Chapter

ADVANCES IN *CANDIDA* SP. BIOFILM MANNANS

Jesus A. Romo[1], Maria Elisa Rodrigues[2], Liliana Fernandes[2], Csaba Papp[3], Attila Gácser[3,4] and Célia F. Rodrigues[5,*]

[1]Department of Molecular Biology and Microbioloy,
Tufts University, Boston, MA, US
[2]Centre of Biological Engineering, Laboratório de Investigação em Biofilmes Rosário Oliveira, University of Minho, Braga, Portugal
[3]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[4]MTA-SZTE "Lendület" "Mycobiome" Research Group, University of Szeged, Szeged, Hungary
[5]LEPABE - Laboratory for Process Engineering Environment Biotechnology and Energy-Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

[*] Corresponding Author address. Email: c.fortunae@gmail.com

ABSTRACT

Candida species belong to the normal human microbiota and are commonly responsible for several clinical manifestations from mucocutaneous overgrowth to severe bloodstream infections. *Candida albicans* is the predominant species involved in disease conditions. Nevertheless, in the last decade, the number of infections due to non-*albicans Candida* (NAC) species has significantly increased. *Candida* species have several virulence factors, such as hyphal growth, secretion of hydrolases, and the ability to form biofilms. Biofilm formation is considered one of the main virulence factors of *C. albicans*. Biofilm production can occur on the host mucosa and on the surface of medical indwelling devices and includes a self-produced polymeric matrix that encloses fungal micro-colonies in a complex structure. The main components of the biofilm matrix are polysaccharides (e.g. β-1,3 glucans and mannans), but proteins, DNA and lipids (e.g. ergosterol) can also be found in variable amounts. In this chapter, we will discuss the role of mannans within *Candida* biofilms. Additionally, the role of *Candida* biofilms in fungal structure, pathogenesis, and resistance will also be addressed.

Keywords: *Candida*, mannan, β-1,3 glucans, biofilm, matrix, pathogenesis, cell wall, NAC

INTRODUCTION TO BIOFILMS AND MANNANS

The majority of microorganisms in the environment and hospitals are able to grow on the surface of various materials in form of biofilms, which are communities attached to either biotic or abiotic surfaces and ecased in a self-secreted polymeric matrix [1]. In the hospital environment, biofilm formation occurs mostly on implanted plastic devices, while in a host biofilm formation can occur on mucosal surfaces [2, 3]. The most common fungal organisms able to form biofilms on clinically relevant surfaces belong to the genus *Candida* and the complexity of the biofilm varies depending on the specific *Candida* species [2, 4]. A common characteristic of most biofilms, including those formed by *Candida*, is the presence of an extracellular matrix (ECM). The main function of the ECM is to preserve

the structure of the biofilm, although the ECM also plays a role in antifungal drug resistance as it prevents antifungal agents to penetrate through the biofilm [4–6]. Generally, the main components of ECMs are proteins (55%), carbohydrates (25%), lipids (15%) and extracellular DNA (5%), all of which are present in *Candida* biofilms [7, 8].

Mannose is a common compound of glycosylated eukaryotic proteins in various species, however the structure and size of mannans on proteins greatly differs between the different organisms. Basically, there are two types of protein glycosylation: O-glycosylation and N-glycosylation – with both present in eukaryotes, including fungi. The glucan chain of O-glycosylated proteins is shorter than in N-glycosylated proteins and in fungi show less diversity compared to mammalian or insect O-glycosylation [9–11]. Both N- and O-glycans are synthetized in the endoplasmic reticulum (ER) and the Golgi. In the next sections we will discuss the structure of the fungal cell wall with a special focus on mannose glycosylation of cell wall proteins in yeast and review how *Candida* biofilms are structured focusing on the biofilm matrix and the mannan portion of ECM.

The Fungal Cell Wall: Role in Structure and in Pathogenesis

The fungal cell wall is a highly complex and dynamic structure composed mainly of glycoproteins and polysaccharides such as glucan and chitin. Furthermore, this structure is involved in wide range of processes in the biology of fungi. These include protection from changes in osmotic pressure and other stressors [12], cell growth and division, development of specialized fungal cell types, and interaction with the environment (i.e., adhesion) [13–16]. The cell wall is also highly dynamic having to adapt to morphological changes such as filamentation, which involve yeast cells becoming hyphae [17–19]. This structure is also highly specific to fungi and therefore represents a valuable target for vaccine [20–22] and antifungal development. Indeed, two of the three main classes of antifungal therapies

target the cell wall [23–25]. In this section, we explore the structural role of the fungal cell wall and its role in pathogenesis.

Cell Wall Components

Although there can be variations in the composition of the fungal cell wall, the main components are present in many fungi [26]. These include chitin, glucans (β-1,3 and β-1,6), and mannans and each plays a distinct role within the cell wall. All of these components are interconnected throughout the cell wall. Closer to the plasma membrane of the fungal cell is chitin, a linear homopolymer of β-1,4-linked N-acetylglucosamine, which only accounts for 1-2% of the cell wall in yeast [27, 28] and up to 20% in filamentous fungi, but the tensile strength it provides significantly contributes to cell wall integrity and stability. The contribution of chitin to the structure and integrity of the cell wall was previously tested by using Nikkomycin Z, a competitive inhibitor of chitin synthase. The investigators reported that the inhibition of chitin-synthase activity resulted in significant alterations in hyphal morphology and an overall reduction in fungal wall thickness [29]. Importantly, in some fungi such as *C. albicans*, proper hyphal formation is required for biofilm formation and pathogenesis [17, 30]. Present in the outer cell wall are glucans, which have been shown to be the major structural constituents of the fungal cell wall accounting for up to 50-60% of the cell wall [31]. These compounds can be found in several forms, but in yeast such as *Saccharimyces cerevisiae* and *C. albicans*, they are found as β-1,3 forming structural scaffolds and β-1,6 forming branches [32]. Lastly, interwoven throughout the chitin and glucan scaffolds are proteins, which are reported to account for 30-50% of cell walls of fungi such as *S. cerevisiae* and *C. albicans* [33]. In this category are the mannans, which are proteins glycosylated with chains rich in mannose [33]. Cell wall proteins play a wide array of roles in the fungal cell wall such as mediating cell adhesion, maintaining cell wall structure by synthesizing cell wall components, maintenance of cell shape, among others. It is important to recognize that although the components described above are found in most

fungi, extensive differences are also present in some fungi [34, 35]. This complex array of structures plays important structural and functional roles within the fungal cell wall, but they are also able to interact with the surrounding environment such as that of the host.

Immune Recognition/Interaction of Host with Fungal Cell Wall Components and Pathogenesis

The immune system of mammals is able to recognize and respond to all of the main components found in the fungal cell wall. Chitin and mannans are both recognized by the mannose receptor (CD206), while β-1,3 glucans are recognized by Dectin-1, CR3 (CD11c/CD18), CD5, CD36, and SCARF1. Additionally, mannans are also recognized by DC-SIGN (CD209), Langerin (CD207), and Dectin-2 [36–39]. Additionally, other receptors can sense more unique structures and components in other fungi [40], a discussion which is beyond the scope of this chapter. The ability of a healthy host to sense and respond to fungi often results in the prevention of pathogenesis. Indeed, most fungal infections are associated with immunocompromised individuals (i.e., AIDS, organ transplants, medically compromised). There are many examples of fungal pathogens devising strategies to overcome the immune response. In this section, we will discuss two examples to illustrate the consequences of these interaction.

Candida glabrata is an opportunistic pathogenic yeast and a common colonizer of the human gastrointestinal (GI) tract. *C. glabrata* is able to disseminate from the GI to cause invasive candidiasis in immune-compromised individuals [41–43]. As described above, the cell wall of fungi is highly complex and dynamic, with the ability to adapt to distinct environments. *C. glabrata* is able to respond to its environment by remodelling cell wall components. Recently, Charlet and co-workers investigated the effect of cell wall remodelling by *C. glabrata* in the context of the GI using a murine dextran sulfate sodium (DSS) colitis mode [44]. In this model, *C. glabrata* was able to remodel the cell wall after passage through the GI with an increase in chitin and α-mannans and a significant

decrease in α-mannans. Additionally, *C. glabrata* was able to persist longer in the GI of mice in the DSS model, which the authors attribute to the remodelling of the cell wall. The authors also studied the impact of improper cell wall structure on the ability of *C. glabrata* to colonize and cause disease. Using a chitin synthetase mutant (Δ*Chs1*) *C. glabrata* strain, which contained higher levels of chitin and α-mannans in the cell wall, the authors described enhanced virulence in the DSS model compared to WT. These results highlight the importance of cell wall structure and composition in the host environment and persistence. Additionally, apart from *C. glabrata* stimulating an immune response, it was also able to impact the composition of the GI microbiota by significantly reducing the population of *Lactobacillus johnsonii*. These effects could be due in part to the immune response that *C. glabrata* induces in the GI, direct antagonistic interactions with *L. johnsonii*, or a change in the metabolite milieu leading to an unfavorable environment for *L. johnsonii*. In summary, this study highlights the importance of the fungal cell wall in the context of colonization and disease. In other studies, genes related to the mannans production where found overexpressed and mannans concentration in the matrices was clearly higher, when *C. glabrata* biofilms were under drug stress [45, 46].

