Polymicrobial Biofilms of *C. albicans* with Bacterial Species: An Insight into Intergenus Interaction

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Purvi Joshi, Pinal Trivedi, Rohit Bhattacharjee, Muskan Sahu, and Devarshi Gajjar

Abstract

Polymicrobial biofilms are more prevalent than reported in the clinical scenario and are more complex and harmful to the host. *C. albicans,* being the most prevalent *Candida* species causing infections, has been found to colonise and infect immunocompromised humans. *C. albicans* is found to interact with various bacterial species like *Streptococcus*, *Staphylococcus*, *Pseudomonas,* and *E. coli.* This chapter focuses on this intergenus interactions and associated antimicrobial resistance.

Keywords

Polymicrobial biofilms · Microbial interactions · Antimicrobial resistance · Candida-bacteria biofilms

11.1 Introduction

Humans are colonised by various population of bacteria and fungi, forming a diverse microbial community. These microorganisms can either positively or negatively influence the host and their interactions play a significant role in determining the overall well-being of the individual.

Candida species are the leading culprits behind fungal infections on a global scale and are widely distributed within the human microbiota. *Candida albicans*, a fungus that typically coexists with humans, can be found naturally on mucosal

P. Joshi · P. Trivedi · R. Bhattacharjee · M. Sahu · D. Gajjar (✉)
Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India
e-mail: devarshi.gajjar-microbio@msubaroda.ac.in

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surfaces. Among all fungal pathogens, *C. albicans* is the most commonly encountered by the human population (Su et al. 2018). This particular species live harmoniously within the gastrointestinal and genitourinary tracts and play a significant role in hospital-acquired infections. It is responsible for about 15% of sepsis cases and contributes to 40% of bloodstream infections (Gulati and Nobile 2016). According to a report from the European Confederation of Medical Mycology (ECMM), in 14 European countries, candidiasis is the primary cause of morbidity in surgical patients, accounting for 54% of cases (Prasath et al. 2019). Reports indicate that as many as 50% of adult patients with systemic candidiasis and roughly 30% of the young population may experience mortality due to candidiasis with potential links to biofilms (Atriwal et al. 2021). Currently, there are more than 150 recognised *Candida* species, with roughly 20 of them known to cause infections in humans. *Candida albicans* notably stands out as the primary agent responsible for candidiasis, foremost fungal infection affecting both adult and paediatric patients (Kaur and Nobile 2023).

Biofilms can be defined as a community of adherent microorganisms that reside within a secreted extracellular matrix. Biofilms are notorious and cause significant health issues, particularly in medical devices like urinary catheters, cardiac devices, and prostheses. The formation of biofilms associated with *C. albicans* is typically observed in immunocompromised patients. These *C. albicans* biofilms consist of a combination of yeast, pseudo-hyphae, and hyphae structures. This transition is a result of complex alterations in phenotype and changes in gene expression (Ponde et al. 2021). The development of *C. albicans* biofilms can be broken down into four stages: (1) Initial attachment, (2) Early biofilm formation, (3) Biofilm maturation, and (4) Dispersal.

Mature *C. albicans* biofilms exhibit remarkable resistance to both stress and antifungal therapies. Key contributors to the pathogenicity of *Candida albicans* are proteins that play pivotal roles in adhesion and invasion. These factors encompass cell wall proteins and physical and chemical attributes of the cell surface (Talapko et al. 2021). There are three principal families of *C. albicans* adhesins that play significant roles in promoting adherence, namely, (1) the Agglutinin-like sequence (Als) family, (2) the Hyphal wall protein (Hwp) family, and (3) the individual protein file family F/hyphally regulated (Iff/Hyr) family (Rosiana et al. 2021).

The process of biofilm maturation occurs subsequent to the initial attachment, resulting in an increased rate of cell proliferation. This progression leads to the establishment of a biofilm matrix that demonstrates a high degree of tolerance to unfavourable conditions. The presence of nucleic acids plays a crucial role in bestowing stability upon the extracellular matrix (ECM). Notably, extracellular DNA (eDNA) released by *C. albicans* within the ECM contributes to maintaining the stability of mature biofilms, although it is not a prerequisite for the initial formation of biofilms. This suggests that eDNA plays a vital role in assembling the ECM (Rajendran et al. 2014). The conditions within a biofilm environment differ significantly from those in the planktonic phase. Various distinctions have been observed between the planktonic phase and the *C. albicans* biofilm matrix, including differences in their transcriptional activities (Yeater et al. 2007; Nobile et al. 2012; Fox et al. 2015).

11.2 Polymicrobial Biofilms of *C. albicans* and Bacteria

In the case of medical devices implanted in immunocompromised patients, there is a tendency to support the formation of polymicrobial biofilms. The interactions involving various microorganisms, including *C. albicans*, on both living tissues and artificial surfaces are intricate and diverse. These interactions can display synergistic, antagonistic, or neutral behaviours. Notably, *C. albicans* is one of the most prevalent fungal pathogens that can establish a commensal relationship with bacteria. Research indicates that when *C. albicans* forms polymicrobial biofilms with bacterial species originating from different body sites, the nature of these interactions can vary. These variations are influenced by signalling molecules that impact the physical interplay between the two species (Atriwal et al. 2021). Various infections occurring due to this polymicrobial interaction are listed in Table 11.1.

Microorganisms enclosed within the ECM exhibit greater resilience to stress when compared to their external surroundings. In polymicrobial biofilms, there is a dynamic exchange of various nutrients, where metabolic waste produced by one species is utilised by another (Ponde et al. 2021). Cells in the biofilm show lower

Table 11.1 Infections caused by polymicrobial biofilms of *Candida* with bacteria

Infections caused	Organisms involved	References
Nosocomial bloodstream infections	*C. albicans* + *S. aureus* *C. albicans* + *S. epidermidis*	Harriott and Noverr (2009)
Oral candidiasis	*Candida* spp. + *Streptococci*	Costa-Orlandi et al. (2017)
Dental plaques	*C. albicans* + *Streptococci gordonii*	Harriott and Noverr (2011)
Denture stomatitis	*C. albicans* + (*P. gingivalis, A. actinomycetemcomitans, F. nucleatum, Lactobacillus* sp., and *Streptococcus* spp.)	O'Donnell et al. (2015)
Wound infections	*C. albicans* + (*S. aureus, P. aeruginosa, E. coli, Enterococcus* sp., *Klebsiella* spp., *Enterobacter* spp., *Staphylococci*)	Clinton and Carter (2015), Bowler et al. (2001) and Bertesteanu et al. (2014)
Diabetic foot ulcers	*C. albicans* + (*S. aureus/ P. aeruginosa/ S. pyogenes*)	Tkaczyk et al. (2022)
Respiratory infections (cystic fibrosis)	*C. albicans* + (*Streptococcus, P. aeruginosa, B. cepacia, S. aureus)* *C. albicans* + *(Streptococcus, Prevotella, Veillonella, Rothia and Actinomyces*)	Acosta et al. (2017) and Ibberson et al. (2018)
Otitis media	*C. albicans* + (*H. influenzae, S. pneumoniae, and M. catarrhalis*)	Leach et al. (2008)
Vulvovaginal diseases	*C. albicans* + *Lactobacillus* spp.	McKloud et al. (2021)
Implanted medical devices	*C. albicans* + (*S. aureus/P. aeruginosa/S. epidemidis/E. faecalis/ E. coli*)	Lohse et al. (2018), Veerachamy et al. (2014) and Khatoon et al. (2018)

growth rate and more resistant to antimicrobial treatment. Polymicrobial biofilms exhibit wide range of genetic regulation to carry out intra- and inter-cellular regulation (Rodríguez-Cerdeira et al. 2020). ECM acts as a protective shelter that can degrade antibiotics. eDNA plays an important role in the resistance by activating cellular processes. High cell density triggers quorum sensing which reacts via gene regulation. Quorum sensing influencing biofilm development regulates virulence factors important for phagocytosis (Li et al. 2020).

