aureus* for 72 hours had the highest average OD value (OD ± SD = 0.903 ± 0.050), while the mixed species treatment of *Candida albicans* with *E. faecalis* for 24 hours had the lowest average OD value (OD ± SD = 0.240 ± 0.032). In conclusion, the parameters observed in this study revealed that biofilms could be formed well by polymicrobial interactions between *Candida albicans* and two Gram-positive bacteria: *S. aureus* and *E. faecalis.*

Keywords: Biofilm, Inter Kingdom, Polymicrobial, Optical Density, Crystal Violet

Introduction

A biofilm is a population of cells adhering to various abiotic and biotic surfaces. These adherent cells would create a matrix of extracellular polysaccharides that further promote adhesion and cohesion.[33] Recently, biofilms associated with polymicrobial infections, especially those originating from bacteria and fungus, have garnered global interest. These infections tend to require patients to undergo costly, prolonged hospital stays. In addition, the mortality rate of polymicrobial infections is substantially higher than monomicrobial infections.[1,5] The interkingdom interaction between polymicrobials can lead to the production of biofilms and cause infections. Such infections can induce virulence traits, evade the immune system, and be resistant to antimicrobial drugs.[32] Additionally, biofilms have been determined to be one of the primary infection mediators, with their development associated with an estimated 80% of illnesses rate.[2] Therefore, it is important to investigate biofilm formation to minimise its negative impacts.

The fungal and bacterial pathogens *C. albicans* and *Staphylococcus spp*, respectively, are the most common causes of approximately 20% of bloodstream infections worldwide.[3] The interactions between these pathogens is called a synergistic interaction because they work together to form dense and architecturally complex biofilms[4] that can lead to increased virulence and disease severity.

*Corresponding author: E-mail: agungimunologi@gmail.com
Tel: +6281252343097

Citation: Puspitasari F, Widodo ADW, Purwanta M. Biofilm Formation by Polymicrobial Interactions between *Candida albicans* with *Staphylococcus aureus* and *Enterococcus faecalis*. Trop J Nat Prod Res. 2024; 8(5):7259-7263. https://doi.org/10.26538/tjnpr/v8i5.30

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Conversely, there is an antagonistic interaction between *C. albicans* and *E. faecalis*. Antagonistic interactions are competing interactions between microbes.[6] *E. faecalis* produces the bacteriocin *EntV*, which promotes the invasion of *C. albicans* tissue and inhibits *C. albicans* biofilm formation. This study aims to investigate the antagonistic or synergistic interactions within polymicrobial interkingdom biofilms involving *S. aureus* with *C. albicans* and *C. albicans* with *E. faecalis*. The parameters observed include measuring Optical Density values obtained by the crystal violet staining method.[7]

Materials and Methods

Ethical approval

Ethical approval was issued by the Health Research Ethical Clearance Commission (KEPK), Faculty of Medicine, Airlangga University, with certificate number 330/EC/KEPK/FKUA/2023 on November 20, 2023.

Sample culture and microbe preparation

The *S. aureus* and *E. faecalis* strains were obtained from Dr Soetomo General Hospital in Surabaya, Indonesia, on November 21, 2023. This research was conducted at the Bacteria and Mycology Laboratory, Faculty of Veterinary Medicine and Institute of Tropical Disease, Airlangga University, Indonesia, from October to November 2023.

All strains were re-cultured from culture stock to the Saboroud Dextrose Agar (SDA) medium for *C. albicans* and Tryptic Soy Agar (TSA) medium for the *S. aureus* and *E. faecalis* strains. Viability was ensured as the re-culturing of the microbes have filled 97% of the agar plate medium. The cultures were incubated for approximately 24 hours in an aerobic incubator at 37˚C. After incubation, each strain was inoculated into 5 ml of TSB (tryptic soy broth) medium (OXOID, CM0129B) and incubated aerobically at 37°C. The bacterial suspension was then compared for turbidity with a 0.5 McFarland standard (1.5 x 10^8 CFU/mL), and the fungal suspension was compared with the 1.0 McFarland standard (3.0 x 10^8 CFU/mL).[9]

