

**TITLE:** ANTIFUNGAL COMBINATION OF *PSEUDOMONAS AERUGINOSA* STRAIN LV SECONDARY METABOLITE WITH BIOGENIC SILVER NANOPARTICLE AGAINST *CANDIDA AURIS*

**AUTHORS:** SPOLADORI, L. F. A.; ANDRIANI, G. M.; GALDINO, A.; NAKAZATO, G.; TAVARES, E. R.; YAMAUCHI, L. M.; YAMADA, S. F. O.

**INSTITUTION:** UNIVERSIDADE ESTADUAL DE LONDRINA, LONDRINA, PARANÁ, PR (RODOVIA CELSO GARCIA CID, PR-445, Km 380 – CAMPUS UNIVERSITÁRIO, CEP 86057-970, LONDRINA, PR, BRAZIL)

**ABSTRACT:**

*Candida auris* is an emerging pathogen associated with high mortality within intensive care environments, the development of multi-resistance to the main classes of antifungal drugs, and the ability to form a biofilm that enables its survival in the most diverse environmental conditions. In this context, the search for new antimicrobial molecules and the combination of their actives have become one of the main strategies used for the development of alternative treatments to conventional ones. Metabolites produced from microorganisms and the synthesis of silver nanoparticles (AgNPs) have been widely studied as promising sources for the isolation of molecules with antimicrobial activity. Therefore, the objective of this work was to evaluate the effect of the combination between the F4a fraction, obtained from the culture of *Pseudomonas aeruginosa* strain LV, and AgNPs on planktonic and sessile cells of the clinical isolates *C. auris* CBS 10913 and *C. auris* CBS 12766. Minimum inhibitory concentration (MIC) of each substance was determined by the broth microdilution assay. F4a and AgNPs showed antifungal activity against both *C. auris* isolates, with MICs of 3.125 µg/mL and 62.5 µM, respectively. The checkerboard test was performed in order to evaluate the activity of the combined substances on the microorganism, and to determine the fractional inhibitory concentration index (FICI), with  $FICI \leq 0.5$  interpreted as synergism between the substances. The combination showed synergistic antifungal activity ( $FICI = 0.5$ ) on *C. auris* planktonic cells, with a 40x reduction in F4a concentration compared to its treatment alone. The metabolic activity of post-treated biofilm with serial concentrations of the combination (F4a: 50 – 1.9 µg/mL; AgNPs: 1000 – 31.25 µM) was evaluated by the MTT reduction method. The combination at concentrations of 250 µM / 12.5 µg/mL was responsible for a significant inhibition of more than 70% of the biofilm. Concentrations lower than 62.5 µM / 3.9 µg/mL showed a reduction in the antibiofilm activity of *C. auris*, showing inhibition of less than 30% of the mature biofilm. The synergistic activity observed between the secondary metabolite of *P. aeruginosa* strain LV and the biogenic silver nanoparticle on planktonic and sessile cells of *C. auris* proved to be a possible source for the development of new antimicrobial agents, aiming at the treatment of infections caused by *C. auris* and the control of its dissemination in hospital environments.

**Keywords:** *Candida auris*, biogenic silver nanoparticles, secondary metabolite, antifungal, antibiofilm.

**Development Agency:** Universidade Estadual de Londrina, CAPES, CNPq.