We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Candida Biofilms: Environmental and Clinical Aspects

Marta Targalska and Alina Kunicka-Styczyńska

Abstract

Candida spp. strains are characterized by their ability to form a biofilm structure on biotic and abiotic surfaces, causing significant problems in many industrial branches and threatening human health. *Candida* biofilm is a heterogeneous, spatially well-organized structure consisting of planktonic and mycelial yeast forms which are interdependent in the *quorum sensing* system and surrounded by an extracellular polysaccharide substance. Biofilm-forming microorganisms are characterized by high invasiveness, the ability to cause dangerous and difficult to treat infections. Furthermore, the cells in the biofilm, compared to planktonic forms, show reduced sensitivity to chemical compounds with antifungal activity and increased survival under unfavorable environmental conditions. The chapter focuses on the emergence of antifungal resistance with the development of biofilms. The work presents the examples of antibiotic resistance of a variety of *Candida*, showing that a group of strains expressing intermediate sensitivity or resistance to the tested antibiotics include both clinical and food-borne isolates. Similarities in enzymatic and biochemical profiles of different origin isolates are discussed. A substantial heterogeneity within *Candida albicans* group is also underlined. Simultaneously, the incidents of biochemical profiles conformity of some clinical and food-borne isolates are presented, which may be a result of *Candida* transmission via food.

Keywords: *Candida albicans*, non-*albicans Candida*, *Candida* biofilm, drug resistance, food-borne *Candida*

1. Introduction

Unicellular forms of yeast are rarely found in nature as single, scattered cells, in the form of plankton but they are rather adsorpt at the solid-liquid, liquid-gas, or liquid-liquid interface. Generally, they form organized, settled structures taking the form of multicellular clusters forming biofilm. Biofilm, also called as the biological membrane, is a complex, multicellular,

and multifunctional structure of one or more species of microorganisms, surrounded by a layer of organic and inorganic substances produced by these microorganisms adhering to both biotic and abiotic surfaces. The form of biofilm enhances the effectiveness of microbial protection against the adverse environmental factors, including antibiotics, reduces the effectiveness of host defense mechanisms, facilitates the acquisition of nutrients, creates the possibility of horizontal gene transfer by providing evolutionary and genetic diversity, and enables the transmission of information between microbial cells [1–3].

Biofilm is most commonly formed on solid surfaces staying in contact with water, living tissues, and liquid-air interface. This ubiquitous structure can be very useful but also dangerous being difficult to be removed. Biofilm plays a key role in a process of self-cleaning of surface-, ground-, and underground water. The biofilm's ability to create a biobarrier has been exploited in water treatment and to reduce a pollution of soil and ground waters. Biofilm also allows biological removal of pollutants from sewage [2]. Biofilm exists not only in the natural environment but also is industrially applied, for example, to catalyze complex chemical reactions. Natural microbiota of the body of a healthy person forms a biofilm modulating some physiological functions, for example, colonic biofilm [4]. Moreover, changing environmental conditions may transform a biofilm from a big friend into a fierce enemy. A good example is the biofilm of the gastrointestinal tract, which, in unfavorable conditions, can become a source of mortal danger. In public facilities such as hospitals, hotels, swimming pools, physiotherapeutic facilities, sanatoria, mass caterers, schools and kindergartens, homes, and enterprise of the cosmetic and food industries, biofilm structure allows saprophytic and pathogenic microorganisms to survive washing, cleaning, and disinfection processes. Biofilm formed in a water supply network poses a sanitary risk to the public. In addition, the pipes water network is subjected to microbiological corrosion. Most food processing plants are struggling with the problem of biofilm formation in water distribution systems, refrigeration systems, and heat exchangers. In the food industry, biofilm can colonize not only sewage systems, but also machine working surfaces and food products. Biofilm on work surfaces, even those made of stainless steel, glass, or Teflon, can lead to food contamination with spoilage microorganisms, including pathogenic ones. Contaminated products of both plant and animal origin can cause serious human illnesses as well as huge losses in the food industry [2]. Biofilm microorganisms are characterized by increased invasiveness and the ability to cause serious infections, even in hospital. Ability to create biofilm is one of the pathogenicity factors of the microorganism. Most often, infections caused by biofilm-building microorganisms are the result of the abiotic surfaces colonization and account about 65% of all infections [1]. Microorganisms inhabiting medical materials both biomaterials within the human body such as vascular and intraperitoneal catheters, artificial valves, prostheses, implants, lenses, stitches, and diagnostic devices such as endoscopes, fibroscopes, and laryngoscopes are also an important problem. Biofilm formation on these devices is the cause of serious infections and also leads to device damage [1, 2, 5–7]. Microorganisms that inhabit the human body also occur mainly in the form of biofilms. These biofilms are mostly composed of symbiotic microorganisms, but also opportunistic ones may occur, which in homeostasis disturbances lead to a development of serious infections. The situation is particularly

dangerous, if the development of infection is accompanied by a dysfunction of the device colonized by biofilm.

