Summary

Candida albicans, as an opportunistic fungus, is the part of the normal microflora of human mucosa, however, in conditions of weakened immunity of the host it may increase virulence abilities, contributing to the development of candidiasis. Due to its common existence in various niches in the human body, it may interact with other microorganisms, including serious pathogens. One of these may be the anaerobic bacterium *Porphyromonas gingivalis*, closely related to the development of periodontal diseases. Literature reports have focused mainly on the mutual interactions of both microorganisms in the background of oral diseases, but this dissertation examines them in relation to occur within the respiratory tract in the context of aspiration pneumonia.

Considering this model, all experiments were conducted in aerobic conditions referring to normal environment in respiratory track. The presence of *P. gingivalis* in such conditions is possible due to the multilayer architecture of the fungal biofilm, which results in the appearance of an oxygen gradient - its low concentration deep in the structure enables the survival of anaerobic bacteria, resulting in the progress of infection.

Gingipains RgpA, RgpB and Kgp with proteolytic activity are one of the main virulence factors of the described bacteria, therefore studies were carried out using the wild-type virulent strain *P. gingivalis* W83 together with strain-deprived genes for gingipain - the ΔKΔRAB strain. Due to the slight differences in the properties (including the protein profile) of different strains of *C. albicans*, the proteomic part was carried out with two of them: 3147 and SC5314.

The aim of the research presented in this thesis was to examine the modifications of the fungal transcriptome (including genes encoding proteins belonging to the group of "master regulators" and others involved in adhesion, remodelling of the fungal cell wall, response to stress and controlling important processes in the *C. albicans* cell), and the fungal matrix caused proteome by *P. gingivalis* together with mammalian serum (FBS) and neutrophils. Furthermore, the importance of selected regulatory proteins for the formation of mixed biofilms, in the context of the activity and survival of anaerobic bacteria was checked. The research was supplemented with verification of the role of the yeast cell wall sugar components in the interaction with *P. gingivalis* cells.

Proteomic analyzes performed using high-performance liquid chromatography coupled with mass spectrometry indicated significant changes occurring in the protein composition (regarding only fungal proteins) of the biofilm matrix formed by yeasts in contact with bacteria,

FBS, and neutrophils. As expected, large changes are observed in the groups of proteins related to virulence, response to stress, processing of genetic information, but primarily in the group related to the metabolism of basic components necessary for living organisms. *P. gingivalis* and serum influence the production and secretion of enzymes involved in carbohydrate metabolism, such as proteins from the "moonlighting" group, but also these important for synthesis and degradation of various fungal cell wall components during the reorganization of its structure. Furthermore, proteomic studies indicated a large number of serous and neutrophilic proteins of mammals embedded in the matrix of homotypic and heterotypic biofilms. These proteins are important in generating an effective immune response of the host organism, and there are identified: elements of the complement cascade, antimicrobial enzymes located in neutrophil granules, components of neutrophil extracellular traps (e.g. histones), proteins from the extracellular matrix (including: collagen, fibronectin), as well as elements of the coagulation system. All of them indicate a high level of interactions between human factors and fungal proteins, as well as the ability to modulate the mutual relations between microorganisms, the serum and neutrophil proteomes.

Knowing the particular importance of the group of "master regulators" during formation of biofilms and in regulation of expression of many other genes, the influence of serum and bacteria on the their expression levels was examined in mixed biofilms created in the aspiration pneumonia model. The results obtained by qPCR indicate significant gene induction by both bacterial strains, with the highest mRNA level observed for *BCR1, also* regulated by contact with FBS. Additionally, the influence of bacteria and serum on the expression of other genes coding transcription factors (*CPH1, FLO8, RLM1, TUP1, UME6*) as well as adhesins (*ALS3, EAP1, HWP1, HYR1, MP65, PRA1*) important at various stages of biofilm formation: initial adhesion, maturation, and dispersion was analyzed. A high level of changes was observed, indicating a significant modification of the fungal transcriptome by bacterial and human factors. Moreover the levels of mRNA for genes encoding various enzymes involved in the synthesis and degradation of glucans *(BGL2, FKS2, KRE5, SKN1, SMI1, ZAP1)* mannans (*MNN1, MNN9, PMT4*), chitin (*CHT2, CHT3)* and the lipid fraction (*CHO1, ERG11)* were checked. Their expression strongly depends on the presence of bacterial and serous factors, with the exception of the *CHO1* and *ERG1* genes, the amount of their mRNA remained at similar levels regardless of the conditions. Both *P. gingivalis* and FBS strongly induce genes related to the stress response (*CTA1, HSP90, SOD5),* possibly as an effect of the natural reaction of organisms to the contact with other cells and their secreted factors. Genetic research was complemented by a survival test of bacteria in biofilms formed with yeast with disturbed cell wall composition, as a result