As described above, the opportunistic pathogenic fungus *C. albicans* is a common colonizer of the GI tract and responsible for a large number of fungal infections [19, 47–50]. As shown by Wagener and co-workers, *C. albicans* is able to use its cell wall to evade and modify the host immune response [51]. In this study, the authors demonstrate that *C. albicans* is able to survive inside macrophages by induction of host arginase activity, which blocks the production of nitric oxide in human-monocyte-derived macrophages. Moreover, *C. albicans* demonstrated the ability to influence macrophage polarization from a classically activated phenotype to an alternatively activated phenotype, which has reduced antimicrobial functions, leading to *C. albicans* enhanced survival. The authors further described that the main component of *C. albicans* leading to the effects observed was chitin. Therefore, fungal cell wall composition affects interaction with host macrophages, which can enhance survival of the pathogen.

The two examples described above underscore the importance of the fungal cell wall in colonization, pathogenesis, and persistence in the host environment.

MANNAN: VARIATIONS IN YEASTS

As explained, mannans form the outermost layer of the yeast cell wall. Although, β-1,3-glucan is the major component of the fungal cell wall compared to the total cell wall dry-weight, mannans occupy much higher volume compared to the volume of other cell wall polysaccharides (β-glucan, chitin) in *C. albicans* [52]. The majority of the mannose in the fungal cell wall is presented as O- or N-linked mannan on specific protein residues.

O-glycans are bound to serine or threonine -OH groups on the surface of proteins. For O-glycosylation there are no specific sequences to initiate glycosylation. Instead, the presence of O-glycan residues is determined by the conformation of the polypeptide chain. O-glycosylation is diverse in higher eukaryotes, but in fungi it contains mannose only without branches [52–54]. The initial O-glycan residue is α-mannose and its binding is directed by Pmt1, Pmt2, Pmt3, Pmt4, Pmt5 and Pmt6 in the endoplasmic reticulum [54–56]. PMT family members bear redundant function, however, Pmt2 appears to be essential for the viability of *C. albica*ns. After the initiation of O-glycosylation, proteins are transported to the Golgi apparatus for subsequent modifications, including the formation of 1,2-bouds between α-mannose monomers by Mnt1 and Mnt2 (Figure 2) [14, 54–56]. Deletion of the corresponding genes resulted in reduced biofilm forming capacity and they also showed adhesion deficiencies (Figure 2) [54, 57, 58]. According to this knowledge O-mannan possibly has an importance in the biofilm forming ability of different *Candida* species.

The polysaccharide chain of N-glycosylated proteins is a branched structure and in fungi it also consists of mannose. The synthesis of N-glycans has two steps that take place in different organelles: the ER and the Golgi apparatus. The first phase takes place in the ER and is a highly conserved biosynthetic pathway among eukaryotic organisms. In this phase the N-

glycosylation core is synthetized on the lipid dolichol-phosphate (Dol-P). Dolichol is a polyisoprene lipid molecule. In yeasts, dolichol consist of 14 isoprene units [59, 60]. During the first part of the synthesis of the N-glycan core, N-Acetyl-D-glucosamine (GlcNAc) and D-Mannose (Man) serve as monomers, after which they are activated by nucleotides UDP and GDP, respectively [61]. This first part is taken place at the outer side of ER until the Man5GlcNAc2-P-P-Dolichol is formed. Then, the molecule is "flipped" into the lumen of the ER by the product of a mammalian RFT1 ortholog, where for the later mannose and glucose incorporation Man-P-Dol and Glc-P-Dol serve as glucan donors. Man-P-Dol and Glc-P-Dol are synthetized in the cytoplasm by Dpm1 and Alg5, respectively. In order to be utilised, further 4 mannose and 3 glucose Man-P-Dol and Glc-P-Dol donor molecules must be "flipped" to the lumen of the ER. From the addition of the first GlcNAc to P-Dol (Glc3Man9Glc NAc2-P-P-Dol) different members of the ALG (Asparagine-Linked Glycosylation) loci catalyse the transfer of sugar residues from the donor molecules (UDP-GlcNAc, GDP-Man, Glc-P-Dol, Man-P-Dol,) to the developing N-glycan core [10, 61]. The subsequent translocation steps include the formation of various types of covalent bounds between sugar residues. Then, two mannose molecules bind to the first one, one by α-1,3- and the other by α-1,6-bound. In the lumen of the ER, two additional mannose molecules bind to the α-1,3-mannose by an α-1,2-bound. Inside the ER, two mannose monomers will bind to the α-1,6-mannose by α-1,3- and α-1,6- bounds [10, 61]. The synthetized glycan precursors are then transferred to the -NH2 group of asparagine at specific Asn-X-Ser/Thr amino acid sequences - where "X" can be any amino acid except for proline - by the oligosaccharyltransferase (OST) complex. Following the transfer of the N-glycan core to the nascent protein, glycosidase I and II trim all the Glc residues for correct protein folding. Before the folded protein is transported to the Golgi, the ER mannosidase I cuts the terminal α-1,2-mannose from the central antenna [62].

In all eukaryotic organisms the maturation of the N-glycan takes place in the Golgi apparatus, however the machinery of glycosylation and the structure of N-glycans are very divergent between different species. In

yeasts the mature N-glycan consists of high amounts of mannose with branches [63]. The donor molecule for mannan extension is GDP-Man in the Golgi apparatus. During the synthesis of the mannan outer chain one glycosyltransferase or a redundant family of glycosyltransferases are responsible for the production of specific bounds between the mannose residues [63]. The first step of outer chain synthesis is the addition of α-1,6-mannose to the basal α-1,3-mannose at the base of the long mannose chain in the N-mannan core, which is catalysed by Och1. Further elongation of the α-1,6-mannose backbone is catalysed by the mannan polymerase I and II complexes, both composed of 2 proteins: Mnn9 in both and Van1 or Anp1, respectively [64]. In *C. albicans* Mnn9 is a key enzyme in α-1,6-polymannose synthesis as Δ/Δ*mnn9* deletion mutants bear significantly less mannan in the cell wall [54]. Operation of Mnn9 results in a long α-1,6-mannose backbone, containing repetitions of 10 α-1,6-mannose residues. These repetitions bear the same mannose branch motifs. The initiative mannose residue of the extensive branches is bound to the α-1,6-mannose backbone via α-1,2-linkages, which is catalysed by Mnn2 and its further 5 gene products (*MNN2*-family). The initial α-1,2-mannose residues are extended with 2 or 3 α-1,2-mannoses due to Mnn5. The absence of Mnn2, Mnn5 and the functionally related proteins results in the deletion of larger mannan fibrils [65]. In yeasts α-1,2-mannose branches are usually capped with an α-1,3-mannose residue. In *C. albicans* α-1,3-mannose caps are formed due to the operation of the *MNN1* gene family, which contains six members and shows redundancy as reported by previous studies [66]. A special aspect of protein glycosylation in *Candida* species is that the α-1,2-mannose branches end with 2 or 3 β-1,2-mannose residues, however β-1,2-mannose is not presented in the cell wall of *S. cerevisiae*. The addition of β-1,2-mannose to N-glycans is performed by β-mannosyltransferases (BMT). In *C. albicans* BMTs are encoded by the *BMT1* and *BMT3* genes. Depletion of β-1,2-mannose does not affect the viability or the cell wall structure of *Candida* cells however, such alteration is recognized by the galectin-3 immune receptor presented on the surface of neutrophils and macrophages [67,68]. Due to this, the importance of the β-1,2-mannose in the cell wall of *Candida* species is unclear (Figure 1).

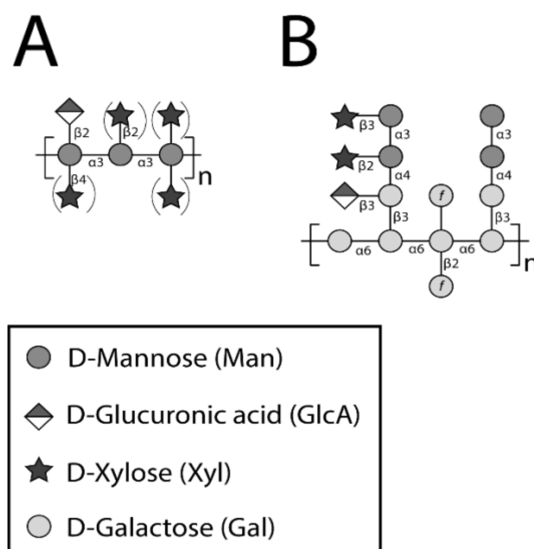

Figure 1. Polysaccharides of yeast cells with significant mannan content. Basic structure of cryptococcal capsule, consisting of glucuronoxylomannan (GXM) (A) and glucuronoxylomannogalactan (GXMGal) (B). Squared brackets [] represent the repetition of polymeric structures. Parentheses () represent the eventuality event of Xyl substitution on the GXM backbone, that determines the serotype of Cryptococcus strains.

Cryptococcus species are basidiomycete yeasts. The unique feature of the cryptococcal cell surface is that it is covered with a capsule [69]. The capsule of *Cryptococcus* species varies in size between the different species; however, the main components remain glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal), which contain mannose residues. Nearly 90% of the capsule dry mass consists of GXM, but GXMGal is also present. The backbone of the GXM consists of an α-1,3-mannose polymer substituted with β-1,2-linked glucuronic acid (GlcA) and β-1,2- or β-1,4-linked xylose (Xyl). An additional modification of the mannose backbone is the 6-O-Acetylation. The substitutions and acetylation on the backbone are repeated in triplets and define the serotype of the different *Cryptococcus* strains (Figure 1A) [69, 70]. In contrast with GXM, the backbone of GXMGal is built up of α-1,6-galactose (Gal), which bears side chains of β-1,2- or β-1,3-linked galactose or galactofuranose (Galf). β-1,3-Gal side

chains are substituted with α-1,4-Man-α-1,3-Man disaccharides, bearing β-1,3-GlcA, β-1,2-Xyl and β-1,3-Xyl, respectively (Figure 1B) [69,70].

