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***Candida albicans* interaction with Gram-positive bacteria within interkingdom biofilms**

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Abstract:

Candida albicans is a commensal of the human body and an opportunistic pathogen frequently responsible for nosocomial bloodstream infections. Most of these infections are linked to the development of a biofilm in or on implanted medical devices. *C. albicans* cells have the capacity to interact with bacteria within biofilms, especially by the way of chemical or metabolic indirect interactions and/or direct physical contacts involving specifically the yeast or hyphal form of the fungal cell, or more rarely involving both forms. According to the species, *C. albicans*-bacteria interactions can be antagonistic or synergistic, competitive or not. The polymicrobial nature of biofilms may deeply influence the physiopathology of infections as well as the efficiency of antimicrobial agents. The present review aims to focus on the current knowledge of interactions between *C. albicans* and major Gram-positive bacteria such as *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Streptococcus* spp. and *Clostridium* spp. within biofilms. A better understanding of this complicated, fast-paced world of multi-kingdom biofilms will contribute to develop new effective ways to fight biofilm-related infections.

Keywords: poly-microbial biofilm, *Candida albicans*, *Streptococcus* spp., *Staphylococcus aureus*, coagulase negative *Staphylococcus*, anaerobic bacteria

Introduction

Candida genus is the fourth most common responsible for nosocomial bloodstream infections (BSI) in the United States of America (USA) [1]. More than 50 % of these infections are caused by *C. albicans* [1,2] and of the main causes of these BSI would be the presence of *C. albicans* on catheters [3,4]. In France, intensive care units candidaemia ranked sixth among BSI, and its average annual incidence was 0.3 per 1000 patients days [5]. According to a study by Klotz *et al.*, many BSI would be polymicrobial and would involve bacterial partners [6].

About 80% of human infections would be linked to a biofilm [7] which is a community of microorganisms formed on biotic and abiotic surfaces and represents the natural mode of life of micro-organisms [8,9]. Biofilm formation can be summarized in three main steps: adhesion, maturation and dispersion [10]. Adhesion is determined by several physico-chemical factors such as pH, ionic strength, nutrient levels, temperature or surface properties. Generally, it involves nonspecific interaction between substrates and microorganisms, then specific interactions through the use of adhesins or different attachment appendages such as pili which allow an irreversible adhesion. Once adhered, the microorganisms multiply, and genes involved in the formation of extracellular matrix are up-regulated. The matrix is a key component of the architecture and stability of the biofilm and also plays a major part in the communication and the exchange of genetic material within these structures [11]. Its composition is heavily influenced by the environment and varies substantially between different species [10], the main components being carbohydrates, proteins, lipids and eDNA. In adverse conditions, such as nutrients depletion, metabolic waste accumulation or shear stress, the dispersion of the biofilms can either be passive (through shear stress) or active. Active dispersion mainly involves the production of enzymes able to degrade biopolymers composing the matrix and the up-regulation of genes responsible for motility. Dispersion allows microorganisms to colonize new substrates and form new biofilms in more suitable conditions [10]. Overall biofilms allow micro-organisms to withstand adverse environmental conditions such as acid stress, osmotic and thermic shock, desiccation, UV radiation [12]... The human immune system is also partially inefficient against these structures [13]. Furthermore, antimicrobial agent efficiency is heavily reduced against micro-organisms under sessile state [14,15]. Concentrations necessary to eradicate microbial agents in biofilm can be a hundred to a thousand-fold higher than minimum inhibitory concentration of planktonic cells [15,16]. Besides, this resistance phenomenon can be

influenced by the poly-microbial nature of biofilms. Hence studies involving several different species of micro-organisms within a same polymicrobial structure are of utmost importance.

C. albicans can form polymicrobial biofilms with both Gram-positive and Gram-negative bacteria and interactions within these biofilms can be antagonistic or synergistic, competitive or not. The interaction between *C. albicans* and bacteria within biofilms has been increasingly investigated in recent years, particularly *in vitro*. Three main different modes of interaction have been described as being: physical, chemical or metabolic interactions [17–19]. The knowledge of such interactions may help to have a better understanding of the physiopathology of biofilm-related diseases and thus may help to propose new prophylactic and/or therapeutic strategies. Indeed, these inter-kingdom interactions can slightly or strongly influence the growth and viability of certain microbial species and in the case of *C. albicans*, they can also affect yeast-to-hyphal transition. As all these species are responsible for major infections, it is also important to be aware that such inter-kingdom interactions may be able to influence the activity of the antimicrobial agents that are mainly used to treat patients suffering from infections related to these bacterial or fungal species. For example, even if echinocandins are among the most active antifungal agents against *C. albicans* biofilms, our team recently showed that the co-presence of *C. albicans* and *Cutibacterium acnes* in a biofilm resulted in a decreased efficiency of micafungin against fungal cells [20]. Thus, this review aims to provide an overview of what is known about the interactions between *C. albicans* and Gram-positive bacteria within polymicrobial biofilms, and of their potential consequences - especially in terms of virulence and susceptibility to antimicrobials.