11.3 Interactions Within Polymicrobial Biofilm: *Candida albicans* and *P. aeruginosa*

The interactions between *Pseudomonas aeruginosa* and *Candida albicans* serve as a model for a wide range of interactions between bacteria and eukaryotic organisms. *Pseudomonas* and *Candida* are frequently found in the sputum of cystic fibrosis patients (Trejo-Hernández et al. 2014). In healthy individuals, both species are typically harmless and exist as commensals. However, individuals with compromised immune systems can trigger aggressive growth, potentially leading to severe diseases and even death (Naglik et al. 2004; Hube 2006; Pfaller and Diekema 2007). In a study involving burned mice, Neely et al. (1986) illustrated that *Pseudomonas* infections can make burned mice more susceptible to deadly candidiasis, with the bacteria's proteolytic activity being the main contributor to lethal fungal infections. Few investigations have explored the underlying physiological mechanisms involved in the interaction between *C. albicans* and *P. aeruginosa*, as well as the broader ecological consequences of this interaction within the *C. albicans-P. aeruginosa* community (Neely et al. 1986), while some studies have hinted that *P. aeruginosa* may hinder the growth of *C. albicans* in the host.

The adhesion between *P. aeruginosa* and *C. albicans* is likely facilitated by the outer layer of the fungal cell wall, which is rich in glycoproteins. Previous research has shown that *P. aeruginosa* relies on glycoproteins for adhesion to host kidney cells (as reported by Apodaca et al. 1995). *Candida* glycans also serve as recognised ligands for pattern recognition receptors of the immune system, as documented by Netea et al. (2006, 2008). Notably, the absence of hypha-specific proteins like Hyr1p, Hwp1p, and Als3p, or the alteration of the wild-type N-linked glycan structure, did not affect resistance or susceptibility to *P. aeruginosa*. In contrast, mutant hyphae with deficiencies in O-glycosylation were notably more susceptible to killing compared to the control strain. The extent of O-mannan truncation appeared to correlate with increased susceptibility, suggesting that O-glycans play a specific role in resistance against *P. aeruginosa*. Truncation of O-glycans may expose high-affinity adhesion sites for the bacterium, or it may lead to the loss, misplacement, or misfolding of specific surface proteins crucial for maintaining the integrity of the fungal cell wall during colonisation by *P. aeruginosa*. Consequently, both soluble secreted compounds and surface factors contribute to the selective elimination of *C. albicans* hyphae by *P. aeruginosa* (Brand et al. 2008).

Colonisation by *C. albicans* and *P. aeruginosa* can be influenced at different levels. Initially, there is a competition for attachment sites on mucosal surfaces, where the normal flora creates a dense layer of mucus, inhibiting *Candida* from adhering. However, some studies have noted that *Candida* can form mixed biofilms with various bacteria found in the oral cavity. In 1973, Hughes and Kim found a positive correlation between *P. aeruginosa* and *C. albicans*, with their growth being reciprocally inhibited based on their respective densities. Laboratory testing demonstrated that substances released by *P. aeruginosa* not only inhibit the growth of *C. albicans* but also other harmful fungi like other *Candida* species and *Aspergillus fumigatus*. Specific substances secreted by *P. aeruginosa* that hinder fungal growth, such as phenazine derivatives, a type of redox-active molecule, were identified. Initially, pyocyanin and 1-Hydroxyphenazine were recognised as antifungal compounds, showing a lower Minimum Inhibitory Concentration (MIC) against *C. albicans* compared to amphotericin B or fluconazole. More recently, a precursor to pyocyanin, 5-methyl-phenazine1-carboxylic acid (5MPCA), was identified as the molecule responsible for *Pseudomonas*-induced *C. albicans* "red death": 5MPCA binds to amino acid residues and cause a red colour shift (Fig. 11.1b). When *C. albicans* hyphae are co-cultured with *P. aeruginosa*, they turn red and undergo cell death. Brand et al. reported significant fungicidal effect of *P. aeruginosa* culture

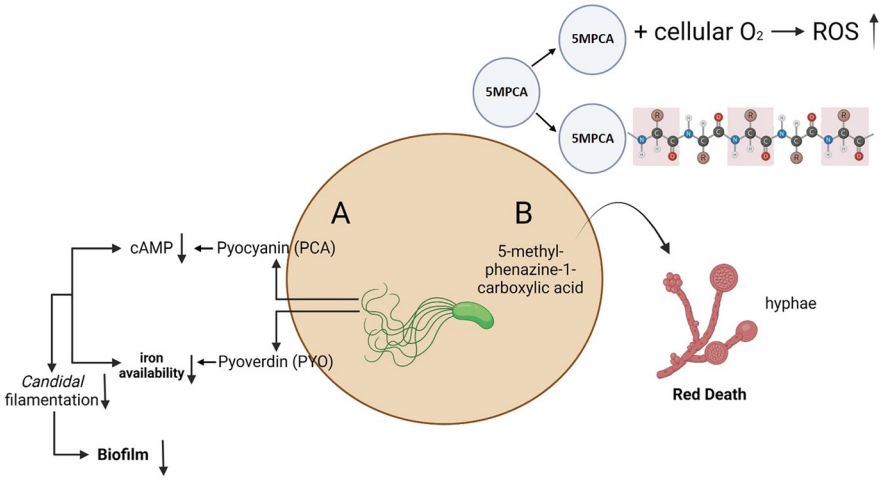

Fig. 11.1 Inhibitory effects of *Pseudomonas* on *C. albicans* biofilms. (**a**) PYO and PCA exhibit inhibitory effects on *Candidal* filamentation, hence reducing biofilm. PCA decreases the cAMP levels of Candida and PYO decreases iron availability in *Candida* causing metabolic failures leading to cell death. (**b**) 5MPCA penetrates *C. albicans* cells, where it may undergo oxidation, resulting in the production of harmful substances like superoxide and H_2O_2 (ROS). Furthermore, within the cells of *C. albicans*, 5MPCA interacts with amines found in large molecules, such as proteins. This interaction leads to the buildup of brightly coloured methylphenazinium derivatives, which could potentially add to the toxicity induced by methylphenazinium causing the inactivation or harm of crucial cellular components leading to cell death. (Created with BioRender.com)

supernatants, resulting in a 97% decrease in *C. albicans* survival rate over 3 days (Morales et al. 2013).

Phenazines also exhibit varying biological effects depending on their concentrations. Kerr and colleagues have demonstrated the ability of *P. aeruginosa* phenazines to combat *Candida* at high concentrations exceeding 500 μM. Additionally, it was observed that these phenazines could hinder respiration at concentrations significantly lower (25- to 200-fold lower) than those required to impede fungal growth. In the context of cystic fibrosis (CF) patients' lungs, where both *P. aeruginosa* and *C. albicans* are commonly found, Pyoverdin (PYO) and Pyocyanin (PCA) concentrations typically range from 5 to 80 μM. These concentrations have been shown to suppress *C. albicans* filamentation and biofilm formation without causing its outright death (Fig. 11.1a). A study conducted by Bhargava and colleagues in 1989, which examined fungal morphology in CF patients' airways, suggests that this fungus predominantly exists in a yeast form within CF lung environments. Furthermore, there was evidence to suggest that P. *aeruginosa* may induce *C. albicans* to secrete more fermentation products by releasing phenazines, which *P. aeruginosa* could utilise to enhance its growth and survival.