CANDIDA SP. BIOFILMS

The virulence of the *Candida* species has been attributed to their ability to form structured aggregates of cells called biofilms [6], which have distinct phenotypes compared to their planktonic cell counterparts [71, 72]. Thus, biofilm cell communities can create a source of persistent infection, more difficult to treat than planktonic cell-induced infections because of an increased resistance to antifungal drugs [71, 73]. In addition, the presence of biofilms reduces the likelihood of removal of organisms by the host defense mechanism, inducing localized pathology and tissue damage [74, 75]. *Candida* species biofilms are among the most common in clinical settings, and can be formed in biotic (e.g., mouth or vaginal mucous membranes) or abiotic (e.g., catheters or prostheses) surfaces [76–78]. One of the most commonly colonized medical devices is the central venous catheter (CVC) used to administer nutrients, fluids and medications to patients [76] infections related to non-medical devices, such as *Candida* endocarditis, may result from the formation of biofilms on damaged vascular endothelium of native heart valves in patients with pre-existing cardiac disease [79]. *Candida* biofilm formation can be explained in four chronological stages (Figure 2): i) surface adhesion/colonization in which the planktonic yeast cells adhere to the surface. The extent of adhesion depends on the characteristics of *Candida* cells and the host and/or abiotic surface properties such as hydrophobicity and cell wall composition (1–3 h); (ii) cell proliferation/invasion and formation of well-organized colonies (11–14 h); (iii) production and maturation of the ECM with differentiation into a mature three-dimensional structure consisting of yeasts, pseudohyphae and/or hyphae (or not, depending on the *Candida* species) embedded in the matrix (20–48h); (iv) dispersion of biofilm cells to promote colonization and infection of distal sites (after 24h) [76, 80–82].

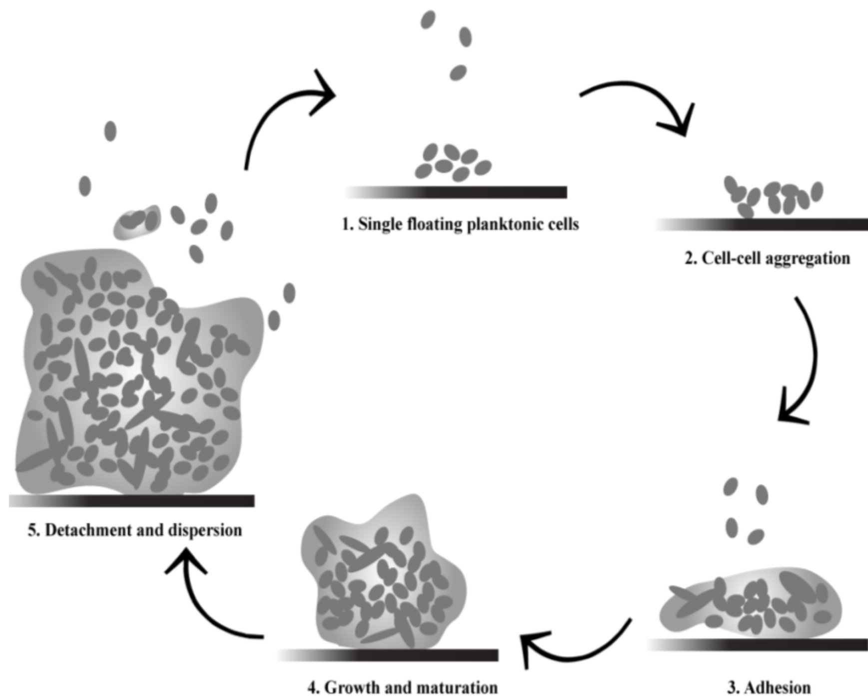

Figure 2. Formation and development of a *Candida* species biofilm on a surface.

As mentioned above, a mature biofilm is comprised of a dense network of cells involved by ECM with water channels between the cells. These channels help in the removal of residues and the diffusion of nutrients from the environment through the biomass to the lower layers [71, 73]. The final architecture of the biofilm is variable and depends, in part, on the growth conditions of the substrate in which it is formed, and, mainly, on the *Candida* species involved and its clinical origin (Table 1) [71, 73]. Cells whitin biofilms usually present several morphologies, from blastospores to true hyphae, depending on the species, e.g., *C. albicans*, can present blastospores, pseudohyphae and hyphae in the same biofilm. Alternatively, species such as *C. glabrata* can only form blastospores. Moreover, several changes can be observed in yeast biofilms induced by changes in environmental conditions, such as oxygen rate, pH and media composition [83]. These changes can affect matrix composition, total biofilm biomass and

yeast morphology. For example, in the presence of media with high glucose content, *C.glabrata* strains form less robust biofilms compared to *Candida tropicalis* and *Candida parapsilosis,* but in poor media, such as artificial urine, the former is a better producer of biofilm than the other species [83]. Furthermore, the morphology of *C. albicans* changes from hyphae to blastospores in response to a pH change, demonstrating here a quick response to environmental stress [83].

Table 1. Characteristics of biofilms of the most common Candida species [74]

<table>
<tr><td>Species</td><td>Biofilm Characteristics</td><td>Biofilm formation</td></tr>
<tr><td>Candida albicans</td><td>Basal blastospores layer.
Dense overlying matrix (exopolysaccharides and hyphae)</td><td>Very high</td></tr>
<tr><td>Candida dubliniensis</td><td>Chains of cells with thin extracellular matrix material.
High variability among clinical isolates.</td><td>High</td></tr>
<tr><td>Candida glabrata</td><td>Forms considerably less biofilm than C. albicans. High in both protein and carbohydrate content.</td><td>High/Normal</td></tr>
<tr><td>Candida krusei</td><td>Thick multilayer with pseudohyphal embedded within the polymeric matrix.</td><td>High</td></tr>
<tr><td>Candida parapsilosis</td><td>Clumped blastospores and less volume.
Large amounts of carbohydrate with less protein.
High variability among clinical isolates</td><td rowspan="2">Normal to high</td></tr>
<tr><td>Candida tropicalis</td><td>Large chains of cells with thin extracellular matrix material.</td></tr>
</table>

From the clinical point of view, the most important feature of *Candida* biofilms is their role in resistance to conventional antifungal therapy [74, 84]. The antifungal resistance can be inducible in reaction to a drug, or a permanent genetic change resulting from prolonged exposure to that drug [45, 85, 86]. Although resistance mechanisms of biofilms to antifungals are not fully understood, the current consensus is that biofilm resistance is a complex multifactorial phenomenon involving different molecular

mechanisms of resistance compared to those exhibited by planktonic cells [75, 80, 87]. These include the structural density; complexity of the biofilm, i.e., the intrinsic metabolic heterogeneity; the impact of sterols content and its correlation with expression of *ERG* genes; the biofilm positive regulation of the efflux pump genes, which may pump out the drugs; the presence of persister cells; and the presence of ECM, which induces the limitation of diffusion [71, 75]. Next section will discuss the density and complexity of the biofilm matrix in *Candida* species and its role in the drug resistance.

CANDIDA SP. BIOFILM MATRIX

In *Candida* species biofilms, the ECM is self-secreted by the cells and completely surrounding the biofilm structure. The ECM is a defining characteristic of all *Candida* species biofilms, providing the cells protection from hostile factors and conferring significant resistance to antifungal therapy and intense host immune responses [85, 88, 89]. Little is known about matrix composition of *Candida* species biofilms. However, it is important to address that the composition of the *Candida* biofilm matrices can vary according to the species [74, 83]. In general, the ECM of *Candida* biofilms is composed by carbohydrates, proteins, phosphorus, hexosamines, DNA and uronic acid [74, 83].

Regarding the general constitution, the ECM of *C. glabrata* biofilms (Table 1) has a high amount of proteins and polysaccharides, *C. parapsilosis* biofilms have more carbohydrates and low protein contents than *C. tropicalis* whose biofilms present low carbohydrates and high protein contents compared to the other *Candida* species [74, 83]. However, the major component quantified in *C. tropicalis* biofilm matrices was hexosamine (27%). Recently, Rodrigues et al. (2016) revealed for the first time the presence of β-glucans in the *C. glabrata* matrices even when treated with azoles, amphotericin B and echinocandins [85, 88, 90]. In *Candida* species, there is scarce knowledge concerning the contribution of extracellular DNA to biofilm matrix and overall structure [74, 90, 91].