This article will successively examine the relationships between the *C. albicans* species, the *Staphylococcus* species and the *Streptococcus* species, which have so far been the most studied. Four main points will be examined: the adhesion between these microorganisms, the structures of polymicrobial biofilms they form together, the inter-kingdom communication they establish and the consequences of all these interactions. A third section will focus on interactions between *C. albicans* and obligate anaerobes which have been less studied until now.

C. albicans* interactions with coagulase-negative *Staphylococcus* and *Staphylococcus aureus

Although being responsible for diseases which are remarkably different in terms of physiopathology and severity, coagulase-negative *Staphylococcus* and *Staphylococcus aureus* are among the most commonly isolated species along with *C. albicans* [6]. *C. albicans* and *S. aureus* can be isolated independently or co-isolated from various medical devices such as dentures, voice prostheses, implants, endo-tracheal tubes, feeding tubes, and, most commonly, catheters [21]. Indeed, according to literature data, 27% of nosocomial *C. albicans* bloodstream infections are poly-microbial, with *S. aureus* being the third most common organism isolated in conjunction with *C. albicans* [6]. As another example, it has also been reported that *Candida* species and *S. aureus* can cause super-infections in the lungs of patients with cystic fibrosis by forming mixed biofilms [22]. Overall, polymicrobial biofilms formed by these microorganisms on implanted medical devices (IMDs) as well as on host tissues are a real concern [21,23]. Figure 1 illustrates the main features of relationships established between *C. albicans* and *S. aureus* (Figure 1).

Adhesion

Adhesion is a physical mode of interaction [19]. *S. aureus* and *Staphylococcus epidermidis* have both the capacity to form biofilms with *C. albicans* [24,25]. *In vitro* experiments showed that *S. aureus* adhesion and biofilm formation on polystyrene was reduced in presence of serum [24,26]. However, Harriott and Noverr observed that in presence of *C. albicans*, *S. aureus* was able to adhere to yeasts and form substantial biofilms in serum in polystyrene wells [24]. *C. albicans* also enhanced the ability of *S. aureus* to form biofilms on silicon surfaces [27,28]. On the contrary *C. albicans* adhesion on preformed *S. aureus* or *S. epidermidis* biofilms was reduced compared to its direct adhesion to abiotic surfaces [29,30].

S. aureus adheres to hyphal forms of *C. albicans* and does not adhere to yeast forms [31,32]. Results obtained by Harriott and Noverr showed that hyphal forms of *C. albicans* were required to form a biofilm with *S. aureus*. Indeed, a *C. albicans* double mutant *efg1/efg1 cph1/cph1* (regulators of morphogenesis in *C. albicans*), which did not produce hyphae, did not allow the formation of the polymicrobial structures [32]. On the contrary, several studies showed that *S. epidermidis* has no specific targets and adheres to both hyphal and yeast forms of *C. albicans* [25,33].

Among fungal factors involved in inter-kingdom adhesion, the Als3 hyphal-specific adhesin has been shown to be a key element of the adhesion of *S. aureus* to *C. albicans*. Indeed Peters *et al.* showed that adhesion of *S. aureus* to *C. albicans* mutant *als3/als3* was greatly reduced in serum free environment [34]. Concerning bacterial factors, some adhesins were identified as partially responsible for the adhesion to *C. albicans*, such as Fibronectin Binding protein B (FnBPB), a putative surface anchored protein (SasF) and a putative N-acetylmuramoyl-L-alanine amidase (Atl) [23].

Biofilm structure and Matrix

Inside *C. albicans-S. aureus* biofilms, bacteria are distributed along hyphae throughout the overall biofilm. The bacteria use fungi as a structural scaffold for their growth [31,35]. Kean *et al.* showed that the biomass of these biofilms increased compared to single-species ones, due to an increase in bacteria population [35].