2. Biofilm definition

Biofilm is defined as a well-organized, three-dimensional social structure surrounded by extra-cellular matrix and irreversibly bound to the surface, built by microorganisms with altered, with respect to planktonic form, genotype properties [5, 6, 8–10]. Biofilm enables microorganisms to survive in a changing and unfavorable environment, and therefore is the dominant form of their existence in the nature. It is characterized by structural heterogeneity, genetic diversity, complexity of interaction, and the presence of extracellular substances. It can be either mono- or multilayer, produced by one species or many different species. The biofilm structure depends on many factors such as hydrodynamic conditions, surface type, pH of the environment, microbial mobility, intercellular communication, nutrient content, exopolysac-charides, proteins, or oxygen. Colonization of various surfaces by microorganisms is possible due to their adhesive properties and extracellular polymeric substances (EPS) stabilizing the biofilm structure. Adjacent microorganisms, in a spatially organized structure, produce a com-mon layer of polymeric substances called extracellular matrix, the complex compounds playing an important role in the formation and functioning of the biofilm. Most EPS polysaccharides are the organic compounds with long linear or branched molecules of 106 Da. The amount of polymers depends on the quantitative and qualitative composition of nutrients. The percentage of water in the biofilm matrix is up to 97%. Polymers ability to cyclical accumulation simultane-ously with donation of water gives the matrix hydrogel features with exceptional viscoelastic properties [2, 11–14]. Matrix hydrogel nature effectively protects biofilm microorganisms from desiccation and provides the cells with protection against environmental stress factors such as UV radiation, temperature shifts, pH fluctuations, or toxic substances [2, 5, 7]. The matrix serves also as a communication system between biofilm cells, where chemical and physical signals are transmitted through a branched open channel system separating individual microcolonies. Thanks to the channel network, oxygen and nutrients are delivered through the channels and the excreted waste products are discharged. Cells in biofilms are present in various metabolic states. On the periphery of the biofilm, where the channel network system is more developed, the cells are large, metabolically active, and its reproducing increases the biofilm thickness. While, microorganisms located inside the biofilm are partially cut off from the water system, which results in their growth rate decreasing. They may also fall in an anabiosis with possible activation in a case of destruction of the outer cell layer, which, no matter how long the biofilm works, uses the features of young biofilm cells [2, 12, 13]. The biofilm cell has different char-acteristics than the planktonic cells. An important determinant of biofilm properties is *quorum sensing*, a specific communication system, strictly controlled by specific genes in response to the abundance of cells in the biofilm—the sense of the piston. The ability of cells to communicate makes the biofilm able to function in a way that resembles an almost one multicellular organ-ism consisting of physiologically diverse subpopulations of microbial cells [2, 4, 5, 12, 13, 15].

3. Biofilm structure

The process of biofilm formation is multistage and depends on the properties of the micro-organisms, the construction, and properties of the colonized materials or the host. There are four basic phases: (I) reversible adhesion, (II) irreversible adhesion, (III) biofilm maturation, and (IV) dispersion (**Figure 1**).
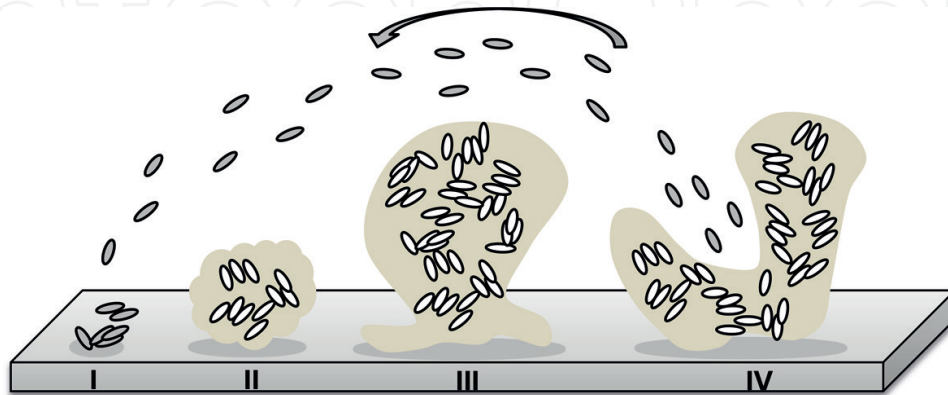
Figure 1. Biofilm formation phases: (I) reversible adhesion, (II) irreversible adhesion, (III) biofilm maturation, and (IV) dispersion (elaborated according to [2]).

Biofilm formation begins with the adhesion of free floating microorganisms to the biotic or abiotic surface. Reversible adhesion is the result of relatively weak physical interactions causing the first cells to attach to a solid surface such as gravitational interaction, electromagnetic surface charge, van der Waals forces, electrostatic, and hydro- and thermodynamic forces (Brownian motion). These forces play a crucial role when the distance between cells and the surface is relatively large. Biofilm is unstable and can easily be removed by both chemical and physical methods. When the cell distance from the surface is less than 1.5 nm, there is irreversible adhesion due to the formation of specific bonds. First microbial cells attached to the surface help attaching another one by the formation of hydrophobic, non-specific or specific hydrogen bonds, and pairs and ionic complexes (carbon-carbon covalent bonds) [2, 5, 12, 13]. An important place in the biofilm-building process is the interaction of specific receptors, adhesives, and ligands on the cell surface of the microorganism or the target host cell extracellular ligand. Initially, the surfaces are covered by a single layer of microbial cells. In the construction of the basic EPS matrix, which gives the biofilm a defined shape and structure, the increased synthesis and secretion of extracellular biopolymers is important. Biofilms expand by increasing the intensity of cell proliferation. While, glycocalyx, a shell composed of polysaccharide residues of glycolipids and glycoproteins, the components of the cell membrane, is produced up to the total surroundings of the microcolonies. At this stage, biofilm, in addition to living microorganisms, also includes dead cells, mineral substances, and organic compounds. These elements are joined by further microbial cells. Irreversible adhesion allows the formation of microcolonies and biofilm maturation [5, 12, 13]. Biofilm maturation is followed by the microorganisms' reproduction, their gradual differentiation and the activation or inhibition of expression of certain genes. Biofilm cells acquire features that are not expressed by planktonic cells and can transmit them to adjacent and progeny cells. When reaching the

critical thickness of the biofilm membrane, cells migrate from peripheral parts of the mature biofilm to the surrounding environment and the process of colonization begins. Disconnecting cells from biofilm and its dispersion is an intentional separation resulting from a reaction to adverse environmental conditions. Biofilm adapts to environmental stresses and the detached cells begin the process of colonization of new surfaces [2, 3, 5, 8, 12, 13].

Both bacterial and fungal biofilms, in medicine and in industry, were first described in 1978 [7]. Since then, it has been the subject of numerous studies that aim to understand the molecular mechanisms of its origins and the role it plays in infections and drug resistance [5]. *Candida albicans* often occurs in the form of biofilm, which is the etiological factor of approximately 90% candidiasis. Among the clinical strains of the genus *Candida*, biofilm formation depends on the type of a strain [16], and *Candida albicans*, even of the same genotype, may differ in biofilm features [1].