The antifungal activity of *P. aeruginosa* against *Candida* spp. was initially documented in the early 90s by Kerr et al. Subsequent research has shown that *P. aeruginosa* could eliminate *C. albicans* by forming a dense film on fungal filaments. Interestingly, *P. aeruginosa* did not bind to or kill the yeast form of *C. albicans*. Thein et al. reported that *P. aeruginosa* ATCC 27853, at varying concentrations, effectively inhibited *C. albicans* biofilms. Research has shown that *P. aeruginosa* influences the growth of *C. albicans* in environments with ample nutrients and normal oxygen levels (Hogan and Kolter 2002; El-Azizi et al. 2004). El-Azizi found that *Pseudomonas* did not significantly affect *C. albicans* adhesion and biofilm growth, regardless of whether preformed *Pseudomonas* biofilms are added to *C. albicans* or vice versa. These investigations have revealed differences in the way *P. aeruginosa* attaches to the yeast form of *C. albicans* versus its filamentous form (El-Azizi et al. 2004). These distinctions in attachment mechanisms may influence the development of mixed biofilms between these two organisms.

It has been established that diminished oxygen levels enhance the development of filaments in *C. albicans* (Dumitru et al. 2004). Additionally, it has been observed that the fungal response to hypoxia resembles the response to low iron, as evidenced by the research of Synnott et al. (2010). This suggests that competition for iron resources may intensify in hypoxic conditions and could be associated with hyphae formation. An extensive analysis of gene expression during the shift from yeast to hyphal forms in *C. albicans*, conducted by Thompson et al. (2011), revealed the upregulation of a substantial number of genes linked to iron utilisation. These findings imply a potential connection between iron metabolism and the yeast-hyphal transition, though this relationship remains unexplored.

The iron acquisition system mediated by siderophores plays a crucial and dominant role in the mixed biofilms of *C. albicans* and *P. aeruginosa*. In the context of microbial interactions, studies have examined iron competition in *Pseudomonas*, and the significance of the pyoverdine siderophore produced by *Pseudomonas*

species has been demonstrated in competition with both fungi and bacteria (Loper and Buyer 1991; Harrison et al. 2008). For instance, Purschke et al. (2012) illustrated that *P. aeruginosa* increases pyoverdine production as a response to iron competition with *C. albicans* in mixed biofilms. In line with these observations, several proteins have been identified with well-known roles in iron uptake through siderophores that were upregulated in *P. aeruginosa* within mixed biofilms when interacting with *C. albicans*. Notable among the highly induced proteins are the group of siderophore receptors, including the ferric pyochelin receptor FptA, the hydroxamate-type ferri-siderophore receptor FiuA, the citrate hydroxamate siderophore receptor ChtA, the ferrienterobactin receptor PfeA, the ferripyoverdine receptor FpvA, the alternative type I ferripyoverdine receptor FpvB, and the putative TonB-dependent receptor CirA. Additionally, the expression of proteins involved in the biosynthesis of the siderophore pyoverdine-PvdH and PvdA, as well as two proteins-HasR and PhuR required for heme and haemoglobin uptake, was significantly induced in mixed biofilms.

11.4 Interactions Within Polymicrobial Biofilm: *Candida albicans* and *Streptococcus* spp.

The interaction of *C. albicans* with *Streptococcus* species is widely studied in the context of oral cavity, dental plaque, and caries. The oral cavity is inhabited by various fungal and bacterial species. Due to various reasons, these microbes become more virulent and form complex biofilms and cause various infections. The interaction of these species is generally reported to be synergistic. For instance, *C. albicans* can't effectively utilise sucrose present in the environment, hence it utilised glucose and fructose from the matrix that are produced via sucrose metabolism by *S. mutans* (Fig. 11.2b) (Ellepola et al. 2019). It has been observed by numerous studies that polymicrobial biofilms of *C. albicans* and *Streptococcus* spp. are strongly adhered, high in biomass, and yeast is highly filamented.

The adhesion of bacteria with *C. albicans* occurs through surface adhesin proteins (Fig. 11.2a). During the interaction, *S. gordonii* expresses cell surface polypeptides-cshA, cshB, and antigen I/II salivary adhesins sspA and sspB, regulated by efg1, that are responsible for adhesion. Along with the bacteria, *C. albicans* also expresses various surface adhesin proteins like Als1, Als3, Als5, Eap1, and Hwp1 (Bernard et al. 2020). Various deletion studies for adhesin proteins have been performed which show impaired adhesion and biofilm formation of the mutants (Holmes et al. 1996; Silverman et al. 2010). Along with physical interaction, *Streptococcus* also secretes lactate, acting as a carbon source, which enhances *C. albicans'* yeast growth (Fig. 11.2c) (Ponde et al. 2021).

Apart from adhesin proteins, glycosyltransferases (Gtf) have also been reported essential for adhesion in *S. mutans* and *S. gordonii*. The adhesion of bacteria is facilitated by bacterial extracellular polysaccharide (EPS). Gtf have a great affinity towards N- or O-linked mannans on cell wall of both yeast and hyphae that convert sucrose (sugar-rich environment) to glucan (Hwang et al. 2017). This increase in

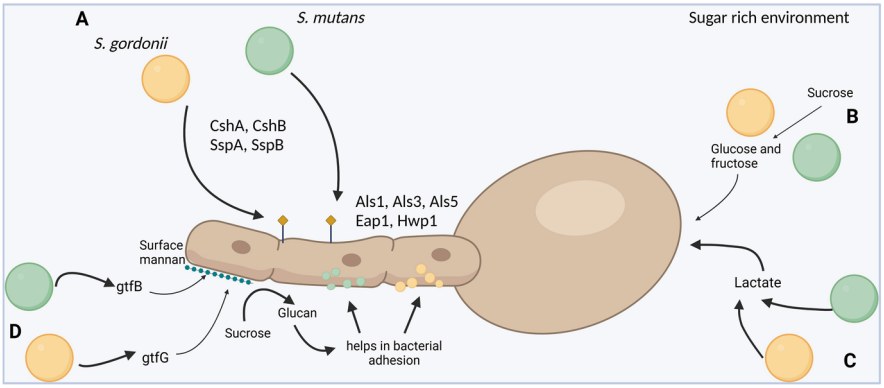

Fig. 11.2 Physical interactions between *C. albicans* and *S. mutans* and *S. gordonii*. (**a**) shows direct interaction between adhesin proteins of *C. albicans* and *Streptococcus* spp., where cshA, cshB, sspA, and sspB are adhesins of *Streptococci* and als1, als3, als5, eap1, and hwp1 are adhesins of *C. albicans*. (**b**) Sucrose present in the environment is metabolised by *Streptococci* to glucose and fructose which are then utilised by *C. albicans*. (**c**) *Streptococci* secretes lactate which is utilised by *C. albicans*. (**d**) Glycosyltransferases (Gtf) are released by *S. mutans* (gtfB) and *S. gordonii* (gtfG) that bind to mannans on *Candida* cell walls and help in conversion of sucrose to glucan. This glucan increases the adhesion of bacteria to *Candida (Created with BioRender.com)*

glucans helps the bacteria to bind firmly to the cell walls coated with glucans (Fig. 11.2d). *S. mutans* secretes gtfB which increases glucan production, hence facilitating bacterial adhesion. (Bernard et al. 2020). Falsetta et al. 2014 in their study have shown drastic decrease in the ability of the bacteria to bind to *C. albicans* in polymicrobial biofilm upon deletion of gtfB and gtfC. Similarly, in case of *S. gordonii*, gtfG is responsible for glucan production leading to increase in adhesion to *C. albicans*.

Quorum sensing molecules play a major role in biofilm formation and maintenance. Farnesol is a quorum sensing molecule secreted by *C. albicans* (Fig. 11.3a). It is known to inhibit the bacteria in their mono-species biofilm. However, in polymicrobial biofilms it is known to promote bacterial colonisation (Kim et al. 2017). *Streptococcus* has various quorum sensing mechanisms (Fig. 11.3b). In *Streptococcus* spp. quorum sensing system, autoinducer 2 (AI-2) is known to enhance hyphal formation. AI-2 is coded by luxS gene, deletion mutant of luxS shown no expression of AI-2 along with decreased biofilm biomass and reduced hyphal growth (Bamford et al. 2009). trans-2-decenoic acid is a fatty acid diffusible factor (DSF) which is another signalling molecule of *S. mutans*, *S. oralis*, *S. sanguinis,* and *S. mitis*. DSF is known to reduce filamentation in *C. albicans* and down-regulate the expression of Hwp1 (Vílchez et al. 2010). Apart from these, mutanobactinA and competence stimulating peptide are other molecules that are known to reduce yeast-to-hyphae transition (Ponde et al. 2021).