Some authors observed no influence of *S. aureus* on *C. albicans* present within biofilms [24,28]. However, Lin *et al.* reported that the average doubling time of mixed *C. albicans-S. aureus* was increased by 20% compared to *C. albicans* single-species biofilms [29].

The polymicrobial biofilms formed by *C. albicans* and *S. epidermidis* *in vitro* tended to be thicker, more voluminous and more complex than those of single-species [33,36]. Moreover, Adam *et al.* showed that growth was impacted within polymicrobial structures: *C. albicans-S. epidermidis* biofilms reached maximum growth after 48h, whereas their single-species counterparts reached the same state after 24h [25].

Fungal β -1,3-glucan is one of the key components of the matrix of *C. albicans-S. aureus* biofilms. Indeed, the inhibition of β -1,3-glucan synthesis by *C. albicans* was recently shown to jeopardize the antibiotic resistance of *S. aureus* in polymicrobial biofilms [37]. Inside biofilms, fungal β -1,3-glucan along with α -mannan and β -1,6-glucan coat *S. aureus* [37]. For both *C. albicans-S. aureus* and *C. albicans-S. epidermidis* biofilms eDNA is also a key element of the extracellular matrix: eDNA is involved in the biofilms' structure and its concentration would be higher in polymicrobial conditions [33,35]. Moreover, Pammi *et al.* showed that the bacterial eDNA prevails in the matrix of *C. albicans-S. epidermidis* polymicrobial biofilms [33].

Communication

Mechanisms involved in cross-kingdom communication are complex and polyfactorial, but they play a role in biofilm formation and microbial virulence. Some teams investigated communication between staphylococci and *C. albicans*

Quorum sensing is another mode of interaction between microbial agents which belongs to the chemical group, according to the classification of Lof *et al.* [19]. Farnesol is a major component of *C. albicans* quorum sensing. This molecule prevents yeast-to-hyphae transition and decreases ability of *C. albicans* to form biofilms [38]. This compound also displays an antibacterial effect on *S. aureus* and can inhibit its biofilm formation [39]. Molecules secreted by *S. aureus* can also influence *C. albicans* as Lin *et al.* reported that cell-free conditioned medium from *S. aureus* biofilm influenced positively *C. albicans* biofilm growth. However, in this study, the medium conditioned from a *C. albicans* biofilm culture had no influence on the bacterial biofilm growth [29].

Several examples of shifts in gene expression in *C. albicans*-*S. aureus* and *C. albicans*-*S. epidermidis* biofilms have been described.

CodY is the transcriptional repressor of several *S. aureus* virulence factors (PIA-dependant biofilm formation, hemolysin production, virulence regulators), and is up-regulated when bacteria are in contact with yeast forms of *C. albicans*. *S. aureus* may thus coexist with *C. albicans* yeast cells by down-regulating its virulence factors. However, in presence of hyphal forms CodY would be down-regulated, thus enhancing toxin production and biofilm formation. In this case, L-lactate dehydrogenase 1 would be also up-regulated, which could increase the resistance toward host derived-oxidative stressors [31].

Peters *et al.* showed that numerous stress-related genes were up-regulated during *C. albicans*-*S. aureus* biofilm formation. Antibacterial-stress, heat-stress and oxidative-stress proteins associated to *S. aureus* were constantly up-regulated in presence of *C. albicans*. Regarding fungi, several stress proteins were also up-regulated, such as Heat Shock Proteins (produced in response to heat, UV exposure, starvation, hypoxia, toxin exposure, dehydration), Mac1p (copper uptake) and Tsa1p (peroxide stress) [31].

In *C. albicans*-*S. epidermidis* polymicrobial biofilms, the transcriptome analysis of *S. epidermidis* showed a repression of two autolysis repressors: *IrgA* and *IrgB*. This down-regulation was

associated with an increase of eDNA concentration in biofilms, possibly linked with autolysis considering that secretion of eDNA by *S. epidermidis* in biofilm had not been reported yet [33].

Consequences of interactions

Interactions between *Staphylococcus* sp and *C. albicans* can have different consequences. Fungal or bacterial single-species biofilms generally enhance drug resistance [14–16]. The polymicrobial nature of some biofilms can further increase this resistance. For example, different teams recently showed that bacteria in *C. albicans*-*S. aureus* polymicrobial biofilms displayed decreased susceptibility toward antibiotics (vancomycin, gentamycin, oxacillin, nafcillin) compared to in *S. aureus* single species biofilms [24,32,37,40]. An increased resistance of *S. epidermidis* toward vancomycin was also reported in polymicrobial biofilms [25]. Interestingly, the susceptibility of *C. albicans* toward amphotericin B in biofilms formed with *S. aureus* remained unaltered [24].