Although the ECM of *Candida* biofilms is far from completely characterized, it is known that it contributes to biofilm resistance to antifungal therapies and recalcitrance [83, 92, 93]. In this sense, studies have been carried out to broaden our knowledge and to clarify the involvement of some of the matrix components in *Candida* biofilm resistance. Recent studies revealed the involvement of the matrix on *C. tropicalis* strains on amphotericin B resistance, namely in increase of the biofilm production [74, 94]. These studies highlight the incapacity of this traditional antifungal to totally prevent biofilm formation and to eradicate *C. tropicalis* biofilms. This probably occurs due to a response of *C. tropicalis* biofilm cells to the drug stress, which determined the overgrowth of the biofilm matrix. Fonseca et al. and Rodrigues et al. (2014 and 2017) revealed a phenomenon similar for *C. glabrata* with an increase of proteins and carbohydrates in the matrices extracted from biofilms treated with fluconazole [95, 96]. In fact, β-1,3 glucans were linked to antifungal resistance and the proposed mechanism is that these polymers make it difficult to the drugs to diffuse through the biofilm matrices and reach the yeast cells [8, 73, 85, 88, 97–99]. When induced, the disruption of β-1,3-glucans or a β-1,3-glucanase treatment have been shown to increase susceptibility of biofilms to fluconazole and the addition of exogenous β-1,3 glucans has been demonstrated to result in the rise of resistance to fluconazole in planktonic cells [80, 100]. Additionally, it is possible that biofilms can also sequester amphotericin B, as it has been shown that β-1,3-glucans can bind specifically to this drug [80, 90, 101]. Research has recently shown that the ECM β-1,3 glucan is synthesized from glucan synthase Fks1 using a defined knockout and over-expressing strain [74, 102]. This study demonstrated that β-1,3 glucan is responsible for sequestering azoles, conferring resistance on *C. albicans* biofilms [74, 102]. Other studies have shown that they are also responsible for sequestering echinocandins, pyrimidines, and polyenes [74, 103]. Following studies have identified a role for the *SMI1* in *C. albicans*, a gene involved in cell-wall glucans synthesis, in biofilm ECM production and development of a drug-resistant phenotype, which appears to act through transcription factor Rlmp and glucan synthase Fks1. In addition to *Fks1*, a zinc-response transcription factor *ZAP1* has been shown to be a negative

regulator of ECM soluble β-1,3 glucan in both *in vitro* and *in vivo C. albicans* biofilm models [74, 104]. Conversely, two glucoamylases, *Gca1* and *Gca2*, are thought to have positive roles in matrix production. A group of alcohol dehydrogenases *ADH5*, *CSH1*, and *LFD6* also have roles in matrix production, with *ADH5* acting positively, and *CSH1* and *LFD6* acting negatively [74, 105]. It is also present on a number of other *Candida* species, including *C. glabrata*, *C. parapsilosis* and *C. tropicalis* [45, 80].

CANDIDA SP. MANNAN: ROLE IN PATHOGENESIS

As mentioned, *Candida* sp. mannoproteins have both N- and O-linked sugars, mainly mannans, gathering up to 200 mannose units [106, 107], attached via a phosphodiester linkage (phosphomannan) (Figure 3) [54]. The number of mannans units and their molecular weights greatly fluctuate between species [108], having significant effects for host-fungus interactions [65, 107]. For example, mannans derived from *C. glabrata* are less closely related to those of *Saccharomyces cerevisiae* than to those of *C. albicans* [109], yet the core of the biosynthetic machinery appears to be conserved in all three organisms [107].

The deletion of Sc*MNN2* has proved to inhibit the accumulation of α-1,2-mannose onto the mannan backbone, preventing any formation of N-mannan outer chains [110]. In *C. albicans*, this conditions growth, cell morphology and immune detection [111]. Numerous mannosyltransferases related to the mannan biosynthesis in *S. cerevisiae*, have been identified, being also conserved in *C. albicans* and other pathogenic fungi. Curiously, various of these fungal mannosyltransferases are not present in human cells, which has been important in development of new targets for novel antifungals and vaccines. This subject has advanced in *S. cerevisiae* and *C. albicans*, but not in NAC, principally in biofilms. Phosphomannans are mainly organised in mannans, and have a crucial role in adhesion and host recognition (host-fungus interactions) [47, 54, 107, 112]. A deletion of any of the *MNN2* family members, disturbs the phosphomannan content of the cell wall [46, 65]. In fact, several authors have demonstrated that *C. glabrata* increases the mannans' content on the cell wall in the presence

of drugs, possibly due to an adaptation of the cells to the drug stress [46]. Other cell walls modifications have been described and explained as a response to antifungal stress/resistance [80, 97, 113, 114].

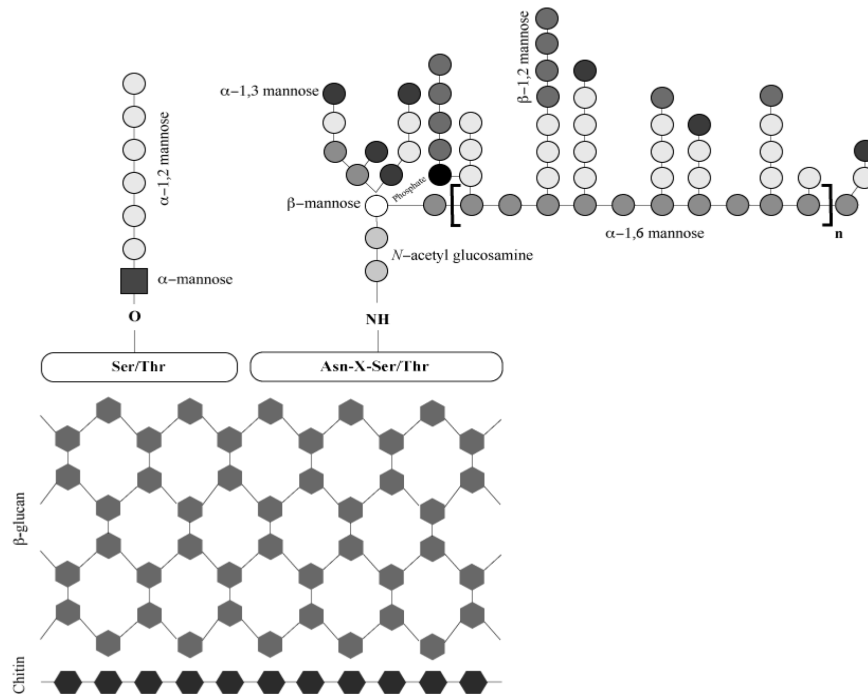

Figure 3. General structure of the *Candida* sp. cell wall, highlighting the mannans.

Besides, it has also been confirmed that strains of *C. glabrata* have less mannans on the matrices with contact to several antifungal drugs, which indicate that matrices with poorer mannans' content are more fragile, more predisposed to environmental stresses and, thus, more susceptible to biomass loss. When this decrease occured, an growth in the β-1,3 glucans' concentration was detected. This observation led to conclude that there was a compensation of the mannans decrease, probably related to resistances profiles [46]. These facts connect directly the mannans and β-1,3 glucans (present in cell wall and in the biofilm matrices) to antifungal drug resistance.

Conclusion

Candida sp. biofilm matrices and cell wall compositions of have been related to higher pathogenicity and virulence states. The mannans evidently influence the drug response profile of biofilm cells, being interconnected to β-glucans in the resistance of *Candida* sp. biofilms to antifungal drugs. Since there is a strong variability between species and strains, other factors are related to this biofilm drug resistance

The identification and blocking of genes directly related to the plasticity of the composition of the matrices and cell walls seem to be important for the search to new antifungal agents, yet other tactics must be investigated, due to the high capacity of gene adjusments in *Candida* genus.

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[113] Ferrari, S.; Sanguinetti, M.; Torelli, R.; Posteraro, B.; Sanglard, D. Contribution of CgPDR1-regulated genes in enhanced virulence of azole-resistant *Candida glabrata*. *PLoS One* 2011, *6*, e17589.

[114] Mathé, L.; Van Dijck, P. Recent insights into *Candida albicans* biofilm resistance mechanisms. *Curr. Genet.* 2013, *59*, 251–264.

BIOGRAPHICAL SKETCHES

Jesus A. Romo

Affiliation: Tufts University, Boston, MA, USA

Education: Cell and Molecular Biology Ph.D.

Business Address: Tufts University
Jaharis 425
150 Harrison Ave. Boston, MA 02111 USA

Research and Professional Experience: Medical mycology/fungal biofilms. Professional Appointments: Postdoctoral Scholar

Publications from the Last 3 Years:

Romo J.A., Pierce C.P., Esqueda M., Hung C.Y., Saville S.P., Lopez-Ribot J. *In vitro* Characterization of an anti-Virulence Compound Targeting *Candida albicans* Filamentation and Biofilm Formation. Frontiers in Cellular and Infection Microbiology. doi: 10.3389/fcimb.2018.00227

Romo J.A., Pierce C.P., Chaturvedi A.K., Lazzell A.L., McHardy S.F., Saville S.P., Lopez-Ribot J. Towards the Development of Anti-virulence Approaches for Candidiasis: a Novel Series of Small Molecule Inhibitors of *Candida albicans* Filamentation. mBio doi:10.1128/mBio.01991-17 Note: Manuscript was recommended on F1000Prime as being of special significance to the field of mycology doi: 10.3410/f.732234524.793540212

Lin Y.H., Romo J.A., Reyes A.N., Smith, T.C., Karna, S.L., Miller, C.L., Van Laar T., Yendapally, R., Chambers, J.P., and J. Seshu. Spermine and spermidine alter gene expression and antigenic profile of *Borrelia burgdorferi.* Infection and Immunity. doi: 10.1128/ IAI.00684-16

Maria Elisa Rodrigues

Affiliation: Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

Education: Biomedical Engineering PhD

Business Address: Centre of Biological Engineering (CEB) - University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