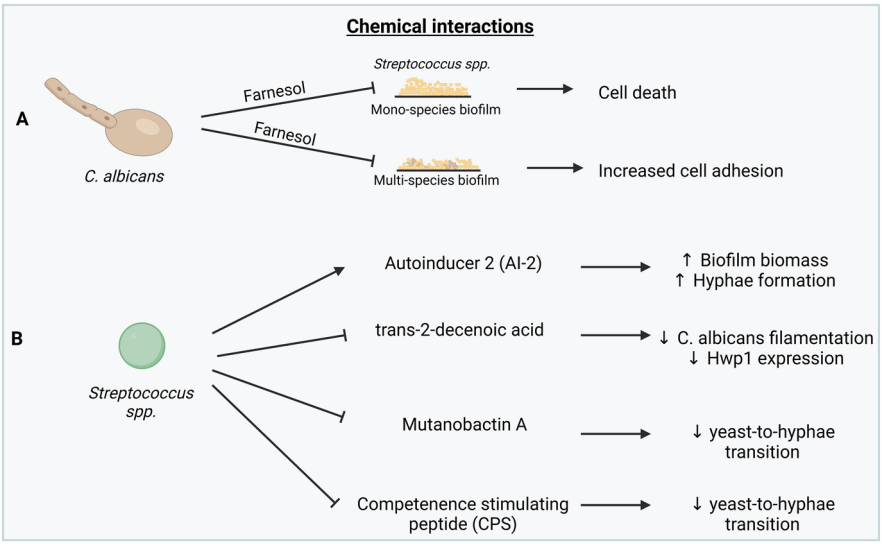

Fig. 11.3 Chemical interactions occur between the organisms and various quorum sensing molecules and small secretory molecules. Farnesol is a quorum sensing molecule released by *C. albicans*, which shows inhibitory effect on *Streptococci* mono-species biofilm, but promotes cell adhesion (**a**). *Streptococcus* secretes several quorum sensing molecules. Autoinducer 2 (AI-2) leads to increase in biofilm biomass and hyphae formation, whereas trans-2-decenoic acid, mutanobactin A, and competence stimulating peptide (CPS) lead to decrease in filamentation and Hwp1 regulation (**b**) *(Created with BioRender.com)*

11.5 Interactions Within Polymicrobial Biofilm: *Candida albicans* and *E. coli*

The majority of research in existing pieces of literature exploring fungal-bacterial biofilms comprising *C. albicans* and *E. coli* is very limited. While a handful of studies have been conducted on this topic, the results have been inconclusive and lack a thorough assessment.

Although *Candida* and *E. coli* are often linked, information is scarce regarding the shared interactions and behaviours of these two microorganisms. Interactions between *Candida* and *E. coli* concerning adhesion and aggregation might be determined by the specific species rather than being influenced by the bacterial strain. Prior research by Nair and Samaranayake (1996) had demonstrated that *Candida* adhesion to acrylic surfaces was enhanced when in the presence of *E. coli* ATCC 25922, regardless of the bacterial density on the surface. On the contrary, a high concentration of *E. coli* (105 and 106 cells/mL) suppressed *C. krusei* adhesion. *E. coli* exerts a notable inhibitory effect on the growth of certain *Candida* species at

different stages of biofilm development, although some *Candida* species display resilience against this effect.

For example, Hummel et al. (1975) observed that various Gram-negative organisms, including *Pseudomonas* and *E. coli*, isolated from burn patients, exhibited a certain level of inhibitory impact on *C. albicans*. Notably, the effect of *E. coli* was consistently more reliable and uniform. They also determined that this inhibitory activity was fungistatic and could be reversed. In laboratory studies, it has been shown that specific Gram-negative bacteria associated with the gut frequently possess inhibitory properties against *C. albicans* (Hummel et al. 1975). Approximately 20% of *E. coli* strains consistently demonstrated inhibitory effects on *C. albicans* growth. These inhibitory *E. coli* strains were consistently more susceptible to a wide range of antibiotics compared to their non-inhibitory counterparts. As a result, antibiotic treatment may potentially promote fungal overgrowth, not only by reducing the population of Gram-negative organisms but also by selectively eliminating those that suppress fungal growth the most (Hummel et al. 1975). The active compound in question seems to hinder the development of hyphae in *C. albicans*, most likely by affecting the differential expression of particular genes and the transcriptional regulatory pathways linked to them. Continuous molecular investigations are being conducted to pinpoint these precise compounds in bacterial supernatants and elucidate their role in the regulation of *Candida* biofilm formation.

Moreover, it is also suggested that viable *E. coli* and *E. coli* LPS (lipopolysaccharides) could increase the virulence of systemic *C. albicans* infections. However, there appears to be a potential protective role of LPS in *Candida* infections that are initiated through the oral route (Bendel et al. 2003).

Even though the precise mechanisms governing interactions between fungi and bacteria are not completely elucidated, recent studies suggest that a variety of molecules secreted by both organisms contribute to these intricate relationships. Notably, molecules associated with bacterial and fungal quorum sensing mechanisms appear to play a primary role in these multispecies interactions. Furthermore, non-quorum sensing molecules such as bacterial peptidoglycan-type compounds, pyocyanin, 1-hydroxyphenazine, lipopolysaccharides (LPS), and purified protein molecules (PPEBL21) also have an impact on the growth and formation of hyphae in *C. albicans*.

Moreover, a previous study has shown that *C. albicans* can modify gene expression in *P. aeruginosa* and affect its iron uptake mechanism. Since *E. coli* also relies on an iron-dependent biofilm formation mechanism, it is plausible that the active compound may have altered gene expression in *E. coli* by influencing the iron-dependent biofilm formation process. Understanding the molecular mechanisms of how the active compound produced by *C. albicans* affects drug resistance in *E. coli* is a significant area of research.

11.6 Interactions Within Polymicrobial Biofilm: *Candida albicans* and *Staphylococcus* spp.

C. albicans and *Staphylococcus* species are commonly found in several biofilm-related infections. Among various blood stream infections caused by *Candida,* about 20% are polymicrobial involving *Staphylococcus epidermidis* and *Staphylococcus aureus* (Carolus et al. 2019). The ability of *C. albicans* to form hyphae, mainly involved in tissue penetration and adhesion, is a leading cause of its virulence and pathogenicity. The interaction of *C. albicans* and *Staphylococcus* spp. has been reported to be synergistic which leads to greater harm to the host. The interaction of *C. albicans* and *S. aureus* is termed as "lethal synergism" which means that the polymicrobial infection leads to mortality of the host which could be prevented in case of mono-species infections of the same organisms (Todd et al. 2019).

C. albicans is known to express various adhesin proteins like Als3, Als5, Als6, and Als7; hyphal wall proteins like Hwp1 are involved in interaction and initial adhesion. Deletion of adhesin genes has led to lesser interaction of bacteria on hyphae resulting in weaker biofilm. Als3 is involved in binding of *S. aureus* and *S. epidermidis* to *C. albicans* hyphae via surface proteins like SasF, FnBPB, and Atl (Ponde et al. 2021). Apart from these adhesin proteins, various other genes have also been reported for mixed formation of *S. epidermidis*-*C. albicans* mixed-species biofilms. Wang et al. have shown increased expression of intercellular adhesion A (icaA), fibrinogen binding protein (fbe), and accumulation-associated protein (aap) genes in S. epidermidis in mixed biofilm with *C. albicans*. It was also observed that thickness of mixed species biofilm was higher with increased growth rate as compared to their individual biofilms (Wang et al. 2015).