4. *Candida* dimorphism and the biofilm formation

Compared to planktonic forms, biofilm cells lead settled lifestyles and have characteristic gene expression associated with the growth rate and synthesis of some of the adhesion and enzyme proteins. Fungal biofilms with cells differing phenotypically and functionally usually are of much more complex structure than the bacterial biofilm. Polymorphism is a characteristic feature of *Candida* yeast. The planktonic *Candida* are usually in a form of blastospores (budding cells), while the biofilm structure is formed by both blastospores and mycelial forms. During biofilm formation, morphological transformation takes place: from blastospores through the germ tubes to the filamentous forms (mycelium or pseudomycelium). The plasticity of *Candida* biofilm indicates that its cell composition may also change depending on the location and characteristics of the biomaterial surface. Individual cell types exhibit differences in antigenic structure and its adhesion and invasive properties, enzymatic activity, and phagocytosis resistance. Blastospores are responsible for the adhesion and spreading of the biofilm, initiating its production by adhering to biotic and abiotic surfaces and its colonization [5]. Adhesion is a signal that induces germination (morphogenesis) of blastospores and the formation of invasive forms, mycelium, or pseudomycelium, that enter epithelial or endothelial cells via endocytosis or active penetration and because of enzymatic activity they contribute to the destruction of colonized tissues. The presence of mycelial forms is not a prerequisite condition for the biofilm formation; however, it seems indispensable in the process of maturation. In the mature *Candida* biofilm, the inner layer is composed of blastospores, and the outer multilayer is mycelium and pseudomycelium. Biofilms created by *Candida* sp. yeast can reach a thickness of 25–450 µm [1, 5, 10, 11, 17]. There are three [6, 11] or four [1, 18] phases of *Candida* biofilm formation. Sometimes the last dispersion phase is included in the third phase, maturation of the biofilm.

5. *Candida* adhesion and the ability to the biofilm formation

The biofilm structure depends on the specific gene expression resulting from yeast contact with biotic or abiotic surface. *Candida albicans* yeast contact with a specific surface and activate the mitogen-activated protein kinase (MAPK) signaling cascade, which carries the extracellular

contact signal to eukaryotic cells, then activating transcription factors and expressing a specific set of genes responsible for adherence. MAPK gene *mkc1* activity level is higher in cells growing on different surfaces than in planktonic cells. After contacting *Candida albicans* with a polystyrene surface, the transcriptional level of the gene coding for methionine and cysteine, and the *cdr1* and *mdr1* codes for the mechanism of active ejection of the drug by efflux are surprisingly increased. Some examples of *Candida* sp. adhesion to polystyrene are presented in **Figure 2**.
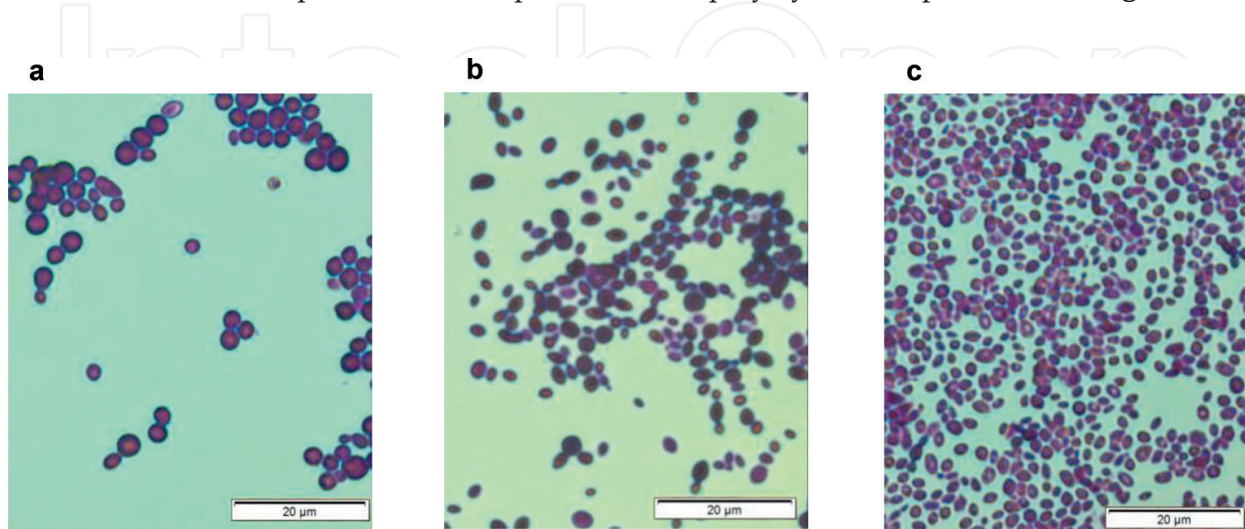

Figure 2. Adhesion to a polystyrene surface of environmental *Candida* sp. strains (a) *Candida albicans* cl/MP/12 clinical isolate; (b) *Candida parapsilosis* Fo/82/03 food-borne isolate; (c) *Candida albicans* cl/MP/08 clinical isolate (photographs by M. Maroszyńska).