Biofilm cultivation

The microbial suspensions were filled into three 96-well microplates (Biologix Europe GmbH, Germany, flat bottom TC sterile) to form the biofilms. All three microplates were used for the CV (Crystal Violet) assay. For the biofilm cultivation step, each microbial suspension was inoculated into 6 treatment wells. One well was filled with the control group (150µL of TSB medium), three wells were filled with a single species suspension (*C. albicans, E. faecalis, S. aureus)*, and two wells were inoculated with 75 µL of mixed-species suspensions (*C. albicans* + *S. aureus* and *E. faecalis* + *C. albicans*), for a total volume of 150 µL in one well. A total of 36 wells were filled due to the 6 repetitions of each treatment. After the wells were filled, the microplate was closed, covered with plastic wrap, and incubated at 37˚C. The three microplates were then incubated for different durations: 24 hours, 48 hours, and 72 hours.[8]

Crystal violet assay

To obtain the total biomass of adherent cells, the microplate wells were rinsed three times with phosphate-buffered saline (PBS, pH 7.2) to remove non-adherent cells. The microplate was then air-dried for approximately 30 minutes. Next, each well was added with 205 µL of a 0.1% (wt/vol) Crystal Violet (CV) solution (Ceristain C.I 42555), and the mixture was left to stand for 30 minutes. Then, the microplate walls added with the CV staining solution were emptied and carefully rinsed with distilled water three times. Next, the three microplates were turned over and dried at room temperature. After the microplate was dry, each treatment was added with 210 µL of 70% ethanol in preparation for the Optical Density measurement at 595 nm using an ELISA reader.[9,33]

Data analysis of biofilm

The biofilms' OD (Optical Density) values at 595 nm were measured using an ELISA reader (Thermo multiscan Go 1510, Thermo Fisher Scientific, Inc) to determine the total biomass of the biofilms after staining. The following formula format[8] was used to calculate the total biomass: ODC = $\bar{X}$ODControl + 3SDControl, ODisolate = $\bar{X}$ODtreatment – ODC. The results were then grouped based on the microbes' biofilm-producing abilities, which consist of several categories: OD < ODcut indicates a non-biofilm former (NBF), ODcut < OD < 2 ODcut indicates a weak biofilm former (WBF), 2 ODcut < OD < 4 ODcut indicates a moderate biofilm former (MBF), and OD > 4 ODcut indicates a high biofilm former (HBF).

Statistical biofilm analysis

The mean ± standard deviation was obtained for each OD result. The OD values represent the output of the CV test. Optical Density data were statistically analysed using the SPSS version 16.0 software with a 95% confidence degree (a P-value of <0.05 is statistically significant). A Shapiro-Wilk normality test analysis was also conducted. If the data is abnormal, the analysis was continued with a non-parametric test using the Friedman Test. If the data is normal, the analysis was continued with the repeated ANOVA (Analysis of Variance) parametric test. If the P-value obtained is <0.05, the result is statistically significant. Meanwhile, *C. albicans* + *S. aureus* and *E. faecalis* + *C. albicans* were compared using the unpaired T-test, where a P-value of <0.05 is considered significant. A descriptive analysis was also conducted, where the data were presented as figures, graphs, and tables.[33]

Results and Discussion

This research cultivated single and mixed fungal bacterial biofilms (*C. albicans, S. aureus, E. faecalis).* The average ± standard deviation of the OD values measured was subtracted from the cut-off OD value so that the final value can be interpreted into four categories of biofilm formation: biofilm not produced, weak biofilm production, moderate biofilm production, and strong biofilm production (Table 1). The cut-off OD value was obtained by calculating the average OD of the control and adding it to three times the standard deviation value of the negative control.

Based on these calculations, almost all biofilm treatments in this study are categorised in the high biofilm-forming category, except for the single species biofilms from *S. aureus* and *E. faecalis* being in the medium biofilm-forming category after 24 hours of incubation.