Concerning virulence, hyphae of *C. albicans* are key components for fungal invasion of epithelial cells [41]. The ability of *S. aureus* to bind hyphae allows it to reach deeper tissue and may facilitate infection [34]. An *in vivo* model of catheter infection showed that the *S. epidermidis* dissemination increased in case of an infection alongside *C. albicans* [33]. Moreover, experiments on a mouse model of peritonitis showed that polymicrobial infections by *C. albicans* and *S. aureus* led to a higher mortality [42–46]. More recently, Kean *et al* showed that the association of these two microorganisms also increased the mortality of *Galleria mellonella* larvae [35]. The association between *C. albicans* and *S. epidermidis* also leads to an increased mortality on *Caenorhabditis elegans* model: in presence of polymicrobial biofilms, the survival rate decreased compared to that in presence of single-species biofilms [47]. Furthermore, Pammi *et al.* showed on a mouse model of catheter biofilm infection that polymicrobial biofilms formed by *C. albicans* and *S. epidermidis* lead to a higher catheter infection and increased dissemination of *S. epidermidis* in mice [33].

***C. albicans* interactions with *Streptococcus* spp.**

Multispecies biofilm models consisting of both *C. albicans* and bacteria belonging to the *Streptococcus* genus were investigated by many labs worldwide in recent years. Interactions between *C. albicans* and *Streptococcus* spp. were especially investigated in the oral context, and here again, bacteria and fungi can interact in both direct and indirect ways. Figure 2

illustrates the main features of relationships established between *C. albicans* and *Streptococcus* spp. (Figure 2).

Adhesion

C. albicans adhesion to streptococcal cells depends on the studied *Streptococcus* species and on the studied *C. albicans* strain [48,49]. The adhesion mechanism is multifactorial and numerous factors influencing the *C. albicans*-*Streptococcus* spp. adhesion are known on both sides.

On the bacterial side, experiments of Holmes *et al.* showed that multiple adhesins were involved in the interaction between *Streptococcus gordonii* and *C. albicans*. They showed that inactivation of *csxA* and *csxB* genes, encoding cell surface polypeptides, reduced adhesion of *C. albicans* to *S. gordonii*. Inactivation of *sspA* and *sspB* genes, encoding antigen I/II salivary adhesins, also altered the capacity of interaction between these microorganisms [50]. Glycosyltransferases (Gtfs) produced by streptococcal cells are a key factor in *C. albicans*-*Streptococcus* spp. coadhesion. The GtfB secreted by *S. mutans* can bind to mannans of the cell-wall of yeast and hyphal forms of *C. albicans* allowing a production of glucan *in situ*. Once produced, the glucan increases *S. mutans* ability to adhere to fungi coated with this polysaccharide [51–54]. Moreover, Falsetta *et al.* reported that *S. mutans* mutants lacking *gtfB* and *gtfC* genes displayed a drastically reduced ability to form polymicrobial biofilms with *C. albicans* [52]. For *S. gordonii*, GtfG may have the same role, leading to glucan coated *C. albicans* cells that would allow an increased adhesion of streptococcal cells [55].

On the fungal side, the hypha-specific Als3 adhesin is a key component for *C. albicans*-*Streptococcus* spp. interaction [56,57]. Silverman *et al.* reported that *S. gordonii* could not adhere to *C. albicans* if Als3 was missing and that SspB and Als3 could interact [56]. Other adhesins from the Als family, Als1 and Als5, were also reported to take part in *C. albicans*-*S. gordonii* adhesion [58]. Furthermore, Eap1 and Hwp1 surface proteins are also involved in the binding of *C. albicans* to *S. gordonii* [57]. Cell-wall associated secreted aspartyl proteinase Sap9 is involved in adhesion too. Dutton *et al.* showed that polymicrobial biofilms formed using *C. albicans* mutant $\Delta sap9$ and *S. oralis*, *S. sanguinis*, *S. parasanguinis* and *S. mutans* taken individually contained more matted hyphae and more bacteria adhered to the substrate compared to biofilms formed with *C. albicans* wild-type. Moreover, these authors reported that hyphae of the mutant strain displayed an increased surface hydrophobicity and increased levels

of Als3 [59]. Finally, Sullivan *et al.* reported that *C. albicans* ability to adhere to *S. gordonii*, *S. oralis* or *S. sanguinis* increased two to three-fold in human saliva. In saliva, basic proline-rich proteins would bind to bacteria providing extra receptors for fungal adhesion [60].