Genes encoding sulfur amino acids are responsible for the amount of biofilm biomass produced, while the expression of the *cdr1* and *mdr1* genes is associated with the acquisition of drug resistance by the biofilm phenotype. What is more, the activity of adh1 alcohol dehydrogenase is higher in plankton cells than in biofilm cells, which influences the biofilm formation [1]. This enzyme is probably responsible for inhibition of biofilm formation and inactivation or mutation of the *adh1* gene results in stronger biofilm formation *in vitro* and *in vivo* [1, 5]. Adhesion to both biotic and abiotic surfaces related to recognition of host cell surface receptors, is a precondition for colonization, biofilm formation, initiation, and development of infection. Lack of adhesion prevents yeast cells from biofilm formation. Phenotypic variability and plasticity of cells in relation to changing environmental conditions allow for the settlement of new surfaces. Numerous *Candida albicans* gene products important for biofilm development have been identified [1, 19]. Adhesive genes can be activated by different environmental signals. The potential adhesives involved in biofilm formation are cell wall surface proteins. The input of surface proteins with GPI (glycosylphosphatidylinositol) module is decisive in the formation of fungal biofilm. Ability of *Candida albicans* to adhesion is an important virulence factor associated with glycoproteins encoded primarily by genes such as *hwp1* and *als*. Such an effect leads to changes in the expression of genes encoding cell wall proteins glycosylphosphatidylinositol dependent. Several key adhesins: als1, als2, als3, als4, als5, eap1, hwp1, hydrolases, lipases, phospholipases, and transcriptional factors bcr1 regulating protein expression are responsible for biofilm formation [1, 5, 19, 20]. Ywp1, mannoprotein with a GPI

module, is both the regulator of adhesion and a marker of *Candida albicans* cells. Blastospores are characterized by weaker intercellular adherence than mycelial forms. In addition, they are probably equipped with mechanisms to regulate the activity of their own adhesins [1]. The ywp1 protein found on yeast cells, a known adherence regulator, can function as an antihistamine and inhibit adherence. The discovery of the ywp1 protein is a scientific proof for the presence of the cell diffusion phase in the biofilm's life cycle. The deletion of the ywp1 gene leads to the enhancement of the blastospor adherence to the various surfaces [1]. On the other hand, in the biofilm layer composed of pseudomycelium *Candida albicans* identified adhesins with GPI, HWP1, EAP1 module, and the ALS adhesin family. Some studies indicate that the genes *als* and *eap1* within the *Candida* genus show similarity to the genes regulating adhesion in *Saccharomyces cerevisiae* [1]. While, the *hwp1* gene, known as the gene coding for the main *Candida albicans* protein, is involved in many functions such as cell wall building, intracellular signaling, and the development of hyphae. In addition, it appears that it is involved in the adhesion of yeast to epithelial cells, which is so important in the initial colonization stage. There is also an evidence for the involvement of the *hwp1* gene in systemic candidiasis pathogenesis on mouse model *in vivo*. It has been shown that strains having the *hwp1* and *hwp1*-null heterologous genes showed, respectively, reduced and no virulence compared to control wild strains. Hwp1 is the first exposed adhesive, required for biofilm formation *in vivo*, which is not present on cells in the form of yeast and plays no role in the formation of microcolonies. However, it expresses during morphogenesis blastospores to pseudomycelium [1].

6. *Candida* germ tubes and the ability to the biofilm formation

Pseudomycelium is formed by a germ tube process and as a key component of the biofilm provides its integrity. Both morphological forms of blastospore and pseudomycelium are capable of a biofilm formation, but strains capable of growth only in the form of blastospores produce only residual biofilm. The transcription factor efg1 plays a key role in regulating the morphology and virulence of yeast *Candida albicans*. It was first identified as an inducer of the development of pseudomycelium in *Saccharomyces cerevisiae* and then as a necessary for the growth of mycelium *Candida albicans*. The consequence of deletion of this gene is a loss of ability to transition into mycelial forms in response to majority of stimulation factors, but may occur in hypoxia and abiotic conditions. The *efg1* gene fulfills many of the important functions in *Candida albicans* yeast cells, and most important is the virulence of vast infection models. Cells with *efg1* gene deletion do not attack the human epithelium. In addition, *efg1* is one of the key regulators of transition from the "white" form to the "opaque" form and essential to keep the default "white" phenotype. Moreover, unlike many other biofilm process regulators, the *efg1* gene is essential for biofilm development under hypoxia and oxygenation conditions. Even when the yeast cells have adhered to the abiotic surface it is necessary to produce resistance to antifungal agents. Efg1 is a part of a network of six transcription factors that regulate the expression of at least 1000 genes involved in the development of the *Candida albicans* biofilm [21]. Several thousands of intergenic regions bound by the transcriptional factor efg1, which binds to promotors at least 53 genes in *Candida albicans*, including many transcription factors have been identified. The binding of efg1 is closely related to the transition from the basic

form of the yeast cell to the pseudomycelium. Mutants with the deletion of *efg1*, *cph1*, and *tec1* genes encoding transcription factors do not form pseudomycelium, and consequently have no ability to form mature biofilm structures. This indicates that transcriptional factors efg1, cph1, and tec1 play a key regulatory role in the formation of mature *Candida albicans* biofilm [1, 21]. Furthermore, the mutations of the genes *suv3*, *nup85*, *mds3*, and *kem1* inhibit the formation of pseudomycelium, which in turn promotes the formation of "immature" biofilm. In addition, mutants with the deletion of the *bcr1* gene produce pseudomycelium, but do not produce biofilm, since the inhibition of gene expression for adhesin als and hwp1, involved in biofilm formation and regulated by *bcr1* [1]. Separation of the filamentation process and biofilm formation showed that the morphogenesis of blastopores to pseudomycelium and consequently the presence of pseudomycelium did not clearly determine the biofilm formation. Pseudomycelium is only a basis, on which under control of the transcription factor bcr1, the adhesins gene gradually express. It is therefore necessary to provide the proper function—filamentous adherence, without which mature biofilm will not be formed. Mutants lacking the activity of tec1, bcr1, als3, or hwp1 proteins exhibit large abnormalities in the biofilm production, which may underline the importance of all these proteins in the early stages of biofilm formation. The ability of residual biofilm formation by these mutants may at the same time indicate that these proteins are not directly involved in adherence to the surface, but in adherence between the blastospore, the mycelium forms, or the adherence mixed between both forms [1, 5, 21].