of inhibition or degradation of selected components by specific compounds, which showed the significant importance of mannans in the mutual interaction and protection of anaerobes against unfavourable environmental conditions.

The next stage of the research focused on the importance of regulatory genes necessary for the process of filamentation and biofilm formation in the context of creating cocultures with *P. gingivalis* in the presence of serum. Already during the first microscopic analyses, it was noticed that deletion of regulatory genes causes unequal modifications of cell morphology, allowing to distinguish two groups of *C. albicans* strains: first similar to the wild strain - with undisturbed hyphae and the second one with clearly inhibited filamentation. Further quantitative characterization confirmed qualitative observations, indicating the great importance of the Efg1, Brg1 and Rob1 proteins in mutual interactions in heterotopic biofilms. These proteins regulate a number of other genes, including the major fungal adhesins, whose interaction with bacterial proteins has been previously demonstrated. The main conclusion of this part of the experiments indicates that the survival of *P. gingivalis* in aerobic conditions is not dependent on the filamentation of *C. albicans* and that the Efg1 protein demonstrate special importance for mutual interaction, concerning, for example, the 10-fold higher proteolytic activity in the supernatants from biofilms formed with *efg1Δ/Δ* strain.

The second part of the study was aimed to characterize the fungal cell surface mannoprotein Mp65 and its importance in adhesion of fungal cells within the biofilm structure, as well as in contact with bronchial epithelial cells BEAS-2B. Literature data indicate the possibility of interaction between Mp65 and gingipain RgpA, thereby the influence of the protein on the formation of heterotypic biofilms and their stability in the presence of drugs: antimycotic amphotericin B and antibacterial levofloxacin were checked. Experiments using the biotin-labelled Mp65 protein and the fluorescent label with subsequent microscopic observations allowed to observe the potential of protein binding not only by epithelial cells but also by *C. albicans* hyphae, indicating the possibility of the protein secreted in the medium during culture to bind afresh to the cell surface. Following the attempt to characterize the interaction of Mp65 with fungi and epithelium, proteins from fungal hyphal cell wall (CWP) and from epithelial cell membrane were isolated, with further chemical crosslinking with Mp65. The results indicated several yeast (e.g. Als3, Als5, Cht3, Pra1) and human (e.g. desmoplakin, ATP synthase subunit and S100-A7) proteins that could interact with this mannoprotein. Furthermore, the chemical cross-linking method also identified plasma proteins that interact with Mp65, followed by a detailed analysis of reaction kinetics carried out with biolayer interferometry (BLI).

In summary, the conducted research allowed for a broad and multi-aspect characterization of mutual interactions occurring in *C. albicans* and *P. gingivalis* biofilms in the model of aspiration pneumonia in the presence of mammalian serum, with particular emphasis on changes occurring in the yeast transcriptome and the matrix proteome. It was shown that anaerobic bacteria can survive within a biofilm formed under aerobic conditions, regardless of yeast filamentation; in addition, the particular importance of the Efg1 protein, a master regulator of many processes in the *C. albicans* cell, was indicated for the creation of heterotypic cultures. Furthermore, the structure of the yeast cell wall was shown to be remarkably important for the interaction with *P. gingivalis*, confirmed by upregulation of various genes involved in biofilm formation and cell wall remodeling, as well as by the CFU test. Although many different experiments were carried out, it was not possible to fully characterize the properties and importance of the Mp65 protein in mutual interactions and interactions with BEAS-2B cells.