Along with physical interaction between the two species, various small secretory molecules/proteins and quorum sensing molecules are also involved. One of the most widely described quorum sensing molecule is farnesol. Farnesol is secreted by *C. albicans* and has been known to increase tolerance of *S. aureus* towards vancomycin (Kong et al. 2017). Kong et al. hypothesised the induction of oxidative stress in *S. aureus*. This oxidative stress was sensed by multiple gene regulator (mgrA) which led to upregulation of drug efflux pump. Vila et al. 2019 observed that frequent exposure to farnesol in polymicrobial biofilm with *C. albicans* leads to loss of the yellow pigment, staphyloxanthin, by *S. aureus* which is an important virulence factor. Farnesol also induces accumulation of intracellular reactive oxygen species (ROS) and higher expression redox sensors. This altered stress response leads to higher H_2O_2 tolerance and phagocytic killing.

The interaction of *C. albicans* and *S. aureus* leads to host cell death as *C. albicans* increases the expression of agr quorum sensing system, leading to increased alpha and delta toxin production and haemolytic activity (Todd et al. 2019).

As per study by Kong et al. (2016), when methicillin-resistant *S. aureus* (MRSA) was grown with *C. albicans* in a polymicrobial biofilm, MRSA showed increased resistance to vancomycin which was earlier susceptible. It was then concluded that

C. albicans matrix acted as a protective shield for MRSA due to the presence of β-1,3-glucan.

11.7 Candida Polymicrobial Biofilms and Antimicrobial Resistance

Drug resistance in biofilms containing bacteria and *Candida* is a complex issue that requires innovative treatments and combination therapies. Biofilms, which form a protective matrix on surfaces, including medical devices, are difficult to treat because they resist antimicrobials. Bacteria and *Candida*, especially *Candida albicans*, may interact and boost each other's resistance mechanisms, making biofilm removal very difficult (Jabra-Rizk et al. 2004; Yuan et al. 2019). Table 11.2 shows

Table 11.2 Interactions between *Candida albicans* and microorganisms leading to antimicrobial resistance

Organism	Name of drug	Phenotypic outcome	Reason for resistance	Reference
P. aeruginosa	Tobramycin Fluconazole Meropenem	Increased polymicrobial resistance	*Candida* has a polysaccharide called β-1,3-glucan *P. aeruginosa* has exopolysaccharides *Pel* and *Psl* that stop many antibiotics, including tobramycin	Pang et al. (2019)
S. aureus	Vancomycin	Increased *S. aureus* drug resistance	*C. albicans* biofilm products, such as extracellular DNA and farnesol	Bruna et al. (2021)
	Gatifloxacin			
Streptococcus gordonii	Fluconazole Amphotericin B Caspofungin Clindamycin Erythromycin Amphicilin	Increased polymicrobial drug resistance		Montelongo-Jauregui et al. (2016) and Chinnici et al. (2019)
Streptococcus mutans	Chlorhexidine Digluconate	Increased *C. albicans and S. mutans* resistance	*Streptococcus mutans* produces glucans that protect the Candida from fluconazole	Lobo et al. (2019)
	Fluconazole	Increased *C. albicans* resistance		Karygianni et al. (2020)
E. coli	Ofloxacin	Ofloxacin tolerance of *E. coli* is significantly increased	Fungal β-1–3 glucan	De Brucker et al. (2015)

the interactions between *Candida albicans* and microorganisms leading to antimicrobial resistance.

Polymicrobial biofilms of *Candida* are associated with bacteria, such as *Staphylococcus, Streptococcus, Enterococcus, Pseudomonas*, *E. coli*, and a few others, presenting a coordinated behaviour. Cross-kingdom interactions are common in all polymicrobial biofilms, and the interacting species may have a beneficial or antagonistic effect (Wang et al. 2023).

Multiple studies have documented that polymicrobial biofilms exhibit greater resistance to antimicrobials in comparison to biofilms composed of a single species. Various mechanisms/ factors through which antimicrobial resistance can develop in diverse populations are detailed below.

- *Composition extracellular matrix*: Biofilms also have a unique extracellular matrix that contributes to drug resistance. This matrix consists of polymers secreted by *Candida* and bacteria, which prevent antimicrobial agents from penetrating the biofilm. The extracellular matrix can act as a physical barrier, preventing drugs from reaching the cells embedded within the biofilm.
- Biofilms exhibit inherent resistance to antimicrobial agents compared to planktonic cells. The extracellular polymeric substance (EPS) matrix of biofilms acts as a protective barrier, making it more difficult to eradicate infections. Bacteria in biofilms can alter their metabolic activity and gene expression, contributing to antibiotic resistance. The biofilm structure and composition also influence the efficacy of antimicrobial agents (Ballén et al. 2022).
- ECM of biofilms from the single species *P. aeruginosa* and *C. albicans* affects the ability of antimicrobial drugs to work. *Candida* has a polysaccharide called β-1,3-glucan that fluconazole sticks to. On the other hand, *P. aeruginosa* has exopolysaccharides *Pel* and *Psl* that stop many antibiotics, including tobramycin (Hattab et al. 2022).
- *C. albicans* forms complex biofilms on living or non-living surfaces, while *S. aureus* prefers to stick to its hyphal parts, creating a mutually beneficial relationship. *S. aureus* shows increased antibiotic resistance in polymicrobial biofilms, including vancomycin drug resistance. *C. albicans* biofilm products, such as extracellular DNA and farnesol, increase *S. aureus* drug resistance. Furthermore, *C. albicans* helps *S. aureus* invade mucosal barriers, causing systemic infection in co-colonised individuals. *C. albicans* biofilms with *S. aureus* or *S. gordonii* are more substantial and drug-resistant than monomicrobial ones.
- Ofloxacin affects *E. coli* axenic biofilm. *E. coli*, a *C. albicans*-associated polymicrobial biofilm, shows ofloxacin resistance via fungal β-1, 3-Glucan, a component of the ECM. It is now clear that biofilm formation involves a complex interaction between fungal and bacterial species. Microbial biofilm communities' resistance pattern is related to cell aggregation on a surface or substratum. The biofilm ECM protects the structure from medication dispersion, hindering disease control (Pohl 2022).
- *Efflux pump activity*: The overexpression of efflux pumps, which actively remove antimicrobial drugs from cells, is a major cause of drug resistance in biofilms.

Both *Candida* and bacteria have efflux pumps, such as ABC transporters CDR1 and CDR2 found in *Candida* and multidrug efflux pumps found in bacteria. Biofilms with upregulated pumps are less susceptible to several antimicrobials. The mechanisms underlying drug resistance in *Staphylococcus aureus* and *Candida albicans* biofilms are complex and multifactorial. Besides the biofilm matrix, other things like increased expression of drug efflux pumps and changes in metabolic pathways also play a role in the development of resistance (Bostanghadiri et al. 2021; Savage et al. 2013).