7. *Candida* communication and the ability to the biofilm formation

For the proper functioning of biofilm, communication between the cells and density regulation is necessary. These tasks are executed by small signaling particles called autoinducers and by responding to the generated signals within population in the *quorum sensing* system. In the culture with a high population density, there are signaling particles which, through diffusion, penetrate other cells running different signals. Exchanging signals lead to specific cell effects and coordination of cellular activity like multicellular behavior. *Candida* yeasts produce several signaling molecules, the accumulation of which determines the development, existence, and breakdown of the biofilm through having a direct influence on the process of mycelial forms creation. The best-known molecule is farnesol ($C_{15}H_{26}O$), a terpene alcohol isolated from *Candida albicans* cells. In the reproduction and maturation phase of the biofilm, the density of cells is relatively small, allowing the formation of mycelium. With the cell concentration increase, the concentration of farnesol, which interacts with the blastospore cell receptors, is increasing, preventing transformation into pseudomycelium and maturation of biofilm. The consequence is the phase of the biofilm dispersion in which individual blastospores and their aggregates are detached [1, 2, 5, 18]. The release of blastospore requires weakening of the adhesive properties, which corresponds to the anti-adhesion ywp1 protein, and the main regulator of the process is the hsp90 protein [5]. Farnesol exogenously inhibits biofilm formation by blocking the expression of many genes responsible for the formation of pseudomycelium and induces expression of the *adh* gene taking part in inhibition of the biofilm formation. The endogenous accumulation of this signaling molecule in biofilm structures may therefore be

a factor initiating the breakdown of the biofilm upon reaching a critical cell concentration. Farnesol also influences the expression of ergosterol metabolism genes. At the time of action of azoles blocking the ergosterol synthesis, the substrate to produce this molecule is increased by *Candida albicans*, and the amount of farnesol may even rise 45 times. Farnesol also affects many other processes, such as production of chlamydospores, iron transport, and activation of genes responsible for antibiotic resistance and oxidative stress. Unfortunately, it also has an adverse effect on host cells by inhibiting macrophage activity in the mouse model [1, 2, 5].

Another *Candida albicans* signaling molecule is an autoinducer thiamazole that stimulates the production of mycelium during the intermediate phase of biofilm growth. Comparing to planktonic cells, biofilm cells produce higher amounts of thiamazole [2, 18]. It protects the cells from a decrease in the expression of DNA replication genes, chromosome segregation, and a cell cycle control [1, 2].

The active regulation of the process of detachment from biofilm surface layers, in the state of achieving critical concentration of cells inside, is a crucial role of signaling molecules [1].

8. *Candida* antibiotic resistance

Candida albicans, like most pathogens, developed a number of mechanisms that regulate their virulence. It has developed different strategies to colonize host tissues and break down and weaken its barriers and defense mechanisms. One of the most important virulence factors of *Candida* sp. is the ability to produce mycelial forms that allow a host tissue invasion, at the same time repelling an effective phagocytes attack. The virulence of *Candida* sp. is strongly related to proteins determining of cell integrity, adherence, colonization, or change of phenotypic forms. These proteins are also an effective weapon in the fight against host defense. Most of them are characterized by the presence of anchored glycosylphosphatidylinositol and represent 88% of all covalently bound *Candida albicans* cell membrane proteins. Increasing clinical drug resistance because of abuse of antimicrobial agents is an important phenomenon hindering the fight against these yeasts. *Candida albicans* drug resistance is closely related to the antifungal activity of the drugs used.

Most drug resistance mechanisms to antifungal agents are the results of gene mutations. Usually, these are point mutations of genes encoding drug-binding molecules, enzymes of metabolic pathways, or transcription factors [22]. Such mutations are stable and their acquisition takes time. It is believed that they are the expression of a cell response to chronic stress, for example, resistance-inducing azoles [23] or genetic aneuploidy [24], which changes the expression of multi-drug pump points or transcription factors. Antifungal drugs can also activate a classic, immediate response to a stress. Resistance acquired on this path does not involve the change of genetic material and is reversible, for example, *Candida* sp. phenotype form change or biofilm formation. This reversible change allows us to obtain the time necessary to induce permanent resistance dependent on genetic mechanisms. For the resistance of one of the oldest antifungal agents, 5-FC, the most responsible is uracil phosphoribosyltransferase mutation preventing conversion of 5-fluorouracil to fluorouradine 5-monophosphate [25].

Resistance to polyene, which is still relatively rare today, is obtained by decreasing ergosterol content in the cell membrane, inter alia by *erg3* gene mutation. Lowering ergosterol content in the cell membrane also leads to azole resistance by the increased expression of the erg11p molecule, the azoles binding point. Point mutations of this molecule are responsible for replacing the toxic ergosterol precursors accumulated in the yeast cell by non-toxic ones [25]. *In vitro* studies show different patterns of drug resistance to azoles, frequently overlapping with clinical trials. *In vitro*, the role of the hsp90 molecule chaperone for calcineurin in promoting the rapid acquisition of *Candida albicans* resistance to fluconazole has been identified [26, 27]. Interestingly, the ability to maintain azole resistance even after treatment has ended [28]. Another effective mechanism of azoles resistance is the high expression of multilayer membrane pumps (MFS Mdr1p drug pump or ATP binding cartridge (ABC) of the Cdr1p or Cdr2p pump). These pumps beside azoles are active against a variety of other drugs, apart from echinocandins [25, 29]. Limited resistance to echinocandin is most likely related to their relatively rare use. Although, in recent years, there have been reports both *in vitro* and *in vivo* on *Candida* sp. resistance to echinocandins. The best-known mechanism of resistance to these antibiotic agents is the mutation of the β-1,3-D-glucan synthase Gsc1p subunit [25, 29].

9. *Candida* biofilm and its drug resistance

Particularly dangerous from a clinical point of view is the ability of most clinically important *Candida* species (*Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis*) to biofilm formation. The clinical significance of biofilm is increasing with the increasing introduction of various medical devices into the human body. Almost all the contaminations of these devices are the results of their colonization by micro-organisms forming organized biofilm structures.

