The widespread and fast development of modern medical techniques, above all of great importance for society, unfortunately also increased the number of individuals whose immune system is weakened. It is due to the prolonged intensive therapy and invasive medical practices related to surgical procedures, the use of parenteral nutrition, intravenous catheters and mechanical ventilation, or the application of long-term antibiotic treatment. Therefore, those patients become more vulnerable to infections caused by opportunistic pathogens including microorganisms belonging to the host physiological microflora or inhabiting human organisms without any harmful symptoms. But when the immunity of an individual is attenuated or the balance of the microflora composition is disturbed, they can pose a serious threat to the patient's health or life. This group of microorganisms includes about twenty fungal species belonging to the genus *Candida* that may be pathogenic for humans with weakened immunity.

Candida yeasts may be responsible for serious systemic diseases with a mortality rate in the range of 30-40%, but they are also the cause of widespread, bothersome and recurrent superficial infections characterized by a high morbidity, high prevalence, nuisance and recurrent deterioration in quality of life. These superficial infections are localized in different host niches, including oral cavity, gastrointestinal tract or lower genital tract and are related to the formation of biofilms. *C. albicans* is the species responsible for about 50% of candidal diseases worldwide; however, the frequency of diagnosed infections caused by other emerging pathogens of this genus—non-albicans *Candida* (NAC) species—is constantly increasing and alarming because of their different pathogenicity mechanisms and resistance to antifungal drugs including fluconazole, itraconazole, voriconazole, nystatin and caspofungin.

The pathogenesis of the mucocutaneous candidiasis development is related to the disturbances in the microbiological balance which is based on the complex interactions between fungal cells of different species and numerous species of bacteria that coexist at specific locations in human organism. This biological imbalance is particularly associated with the insufficient growth of probiotic bacteria of the genera *Lactobacillus* and *Bifidobacterium*, resulting in fungal overgrowth, biofilm formation and further production of fungal virulence factors involved in the subsequent candidal invasion on host tissues. One of the important mechanisms of the physiology of fungal and bacterial cells is the production of extracellular vesicles - small structures with dimensions of 30-300 nm surrounded by a lipid bilayer, in which proteins, carbohydrates, nucleic acids and low-molecular compounds are contained and secreted extracellularly. These vesicles may be involved in the virulence of pathogens and in communication between microorganisms.

This can be a particularly important and novel issue to investigate the influence of extracellular vesicles produced by probiotic bacteria on *Candida* biofilms as well as testing how vesicles produced by one *Candida* species can affect biofilms produced by other species of these fungi. Both competition as well as cooperation between different species is possible. Data obtained during the implementation of the project may contribute to a promising complement to the antifungal therapies and to the usage of vesicle-containing active substances protected against external factors in supporting the treatment of candidiases. The use of vesicles from pro-health bacteria might be a great novelty in application not only in medicine but also in industry. Hence, the general aim of the proposed study is to characterize the participation of fungal and bacterial extracellular vesicles in the process of biofilm formation by *Candida* yeasts and to identify the molecular mechanisms of this phenomenon.

These studies will be performed in vitro using different *Candida* species and different strains of probiotic bacteria selected from *Lactobacillus* and *Bifidobacterium* species. The extracellular vesicles produced by these microorganisms will be obtained by ultracentrifugation of the concentrated supernatants collected after fungal or bacterial growth in different culture media. The content of vesicles will be characterized with the broad spectrum of spectrophotometric and fluorometric methods and protein identification with mass spectrometry. Then, the mechanism of influence of vesicles produced by *Candida* yeasts or produced by selected species of probiotic bacteria on the formation of biofilms by *Candida* species will be investigated with the analysis of the gene expression. The effect of bacterial and fungal vesicles on biofilm resistance to antifungal drugs including fluconazole, nystatin and caspofungin will also be studied. Moreover, the analysis of the interactions and binding of vesicles or proteins originating from vesicles to the fungal cells forming biofilm and investigating the impact of these interactions on adhesion of fungi to human epithelial cells will be performed.