Table 1: Optical Density values of biofilm 24, 48 and 72 hours incubation time

Time(h)	Species	Optical Density Values	Crystal Violet Category
24	*C. albicans*	0.355 ± 0.022	HBF
	S. aureus	0.143 ± 0.032	MBF
	E. faecalis	0.139 ± 0.012	MBF
	C. albicans+S.aureus	0.651 ± 0.035	HBF
	C. albicans+E. faecalis	0.240 ± 0.032	HBF
	Control group	0.038 ± 0.001	-
48	*C. albicans*	0.760 ± 0.038	HBF
	S. aureus	0.599 ± 0.034	HBF
	E. faecalis	0.610 ± 0.065	HBF
	C. albicans+S. aureus	0.826 ± 0.007	HBF
	C. albicans+E. faecalis	0.484 ± 0.061	HBF
	Control group	0.038 ± 0.004	-
72	*C. albicans*	1.305 ± 0.114	HBF
	S. aureus	0.965 ± 0.027	HBF
	E. faecalis	0.817 ± 0.057	HBF
	C. albicans+S.aureus	0.903 ± 0.050	HBF
	C. albicans+E.faecalis	0.778 ± 0.044	HBF
	Control group	0.038 ± 0.003	-

Notes* OD Values = displays the absorbance value with Crystal violet assay with *P-value < 0.05 from the data considered statistically significant; HBF = high biofilm-forming; MDF = moderate biofilm-forming; OD = optical density; CV= crystal violet

The biofilms categorised as high-biofilm forming were grouped as such because they had final OD values over four times greater than the OD cut-off values at 24 hours of incubation (0.041), at 48 hours of incubation (0.052), and at 72 hours of incubation (0.046). The research results also found that of the other single species treatments, *C. albicans*, and of the other mixed-species treatments, *C. albicans + S. aureus*, at 72 hours had the highest OD values. Conversely, of the other single species treatments, *E. faecalis*, and of the other mixed-species treatments, *E. faecalis + C. albicans*, at 24 hours had the lowest OD values.

Figure 1 shows significant differences in OD in all strains of *S. aureus + C. albicans* and *E. faecalis + C. albicans* at 24, 48, and 72 hours. This study proved that the interaction of *C. albicans* with Gram-positive bacteria (*E. faecalis* and *S. aureus*) affects the biofilm formation of each species as the crystal violet staining results indicated significant differences with P-values = <0.05. The crystal violet method was applied to calculate the total biomass of living cells and dead cells, the total biofilm formed, and the exopolysaccharides formed. The crystal violet binded to the biofilm is proportional to the number of cells in the biofilm, enabling us to quantify the biofilm's biomass by measuring Optical Density values at 595 nm on the ELISA reader.[9]

Biofilms are formed when single-species and mixed-species microbes interact with each other. The interactions between bacteria and fungi include physical, chemical, differential regulation, protein expression, and other interactions.[10] Bacteria and fungi have coexisted for a long time in the same ecological niche and have interacted in various ways to improve survival. Antagonistic or synergistic interactions depend on the host, microorganism, and environment. The interactions between fungi and bacteria influence factors that induce and increase virulence.[11]

Interactions between C. albicans and S. aureus

Physical Interactions

In Figure 2, the highest biofilm biomass was observed in the single-species *C. albicans*, *S. aureus*, and the *C. albicans + S. aureus* treatments with an incubation time of 72 hours. Data from this study shows that *C. albicans* combined with *S. aureus* has the highest biofilm thickness with a synergistic interaction. These findings align with previous studies that describe how *S. aureus* physically bonds firmly to the hyphae of *C. albicans*, resulting in a dense and unique biofilm architecture. *Candida* hyphae can also penetrate the epithelial layer to help it adhere to other microbes.[17] Additionally, *Candida* hyphae plays a role in preparing exopolymeric substances and blastopores, contributing to *Candida* biofilms' strong structure.[18]

Moreover, *C. albicans* hyphae express cell wall proteins comprising *Als3p*, *Hwp1p* and *O-linked mannosylation*, which are specialised receptors for binding specific peptide ligands and attaching to the *SspA* and *SspB* cell surfaces of *S. aureus* [16]. *O-linked mannosylation* of cell surface and hyphal proteins of *C. albicans*, *Als1p*, *Als3p*, and *Hwp1p* are involved in the interactions between *C. albicans* and *S. aureus*. A previous study also found that this protein is a virulence factor in *S. aureus*, indicating that the *C. albicans* process can enhance *S. aureus* pathogenesis.[12]