Biofilm *Candida* sp. is characterized by high resistance to all antifungal agents currently used: azoles (fluconazole, itraconazole, voriconazole, posaconazole), echinocandins (kaspofungin, mikafungin, anidulafungin), amphotericin B, flucytosine, but the level of this resistance is different for different drugs. Studies have shown that *Candida* sp. biofilm is resistant to fluconazole at a concentration of 2000 times higher than the MIC value for the planktonic form. The liposomal form of amphotericin B and echinocandin are the most active against *Candida* sp. biofilm. These antibiotic agents exhibit anti-biofilm activity in concentrations 2–25 times higher than the MIC values against planktonic forms [25, 30, 31]. Biofilm resistance is a complex, multi-factor phenomenon that uses the different mechanisms generated by planktonic forms at different stages of biofilm formation. There is also the possibility of generating different mechanisms of drug resistance by individual cell in the biofilm. For example, in the early stages of biofilm-building with low cell concentration, the increase in the activity of drug pumps, lowering the intracellular concentration of azoles, is noted. In mature biofilms characterized by greater cell concentration and many extracellular substances, resistance to amphotericin, azoles, and echinocandins is generated [11, 25, 30]. In addition, it appears that the lower content of ergosterol in mature biofilm is also one of the mechanisms of defense against antifungal agents [22]. The change from planktonic forms to biofilm is a response to

unfavorable environmental conditions, which starts a rapid response to stress, which generates, for example, drug resistance. Acquired by acute stress resistance, it is associated with protein kinase activity, calcineurin or hsp90p heat shock protein. Drug-resistant subpopulation protects the pool of cells needed to rebuild the biofilm [25]. An extracellular matrix (ECM) is an important factor in the generation of multi-drug resistance. β-1,3-ᴅ-glucan, one of the ECM components, is responsible for drug resistance to fluconazole and amphotericin B. While the role of ECM in generating multi-drug resistance is unquestioned, the mechanisms leading to it remain unexplained.

In the fight against *Candida* biofilm, there are two main problems: a penetration of the drug into the biofilm structure and to overcome the yeast resistance produced by the cells organized in the biofilm. A method of "lock therapy" is conformed to deliver the antifungals directly into the places colonized by the biofilm. To conquer the growing antibiotic biofilm resistance the following strategies are applied: (i) novel antifungal agents in the forms of conjugates, (ii) a multi-drug therapy, (iii) a combination of antifungal agents with nonsteroidal anti-inflammatory drugs, and (iv) agents interfering the communication of cells in the biofilm.

The use of high drug concentrations, for example, higher echinocandin doses used to treat endocarditis, is one of the proposed methods of fighting against *Candida* sp. biofilm. "Lock therapy" uses medical devices (e.g., vascular catheters) for treatment, where high drug doses are introduced into the catheter [32, 33].

Hudson et al. [34] describe a novel form of amphotericin B, dextran aldehyde conjugate with amphotericin B, preservative gel formulation used in local treatment of infections (ligaments, vascular catheters, bones) caused by *Candida* sp. biofilm. *In vitro*, also other compounds: EDTA, ethanol, and high doses of monocycline, are effective in the fight against *Candida* sp. biofilm as "lock therapy" [32, 35].

Another method of fighting infections caused by *Candida* sp. biofilm is the combination therapy of antifungal agents (fluconazole, echinocandin, and amphotericin B) with calcineurin inhibitors such as cyclosporin A or tacrolimus. Such therapy exhibited good *in vivo* activity in the treatment of rat-associated venous catheter infections [35]. Other promising preparations used in "lock therapy" in combination with antifungal agents are compounds that target hsp90 heat shock proteins such as geldanamycin [25, 35]. However, none of these preparations are suitable for systemic use due to their toxicity or lack of confirmed safety in clinical trials.

An interesting proposal seems to be the combination of antifungal preparations with widely used nonsteroidal anti-inflammatory drugs (NSAIDs). Their activity by inhibiting cyclooxygenase prevents yeasts filamentation and thus biofilm formation [32].

Recently, the synergistic effects of 2-adamantanamine, a structural analogue of antiviral amantadine, with fluconazole have been discovered. The mechanism of action is unknown, but it appears that 2-adamantanamine inhibits lanosterol 14-α-demethylase in the ergosterol cycle [32]. The patients' safety of such association has not been established.

Attempts are also being made to use molecules responsible for biofilm communication. One of them is farnesol, which, more than in physiological concentration, leads to biofilm degradation.

Its activity in mouse model studies *in vitro* was comparable to that of azoles. However, *in vivo* studies on animal models have not been conducted [32, 36].

Pulmozyme preparation, comprising recombinant human deoxy ribonuclease (rkDNase), is currently used in inhalation therapy of patients with cystic fibrosis, which targets bacterial biofilm DNA [32].

10. Probable environmental circulation of *Candida* strains

Besides the most frequent fungal pathogen *Candida albicans*, non-*albicans Candida* strains are isolated from the patients and clinical environments. Among non-*albicans*, the common clinical isolates are *Candida glabrata*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida auris*, *Candida tropicalis*, and *Candida dubliniensis*. *Candida* sp. are the widespread yeasts in food products serving as natural flora members or food-contaminants. The examples of food-associated *Candida* yeasts are *Candida lusitaniae*, *Candida famata*, *Candida parapsilosis*, *Candida colliculosa*, *Candida tropicalis*, *Candida krusei*, *Candida boidinii*, and *Candida pelliculosa*. Considering the possibilities of *Candida* strains' natural circulation between food and clinical environments, a question arises if the food-borne strains can be a threat for specific groups of patients. Our previous work presented the examples of antibiotic resistance of a variety of *Candida* clinical and food-borne isolates [37]. Within the study, 24 clinical strains of *Candida albicans* and 1 *Candida glabrata* strain as well as a *Candida lusitaniae* strain were compared with 18 non-*albicans* food-borne candidas. The set of food-borne isolates consisted of *Candida lusitaniae* (four strains), *Candida famata* (two strains), *Candida parapsilosis* (one strain), *Candida colliculosa* (one strain), *Candida tropicalis* (one strain), *Candida krusei* (four strains), *Candida boidinii* (three strains), *Candida rugosa* (one strain), and *Candida pelliculosa* (one strain). The strains sensitivity to the nystatin (polyenes), fluconazole (triazoles I generation), voriconazole (triazoles II generation), and caspofungin (echinocandins) were checked. It was found that all the tested strains were sensitive to caspofungin but 15 strains differed in sensitivity to nystatin, fluconazole, and voriconazole irrespective of their origin. Interestingly, two of four tested food-borne strains of *Candida krusei* were not susceptible to fluconazole, and the third one was classified as intermediate. All *Candida krusei* isolates were sensitive to fluconazole. One clinical isolate of *Candida glabrata* was not sensitive to fluconazole. Triazoles were the last effective not totally inhibiting the growth of the clinical isolates and five food-borne strains. The results proved that a group of strains expressing intermediate sensitivity or resistance to the tested antibiotics include both clinical and food-borne isolates.