- *Adaptive stress response*: Microorganisms within biofilms can activate stress responses, triggering mechanisms that enhance their resistance to antimicrobial agents. The slow growth of cells in biofilms contributes to drug resistance, as slow growth reduces antimicrobial sensitivity since several drugs rely on active cell division.
- *Cross resistance*: The presence of *Staphylococcus aureus* in a polymicrobial biofilm with *C. albicans* led to cross-resistance, where the biofilm became resistant not only to antibiotics but also to antifungal agents. A mixed biofilm of *C. albicans* and *Staphylococcus epidermidis* increases *S. epidermidis* growth and vancomycin resistance. *C. candida* may make *S. gordonii* clindamycin-tolerant. *Streptococcus mutans* exopolysaccharides bind and sequester fluconazole in dual-species biofilms, reducing its efficacy against *C. albicans* (Pohl 2022; Tabassum et al. 2023; Harriott et al. 2009).
- *Enhanced biofilm structure*: The risk of mortality is higher in biofilms made up of both *C. albicans* and *E. coli* because they are more resistant to antibiotics. The supernatant of *E. coli* biofilm can restrain the growth and biofilm formation of *C. albicans*. (Sadanandan et al. 2022).
- *Commensal effect*: An *E. coli/C. albicans* biofilm does not impact *C. albicans* tolerance to amphotericin or caspofungin, according to one investigation. In contrast, polymicrobial *E. coli-C. albicans* biofilms have far higher ofloxacin tolerance than axenic biofilms. *E. coli's* higher ofloxacin tolerance is biofilm-specific, while polymicrobial *E. coli-C. albicans* planktonic cultures had reduced tolerance. In addition, treating *E. coli-C. albicans* biofilms with matrix-degrading enzymes like lyticase dramatically reduced ofloxacin tolerance. *E. coli-C. albicans* biofilms with a high-β-1,3-glucan-producing mutant showed higher ofloxacin tolerance, supporting a role for β-1,3-glucan in biofilms. Exogenous addition of laminarin, a polysaccharide in *E. coli* biofilms, enhanced ofloxacin tolerance. Research suggests that β-1,3-glucan from *C. albicans* enhances *E. coli's* ofloxacin tolerance in a biofilm (De Brucker et al. 2015).

11.8 Strategies to Combat AMR in Polymicrobial Biofilms

To overcome drug resistance, alternative therapeutic strategies are being explored, including the development of new antibiotics, combination therapies targeting multiple resistance mechanisms, and the use of antimicrobial peptides and

nanoparticles. Understanding the complex mechanisms of drug resistance in these biofilms is crucial for developing effective therapeutic interventions.

To combat drug resistance in *E. coli* and *Candida albicans* biofilms, researchers are exploring various approaches. A research study found that AGE could stop biofilms from being made by ampicillin-resistant *E. coli* and multidrug-resistant *C. albicans*. Other potential strategies include using natural compounds in conjunction with conventional antimicrobials to enhance treatment efficacy (Sadanandan et al. 2022). Further research is needed to fully understand the mechanisms of drug resistance in *E. coli* and *Candida albicans* biofilms and develop effective treatment strategies.

Luteolin was tested against single and dual-species *Candida albicans* and *Enterococcus faecalis* biofilms in Frontiers in Microbiology. Luteolin effectively inhibited biofilm formation and metabolic activity in single and dual-species biofilms.

A different study looked at how well a membranotropic peptide called gH625-M could break down a biofilm of *C. albicans* and *K. pneumoniae*. (Maione et al. 2021).

Gold nanoparticles, caspofungin, silver nanoparticles, and echinocandins have shown potential in inhibiting the formation and eradicating mixed biofilms of *Staphylococcus* species with *Candida albicans* (Scheunemann et al. 2021; Daniel et al. 2021; Humberto and Jose 2020; Nazia et al. 2023). However, each drug may have specific concentration ranges at which it is effective, and further research is necessary to optimise treatment strategies and address drug resistance in these complex infections.

11.9 Concluding Remarks

Polymicrobial biofilms are quite prevalent and understudied. The virulence of a polymicrobial biofilm increases (1) due to protection provided by ECM of biofilm and (2) due to multiple organisms and their interactions. Hence, understanding the morphology and the interaction within the complex polymicrobial community would help in designing the strategies to inhibit and degrade the polymicrobial biofilms. To conclude, more research is required for interactions and better clinical scenario should be understood.

References

Acosta N, Whelan FJ, Somayaji R, Poonja A, Surette MG, Rabin HR, Parkins MD (2017) The evolving cystic fibrosis microbiome: a comparative cohort study spanning 16 years. Ann Am Thorac Soc 14(8):1288–1297

Apodaca G. et al. (1995) 'Characterization of Pseudomonas aeruginosa-induced MDCK cell injury: glycosylationdefective host cells are resistant to bacterial killing', Infection and Immunity, 63(4), pp. 1541–1551. Available at: https://doi.org/10.1128/iai.63.4.1541-1551.1995

Atriwal T, Azeem K, Husain FM, Hussain A, Khan MN, Alajmi MF, Abid M (2021) Mechanistic understanding of *Candida albicans* biofilm formation and approaches for its inhibition. Front Microbiol 12:638609

Ballén V, Cepas V, Ratia C, Gabasa Y, Soto SM (2022) Clinical Escherichia coli: from biofilm formation to new antibiofilm strategies. Microorganisms 10(6):1103

Bamford CV, d'Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF (2009) *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. Infect Immun 77(9):3696–3704

Bendel CM (2003) 'Colonization and epithelial adhesion in the pathogenesis of neonatal candidiasis', Seminars in Perinatology, 27(5), pp. 357–364. Available at: https://doi.org/10.1016/S0146-0005(03)00059-4

Bernard C, Girardot M, Imbert C (2020) *Candida albicans* interaction with gram-positive bacteria within interkingdom biofilms. J Mycol Med 30(1):100909

Bertesteanu S, Triaridis S, Stankovic M, Lazar V, Chifiriuc MC, Vlad M, Grigore R (2014) Polymicrobial wound infections: pathophysiology and current therapeutic approaches. Int J Pharm 463(2):119–126

Bostanghadiri N, Ardebili A, Ghalavand Z, Teymouri S, Mirzarazi M, Goudarzi M, Ghasemi E, Hashemi A (2021) Antibiotic resistance, biofilm formation, and biofilm-associated genes among Stenotrophomonas maltophilia clinical isolates. BMC Res Notes 14:1–6

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14(2):244–269

Brand A, Barnes JD, Mackenzie KS, Odds FC, Gow NA (2008) Cell wall glycans and soluble factors determine the interactions between the hyphae of *Candida albicans* and *Pseudomonas aeruginosa*. FEMS Microbiol Lett 287(1):48–55

Bruna T, Maldonado-Bravo F, Jara P. and Caro N. (2021) Silver nanoparticles and their antibacterial applications. International journal of molecular sciences 22(13):7202

Carolus H, Van Dyck K, Van Dijck P (2019) *Candida albicans* and *Staphylococcus* species: a threatening twosome. Front Microbiol 10:2162

Chinnici J, Yerke L, Tsou C, Busarajan S, Mancuso R, Sadhak ND, Kim J, Maddi A (2019) Candida albicans cell wall integrity transcription factors regulate polymicrobial biofilm formation with Streptococcus gordonii. PeerJ 7:e7870

Clinton A, Carter T (2015) Chronic wound biofilms: pathogenesis and potential therapies. Lab Med 46(4):277–284

Costa-Orlandi CB, Sardi JC, Pitangui NS, De Oliveira HC, Scorzoni L, Galeane MC, Medina-Alarcón KP, Melo WC, Marcelino MY, Braz JD, Fusco-Almeida AM (2017) Fungal biofilms and polymicrobial diseases. J Fungi 3(2):22

Daniel FM, Catherine RF, Joana SP, Ronan M, Lundy FT (2021) Targeting *C. albicans* in dual-species biofilms with antifungal treatment reduces *S. aureus* and MRSA in vitro. PLoS One 16:e0249547

De Brucker K, Tan Y, Vints K, De Cremer K, Braem A, Verstraeten N, Michiels J, Vleugels J, Cammue BP, Thevissen K (2015) Fungal β-1, 3-glucan increases ofloxacin tolerance of *Escherichia coli* in a polymicrobial *E. coli*/*Candida albicans* biofilm. Antimicrob Agents Chemother 59(6):3052–3058

Dumitru R, Hornby JM and Nickerson KW (2004) 'Defined Anaerobic Growth Medium for Studying Candida albicans Basic Biology and Resistance to Eight Antifungal Drugs', Antimicrobial Agents and Chemotherapy, 48(7), pp. 2350–2354. Available at: https://doi.org/10.1128/AAC.48.7.2350-2354.2004

El-Azizi MA, Starks SE, Khardori N (2004) Interactions of *Candida albicans* with other *Candida* spp. and bacteria in the biofilms. J Appl Microbiol 96(5):1067–1073