Research and Professional Experience: I studied Biomedical Engineering, completing the Master Integrated cycle in Clinical Engineering in 2007. After my master degree, my professional career was mostly focused in the research field. I trained for three months at Hospital de São Marcos de Braga in the scope of "MIC Evaluation of *Candida* Reference Strains and Clinical Isolates by E-test", before starting working as researcher member in a collaboration project between University of Minho, the

biopharmaceutical company Biotecnol, Immutherapies for life®, and the Institute of Molecular Pathology and Immunology of the University of Porto, in 2008. This project, aimed for the optimization of monoclonal antibody production in large-scale cultures, and gave me experience in the biopharmaceutical industry and a sufficient know-how to develop my PhD project. In 2009, I initiated my PhD project in animal cell cultures to optimize a monoclonal antibody production and to assure the antibody quality and functionally. My PhD research activity has been mostly performed at the Centre of Biological Engineering (CEB) at University of Minho, in Biofilm´s researcher group (Supervisors: Professor Rosário Oliveira and Professor Mariana Henriques), having an additional training at Professor Ian Marison's research group, at Biotechnology Engineering School - Dublin City University. In 2013, I obtained my PhD degree in Biomedical Engineering. In 2013, I was hired as a post-doctoral researcher to work in a project entitled "Consolidating Research Expertise and Resources on Cellular and Molecular Biotechnology at CEB/IBB" in molecular biotechnology research at the Centre of Biological Engineering, University of Minho. From 2014 until 2018, I was working on a new Pos-doc research as a part of the CEB-*Candida* group, funded by "Fundação para a Ciência e Tecnologia" (FCT), entitled "Novel strategies to fight *Candida* species infection". From December 2018 until now I am working as Junior Researcher at the CEB at University of Minho.

In the last 5 years, I was a member of the project ESCMID Research Grants 2014 (2014-2015, reference EXPL/BEX-BCB/0482/2013), entitled "Reconstruction of polymicrobial interaction in infections: the case of *Pseudomonas aeruginosa* and *Candida albicans* cross talk in ventilator associated pneumonia", and a member of the project COMPETE-FCT (2014-2015, reference PTDC/SAU-MIC/119069/2010), entitled "Mechanisms of *Candida glabrata* biofilms tolerance to antifungal agents". Additional, at the moment, I´m a member of the project FCT/Portugal2020/Compete2020 (2018-2021, reference POCI-01-0145-FEDER-028893), entitled " Application of antisense oligomers for controlling *Candida* species biofilm formation on medical surfaces". Even more, I have supervised and co-supervised the work of a total of 10 students (9 master students and support in the supervision of the 1 PhD student) and, also, I developed activities in the scope of the examination of master´s thesis and projects of several scientific works. Other activities, as well as the revision of papers in international peer-reviewed scientific journals, participation on international congresses and on scientific courses and workshop were noted. I, also, was invited auxiliary professor of the practical/theoretical-practical classes "Fenómenos Interfaciais" (2nd year students of the Integrated Masters in Biomedical), at University of Minho in 2015. Also, now as Junior Researcher, I am professor of the practical/theoretical-practical classes "Laboratórios de Fenómenos de Transferência" (2nd year students of the Integrated Masters in Biological Engineering), at University of Minho since 2018. Additionally, I occupy (2017-2018) the position of the assistant director of LIBRO (Laboratory of Investigation on Biofilms Rosário Oliveira). It is important to emphasize that the last five years include two periods of maternity license of 5 months each (total 10 months) (from 1st October 2015 until 29th February 2016 and from 12th May 2018 until 11th October 2018).

Using objective indicators, from 2009 to 2019, I have published 22 papers and 6 book chapters, submitted 3 papers, and prepared 2 more articles for publication. Also, 17 posters and 1 oral communication have been published in national and international scientific conferences. All of my research has a high impact of scientific innovation, evidenced by the quality of publications in internationally renowned scientific journals, in Q1 (65% of publications) and Q2 (35% of publications) quartilhes. The impact factor sum of my articles is around 60 (47% of publications above the impact factor of 3 and 11% of publications above the impact factor of 6) and the sum of citations exceeds 310 (Scimago Journal & Country Rank). My h-index according to ResearchID is 7 (F-2350-2010). In addition, the diversity of my published works demonstrates my capacities to work in different areas of research and to collaborate in different research teams. These highlight the important contribute of my work to the development of the scientific areas of microbiology and medical health.

Professional Appointments:

2019- until now
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Junior Research
Activity: Virulence of polimicrobial yeasts-bacteria biofilms. FOS / subFOS: Medical and Health Sciences / Health Biotechnology.

2019- until now
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Junior Research
Activity: Professor of the practical/theoretical-practical classes "Laboratórios de Fenómenos de Transferência" (2nd year students of the Integrated Masters in Biological Engineering), at University of Minho.

2017- 2018
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Assistant director of LIBRO (Laboratory of Investigation on Biofilms Rosário Oliveira).
Activity: Management of all kinds of issues related with the maintenance of the laboratory.

2014-2018
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Research fellow
Activity: FCT Post-Doctoral Fellowship under the theme "Novel strategies to fight *Candida* species infection - Natural compounds to improve polymicrobial VVC infections treatment".

2014-2015
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Invited auxiliary professor
Activity: Invited auxiliary professor of the practical/theoretical-practical classes "Fenómenos Interfaciais" (2nd year students of the Integrated Masters in Biomedical), at University of Minho.

2013-2014
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Research fellow
Activity: FCT Post-Doctoral Fellowship under the project FCOMP-01-0124-FEDER-027462 (Ref. FCT RECI/BBB-EBI/0179/2012) "Consolidating Research Expertise and Resources on Cellular and Molecular Biotechnology at CEB/IBB", in the area of Molecular Biotechnology.

2009-2013
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Research fellow
Activity: FCT PhD Fellowship under the theme "Optimization of monoclonal antibody production".

2009

Institution: Dublin City University – Laboratory of Integrated Bioprocessing
Employment bond: Research fellow - complementary training under the phD project
Activity: Training in mammalian cell culture and bioreactor operation under the theme "Optimization of monoclonal antibody production".

2008
Institution: University of Minho, Centre of Biological Engineering
Employment bond: Research fellow
Activity: MAbExpress Project (a collaboration with Biotecnol S.A., IPATIMUP and University of Edinburgh, funded by *Agência de Inovação* (ADI))

2007
Institution: Hospital de São Marcos de Braga
Employment bond: Researcher trainee
Activity: Comparison of methods to determine antifungal susceptibility in microbiology laboratory.

2007
Institution: Hospital da Santa Casa da Misericórdia de Vila Verde
Employment bond: Researcher trainee
Activity: Medical waste managment

Honors:

2011
Award for first participation at conference Meeting on Cell Based Technologies, Vienna, Austria
Promoting entity: European Society for Animal Cell Technology (ESACT).

2007
Award *Caixa Geral de Depósitos*.
Promoting entity: Caixa Geral de Depósitos and University of Minho.

2003
Merit award
Promoting entity: University of Minho.

Publications from the Last 3 Years:
*In the last three years (from 2016 to 2019) Maria Elisa Rodrigues published **11 papers** in international scientific journals. Additionally, more **3 papers** is currently submitted and under review in an international journal and **2 papers** are in final preparation phase to submission. Also of note is the forthcoming publishing of **1 book chapters**. Maria Elisa Rodrigues has also presented **9 posters** in international scientific conferences.*

Fernandes L., Oliveira A., Henriques M., Rodrigues M.E. (2019). Honey as a strategy to fight Candida tropicalis in mixed-biofilms with Pseudomonas aeruginosa. Journal of Applied Microbiology, JAM-2019-0927 (Status – under review).

Gomes F., Dias M.I., Lima Â., Barros L., Rodrigues M.E., Ferreira I.C.F.R., Henriques M. (2019). Antimicrobial activity and phenolic characterization of different medicinal and aromatic plants: study of their decoction and hydroalcoholic extracts. Scientific Reports, SREP-19-08049 (Status - peer review).

Gomes F., Rodrigues M.E., Martins N., Ferreira I.C.F.R., Henriques M. (2019). Phenolic plant extracts versus penicillin G: in vitro susceptibility of Staphylococcus aureus isolated from bovine mastitis. HELIYON_2019_700 (Status - major revisions).

Rodrigues C.F., Rodrigues M.E., Henriques M. (2018). *Candida* spp. infections in patients with diabetes mellitus. Journal of Clinical Medicine, 8(1), 76. (JCR: 3.08; Q1).

Rodrigues C.F., Rodrigues M.E., Henriques M. (2018). Promising alternative therapeutics for oral candidiasis. Current Medicinal Chemistry, 31. (JCR: 3.853; Q1).

Rodrigues C.F., Rodrigues M.E., Henriques M. (2018). Susceptibility of *Candida glabrata* biofilms to echinocandins: alterations on the matrix composition. Biofouling, 34, 569-578. (JCR: 3.08; Q1).

Gomes F., Martins N., Barros L., Rodrigues M.E., Oliveira M.B., Henrique M., Ferreira I.C.F.R. (2018). Plant phenolic extracts as an effective strategy to control Staphylococcus aureus, the dairy industry pathogen. Industrial Crops & Products, 112, 515-520. (JCR: 3.181; Q1).

Castro J., Martins A.P., Rodrigues M.E., Cerca N. (2018). Lactobacillus crispatus represses vaginolysin expression by BV associated Gardnerella vaginalis and reduces cell cytotoxicity. Anaerobe, 50, 60-63. (JCR: 2.685; Q2).