Structure and Matrix

C. albicans and Streptococci would have mainly synergistic relationship that would lead to biofilms with enhanced biomasses.

Diaz *et al.* noticed an impressive difference for *S. oralis* behavior, which alone nearly did not form mucosal biofilm under flow conditions contrary to associated with *C. albicans*. Several authors demonstrated that *S. oralis* did not influence *C. albicans* growth [61,62]. Another report from Cavalcanti *et al.* mentioned that *C. albicans*-*S. oralis* biofilms displayed increased biovolumes. *S. oralis* would also increase hyphal formation of *C. albicans* [63].

Regarding *C. albicans*-*S. gordonii* biofilms, it was also reported that bacteria promoted filamentation, whether bacteria were added before or after yeasts. Bacteria enhanced the biofilm formation by fungi [64]. Ricker *et al.* also reported a higher biomass in polymicrobial biofilms formed by *C. albicans* and *S. gordonii* under static conditions compared to single species conditions [55]. In this polymicrobial biofilm, matrix is formed partially by high amounts of *S. gordonii* α -glucans [55].

C. albicans-*S. mutans* biofilms also displayed increased biomasses [52,65–67]. The proportion of viable *S. mutans* cells would increase in polymicrobial conditions [52]. The production of EPS increased in polymicrobial conditions [52,54]. However Sztajer *et al.* reported that EPS secretion by *S. mutans* would strongly decrease [65]. The EPS produced by *S. mutans* (α -glucans) and *C. albicans* (β -glucans, chitins...) would act as a scaffold for biofilm formation and the α -glucans would prevail in the matrix [52,54].

Communication

Several molecules from bacteria were identified responsible for inter-kingdom communications. *LuxS* gene is involved in synthesis of autoinducer 2 (AI-2) which is implied in the quorum sensing of *Streptococcus* spp. Bamford *et al.* used a $\Delta luxS$ *S. gordonii* mutant, without AI-2 production, and reported that biomasses of polymicrobial biofilms were reduced of 35% compared to using the wild strain. The hyphae/yeast form ratio was also reduced, with a 30% reduction of hyphae which appeared shorter compared to those observed using the wild

bacterial strain. The addition of exogenous DPD (precursor of AI-2) did not compensate the lack of AI-2 production by the Δlux mutant. Authors suggested that AI-2 and other signal molecules could be involved in the enhancement of the hyphal formation [64].

The fatty acid diffusible signal factors (DSF) represent another class of signaling molecules for streptococcal cells. It includes *trans*-2-decenoic acid which was reported to play a role in inter-kingdom communication and was found secreted by *S. mutans*, *Streptococcus mitis*, *S. oralis* and *S. sanguinis*: DSF can suppress *C. albicans* filamentation and repress the expression of *HWP1*, hyphal-specific gene [68].

Competence-Stimulating Peptide (CSP) is another molecule involved in *Streptococcus* spp. quorum sensing and inter-kingdom communication. The conditioned cell-free medium from a *S. mutans* culture was reported to decrease the filamentation of *C. albicans* [66,69]. Jarosz *et al.* used the mutant *S. mutans* $\Delta comC$ strain unable to produce CSP and showed that conditioned medium of this mutant strain did not inhibit filamentation. This inhibition was restored by adding synthetic CSP in the spent medium of the mutant strain. Moreover, CSP may also force hyphae to yeast transition [69]. In addition, Jack *et al.* observed that mutants *S. gordonii* $\Delta comCDE$ or $\Delta comC$ formed biofilms with *C. albicans* with increased biomasses compared to those obtained using the wild-type strain. Polymicrobial biofilms formed with $\Delta comCDE$ also tended to contain more eDNA [70].

Xu *et al.* reported that *S. oralis* increased Efg1 expression (master regulator of *C. albicans* biofilm formation [71]) in *C. albicans* late biofilm which lead to an increased expression of *ALS1* which promoted coaggregation and mucosal biofilm growth. However the mechanism remains unknown [72]. Bcr1 is another master regulator of *C. albicans* biofilm formation which is influenced by Streptococcal proteins [73]. GtfB would also increase polymicrobial *C. albicans*-*S. mutans* biofilm formation by a *Bcr1*-independent mechanism [74].