Furthermore, a previous study revealed that hyphae and yeast cells play distinct roles in biofilm production.[13] This co-infection of *C. albicans + S. aureus* mediates increased mortality compared to infection by monomicrobials. Another study using a mouse model also found an 80-100% increase in mortality at 48-72 hours post-inoculation of *C. albicans + S. aureus*.[13] This co-infection also results in local and systemic inflammation and a significant increase in the virulence of the infection.[14,15]

Chemical interactions

The mechanism utilised by *C. albicans* and *S. aureus* to produce biofilms is facilitated by quorum sensing as a chemical signal is incorporated into the extracellular polysaccharide matrix (EPM). Quorum sensing (*QS*) facilitates cell formation, adhesion, and detachment from biofilms, as well as gene expression regulation within a population and cell-to-cell communication.[21] *C. albicans* secretes *QS* farnesol, tyrosol, and prostaglandin E2. Farnesol and pheromone signals in *C. albicans* regulate morphological and phenotypic conditions, mediate hyphal formation into yeast, and elicit gene expression and virulence.[22] Farnesol can also prevent hyphae filamentation by suppressing *adenylyl cyclase (Cyr1p*) in the *Ras1-cyclic AMP-protein kinase-A* pathway.[23] Meanwhile, tyrosol induces the transition from yeast to hyphae,[24] whereas the prostaglandin *E2* produced by *C. albicans* stimulates *S. aureus* biofilm formation in the polymicrobial biofilm between *C. albicans* and *S. aureus*.[25]

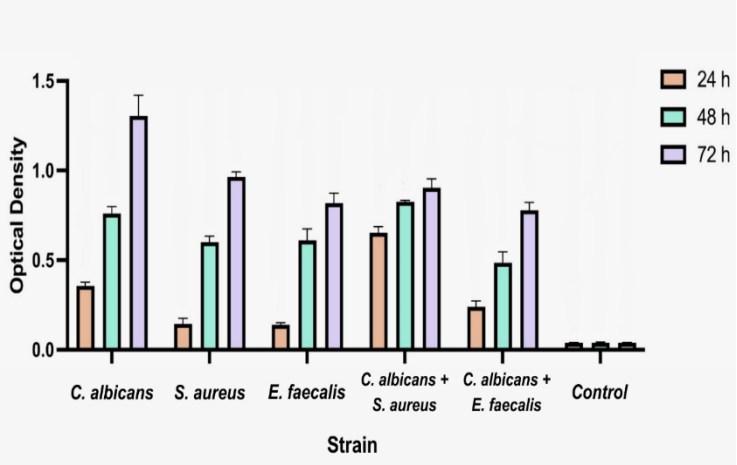

Figure 1: Graph of biofilm formation based optical density by single species and mixed species of *C. albicans*, *S. aureus* and *E. faecalis* at 24, 48, and 72 hours.

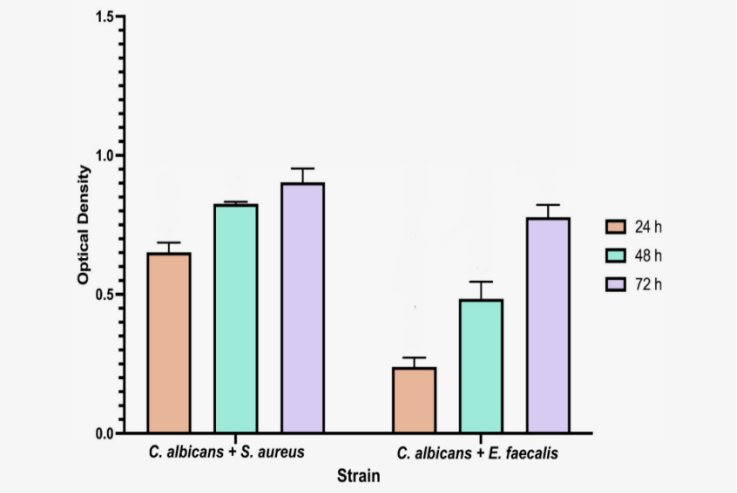

Figure 2: Graph of biofilm formation based optical density by mixed polymicrobial species of *C. albicans* with *S. aureus* and *C. albicans* with *E. faecalis* at 24, 48, and 72 hours

