"GEORGE EMIL PALADE" UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE, AND TECHNOLOGY OF TÂRGU MUREȘ - SCHOOL OF DOCTORAL STUDIES

Summary of the doctoral thesis „*Candida* spp. virulence traits and their relations with the macroorganism"

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With an increased pool of immunocompromised patients and with a rising number of invasive medical maneuvers, infections caused by *Candida* spp. are becoming more and more prevalent. Despite the fact that *Candida albicans* is the most isolated *Candida* spp., a progressive shift to non-albicans species has been reported. As *Candida* spp. are opportunist pathogens, the occurrence of infection is closely linked to the expression of virulence characters. In this context, the doctoral thesis aims to analyze the virulence traits of the most frequently isolated *Candida* spp. (*C. albicans*) and, also, of some rare, insufficiently studied non-albicans species (*Candida parapsilosis, Candida krusei, Candida guilliermondii,* and the multi-drog resistant *Candida auris*).

The first study retrospectively analyzes the prevalence of *Candida* spp. in lower respiratory tract secretions, thus providing local epidemiological data, over a timeframe of 10 years. The study included a total of 3199 sputum and tracheal aspirate samples, 1144 (35.76%) of which were positive for *Candida* spp. growth. In the analyzed samples, *C. albicans* is the most prevalent species, and male patients over 60 years of age are more prone to be colonized with *Candida* spp.

The second study analyzes the influence of two Quorum Sensing Molecules (QSMs) (farnesol and tyrosol), on *Candida* spp. virulence traits. Both farnesol and tyrosol influence the growth rate of *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. auris,* and *C. guilliermondii*. Their effects on *Candida* cells are species-dependent. Generally, farnesol inhibits the *Candida* spp. growth rate, especially in the first 12 hours of incubation. The inhibitory effects cease after 48 hours of incubation, probably due to adaptation mechanisms. Compared with farnesol, tyrosol has fewer effects on the *Candida* spp. growth rate. Both farnesol and tyrosol inhibit the growth rate of *C. auris* in the first 12 hours of incubation. Farnesol (100 μM) enhances the production of biofilms by *C. albicans*, while tyrosol (100 μM) inhibits it. Also, tyrosol enhances the production of biofilms by *C. krusei*. Both QSMs affect the expression of the *ALS3*, *HSP70,* and *SAP2* genes in *C. albicans*.

The third study analyzes the influence of glucose, fructose, lactose, glibenclamide, and metformin on the virulence traits of *Candida* spp. The main objectives are to determine the effects of three carbon sources (glucose, fructose, and lactose) and two widely prescribed antidiabetic drugs (metformin and glibenclamide) on the growth rates and the biofilm formation abilities of five *Candida* species: *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. auris,* and *C. guilliermondii*. Moreover, the study

analyzes the effects of the mentioned substances on the expression of *ALS3*, *HSP70,* and *SAP2* genes and germ tube formation in *C. albicans*. The nutrient sources affect the growth rate and the biofilm formation ability of *C. albicans*, *C. krusei*, *C. auris*, *C. guilliermondii,* and *C. parapsilosis*, in a species-dependent way. Fructose (100-400 mg/dL), glibenclamide (10-40 ng/dL) and glucose (50-400 mg/dL) inhibit the formation of biofilms by *C. albicans*. As *Candida* genus is extremely heterogeneous, every species should be studied individually.

The fourth study analyzes the direct influence of a pro-inflammatory cytokine (IL-6) on the virulence traits of *Candida* spp. During the microbial-host agent interaction, cytokines can act directly on *Candida* cells, influencing growth rate, biofilm formation depending on the structural and physiological characteristics of the *Candida* spp. Moreover, IL-6 can act as a stress factor, inducing overexpression of the *HSP70* gene in *C. albicans*.

The fifth and the sixth studies focus on analyzing the antifungal and/or the virulence modulator effects of silver nanoparticles (AgNPs) synthesized using extracts of *Fagus sylvatica* L and *Picea abies* L and six essential oils (EO) extracted from cinnamon, clove, melaleuca, basil, oregano, and thyme. The antifungal effect was determined by using the microdilution method. To determine if the substances can be used as therapeutic adjuvants, fluconazole synergy tests were performed.

All studied AgNPs present different degrees of inhibition for *Candida* spp. (the 50%MIC and 100%MIC values indicate antifungal activity). Silver nanoparticles biosynthesized using spruce bark extract (AgNP SBEs) have lower MIC values compared with silver nanoparticles biosynthesized using beech bark extract (AgNP BBEs). Also, the AgNPs have an antifungal effect synergic with fluconazole, against *C. parapsilosis* and *C. guilliermondii*. AgNPs BBE and AgNPs SBE have mostly an inhibitory effect on the biofilm formation ability of *C. albicans* and *C. auris*, while they enhance the biofilm formation by *C. parapsilosis*. AgNPs SBE inhibit the formation of biofilms for *C. guilliermondii*. The studied AgNPs have antifungal properties and, also, can influence the virulence of *Candida* spp.

EOs, both in a micellar and aqueous form, have antifungal activity against *Candida* spp. and the ability to modulate the virulence traits of *Candida* spp. Cinnamon, cloves, and thyme EOs in particular showed the most significant biological activities in terms of antifungal effect, inhibition of growth, reduction of biofilm, regulation of virulence genes, and formation of germ tubes. The antifungal effects exerted by cinnamon and cloves may be due to high concentrations of water-soluble compounds such as cinnamaldehyde and eugenol, compounds with potential therapeutic usage. The chemical compounds contained in the EO have a promising utility in the prevention and treatment of *Candida* spp. infections.