Experiments involving a mutant strain of *S. mutans* carrying a transcriptional fusion between a green fluorescent protein-encoding gene and the promoter for *sigX* (alternative sigma factor of *S. mutans*), showed that *S. mutans* quorum sensing was activated by *C. albicans* [65]. In polymicrobial biofilms, *sigX* was strongly induced, it was also induced by conditioned media from polymicrobial cultures. However, *sigX* was not induced in single-species biofilm conditions or by conditioned media originating from single-species biofilm of *C. albicans* or *S. mutans*. Transcriptome analysis showed that *comS*, *sigX*, bacteriocins and the down streams late

competence genes were induced in polymicrobial biofilms. Actually complete quorum sensing system of *S. mutans* was activated by *C. albicans* [65].

As for *S. aureus*, farnesol was also reported as being responsible for inter-kingdom communications between *Streptococcus* spp. and *C. albicans*. Farnesol can inhibit glucosyl transferase of *S. mutans* [75]. Fernandez *et al.* reported that farnesol decreased biofilm formation, metabolic activity and cell viability in single and polymicrobial *C. albicans*-*S. mutans* biofilms but had no effect on *S. mutans* metabolic activity in single-species biofilms [76,77]. The acid production of *S. mutans* in single-species biofilms was also reduced by this molecule [77]. Farnesol displayed a concentration-dependent effect on *S. mutans*. Kim *et al.* reported that at 25-50 μ M the farnesol of conditioned medium of *C. albicans*-*S. mutans* polymicrobial culture enhanced the *S. mutans* biofilm cell growth, microcolony formation and Gtf activity [67].

Streptococcus spp. can also modulate the effect of farnesol. Bamford *et al.* reported that the addition of farnesol 30 μ M to a *C. albicans* culture was enough to inhibit the hyphal formation whereas this concentration had no effect when *S. gordonii* was cocultured with *C. albicans*. Authors suggested that *S. gordonii* could block or inactivate the farnesol signal, and/or induce another signaling path in *C. albicans* that could override the farnesol signal [64].

Consequences of interactions

Recent articles suggest that the co-presence of *C. albicans* and *Streptococcus* spp. in biofilms would influence the susceptibility of *C. albicans* and *Streptococcus* spp. to antimicrobial agents. Montelongo-Jauregui *et al.* recently reported that *C. albicans*-*S. gordonii* biofilms induced increased resistance on both sides. Fungal cells were more resistant to amphotericin B, echinocandins (caspofungin) and azoles (fluconazole) and *S. gordonii* was more tolerant to clindamycin [78].

Polymicrobial interactions can also influence virulence of microorganisms present in biofilms. The mitis group streptococci are commensal of healthy oral human microbiome, however there are considered as “accessory pathogens” because they reveal their pathogenic potential in polymicrobial communities [79,80]. *S. oralis* belongs to this group. Studying a murine oral mucosa model, Xu *et al.* showed that concomitant presence of *S. oralis* and *C. albicans* increased frequency and size of oral thrush lesions, although *S. oralis* strain used alone did not; in polymicrobial conditions *C. albicans* cells number was similar to that in single-species ones. Moreover, the dissemination of *C. albicans* in deep organs was enhanced and presence of *S.*

oralis in oral and gastrointestinal tracts was increased in polymicrobial conditions. In mice, an enhanced inflammatory response was observed [62,81]. *In vitro* mucosal tissue models also showed that *S. oralis* promoted fungal invasion and tissue damages [72,82]. Furthermore, on oral epithelial models *C. albicans* invasion through epithelial junctions was observed when cultured with *S. oralis*. Authors suggested that the decrease in epithelial E-cadherin levels by a synergistic activation of μ -calpain (proteolytic enzyme which targets E-cadherin) by *C. albicans* and *S. oralis* would be responsible of their results [81].

However, this enhanced virulence was also observed with *Streptococcus* species which did not belong to the mitis group. For example, with a rat model of dental carries involving *C. albicans* and *S. mutans*, Falsetta *et al.* showed that this coinfection enhanced the virulence of these two organisms, hence potentially carries formation [52].

***C. albicans* interactions with obligate anaerobic bacteria**

C. albicans and different strict anaerobes can be encountered in similar environment on the Human body. For example, *C. albicans* and *Clostridium difficile* are part of the subgingival-associated biofilm microbiota [83]. *C. difficile*, *Clostridium perfringens* and *C. albicans* are normal resident of the intestinal flora [84–86]. Therefore, different authors investigated interactions between *C. albicans* and some of these obligate and facultative anaerobic bacterial species within biofilms.