According to the biochemical profiles, the tested strains were classified in two groups: (i) 24 *Candida albicans* clinical isolates and 1 strain of food-borne yeast *Candida tropicalis*, which was isolated from pickled cucumbers; (ii) 17 food-borne strains and 2 clinical isolates *Candida glabrata* and *Candida lusitaniae*. What is more, *Candida albicans* isolates expressed vast biochemical heterogeneity. A yeast adaptation to the host organism may explain these differences.

Both *Candida albicans* and *Candida glabrata*, typical human pathogens, were not found in food [37]. The noted biochemical profiles conformity of some clinical and food-borne isolates may be a result of *Candida* transmission via food. The similarity of food-borne *Candida tropicalis* to

the *Candida albicans* strains isolated from clinical patients implies the possibility of circulating of antibiotic-resistant strains outside the hospital environment and the possible yeast infection caused by yeasts entered into the body with food.

The plasticity of *Candida* yeasts subjected to non-conventional antifungal compounds like essential oils were also proved [38, 39]. Both *Candida albicans* and food-borne isolates, *Candida rugosa*, *Candida famata*, and *Candida krusei*, have changed their properties at the presence of tea tree oil (*Melaleuca alternifolia* Maiden & Betche Cheel), thyme oil *(Thymus vulgaris* ʟ.), and clove oil (*Syzygium aromaticum* ʟ. Merr. & L.M. Perry).

11. Conclusions

Biofilm-forming microorganisms, including *Candida* species, are characterized by high invasiveness, the ability to cause dangerous, and difficult to treat infections. Furthermore, the cells in the biofilm, compared to planktonic forms, show reduced sensitivity to chemical compounds with antifungal activity and increased survival under unfavorable environmental conditions. The morphological diversity of the biofilm structures formed by *Candida albicans* and non-*albicans* strains allows these yeasts to colonize both biotic and abiotic surfaces. The emergence of antifungal resistance with the development of biofilms is still a problem. The incidences of medical equipment colonization by *Candida* yeasts are constantly noted. Moreover, the proven biochemical profiles conformity of some clinical and food-borne isolates may be a result of *Candida* transmission via food.

Author details

Marta Targalska and Alina Kunicka-Styczyńska*

*Address all correspondence to: alina.kunicka@p.lodz.pl

Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Lodz, Poland

References

[1] Mnichowska-Polanowska M, Kaczała M, Gierdys-Kalemba S. Charakterystyka biofilmu *Candida*. Mikologia Lekarska. 2009;**16**:159-164

[2] Kołwzan B. Analiza zjawiska biofilmu—Warunki jego powstawania i funkcjonowania. Ochrona Środowiska. 2011;**33**:3-14

[3] Budzyńska A, Różalska B. Potencjalne wykorzystanie roślinnych olejków eterycznych w zwalczaniu zakażeń z udziałem biofilmów drobnoustrojów. Życie Weterynaryjne. 2012;**87**:213-215

[4]   Matejczyk M, Suchowierska M. Charakterystyka zjawiska *quorum sensing* i jego znaczenie w aspekcie formowania i funkcjonowania biofilmu w inżynierii środowiska, budownictwie, medycynie oraz gospodarstwie domowym. Budownictwo i Inżynieria Środowiska. 2011;**2**:71-75

[5]   Nawrot U. Znaczenie biofilmu w patogenezie i leczeniu grzybic. Zakażenia. 2013;**4**:56-59

[6]   Mazaheritehrani E, Sala A, Orsi CF, Neglia RG, Morace G, Blasi E, et al. Human pathogenic viruses are retained in and released by *Candida albicans* biofilm *in vitro*. Virus Research. 2014;**179**:153-160

[7]   Shao X, Cao B, Xu F, Xie S, Yu D, Wang H. Effect of postharvest application of chitosan combined with clove oil against citrus green mold. Postharvest Biology and Technology. 2015;**99**:37-43

[8]   Reśliński A, Mikucka A, Szczęsny W, Szmytkowski J, Gospodarek E, Dąbrowiecki S. Wykrywanie biofilmu *in vivo* na powierzchni siatki chirurgicznej—Opis przypadku. Chirurgia Polska. 2008;**10**:181-188

[9]   Sadowska B, Budzyńska A, Więckowska-Szakiel M, Paszkiewicz M, Stochmal A, Moniuszko-Szajwaj B, et al. New pharmacological properties of *Medicago sativa* and *Saponaria officinalis* saponin-rich fractions addressed to *Candida albicans*. Journal of Medical Microbiology. 2014;**63**:1076-1086

[10] Rosseti IB, Rochab JBT, Costa MS. Diphenyl diselenide (PhSe)2 inhibits biofilm formation by *Candida albicans*, increasing both ROS production and membrane permeability. Journal of Trace Elements in Medicine and Biology. 2015;**29**:289-295

[11] Dorocka-Bobkowska B, Konopka K. Biofilm formation by *Candida* and its role in the pathogenesis of chronic infections—Review. Dental and Medical Problems. 2003;**40**:405-410