Differential regulation

The change in *Candida* morphology from yeast to hyphae plays a role in adhesion and colonisation. Additionally, *S. aureus'* ability to recognise adhesive matrix molecules *(MSCRAMMs)* is due to several factors, such as the upregulation of the *lrg* operon, autolysis repression, the upregulation of the *ica locus* operon, *hemB* mutations,[19] and *polysaccharide intercellular adhesin (PIA)* production, also known as *polyN-acetylglucosamine (PNAG)*, which is regulated by the *icaADBC* gene. This gene mediates bacterial cells' adhesion ability and increases eDNA production to produce biofilms and their extracellular polysaccharide matrix (EPM). Additionally, *α-glucan* forms the extracellular polysaccharide matrix (EPM) of *Staphylococcus* biofilms and *β-glucan* is present in *Candida* biofilms.[20]

Interactions between C. albicans with E. faecalis

The lowest total biomass value was obtained from the *C. albicans* and *E. faecalis* treatment. This finding aligns with previous studies as research has shown that *E. faecalis* and *Candida*

albicans have an antagonistic physical interaction that can inhibit the virulence caused by *C. albicans* infection.[6]

The interaction between *E. faecalis* and *C. albicans* inhibits the development of *C. albicans* hyphae on abiotic substrates.[6] Studies have demonstrated that *E. faecalis* can integrate itself into *C. albicans* biofilms after their hyphal and yeast develop. *E. faecalis* then produces *EntV* to inhibit *C. albicans* hyphal growth in-vitro.[6] Although *EntV* bacteriocin protein exhibits anti-hyphal and anti-virulence properties, it does not inhibit fungal growth. *EntV* bacteriocin increases *C. albicans* tissue invasion,[30] then inhibits *C. albicans* morphogenesis and biofilm formation from yeast to hyphae.[31] Even at high concentrations of *EntV*, the bacteriocin only reduces fungal virulence, and it does not kill *E. faecalis*. However, in patients with impaired immune systems, *C. albicans* and *E. faecalis* pose a severe risk of opportunistic infections.[30]

Quorum sensing is the cell-to-cell chemical signalling mechanism that controls the biofilm production process. This mechanism influences cell development, adhesion, and detachment from biofilms, regulating gene expression within a population and promoting communication between individual cells.[21] A signal from the matrix exopolysaccharide (EPS) and eDNA is sent through quorum to surrounding cells to gather, increasing biofilm development and adherence.[26] A the primary regulator of virulence in *E. faecalis*, the *FsrB* transcriptional regulator is used by the bacteria to activate the quorum sensing molecule (QSM) system via activating the *fsr* quorum sensing system through the gelatinase biosynthesis-activating cluster (GBAP) peptide. There are also other types of quorum sensing in *E. faecalis*, such as the *luxS* gene[27] and the *fsr* gene (*fsrA, fsrB, fsrC*) to regulate virulence.[28] The *fsr* gene also produces proteases gelatinase (*gelE*) and serine protease (*SprE*) to activate *EntV*.[29] Overall, the activation of quorum sensing as a chemical signal regulator in the interactions between microbial species influenced the biofilm growth seen in *E. faecalis* and *C. albicans*.

Conclusion

This study observed that biofilms can be formed from a single microbe species and combined species too. These synergistic and antagonistic interactions between fungi and bacteria are influenced by physical, chemical, differential regulations and other factors. Antagonistic or synergistic interactions depend on the host, microorganism, and environment. Interactions between fungi and bacteria influence factors that induce and increase virulence. The total biomass of biofilm obtained from the optical density value indicates the synergistic interaction between *C. albicans* and *S. aureus*, which has the highest average OD value at 72 hours of incubation (0.903 ± 0.050). In contrast, the antagonistic interaction between *C. albicans* and *E. faecalis* has the lowest average OD value at 24 hours of incubation (0.240 ± 0.032). This study's limitation is its inability to distinguish the constituent components of the biofilm formed. Therefore, further research should add parameters for observing the biofilm layer. Scanning electron microscopy can be used to obtain a visualization of the layer structure of the biofilm formed from the combination of these species. In addition, future research can add variations in the biofilm growth media, used and clinical research can be conducted on the interaction between *C. albicans* and *E. faecalis*. Further studies can also be done to explore biofilm inhibition methods with various doses of antimicrobials and the use of *E. faecalis EntV* bacteriocin as a new antifungal agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We would like to thank the Faculty of Veterinary Medicine and Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia.

