Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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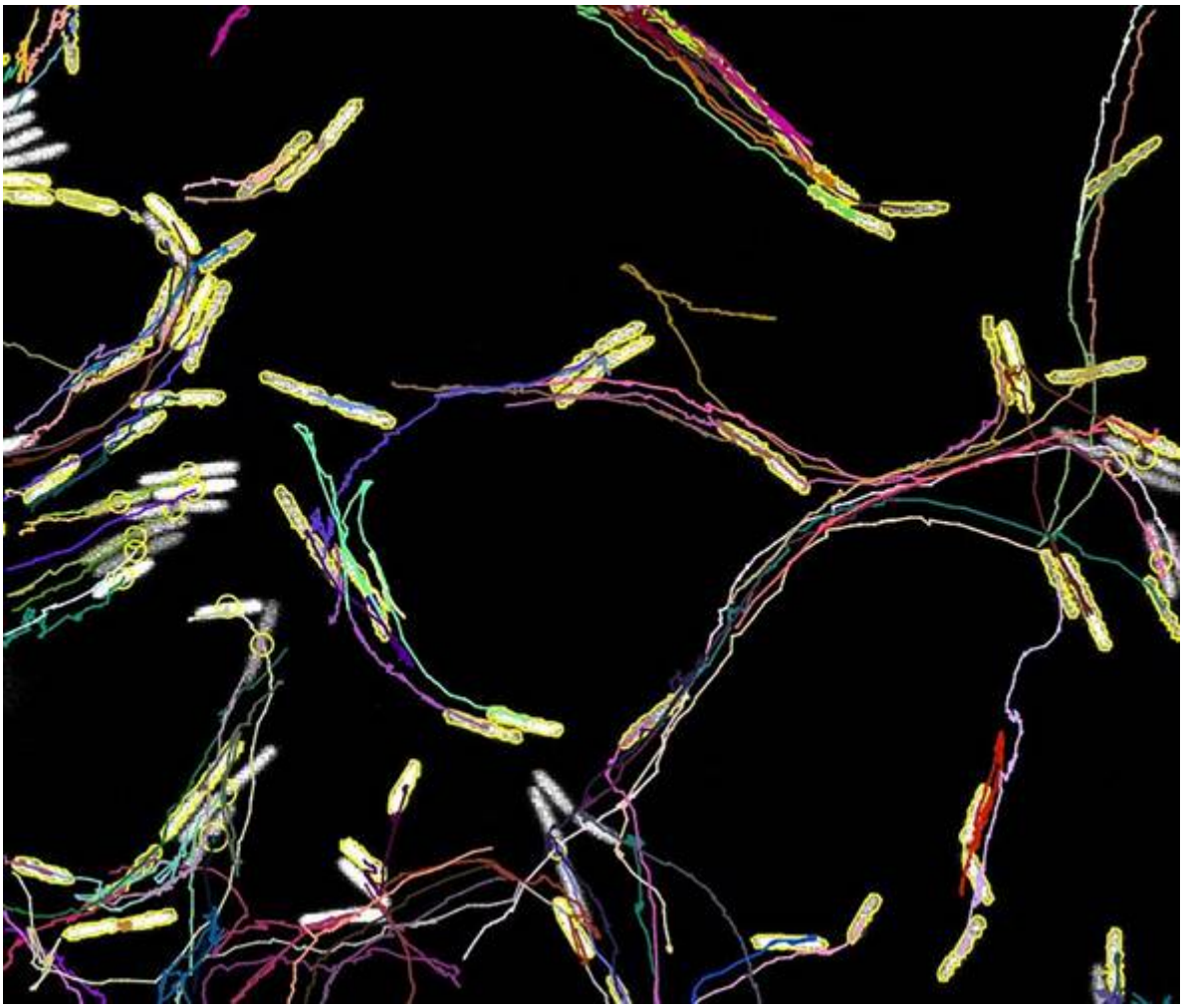

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

183 - Microbiología Molecular y Fisiología

ANALYSIS OF THE TYPE OF INTERACTION BETWEEN AMPHOTERICIN B AND SILVER NANOPARTICLES ON BIOFILM OF *Pichia kudriavzeveii (Candida krusei)*

Galera Ivana L[1,3] - Páez Paulina L[2,4] - Paraje María G[1,3]

1) Universidad Nacional de Córdoba, Facultad de Ciencias Exactas Físicas y Naturales, Departamento de Fisiología, Cátedra de Microbiología, Córdoba, Argentina.
2) Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Departamento de Ciencias Farmacéuticas, Córdoba, Argentina.
3) CONICET, Instituto Multidisciplinario de Biología Vegetal (IMBIV).
4) CONICET, Unidad de investigación y desarrollo en Tecnología Farmacéutica (UNITEFA).
Contacto: ivana.galera@unc.edu.ar

Pichia kudriavzeveii (formerly *C. krusei)* is one of the 5 pathogens that cause most invasive fungal infections in humans. Its ability to form biofilm represents an important virulence factor. It is currently a challenge to reduce the toxicity of amphotericin B (AmB) when treating biofilms of these pathogens, which opens a challenge for nanomedicine. In this work, the type of interaction between AmB and silver nanoparticles (AgNPs) on *P. kudriavzeveii* biofilm was investigated. The antifungal effect of the combinations between AmB and AgNPs on the biofilm was studied by the microdilution method in Checkerboard. From these results, the agar diffusion test was performed to determine the sensitivity to different concentrations of AmB, AgNPs and the selected combination (3.4 x 104 pM AmB + 13 pM AgNPs). The structure of the biofilm was analyzed by Fluorescence Microscopy, after exposure to the ATF compounds and the selected combination. Through confocal laser scanning microscopy (MCEL), the morphology, grouping and architecture of the biofilm were analyzed through the FIJI-ImageJ program and the images were analyzed by COMSTAT, evaluating its structure. The t-Student test was used for statistical analysis. Combinations of AgNPs and AmB were found that managed to eradicate 50% of the mature biofilm (CEB50). For the combination detailed above, the percentage of biofilm reduction was 58%. The inhibitory activity of the combinations that achieved CEB50 was greater, compared to the effect of each compound individually at the same concentration. According to the agar diffusion method, it was observed that the combination achieved the greatest inhibition halo (*$p<0.05$) with a diameter of 17.79 ± 0.06 mm, compared to the same concentrations separately and higher concentrations. The images by Fluorescence Microscopy revealed changes in the morphology and grouping of macro and microcolonies when exposed to the compounds and their combination. The 3D reconstruction of the images by MCEL showed a decrease in biomass in the three conditions studied compared to the control, being greater in the combination of compounds. From COMSTAT,

significant differences (*p<0.05) were observed in the biofilm parameters: Biomass, surface volume ratio and average thickness, compared to the untreated control. Antibiofilm fungicidal activity of AgNPs and its combination with AmB was demonstrated. The combination decreased the biomass of the biofilm in *P. kudriavzeveii* and caused alterations in its morphology, grouping and architecture. In addition, AgNPs decreased the concentration of AmB when combined, which could allow reducing the concentration of this ATF and thus its toxicity against new antibiofilm strategies.

Palabras clave: Amphotericin B - silver nanoparticles - Synergistic combination - biofilm - Pichia kudriavzeveii