Ellepola K, Truong T, Liu Y, Lin Q, Lim TK, Lee YM, Cao T, Koo H, Seneviratne CJ (2019) Multi-omics analyses reveal synergistic carbohydrate metabolism in *Streptococcus mutans-Candida albicans* mixed-species biofilms. Infect Immun 87(10):10–1128

Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, Koo H (2014) Symbiotic relationship between *Streptococcus*

mutans and *Candida albicans* synergizes virulence of plaque biofilms in vivo. Infect Immun 82(5):1968–1981

Fox EP, Bui CK, Nett JE, Hartooni N, Mui MC, Andes DR, Nobile CJ, Johnson AD (2015) An expanded regulatory network temporally controls C andida albicans biofilm formation. Molecular microbiology 96(6):1226–1239

Gulati M, Nobile CJ (2016) *Candida albicans* biofilms: development, regulation, and molecular mechanisms. Microbes Infect 18(5):310–321

Harriott MM, Noverr MC (2009) *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrob Agents Chemother 53(9):3914–3922

Harriott MM, Noverr MC (2011) Importance of Candida–bacterial polymicrobial biofilms in disease. Trends Microbiol 19(11):557–563

Harrison F. et al. (2008) 'Interspecific competition and siderophore-mediated cooperation in Pseudomonas aeruginosa', The ISME Journal, 2(1), pp. 49–55. Available at: https://doi.org/10.1038/ismej.2007.96

Hattab S, Dagher AM, Wheeler RT (2022) Pseudomonas synergizes with fluconazole against Candida during treatment of polymicrobial infection. Infect Immun 90(4):e00626–e00621

Hogan DA and Kolter R (2002) 'Pseudomonas - Candida Interactions: An Ecological Role for Virulence Factors', Science, 296(5576), pp. 2229–2232. Available at: https://doi.org/10.1126/science.1070784.

Holmes AR, McNAB R, Jenkinson HF (1996) *Candida albicans* binding to the oral bacterium *Streptococcus gordonii* involves multiple adhesin-receptor interactions. Infect Immun 64(11):4680–4685

Hube B. (2006) 'Infection-Associated Genes of Candida Albicans', Future Microbiology, 1(2), pp. 209–218. Available at: https://doi.org/10.2217/17460913.1.2.209

Hummel RP, Maley MP, Miskell PW, Altemeier WA (1975) Suppression of Candida albicans by Escherichia coli. Journal of Trauma and Acute Care Surgery 15(5):413–418

Lara Humberto H, and Jose L. Lopez-Ribot (2020) "Inhibition of mixed biofilms of Candida albicans and methicillinresistant Staphylococcus aureus by positively charged silver nanoparticles and functionalized silicone elastomers."Pathogens 9.10: 784.

Hwang G, Liu Y, Kim D, Li Y, Krysan DJ, Koo H (2017) *Candida albicans* mannans mediate *Streptococcus mutans* exoenzyme GtfB binding to modulate cross-kingdom biofilm development in vivo. PLoS Pathog 13(6):e1006407

Ibberson CB, Stacy A, Fleming D, Dees JL, Rumbaugh K, Gilmore MS, Whiteley M (2018) Co-infecting microbes dramatically alter pathogen gene essentiality during polymicrobial infection. Nat Microbiol 2:17079

Jabra-Rizk, Mary Ann et al. "Fungal biofilms and drug resistance." Emerging infectious diseases vol. 10,1 (2004): 14-19. https://doi.org/https://doi.org/10.3201/eid1001.030119

Karygianni L, Ren Z, Koo H, Thurnheer T (2020) Biofilm matrixome: extracellular components in structured microbial communities. Trends Microbiol 28(8):668–681

Kaur J, Nobile CJ (2023) Antifungal drug-resistance mechanisms in Candida biofilms. Curr Opin Microbiol 71:102237

Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI (2018) Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4(12):e01067

Kim D, Sengupta A, Niepa TH, Lee BH, Weljie A, Freitas-Blanco VS, Murata RM, Stebe KJ, Lee D, Koo H (2017) *Candida albicans* stimulates *Streptococcus mutans* microcolony development via cross-kingdom biofilm-derived metabolites. Sci Rep 7(1):41332

Kong EF, Tsui C, Kucharíková S, Andes D, Van Dijck P, Jabra-Rizk MA (2016) Commensal protection of *Staphylococcus aureus* against antimicrobials by *Candida albicans* biofilm matrix. mBio 7(5):10–1128

Kong EF, Tsui C, Kucharíková S, Van Dijck P, Jabra-Rizk MA (2017) Modulation of *Staphylococcus aureus* response to antimicrobials by the *Candida albicans* quorum sensing molecule farnesol. Antimicrob Agents Chemother 61(12):10–1128

Leach AJ, Morris PS, Mathews JD, Chronic Otitis Media Intervention Trial–One (COMIT1) Group (2008) Compared to placebo, long-term antibiotics resolve otitis media with effusion

(OME) and prevent acute otitis media with perforation (AOMwiP) in a high-risk population: a randomized controlled trial. BMC Pediatr 8:1–9

Li Y, Xiao P, Wang Y, Hao Y (2020) Mechanisms and control measures of mature biofilm resistance to antimicrobial agents in the clinical context. ACS Omega 5(36):22684–22690

Lobo CIV, Rinaldi TB, Christiano CMS, De Sales Leite L, Barbugli PA, Klein MI (2019) Dual-species biofilms of Streptococcus mutans and Candida albicans exhibit more biomass and are mutually beneficial compared with single-species biofilms. Journal of Oral Microbiology 11(1):1581520

Loper JE (1991) 'Current Review Siderophores in Microbial Interactions on Plant Surfaces', Molecular Plant-Microbe Interactions, 4(1), p. 5. Available at: https://doi.org/10.1094/MPMI-4-005

Lohse MB, Gulati M, Johnson AD, Nobile CJ (2018) Development and regulation of single-and multi-species *Candida albicans* biofilms. Nat Rev Microbiol 16(1):19–31

Maione A, de Alteriis E, Carraturo F, Galdiero S, Falanga A, Guida M, Di Cosmo A, Maselli V, Galdiero E (2021) The membranotropic peptide gH625 to combat mixed *Candida albicans*/*Klebsiella pneumoniae* biofilm: correlation between in vitro anti-biofilm activity and in vivo antimicrobial protection. J Fungi 7(1):26

McKloud E, Delaney C, Sherry L, Kean R, Williams S, Metcalfe R, Thomas R, Richardson R, Gerasimidis K, Nile CJ, Williams C (2021) Recurrent vulvovaginal candidiasis: a dynamic interkingdom biofilm disease of Candida and Lactobacillus. mSystems 6(4):10–1128

Montelongo-Jauregui D, Srinivasan A, Ramasubramanian AK, Lopez-Ribot JL (2016) An in vitro model for oral mixed biofilms of Candida albicans and Streptococcus gordonii in synthetic saliva. Frontiers in microbiology 7:686

Morales DK, Grahl N, Okegbe C, Dietrich LE, Jacobs NJ, Hogan DA (2013) Control of *Candida albicans* metabolism and biofilm formation by *Pseudomonas aeruginosa* phenazines. mBio 4(1):10–1128

Naglik J. et al. (2004) 'Candida albicans proteinases and host/pathogen interactions', Cellular Microbiology, 6(10), pp. 915–926. Available at: https://doi.org/10.1111/j.1462-5822.2004.00439.x

Nair RG. and Samaranayake LP. (1996) 'The effect of oral commensal bacteria on candidal adhesion to denture acrylic surfaces', APMIS, 104(1–6), pp. 339–349. Available at: https://doi.org/10.1111/j.1699-0463.1996.tb00725.x

Netea MG (2006) 'Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors', Journal of Clinical Investigation, 116(6), pp. 1642–1650. Available at: https://doi.org/10.1172/JCI27114