References

1. Wu PF, Liu WL, Hsieh MH, Hii IM, Lee YL, Lin YT, Ho MW, Liu CE, Chen YH, Wang FD. Epidemiology and antifungal susceptibility of candidemia isolates of non-albicans *Candida* species from cancer patients. Emerg Microbes Infect. 2017;6(10):e87.
2. Carolus H, Van DK, Van DP. *Candida albicans* and *Staphylococcus* Species: A Threatening Twosome. Front Microbiol. 2019;10:2162.
3. O'Donnell LE, Millhouse E, Sherry L, Kean R, Malcolm J, Nile CJ, Ramage G. Polymicrobial *Candida* biofilms: friends and foe in the oral cavity. FEMS Yeast Res. 2015;15(7):fov077.
4. Kong EF, Tsui C, Kucharíková S, Andes D, Van DP, Jabra RMA. Commensal Protection of *Staphylococcus aureus* against Antimicrobials by *Candida albicans* Biofilm Matrix. mBio. 2016;7(5):e01365-16.
5. Costa-Orlandi CB, Sardi JCO, Pitangui NS, de Oliveira HC, Scorzoni L, Galeane MC, Medina-Alarcón KP, Melo WCMA, Marcelino MY, Braz JD, Fusco-Almeida AM, Mendes-Giannini MJS. Fungal Biofilms and Polymicrobial Diseases. J Fungi (Basel). 2017;3(2):22.
6. Graham CE, Cruz MR, Garsin DA, Lorenz MC. *Enterococcus faecalis* bacteriocin *EntV* inhibits hyphal morphogenesis, biofilm formation, and virulence of *Candida albicans*. Proc Natl Acad Sci U S A. 2017;114(17):4507-4512.
7. Präbst K, Engelhardt H, Ringgeler S, Hübner H. Basic Colorimetric Proliferation Assays: MTT, WST, and Resazurin. Methods Mol Biol. 2017;1601:1-17.
8. Corte L, Casagrande Pierantoni D, Tascini C, Roscini L, Cardinali G. Biofilm Specific Activity: A Measure to Quantify Microbial Biofilm. Microorganisms. 2019;7(3):73.
9. Haney EF, Trimble MJ, Cheng JT, Vallé Q, Hancock REW. Critical Assessment of Methods to Quantify Biofilm Growth and Evaluate Antibiofilm Activity of Host Defence Peptides. Biomolecules. 2018;8(2):29.
10. Zuza ADL, Silva RWP, Chaves GM. An Update on *Candida tropicalis* Based on Basic and Clinical Approaches. Front Microbiol. 2017;8:1927.
11. Bisht K, Baishya J, Wakeman CA. *Pseudomonas aeruginosa* polymicrobial interactions during lung infection. Curr Opin Microbiol. 2020;53:1-8.
12. Peters BM, Jabra RMA, O'May GA, Costerton JW, Shirtliff ME. Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev. 2012;25(1):193-213.
13. Todd OA, Fidel PL Jr, Harro JM, Hilliard JJ, Tkaczyk C, Sellman BR, Noverr MC, Peters BM. *Candida albicans* Augments *Staphylococcus aureus* Virulence by Engaging the *Staphylococcal agr* Quorum Sensing System. mBio. 2019;10(3):e00910-19.
14. Hager CL, Isham N, Schrom KP, Chandra J, McCormick T, Miyagi M, Ghannoum MA. Effects of a Novel Probiotic Combination on Pathogenic Bacterial-Fungal Polymicrobial Biofilms. mBio. 2019;10:10.1128/mbio.00338-19.
15. Prasad, R. *Candida albicans*: Cellular and Molecular Biology (2nd ed.). India: Springer Cham; 2017.
16. Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, Bindschedler S, Hacquard S, Hervé V, Labbé J, Lastovetsky OA, Mieszkin S, Millet LJ, Vajna B, Junier P, Bonfante P, Krom BP, Olsson S, van Elsas JD, Wick LY. Bacterial-fungal interactions: ecology, mechanisms and challenges. FEMS Microbiol Rev. 2018;42(3):335-352.
17. Kong EF, Kucharíková S, Van Dijck P, Peters BM, Shirtliff ME, Jabra-Rizk MA. Clinical implications of oral