[12] Czaczyk K. Czynniki warunkujące adhezję drobnoustrojów do powierzchni abiotycznych. Postępy Mikrobiologii. 2004;**43**:267-283

[13] Karatan E, Watnick P. Signals, regulatory networks, and materials that build and break bacterial biofilm. Microbiology and Molecular Biology Reviews. 2009;**73**:310-347

[14] Strużycka I, Stępień I. Biofilm—Nowy sposób rozumienia mikrobiologii. Nowa Stomatologia. 2009;**3**:85-89

[15] Gospodarek E. *Quorum sensing*—Chemiczne komunikowanie się drobnoustrojów. Postępy Mikrobiologii. 2004;**43**(S):12

[16] Sanchez-Vargas LO, Estrada-Barraza D, Pozos-Guillen AJ, Rivas-Caceres R. Biofilm formation by oral clinical isolates of *Candida* species. Archives of Oral Biology. 2013; **58**:1318-1326

[17] Rossi BP, Garcia C, Alcaraz E, Franco M. *Stenotrophomonas maltophilia* interferes via the DSF-mediated *quorum sensing* system with *Candida albicans* filamentation and its planktonic and biofilm models of growth. Revista Argentina de Microbiología. 2014;**46**:288-297

[18] Tsai PW, Chen YT, Yang CY, Chen HF, Tan TS, Lin TW, et al. The role of Mss11 in *Candida albicans* biofilm formation. Molecular Genetics and Genomics. 2014;**289**:807-819

[19] Samaranayake YH, Cheung BPK, Yau JYY, Yeung SKW, Samaranayake LP. Human serum promotes *Candida albicans* biofilm growth and virulence gene expression on silicone biomaterial. PLoS One. 2013;**8**:e62902

[20] Orsi CF, Borghi E, Colombari E, Neglia RG, Quaglino D, Ardizzoni A, et al. Impact of *Candida albicans* hyphal wall protein 1 (HWP1) genotype on biofilm production and fungal susceptibility to microbial cells. Microbial Pathogenesis. 2014;**69-70**:20-27

[21] Connolly LA, Riccombeni A, Grozer Z, Holland LM, Lynch DB, Andes DR, et al. The APSES transcription factor Efg1 is a global regulator that controls morphogenesis and biofilm formation in *Candida parapsilosis*. Molecular Microbiology. 2013;**90**:36-53

[22] Ferreira C, Silva S, Oliveira FF, Pinho E, Henriques M, Lucas C. *Candida albicans* virulence and drug-resistance requires the O-acyltransferase Gup1p. Microbiology. 2010;**10**:238-251

[23] Perepnikhatka V, Fischer FJ, Niimi M, Baker RA, Cannon RD, Wang YK, et al. Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. Journal of Bacteriology. 1999;**181**:4041-4049

[24] Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. Science. 2006;**313**:367-370

[25] Cannon RD, Lamping E, Holmes AR, Niimi K, Tanabe K, Niimi M, et al. *Candida albicans* drug resistance—Another way to cope with stress. Microbiology. 2007;**153**:3211-3217

[26] Cowen LE, Carpenter AE, Matangkasombut O, Fink GR, Linquist S. Genetic architecture of Hsp90-dependent drug resistance. Eukaryotic Cell. 2006;**5**:2184-2188

[27] Cowen LE, Lindquist S. Hsp90 potentiates the rapid evolution of new traits: Drug resistance in diverse fungi. Science. 2005;**309**:2185-2189

[28] Anderson JB. Evolution of antifungal-drug resistance: Mechanisms and pathogen fitness. Nature Reviews. Microbiology. 2005;**3**:547-556

[29] Baixench MT, Desnos-Ollivier M, Garcia-Hermoso D, Bretagne S, Ramires S, Piketty C, et al. Acquired resistance to echinocandins in *Candida albicans*: Case report and review. The Journal of Antimicrobial Chemotherapy. 2007;**59**:1076-1083

[30] Kojic EM, Darouiche RO. *Candida* infections of medical devices. Clinical Microbiology Reviews. 2004;**17**:255-267

[31] Heizmann P, Klefisch F, Heizmann WR. Basic research—Significance of detection and clinical impact of *Candida albicans* in non-immunosupressed patients. Pharmacology & Pharmacy. 2011;**2**:354-360

[32] Walvaren CJ, Leea SA. Antifungal lock therapy. Antimicrobial Agents and Chemotherapy. 2013;**57**:1-8. DOI: 10.1128/AAC.01351-12

[33] DiMondi VP, Townsend ML, Johnson M, Durkin M. Antifungal catheter lock therapy for the management of a persistent *Candida albicans* bloodstream infection in an adult receiving hemodialysis. Pharmacotherapy. 2014;**34**:120-127

[34] Hudson SP, Langer R, Fink GR, Kohane DS. Injectable *in situ* cross-linking hydrogels for local antifungal therapy. Biomaterials. 2010;**31**:1444-1452

[35] Nett JE. Future directions for anti-biofilm therapeutics targeting *Candida*. Expert Review of Anti-Infective Therapy. 2014;**12**:375-382

[36] Ramage G, Saville SP, Wickes BL, Lopez-Ribot JL. Inhibition of *Candida albicans* biofilm formation by farnesol, a *quorum sensing* molecule. Applied and Environmental Microbiology. 2002;**68**:5459-5463

[37] Maroszyńska M, Kunicka-Styczyńska A, Rajkowska K, Maroszyńska I. Antibiotic sensitivity of *Candida* clinical and food-borne isolates. Acta Biochimica Polonica. 2013;**60**:719-724

[38] Rajkowska K, Kunicka-Styczyńska A, Maroszyńska M, Dąbrowska M. The effects of thyme and tea tree oils on morphology and metabolism *Candida albicans*. Acta Biochimica Polonica. 2014;**61**:305-310

[39] Rajkowska K, Kunicka-Styczyńska A, Pęczek M. Hydrophobic properties of *Candida* spp. under the influence of selected essential oils. Acta Biochimica Polonica. 2015;**62**:663-668