Alves S.A., Ribeiro A.R., Gemini-Piperni S., Silva R.C., Saraiva A.M., Leite P.E., Perez G., Oliveira S.M., Araujo J.R., Archanjo B.S., Rodrigues M.E., Henriques M., Celis J.-P., Shokuhfar T., Borojevic R., Granjeiro J.M., Rocha L.A. (2017). TiO2 nanotubes enriched with calcium, phosphorous and zinc: promising bio-selective functional surfaces for osseointegrated titanium implants. RSC Advances, 7, 49720-49738. (JCR: 3.108; Q1).

Rodrigues C.F., Gonçalves B., Rodrigues M. E., Silva S., Azeredo J., Henriques M. (2017). The effectiveness of voriconazole in therapy of *Candida glabrata*'s biofilms oral infections and its influence on the matrix composition and gene expression. Mycopathologia, 182(7-8):653-664. (JCR: 0.746; Q2).

Silva S., Rodrigues C.F., Araújo D., Rodrigues M.E., Henriques M. (2017). *Candida* species biofilms' antifungal resistance. Journal of Fungi, 3(1, 8), 1-17.

Rodrigues C.F., Rodrigues M.E., Silva S., Henriques M. (2017). *Candida glabrata* biofilms: How far have we come? Journal of Fungi, 3(1), 11.

Rodrigues M.E., Lopes S.L., Pereira C.R., Azevedo N.F., Lourenço A., Henriques M., Pereira M.O. (2017). Fighting mixed bacterial-fungal biofilms in ventilator-associated pneumonia with antifungal-antibacterial combination therapy. PLOS ONE Journal, 12(1): e0170433. (JCR: 2.806; Q1).

Rodrigues M.E., Silva, S., Azeredo J., Henriques M. (2016). Novel strategies to fight *Candida* species infection. Critical Reviews in Microbiology, 42(4): 594-606. (JCR: 6.02; Q1).

Rodrigues M.E., Henriques M., Silva S. (2016). Disinfectants to fight oral *Candida* biofilms. Chapter - Fungal Biofilms and related infections. Advances in Microbiology, Infectious Diseases and Public Health. Vol. 931, pp. 83-93.

Rodrigues M.E., Fernandes L., Rodrigues C.F., Oliveira A., Henriques M. (2017). Natural Honey To Fight *Candida* Infections. Eurobiofilms 2017 – 5th European Congress on Microbial Biofilms, Netherlands, September 19-22.

Martins P., Castro J., Rosca A., Rodrigues M.E., Cerca N. (2017). L. Crispatus Protects Hela Cells Against G. Vaginalis Cytotoxicity. Eurobiofilms 2017 – 5th European Congress on Microbial Biofilms, Netherlands, September 19-22.

Rodrigues C.F., Rodrigues M.E., Henriques M. (2017). Portrait Of Gene Expression In C. Glabrata With Stress Induced By Drugs. Eurobiofilms 2017 – 5th European Congress on Microbial Biofilms, Netherlands, September 19-22.

Rodrigues C.F., Rodrigues M.E., Ames L., Haynes K., Henriques M. (2017). Mnn2 gene affects drug resistance In *Candida glabrata*'s ?Biofilms. Eurobiofilms 2017 – 5th European Congress on Microbial Biofilms, Netherlands, September 19-22.

Gomes F., Rodrigues M.E., Martins N., Ferreira I., Henriques M. (2017). Management Of Dairy Industry Contaminations: Use Of Phenolic Extracts. Eurobiofilms 2017 – 5th European Congress on Microbial Biofilms, Netherlands, September 19-22.

Gomes F., Rodrigues M.E., Martins N., Ferreira I., Henriques M. (2017). Antimicrobial activity of phenolic extracts of Eucalyptus globulus and Juglans regia against dairy industry pathogens. Book of Abstracts of CEB Annual Meeting 2017. Braga, 6 July, 58, 2017. ISBN: 978-989-97478-8-3.

Rodrigues M.E., Lopes S.P., Pereira C.R., Azevedo N. F., Lourenço A., Henriques M., Pereira M.O. (2016). Antifungal-Antibacterial combination therapy as an attractive option to fight inter-kingdom polymicrobial biofilms in ventilator-associated pneumonia. Biofilms 7 - International Conference on Microbial Biofilms, Porto, Portugal, June 25-28.

Rodrigues C.F., Rodrigues M.E., Silva S.C., Azeredo J., Henriques M. (2016). The chemical structure of the biofilm matrices of *Candida glabrata* induces resistance to antifungal drugs. Biofilms 7 - International Conference on Microbial Biofilms, Porto, Portugal, June 25-28.

Grainha T., Rodrigues M.E., Lopes S.P., Anália L., Henriques M., Pereira M.O. (2016). Insights into Pseudomonas aeruginosa and *Candida albicans* consortia challenged by antimicrobials. Biofilms 7 - International Conference on Microbial Biofilms, Porto, Portugal, June 25-28.

Liliana Fernandes

Affiliation: Centre of Biological Engineering, University of Minho, Braga, Portugal

Education: Master in Biotechnology, University of Minho (Portugal)

Business Address: lilianafernandes@ceb.uminho.pt

Research and Professional Experience:
Research Fellow, Department of Biological Engineering, University of Minho

Publications from the Last 3 Years:

Rodrigues, M Elisa; Fernandes, Liliana; Rodrigues, Célia F; Oliveira, Ana; Henriques, Mariana. 2017. "Novel strategies to fight Candida infections: natural honey". Trabalho apresentado em *CEB Annual Meeting 2017*.

Rodrigues, M Elisa; Fernandes, Liliana; Rodrigues, Célia F; Oliveira, A.; Henriques, Mariana. 2017. "Natural honey to fight Candida infections". Trabalho apresentado em *Eurobiofilms 2017 - 5th European Congress on Microbial Biofilms.*

Csaba Gergő Papp

Affiliation: Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Education: University of Szeged, Szeged, Hungary
Business Address: 52. Közép fasor, 6726 Szeged, Hungary

Research and Professional Experience: Dr. Csaba Papp finished his Ph.D. in 2019. in the field of *Candida parapsilosis* antifungal drug resistance and the connection between resistance and virulence properties of this species. During his master and Ph.D. studies, he spent a year in Dr. Joshua D. Nosanchuk's laboratory at the Albert Einstein College of Medicine in New York, US and five months in Dr. Leonardo Nimrichter's laboratory at the Federal University of Rio de Janeiro.

Publications from the Last 3 Years:

Kovacs R, Holzknecht J, Hargitai Z, Papp C, Farkas A, Borics A, Toth L, Varadi G, Toth GK, Kovacs I, Dubrac S, Majoros L, Marx F, Galgoczy L. 2019. *In Vivo* Applicability of Neosartorya fischeri Antifungal Protein 2 (NFAP2) in Treatment of Vulvovaginal Candidiasis. Antimicrob Agents Chemother 63.

Papp C, Kocsis K, Toth R, Bodai L, Willis JR, Ksiezopolska E, Lozoya-Perez NE, Vagvolgyi C, Mora Montes H, Gabaldon T, Nosanchuk JD, Gacser A. 2018. Echinocandin-Induced Microevolution of Candida parapsilosis Influences Virulence and Abiotic Stress Tolerance. mSphere 3.

Zoppo M, Lombardi L, Rizzato C, Lupetti A, Bottai D, Papp C, Gacser A, Tavanti A. 2018. CORT0C04210 is required for Candida orthopsilosis adhesion to human buccal cells. Fungal Genet Biol 120:19-29.

Ronavari A, Igaz N, Gopisetty MK, Szerencses B, Kovacs D, Papp C, Vagvolgyi C, Boros IM, Konya Z, Kiricsi M, Pfeiffer I. 2018. Biosynthesized silver and gold nanoparticles are potent antimycotics against opportunistic pathogenic yeasts and dermatophytes. Int J Nanomedicine 13:695-703.

Toth R, Cabral V, Thuer E, Bohner F, Nemeth T, Papp C, Nimrichter L, Molnar G, Vagvolgyi C, Gabaldon T, Nosanchuk JD, Gacser A. 2018. Investigation of Candida parapsilosis virulence regulatory factors during host-pathogen interaction. Sci Rep 8:1346.

Attila Gacser

Affiliation: University of Szeged, Department of Microbilogy, MTA-SZTE "Lendület" "Mycobiome" Research Group

Education: Biologist
Business Address: 6726 Szeged, Kozep fasor 52

Research and Professional Experience: Dr. Gácser completed his Ph.D. at the University of Szeged, working on Cryptococcus hungaricus. During his first postdoctoral training at the University of Hamburg, Germany, as a Marie Curie Fellow, he studied opportunistic Candida species. In 2005, he joined Prof. Joshua D. Nosanchuk's laboratory at the Albert Einstein College of Medicine in New York, where he made important advances in our understanding of the virulence and pathogenesis of several different fungal pathogens, such as Histoplasma capsulatum, Cryptococcus neoformans, and Candida parapsilosis. In 2008, he returned to Hungary, and the current research in

his laboratory focuses on the development of tools for genetic manipulation of C. parapsilosis and the study of Candida virulence and pathogenesis, the host response, and the immunology of fungal infections.

Professional Appointments: Professor of Microbiology and Immunology at the University of Szeged, Head of the Institute of Biology.

