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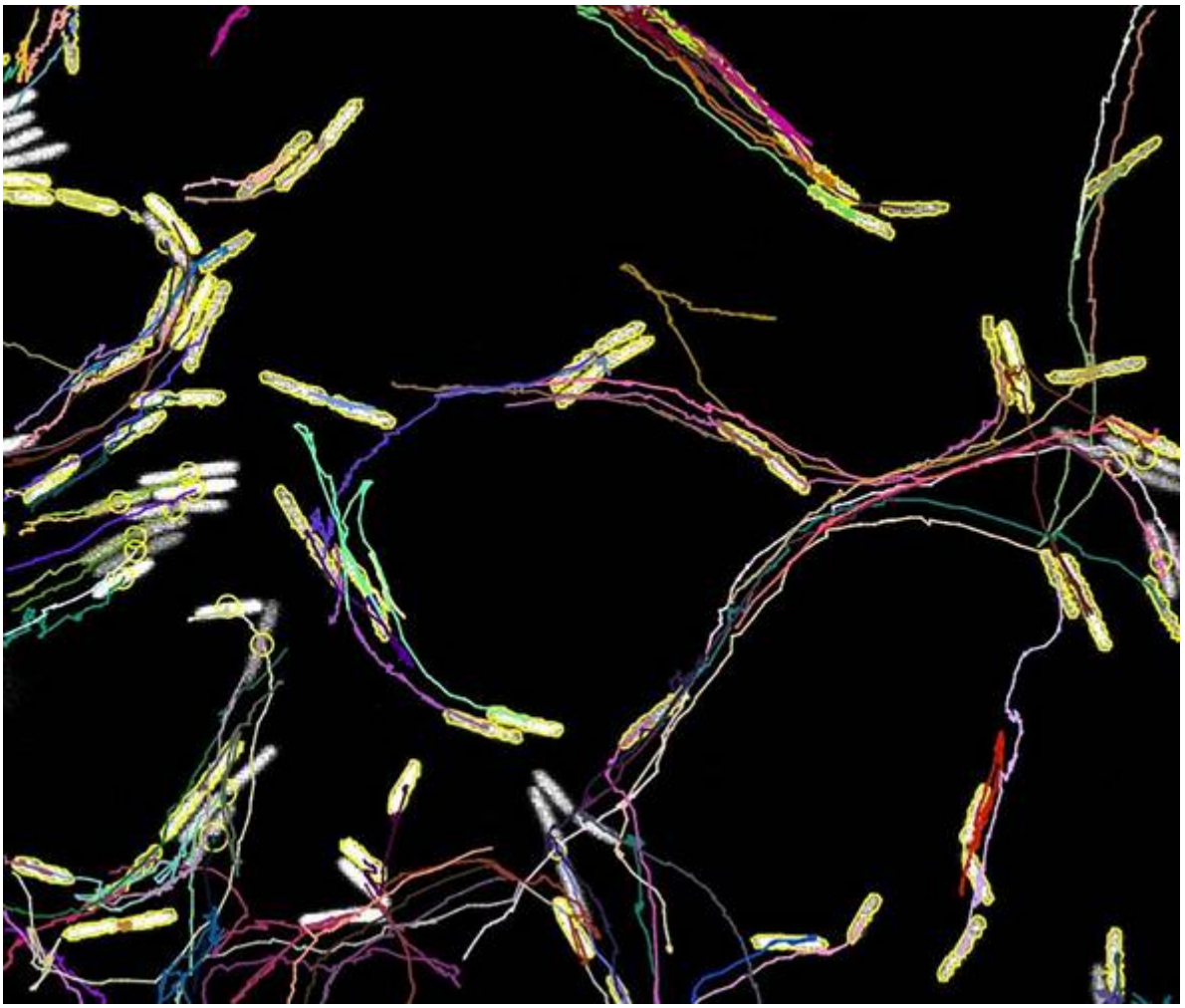

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

OPTIMIZATION OF SURFACTIN PRODUCTION IN INDIGENOUS STRAINS OF BACILLUS AND EVALUATION OF ITS CYTOTOXICITY IN RED BLOOD CELLS AND NORMAL AND TUMOR CELL LINES

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Surfactin (Srf) is a biosurfactant produced by certain species of Bacillus that is characterized by having an amphipathic nature and is composed of a peptide ring of 7 amino acids, intertwined with a chain of ?-hydroxy fatty acid containing 12 to 16 carbon atoms. Srf is capable of inserting itself into biological membranes, disrupting their integrity in a dose-dependent manner. This property grants it antimicrobial, antifungal, anti mycoplasma, antiviral, and antitumor effects. In this study, the effect of different cultivation conditions was evaluated, such as temperature (30ºC vs 37ºC), incubation time (48h vs 72h), and air volume (50% vs 90%) on the production of Srf in four Indigenous strains isolated from soils of Córdoba: *B. amyloliquefaciens* ARP23, *B. subtilis* A7, and *B. velezensis* MEP218 and A6. From each condition, cyclic lipopeptides were extracted, and surfactins were separated and purified using RP-HPLC. It was observed that an air volume of 90% was critical for the production of Srf in strains MEP218, ARP23, and A6. In contrast, strain A7 was the only one that increased the production of Srf under the condition of 50% air volume. The highest yield of Srf obtained under optimal conditions was 0.89 mg/ml for MEP218, 0.39 mg/ml for ARP23, 0.16 mg/ml for A6, and 0.26 mg/ml for A7. To evaluate the cytotoxic effect of Srf, in vitro cytotoxicity assays were conducted on cultures of a normal fibroblast cell line (MRC-5) and a cancer cell line from glioblastoma (U-87 MG). For this, three concentrations of purified Srf from the four strains (10, 30, and 80 µM, equivalent to 0.01, 0.03, and 0.08 mg/ml, respectively) were applied, and the percentage of cell viability was calculated using a fluorometric assay with resazurin. The results showed that at the concentration of 80 µM, Srf reduced the viability of both cell lines, with Srf from ARP23 being more cytotoxic for the U-87 MG cell line. The concentration of 30 µM of Srf from MEP218 and ARP23 proved to be more cytotoxic to the U-87 MG line, while the concentration of 10 µM did not show cytotoxic effects. Additionally, the cytotoxicity of Srf was analyzed in red blood cells (RBCs) from BALB/c mice (Mus musculus) to determine the released hemoglobin through spectrophotometry, along with an analysis by flow cytometry to demonstrate the effects of Srf on the cell membrane and on the cell volume of the RBCs. The results showed that Srf from the four strains is hemolytic at concentrations of 0.5 and 0.25 mg/ml; however, in most strains, hemolysis was not observed at concentrations of 0.05 and 0.01 mg/ml. The production of Srf was optimized, and a differential cytotoxic effect of Srf on both

cell lines was evidenced, resulting in greater cytotoxicity in the U-87 MG cell line. Furthermore, Srf did not show hemolytic effects at the lower concentrations.

Palabras clave: Keywords: Bacillus– surfactin – antitumor – cytotoxicity