Fox *et al.* performed experiments on polymicrobial biofilms formed by *C. albicans* and some bacteria from the gut microbiome, including *C. perfringens* [87]. They showed that *C. perfringens* cells adhered to both yeast- and hyphal-forms and were able to form biofilms with *C. albicans* in both ambient oxic and anoxic conditions, without triggering a notable change in its architecture. Moreover, the presence of *C. perfringens* did not influence yeasts adherence, but bacterial adherence increased tenfold in presence of yeasts. In ambient oxic condition, *C. perfringens* viability decreased drastically within the first 24h when cultivated alone. However, Fox *et al.* also demonstrated that *C. albicans* and *C. perfringens* formed dense aggregates similar to miniature biofilms in suspension in ambient-oxic conditions and, in so doing, aggregates provided a hypoxic micro-environment that sustained survival and growth of the anaerobic bacteria [87].

Leuween *et al.* reported that *C. albicans* also allowed *C. difficile* growth in aerobic conditions, normally toxic for this bacterium, this phenomenon being not linked to adherence to hyphae nor

biofilm formation as *C. difficile* does not adhere to *C. albicans* hyphae nor forms biofilm with it. Furthermore, in anaerobic conditions, yeast-forms were predominant in coculture with *C. difficile*, whereas hyphae prevailed in single species cultures. Leuween *et al.* showed afterward that conditioned medium of *C. difficile* inhibited *C. albicans* filamentation and induced hypha-to-yeast conversion. The *P*-Cresol, a resulting product of tyrosine fermentation, secreted by *C. difficile* may be at least partially responsible for the effect of conditioned medium [88].

By studying the influence of *C. albicans* on oral biofilms, Janus *et al.* showed that Shannon diversity increased in presence of fungi. Presence of strictly anaerobic bacteria (*Fusobacterium*, *Prevotella*, *Veillonella* genera...) in aerobic conditions was enhanced when cocultivated with *C. albicans* [89].

The positive influence of *C. albicans* on the presence, survival and growth of anaerobic bacteria could be linked to its faculty to consume rapidly the environmental oxygen. A recent report by Lambooij *et al.* showed that the consumption of dissolved oxygen by fungal mitochondrial respiration was very rapid. This depletion allows the creation of microaerophilic niches that favour anaerobic bacteria presence [90].

Conclusion

C. albicans is able to form polymicrobial biofilms with many different partners. Here the focus was set on the capacity of the fungi to interact with different Gram-positive bacteria: recent data on *S. aureus*, coagulase negative *Staphylococcus*, *Streptococcus* spp. and *Clostridium* spp. were reported. Many of these bacterial species have been shown as setting-up a synergetic relationship with *C. albicans*: for example, in some cases *C. albicans* can enhance bacterial resistance towards antibiotic agents, or permit the survival and growth of anaerobic bacteria in aerobic conditions. The virulence of these opportunistic pathogens can also be influenced by inter-kingdom interactions: for example, the association between *C. albicans* and *S. aureus* increases the lethality of infections on mouse model.

These observations point out that study of inter-kingdom interactions within biofilms is of utmost importance. New and more complex polymicrobial-biofilms models are required. They would need to associate several microbial species in accordance with what occurs in nature or in the human body, as physical and chemical interactions can deeply influence the physiopathology and the treatment of biofilm-related infections.

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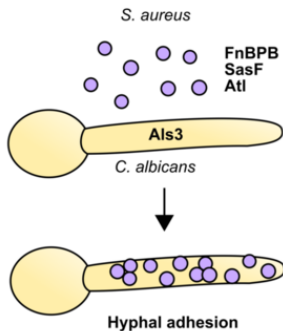
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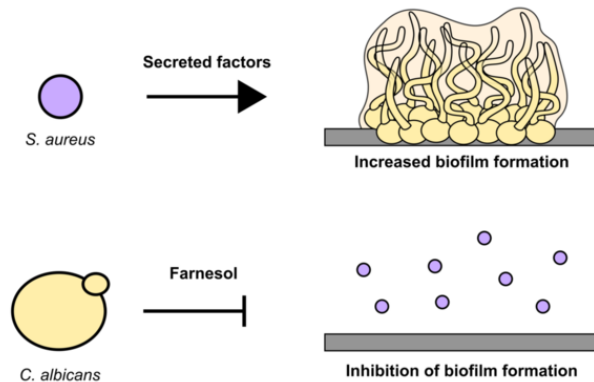
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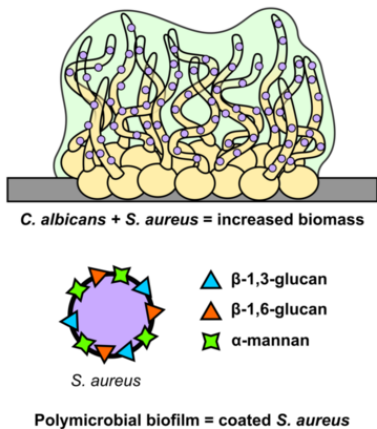
Adhesion