Netea MG et al. (2008) 'An integrated model of the recognition of Candida albicans by the innate immune system', Nature Reviews Microbiology, 6(1), pp. 67–78. Available at: https://doi.org/10.1038/nrmicro1815

Nazia T, Geum-Jae J, Won-Kyo J, Young-Mog K (2023) Inhibition of mixed biofilms of *Candida albicans* and *Staphylococcus aureus* by β-Caryophyllene-gold nanoparticles. Antibiotics 12:726

Neely AN, Law EJ, Holder IA (1986) Increased susceptibility to lethal Candida infections in burned mice preinfected with Pseudomonas aeruginosa or pretreated with proteolytic enzymes. Infection and Immunity 52(1):200–204

Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM, Hernday AD, Tuch BB, Andes DR, Johnson AD (2012) A recently evolved transcriptional network controls biofilm development in Candida albicans. Cell 148(1):126–138

O'Donnell LE, Millhouse E, Sherry L, Kean R, Malcolm J, Nile CJ, Ramage G (2015) Polymicrobial Candida biofilms: friends and foe in the oral cavity. FEMS Yeast Res 15(7):fov077

Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z (2019) Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology advances 37(1):177–192

Pfaller MA. and Diekema DJ. (2007) 'Epidemiology of Invasive Candidiasis: a Persistent Public Health Problem', Clinical Microbiology Reviews, 20(1), pp. 133–163. Available at: https://doi.org/10.1128/CMR.00029-06

Pohl CH (2022) Recent advances and opportunities in the study of *Candida albicans* polymicrobial biofilms. Front Cell Infect Microbiol 12:125

Ponde NO, Lortal L, Ramage G, Naglik JR, Richardson JP (2021) *Candida albicans* biofilms and polymicrobial interactions. Crit Rev Microbiol 47(1):91–111

Prasath KG, Sethupathy S, Pandian SK (2019) Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in *Candida albicans*. J Proteomics 208:103503

Purschke FG et al. (2012) 'Flexible Survival Strategies of Pseudomonas aeruginosa in Biofilms Result in Increased Fitness Compared with Candida albicans', Molecular & Cellular Proteomics, 11(12), pp. 1652–1669. Available at: https://doi.org/10.1074/mcp.M112.017673.

Rajendran R, Sherry L, Lappin DF, Nile CJ, Smith K, Williams C, Munro CA, Ramage G (2014) Extracellular DNA release confers heterogeneity in *Candida albicans* biofilm formation. BMC Microbiol 14(1):1–9

Rodríguez-Cerdeira C, Martínez-Herrera E, Carnero-Gregorio M, López-Barcenas A, Fabbrocini G, Fida M, El-Samahy M, González-Cespón JL (2020) Pathogenesis and clinical relevance of Candida biofilms in vulvovaginal candidiasis. Front Microbiol 11:544480

Rosiana S, Zhang L, Kim GH, Revtovich AV, Uthayakumar D, Sukumaran A, Geddes-McAlister J, Kirienko NV, Shapiro RS (2021) Comprehensive genetic analysis of adhesin proteins and their role in virulence of *Candida albicans*. Genetics 217(2):iyab003

Sadanandan B, Ashrit P, Nataraj LK, Shetty K, Jogalekar AP, Vaniyamparambath V, Hemanth B (2022) High throughput comparative assessment of biofilm formation of Candida glabrata on polystyrene material. Kor J Chem Eng 39(5):1277–1286

Savage VJ, Chopra I, O'Neill AJ (2013) *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. Antimicrob Agents Chemother 57(4):1968–1970

Scheunemann G, Fortes BN, Lincopan N, Ishida K (2021) Caspofungin inhibits mixed biofilms of *Candida albicans* and methicillin-resistant *Staphylococcus aureus* and displays effectiveness in coinfected galleria mellonella larvae. Microbiol Spectr 9(2):e00744–e00721

Silverman RJ, Nobbs AH, Vickerman MM, Barbour ME, Jenkinson HF (2010) Interaction of *Candida albicans* cell wall Als3 protein with *Streptococcus gordonii* SspB adhesin promotes development of mixed-species communities. Infect Immun 78(11):4644–4652

Su H, Han L, Huang X (2018) Potential targets for the development of new antifungal drugs. The journal of antibiotics 71(12):978–991

Synnott JM, Guida A, Mulhern-Haughey S, Higgins DG, Butler G (2010) Regulation of the hypoxic response in Candida albicans. Eukaryotic cell 9(11):1734–1746

Tabassum N, Khan F, Kang MG, Jo DM, Cho KJ, Kim YM (2023) Inhibition of polymicrobial biofilms of *Candida albicans–Staphylococcus aureus*/*Streptococcus mutans* by Fucoidan–Gold nanoparticles. Mar Drugs 21(2):123

Thompson DS, Carlisle PL and Kadosh D (2011) 'Coevolution of Morphology and Virulence in Candida Species', Eukaryotic Cell, 10(9), pp. 1173–1182. Available at: https://doi.org/10.1128/EC.05085-11

Tkaczyk C, Jones-Nelson O, Shi YY, Tabor DE, Cheng L, Zhang T, Sellman BR (2022) Neutralizing *Staphylococcus aureus* virulence with AZD6389, a three mAb combination, accelerates closure of a diabetic polymicrobial wound. mSphere 7(3):e00130–e00122

Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Škrlec I (2021) Candida albicans—the virulence factors and clinical manifestations of infection. Journal of Fungi 7(2):79

Todd OA, Fidel PL Jr, Harro JM, Hilliard JJ, Tkaczyk C, Sellman BR, Noverr MC, Peters BM (2019) *Candida albicans* augments *Staphylococcus aureus* virulence by engaging the staphylococcal *agr* quorum sensing system. mBio 10(3):10–1128

Trejo-Hernández A, Andrade-Domínguez A, Hernández M, Encarnacion S (2014) Interspecies competition triggers virulence and mutability in *Candida albicans–Pseudomonas aeruginosa* mixed biofilms. ISME J 8(10):1974–1988

Veerachamy S, Yarlagadda T, Manivasagam G, Yarlagadda PK (2014) Bacterial adherence and biofilm formation on medical implants: a review. Proc Inst Mech Eng H J Eng Med 228(10):1083–1099

Vila T, Kong EF, Ibrahim A, Piepenbrink K, Shetty AC, McCracken C, Bruno V, Jabra-Rizk MA (2019) *Candida albicans* quorum-sensing molecule farnesol modulates staphyloxanthin production and activates the thiol-based oxidative-stress response in *Staphylococcus aureus*. Virulence 10(1):625–642

Vílchez R, Lemme A, Ballhausen B, Thiel V, Schulz S, Jansen R, Sztajer H, Wagner-Döbler I (2010) *Streptococcus mutans* inhibits *Candida albicans* hyphal formation by the fatty acid signaling molecule trans-2-decenoic acid (SDSF). ChemBioChem 11(11):1552–1562

Wang X, Chen Y, Huang Y, Zhou Y, Zhao G, Ye L, Lei Y, Tang Q (2015) Function of intercellular adhesion a, fibrinogen binding protein, and accumulation-associated protein genes in formation of *Staphylococcus epidermidis-Candida albicans* mixed species biofilms. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 29:63–68

Wang F, Wang Z, Tang J (2023) The interactions of Candida albicans with gut bacteria: a new strategy to prevent and treat invasive intestinal candidiasis. Gut Pathogens 15(1):30

Yeater KM, Chandra J, Cheng G, Mukherjee PK, Zhao X, Rodriguez-Zas SL, Kwast KE, Ghannoum MA, Hoyer LL (2007) Temporal analysis of *Candida albicans* gene expression during biofilm development. Microbiology 153(8):2373–2385

Yuan L. et al. (2019) 'Mixed-species biofilms in the food industry: Current knowledge and novel control strategies', Critical Reviews in Food Science and Nutrition, 60(13), pp. 2277–2293. https://doi.org/10.1080/10408398.2019.1632790