Honors: Manninger Memorial Prize by the Hungarian Microbiology Society, Fulbright Research fellowship at Einstein College of Medicine, Bronx, Scientific Award of the University of Szeged, Faculty of Natural Sciences and Informatics, Special Guest Scientist selected by the Federal Government of Barzil, "Outstanding Postdoctoral Research Scholar" Albert Einstein College of Medicine.

Publications from the Last 3 Years:

2019

Tóth R, Nosek J, Mora-Montes HM, Gabaldon T, Bliss JM, Nosanchuk JD, Turner SA, Butler G, Vágvölgyi C, Gácser A. Candida parapsilosis: from Genes to the Bedside. *Clin Microbiol Rev*. 2019 Feb 27;32(2). pii: e00111-18. doi: 10.1128/CMR.00111-18. Print 2019 Mar 20. Review.

Chakraborty, Tanmoy; Tóth, Renáta; Gácser, Attila. Eicosanoid production by Candida parapsilosis and other pathogenic yeasts. *Virulenc*e Epub p. https://doi.org/10.1080/21505594.2018.1559674.

2018

Chakraborty, T; Thuer, E; Heijink, M; Toth, R; Bodai, L; Vagvolgyi, C; Giera, M; Gabaldon, T; Gacser, A. Eicosanoid biosynthesis influences the virulence of Candida parapsilosis. *Virulence* 9: 1 pp. 1019-1035,
17 p.

Csepregi, Janka Zsófia; Orosz, Anita; Zajta, Erik; Kása, Orsolya; Németh, Tamás; Simon, Edina; Fodor, Szabina; Csonka, Katalin; Barátki, Balázs L; Kövesdi, Dorottya He You-Wen; Gácser Attila; Mócsai Attila Myeloid-Specific Deletion of Mcl-1 Yields Severely Neutropenic Mice That Survive and Breed in Homozygous Form. *Journal of Immunology* 201: 12 pp. 3793-3803, 11 p.

Papp, Cs; Kocsis, K; Tóth, R; Bodai, L; Willis, J R.; Ksiezopolska, E; Lozoya-Pérez, N E.; Vágvölgyi, Cs; Mora Montes, H;Gabaldón, T; Nosanchuk J D., Gácser A Echinocandin-Induced Microevolution of Candida parapsilosis Influences Virulence and Abiotic Stress Tolerance mSPHERE 3: 6 Paper: e00547-18.

Toth R, Cabral V, Thuer E, Bohner F, Nemeth T, Papp C, Nimrichter L, Molnar G, Vagvolgyi C, Gabaldon T, Nosanchuk JD, Gacser A Investigation of Candida parapsilosis virulence regulatory factors during host-pathogen interaction. *Scientific Reports* 8: Paper 1346.

2017

Andrea Cillingová, Igor Zeman, Renáta Tóth, Martina Neboháčová, Ivana Duncková, Mária Hölcová, Michaela Jakúbková, Gabriela Gérecová, Leszek P Pryszcz, L'ubomír Tomáska, Toni Gabaldón, Attila Gácser, Jozef Nosek Eukaryotic transporters for hydroxyderivatives of benzoic acid. *Scientific Reports* 7: Paper 8998.

Csonka K, Vadovics M, Marton A, Vagvolgyi C, Zajta E, Toth A, Toth R, Vizler C, Tiszlavicz L, Mora-Montes HM, Gacser A Investigation of OCH1 in the Virulence of Candida parapsilosis Using a New Neonatal Mouse Model. *Frontiers in Microbiology* 8: Paper 1197.

László G Nagy, Renáta Tóth, Enikő Kiss, Jason Slot, Attila Gácser, Gábor M Kovács Six Key Traits of Fungi: Their Evolutionary

Origins and Genetic Bases *Microbiology Spectrum* 5:(4) Paper FUNK-0036-2016.

Toth A, Zajta E, Csonka K, Vagvolgyi C, Netea GM, Gacser A Specific pathways mediating inflammasome activation by Candida parapsilosis. *Scientific Reports I*n press (2017)

Tóth R, Tóth A, Vágvölgyi C, Gácser A Candida parapsilosis secreted lipase as an important virulence factor Current Protein and Peptide Science. In press: Paper In press.

Célia Fortuna Rodrigues

Affiliation: LEPABE - Laboratory for Process Engineering Environment Biotechnology and Energy-Department of Chemical Engineering, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal

Education: PharmD, PhD

Business Address: Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Research and Professional Experience:

I am a pharmacist and have concluded my PhD in Chemical and Biological Engineering in May 2018, unanimously approved with the highest mention (Very Good). My PhD was mainly developed at the Centre of Biological Engineering/UMinho, with one year at Immunobiology Group, Abel Salazar Institute of Biomedical Sciences and i3S (Institute for Innovation and Health Research)/UPorto and one month internship in the Laboratory of Physiology and Functional Genomics of Eukaryotes/INSA Toulouse, France), under the theme “Candida glabrata biofilms: mechanisms of antifungal resistance and matrix role” (FCT SFRH/BD/93078/2013).

From January 2019, I am an Invited Assistant Professor of the classes “Análise do Medicamento” (Drug Analysis) and “Virologia” (Virology) to the 3rd and 4nd year students of the Integrated Masters in Pharmaceutical Sciences, respectively, at IUCS(Instituto Universitário de Ciências da Saúde). Also, since October 2017, I am an Invited Professor of UEuropeia, where I teach a Bioethics Masterclass under the scope of the disciplines of Research

Methodologies in the courses of Psychology and Sports Management. Recently, I have been developing research activity under my FCT Post-doctoral fellowship (POCI-01-0145-FEDER-031011), entitled “Integration of fluorescence in situ hybridization with microfluidics for the quasi real-time detection of food pathogens”.

I have actively collaborated in several projects (1F/10/2005/CESPU, AL/14/2006/CESPU, PTDC/SAU- MIC/119069/2010), which has allowed me to acquire additional competences and expand my research to other scientific areas. Over this period, relevant scientific output is highlighted: 19 scientific papers published in high- quality, international peer reviewed journals, resulting in 259 citations and a h-index of 8 (https://www.researchgate.net/profile/Celia_Rodrigues2). In addition, I have published 5 book chapters and my work has been selected for oral communications and poster presentations in relevant national/international scientific meetings in the area.

Over the last 5 years, I have been also involved in other scientific-related activities: support of a PhD Student (UHull); supervision/co-supervision of 5 Master Students of Bioengineering or Biomedical Engineering; support of 5 Master Students from other research groups (Biofilm Group of Centre of Biological Engineering, UMinho); revision of several papers in different international peer-reviewed

scientific journals and reviewer of the abstracts of the ASM Microbe 2019. I was also one of the organizers and instructor of practical sessions of the 2014 CEB International Biofilm Course (UMinho). I was invited to contribute as a science communicator for the Nature Microbiology Community (https://naturemicrobiologycommunity.nature.com/users/16248-celia-fortuna-rodrigues) and I am also a member of the FEMS Microbiology Events Taskforce Board and FEMS' ALAM Project (https://fems-microbiology.org/network/types-of-involvement/volunteering/volunteer-celia-fortuna-rodrigues/).

Professional Appointments:

2019 - Invited Professor. Instituto Universitário de Ciências da Saúde (IUCS-CESPU) Virology and Drug Analysis - MSc Pharmaceutical Sciences and BSc Biochemistry.

2018 - Post-Doctoral Researcher. LEPABE – Dep. Chemical Engineering, Fac. Engineering, University of Porto Research activities regarding pathogen detection through microfluidics devices (Project: "uFISH - Integration of fluorescence in situ hybridization with microfluidics for the quasi real-time detection of food pathogens"), biofilms and help in coordination of Master Students projects @ FISH Group.

2018 - Post-Doctoral Researcher. Biofilm Group, Centre of Biological Engineering – University of Minho. Research activities regarding biofilms and help in coordination of Master Students projects.

2018 - Science Communicator @ Nature Microbiology Community, Nature Group (Publishing), Forum for the sharing and discussion of ideas and opinions about microbiology. https://naturemicrobio-logycommunity.nature.com/https://naturemicrobiologycommunity.nature.com/users/16248-celia-fortuna-rodrigues.

2017 - FEMS Microbiology Events Taskforce Board, FEMS – Federation of European Microbiology Societies, Science communication volunteer. Microbiology events dissemination from Asia, Oceania and American continents, (https://www.fems-microbiology.org/). Transla-tion of projects/abstracts PT-EN/EN-PT (ALAM Project).

2014 - 2018 - PhD Student (FCT SFRH/BD/93078/2013). Biofilm Group, Centre of Biological Engineering – University of Minho Candida glabrata biofilms: mechanisms of antifungal resistance and matrix role. Assistance in other research projects and in coordination of Master Students projects.

2017 – 2018 - Science Communicator @ npj Biofilms and Microbiomes Community, Nature Group (Publishing). Interactive online community that progress the journal's aim to host cross-disciplinary discussions and allows the understanding of mechanisms governing the social behaviour of microbial biofilm populations and communities, and their impact on life, human health, and the environment, both natural and engineered. https://www.nature.com/npjbiofilms/community. https://npjbiofilmscommunity.nature.com/users/16248-celia-fortuna-rodrigues

2017 - 2018 - Visiting PhD Candidate, Immunobiology Group, Abel Salazar Institute of Biomedical Sciences and Institute for Innovation and Health, Research (i3S) - University of Porto, Project FCT SFRH/BD/93078/2013 - Candida glabrata biofilms: mechanisms of antifungal resistance and matrix role. - *in vivo* infection assays.