Communication



Biofilm structure and matrix



Consequence of interactions

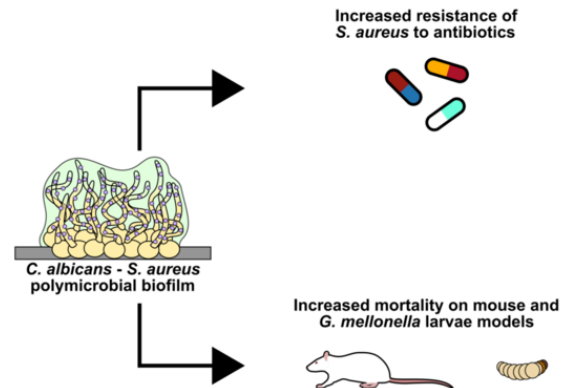
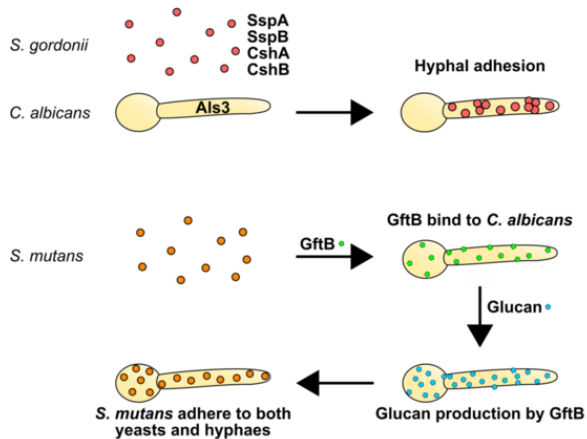
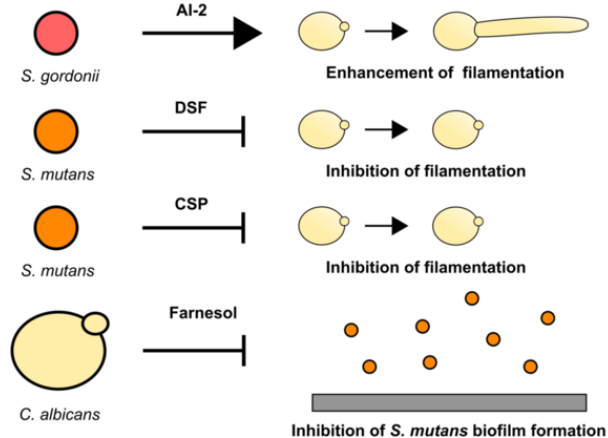


Figure 1: Main features of *C. albicans*-*S. aureus* relationship related to biofilm lifestyle

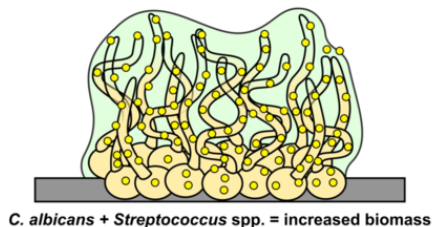
Adhesion



Communication



Biofilm structure and matrix



Consequence of interactions

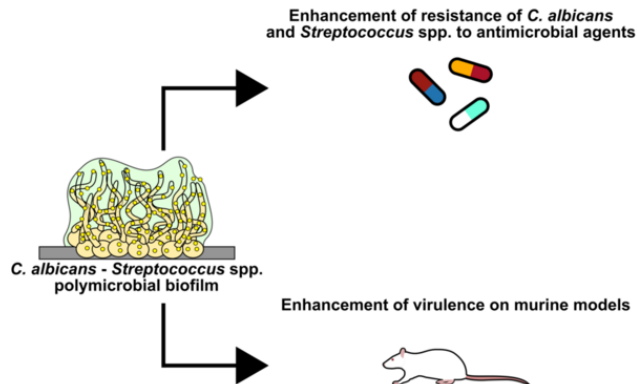


Figure 2: Main features of *C. albicans*-*Streptococcus* spp. relationship related to biofilm