candidiasis: host tissue damage and disseminated bacterial disease. Infect Immun. 2015;83(2):604-613.
18. Pires RH, Montanari LB, Martins CH, Zaia JE, Almeida AM, Matsumoto MT, Mendes-Giannini MJ. Anticandidal efficacy of cinnamon oil against planktonic and biofilm cultures of *Candida parapsilosis* and *Candida orthopsilosis*. Mycopathologia. 2011;172(6):453-464
19. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. 2008;46 Suppl 5(Suppl 5):S350-S359.
20. Vila T, Kong EF, Montelongo-Jauregui D, Van Dijck P, Shetty AC, McCracken C, Bruno VM, Jabra-Rizk MA. Therapeutic implications of *C. albicans*-*S. aureus* mixed biofilm in a murine subcutaneous catheter model of polymicrobial infection. Virulence. 2021;12(1):835-851.
21. Abisado RG, Benomar S, Klaus JR, Dandekar AA, Chandler JR. Bacterial Quorum Sensing and Microbial Community Interactions [published correction appears in MBio. 2018 Oct 2;9(5):]. mBio. 2018;9(3):e02331-17.
22. Ramage G, Saville SP, Wickes BL, López-Ribot JL. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl Environ Microbiol. 2002;68(11):5459-5463.
23. Bandara HMHN, Wood DLA, Vanwonterghem I, Hugenholtz P, Cheung BPK, Samaranayake LP. Fluconazole resistance in *Candida albicans* is induced by *Pseudomonas aeruginosa* quorum sensing. Sci Rep. 2020;8;10(1):7769.
24. Kovács R, Majoros L. Fungal Quorum-Sensing Molecules: A Review of Their Antifungal Effect against *Candida Biofilms*. J Fungi (Basel). 2020;6(3):99.
25. Krause J, Geginat G, Tammer I. Prostaglandin E2 from *Candida albicans* Stimulates the Growth of *Staphylococcus aureus* in Mixed Biofilms. PLoS One. 2015;10(8):e0135404.
26. Pammi M, Liang R, Hicks J, Mistretta TA, Versalovic J. Biofilm extracellular DNA enhances mixed species biofilms of *Staphylococcus epidermidis* and *Candida albicans*. BMC Microbiol. 2013;13:257.
27. He Z, Liang J, Zhou W, Xie Q, Tang Z, Ma R, Huang Z. Effect of the quorum-sensing *luxS* gene on biofilm formation by *Enterococcus faecalis*. Eur J Oral Sci. 2016;124(3):234-240.
28. Ali IAA, Lévesque CM, Neelakantan P. *Fsr* quorum sensing system modulates the temporal development of *Enterococcus faecalis* biofilm matrix. Mol Oral Microbiol. 2022;37(1):22-30.
29. Ali L, Goraya MU, Arafat Y, Ajmal M, Chen JL, Yu D. Molecular Mechanism of Quorum-Sensing in *Enterococcus faecalis*: Its Role in Virulence and Therapeutic Approaches. Int J Mol Sci. 2017;18(5):960.
30. Krishnamoorthy AL, Lemus AA, Solomon AP, Valm AM, Neelakantan P. Interactions between *Candida albicans* and *Enterococcus faecalis* in an Organotypic Oral Epithelial Model. Microorganisms. 2020;8(11):1771.
31. Zeise KD, Woods RJ, Huffnagle GB. Interplay between *Candida albicans* and Lactic Acid Bacteria in the Gastrointestinal Tract: Impact on Colonization Resistance, Microbial Carriage, Opportunistic Infection, and Host Immunity. Clin Microbiol Rev. 2021;34(4):e0032320.
32. Hamzah H, Pratiwi SUT, Nur A, Nuryastuti T, Pratama VY, Riza Maulana R. Antibacterial and Antibiofilm Activities of Ternate Blue Pea *Clitoria ternatea* Flower Extract against *Staphylococcus aureus*. Trop J Nat Prod Res. 2024; 8(1):5992 5996.
33. Helmi M, Ibrahem H. Effect of Lactobacillus Species on the Expression of Gene Related to Biofilm Formation by *Streptococcus mutans*. Trop J Nat Prod Res. 2021; 5(3):445-447.