2014 - 2018 - PhD Student (FCT SFRH/BD/93078/2013), Biofilm Group, Centre of Biological Engineering – University of Minho Candida glabrata biofilms: mechanisms of antifungal resistance and matrix role. Assistance in other research projects and in coordination of Master Students projects.

2018, 2017 – Invited Professor, Universidade Europeia, Bioethics class (general concepts and techniques, animal and human experiments, scope of the disciplines of Research Methodologies) - BSc Psychology and BSc Sports Management.

2016 - Visiting PhD Candidate, LISBP, Institut National des Sciences Appliquées (INSA), Université Fédérale de Toulouse, France. Project FCT SFRH/BD/93078/2013 - Candida glabrata biofilms: mechanisms of antifungal resistance and matrix role – HPLC techniques to determine C. glabrata biofilm matrices composition.

2014 - Tutor of practical classes, Biofilm Group, Centre of Biological Engineering – University of Minho. IV Advanced Practical Course on Biofilms Science.

2013 - 2014 - Research Assistant, Biofilm Group, Centre of Biological Engineering – University of Minho. Project: Mechanisms of Candida glabrata biofilms tolerance to antifungal agents - FCT PTDC/SAU- MIC/119069/2010. Assistance in other projects and in coordination of Master Students projects.

2011 - 2012 - Voluntary Research Fellow, Centre of Research in Health Sciences (CICS/CESPU) – IUCS, Bioencapsulation: micro e nanoencapsulation, HPLC, Bifidobacterium, Caco-2 e HT-29 cell cultures.

Honors:

AWARD
Golden CEB-onion-Awards: 2017 Paper with most pages
Centre of Biological Engineering – University of Minho
09.2017

GRANT
ESCMID Attendance Grant
Eurobiofilms Congress - Amsterdam, The Netherlands
06.2015

GRANT
FEMS Young Scientist Meeting Grant
6th Congress of European Microbiologists - Maastricht, The Netherlands
06.2015

GRANT
Materials Transfer Agreement Program, Ref: MTR18401.02 - liposomal amphotericin B Gilead Sciences, Inc. - Foster City, USA
09.2014

GRANT
ESCMID Attendance Grant – 3rd Workshop on Antimicrobial Susceptibility Testing. Linz, Austria
05.2014 - 05-2018

GRANT
PhD Grant. Ref: SFRH/BD/93078/2013
Fundação para a Ciência e Tecnologia - Portugal
09.2014

GRANT
Provision of Materials Program, Ref: PMA201404 – micafungin
Astellas Pharma, Inc. - Obaraki, Japan
01.2014

GRANT
Merck Investigator Studies Program, Ref: MISP51371 - caspofungin and posaconazole
Merck Sharp & Dohme Corp. - New Jersey, USA
05.2013

GRANT
Pfizer Investigator Initiated Research, Ref: PF-00579955 – voriconazole, Pfizer, Inc New York, USA

05.2013
GRANT
Pfizer Investigator Initiated Research, Ref: PF-00345508 - fluconazole
Pfizer, Inc - New York, USA

10.2010
AWARD
Best Student of the Master in Pharmaceutical Sciences 2004/2009
CESPU - Instituto Universitário de Ciências da Saúde

09.2010
AWARD
"Sociedade Farmacêutica Lusitana" Award for the Best Portuguese Pharmaceutical Sciences Student
Ordem dos Farmacêuticos - Lisboa, Portugal

Publications from the Last 3 Years:

Submitted:

Novel therapies for biofilm-related Candida spp. Infections (invited review) - Accepted for publication. Černáková, L; Light, L; Salehi, B; Rogel-Castillo, C; Victoriano, M; Martorell, M; Sharifi-Rad, J; Martins, N; Rodrigues, CF. Advances in Microbiology, Infectious Diseases and Public Health

Phytochemicals in prostate cancer: From bioactive molecules to upcoming therapeutic agents. Salehi, B; Fokou, PVT; Yamthe, PRT; Tali, BT; Oluwaseun, AC; Rahavian, A; Mudau, FN; Martorell, M; Setzer, WN; Rodrigues, CF; Martins, N; Sharifi-Rad, J.

Silymarin Anticancer Activity: Key Emphasis on Molecular Mechanisms and Therapeutic Potentials. Tabarzad, M; Iorigooini, Z; Hosseinabadi, T; Salehi, B; Sharifi-Rad, J; Rodrigues, CF; Natália Martins.

Published:

2019

Advances in chemical and biological methods to identify microorganisms – from past to present - Accepted for publicatiion. Franco-Duarte, R; Černáková, L; Kadam, S; Salehi, K; Salehi, B; Bevilacqua, A; Corbo, MR; Antolak, H; Dybka-Stępień, K; Leszczewicz, M; Tintino, RS; Souza, VCA; Coutinho, HDM; Sharifi-Rad, J; Martins, N; Rodrigues, CF. Microorganisms. doi: 10.3390/microorganisms7050130.

Candida spp. Infections in patients with diabetes mellitus. Rodrigues, CF; Rodrigues, ME; Henriques, M. Journal of Clinical Medicine. doi.org/10.3390/jcm8010076.

Inflammatory cell recruitment in Candida glabrata biofilm cells-infected mice receiving antifungal chemotherapy. Rodrigues, CF; Costa, AC; Vilanova, M; Henriques, M. Journal of Clinical Medicine. doi.org/10.3390/jcm8020142.

Measurement of Off-Flavoring Volatile Compounds and Microbial Load as a Probable Marker for Keeping Quality of Pasteurized Milk. Rashid, A; Javed, I; Rasco, B; Sablani, S; Ayaz, M;

Ali, MA; Abdullah, M; Imran, M; Gondal, TA; Afzal, MI; Atif, M; Salehi, B; Rodrigues, CF; Sharifi-Rad, J; Martins, N. Applied Sciences. oi.org/10.3390/app9050959.

Plant-Derived Bioactives in Oral Mucosal Lesions: A Key Emphasis to Curcumin, Lycopene, Chamomile, Aloe vera, Green Tea and Coffee Properties. Rashid, A; Javed, I; Rasco, B; Sablani, S; Ayaz, M; Ali, MA; Abdullah, M; Imran, M; Gondal, TA; Afzal, MI; Atif, M; Salehi, B; Rodrigues, CF; Sharifi-Rad, J; Martins, N. Biomolecules. doi.org/10.3390/biom9030106.

2018

Association of posaconazole and amphotericin B in the treatment of biofilms of Candida glabrata. Rodrigues, CF; Alves, D; Henriques, M. Microorganisms. doi: 10.3390/microorganisms 6040123.

Portrait of matrix genes expression in C. glabrata biofilms with stress induced by different drugs. Genes. Rodrigues, CF; Henriques, M. doi: 10.3390/genes9040205.

Promising therapeutics for Candida oral biofilms' infections. Rodrigues, CF; Rodrigues, ME; Henriques, M. Current Medicinal Chemistry. doi: 10.2174/0929867325666180601102333.

Susceptibility of Candida glabrata biofilms to echinocandins: alterations on the matrix composition. Biofouling. Rodrigues, CF; Rodrigues, ME; Henriques, M. doi: 10.1080/08927014.2018.
1472244.

The MNN2 gene knockout modulates the antifungal resistance of biofilms of Candida glabrata. Rodrigues, CF; Vilas. Boas, D; Haynes, K; Henriques, M. Biomolecules. doi.org/10.3390/ biom8040130.

2017

Candida glabrata Biofilms: How Far Have We Come? Rodrigues, CF; Rodrigues, ME; Silva, S; Henriques, M. d Journal of Fung. oi: 10.3390/jof3010011.

Candida Species Biofilms' Antifungal Resistance. Silva, S; Rodrigues, CF; Araújo, D; Rodrigues, ME; Henriques, M. Journal of Fungi. doi: 10.3390/jof3010008.

Liposomal and Deoxycholate Amphotericin B Formulations: Effectiveness against Biofilm Infections of Candida spp. Rodrigues, CF; Henriques, M. Pathogens. doi: 10.3390/pathogens6040062.

Oral mucositis caused by Candida glabrata biofilms: failure of the concomitant use of fluconazole and ascorbic acid. Rodrigues, CF; Henriques, M. Therapeutic Advances in Infectious Disease. doi: 10.1177/2049936116684477.

Synergistic Antimicrobial Interaction between Honey and Phage against Escherichia coli Biofilms. Oliveira, A; Ribeiro, H; Silva, AC; Silva, D; Sousa, J; Rodrigues CF; Melo, LDR; Sillankorva, S. Frontiers in Microbiology doi: 10.3389/fmicb.2017.02407.

The carboxylic acid transporters Jen1 and Jen2 affect the architecture and fluconazole susceptibility of Candida albicans biofilm in the presence of lactate. Alves, R; Mota, S; Silva, S; Rodrigues, CF; Brown, AJP; Henriques, M; Casal, M; Paiva, S. Biofouling. doi: 10.1080/08927014.2017.1392514.

The effectiveness of voriconazole in therapy of C. glabrata's biofilms oral infections and its influence on the matrix composition and gene expression. Rodrigues, CF; Gonçalves, B; Rodrigues, ME; Silva, S; Azeredo, J; Henriques, M. Mycopathologia. doi: 10.1007/s11046-017-0135-7.

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