Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

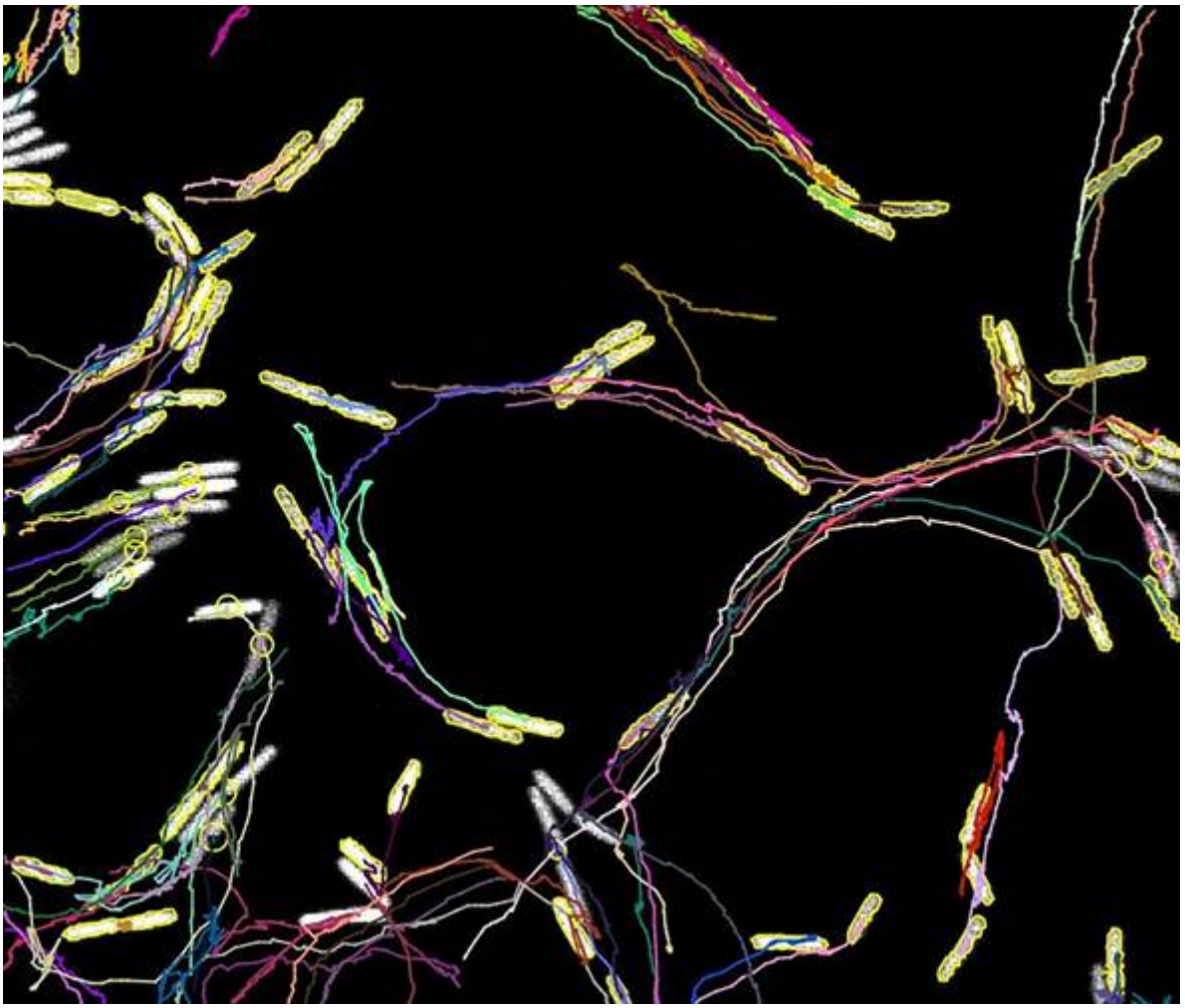

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

DEPOLARIZING EFFECT OF PATHOGENS MEMBRANE ACTIVATED BY C16-C17 FENGYCINS PRODUCED BY Bacillus velezensis MEP218

Medeot, Daniela[1] - Fernández, Marilina[1] - Bazán, Candela[1] - Basso, Vanesa[1] - Puche, Ricardo[1] - Ferrari, Walter[1] - Alejandra Bosch[2] - Manrique-Moreno, Marcela[3] - Jofré, Edgardo[1]

1) Instituto de Biotecnología Ambiental y Salud INBIAS-CONICET-UNRC - Río Cuarto - Córdoba - Argentina.
2) Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI) - La Plata - Buenos Aires - Argentina.
3) Grupo de Bioquímica Estructural de Macromoléculas - Universidad de Antioquia - Medellín - Antioquia - Colombia.
Contacto: dmedeot@exa.unrc.edu.ar

To combat the growing threat of antibiotic resistance, there is an urgent need to develop new antimicrobials. These new drugs should target bacteria novelty, bypassing existing resistance mechanisms. Fengycins C16-C17 (FENG) are a specific cyclic lipopeptide (CLPs) fraction produced by *Bacillus velezensis* MEP218, with well-characterized antibacterial activity against several pathogens including *A. baumanii*, *P. aeruginosa*, *A. xylosoxidans*, and *Burkholderia* sp. FENG were obtained by acid precipitation of supernatant from the MEP218 culture after 72 h of growth in MMOLP medium, purified by HPLC and lyophilized. Then, FENG was dissolved in chloroform:methanol (1:1) and 20 ?L were added in a BioATRII cell of a Tensor II with an MCT detector. Fourier transformation infrared (FT-IR) spectroscopy analysis was performed to identify the structural groups of the FENG. For determination of the vibrational bands of amino acids, the spectra were divided in four zones and cut (zone 1: 1750–1490 cm?¹, zone 2: 1470-1300 cm-1, zone 3: 1285-1145 cm-1, zone 4: 1140-1005 cm-1). After baseline correction, the curve fitting was estimated using Local Least Squares in OPUS software. Absorbance was plotted as a function of wavenumber and characteristic infrared bands of amino acids side chains from FENG were assigned by comparison with Spectral Database for Organic Compounds (SDBS) and references. The fluorometric measurements of the membrane potential of *A. xylosoxidans* CAMPA 1650 and *B. cepacia* CAMPA 886 were carried out using DiSC3(5), a voltage-sensitive dye. DisC3(5) accumulates and self-quenched in intact cytoplasmic membrane cells and fluoresces when the membrane is disrupted. Attenuated FT-IR spectrum from 3000 to 1000 cm-1 confirms lipids and amino acids on FENG composition. Specifically, peaks at 2970-2820, 1453, and 1403 cm–1 confirm the –C–H stretching (?CH3, ?CH2) of the aliphatic chain of the lipid. The presence of the carbonyl group (C = O) of amide (1661 cm–1) confirms the peptide fraction in the sample, whereas C–O bending of esters was characterized by the peak at 1068

cm–1. The peak at 1734 cm–1 indicates the lactone carbonyl cyclizing the peptide. Moreover, detailed amino acids assigned from FT-IR peaks were coincident with those reported for FENG. These data contribute to elucidating the molecular structure of FENG. FENG induced dose-dependent membrane depolarization in both pathogens tested. Furthermore, DisC3-5 fluorescence immediately increased to the highest level after FENG addition, leading to rapid membrane depolarization even at the lowest concentrations tested (5 µg ml-1). Triton-X100 produced maximum fluorescence due to complete membrane disruption, whereas ceftazidime (a third-generation cephalosporin that disrupts peptidoglycan synthesis) did not produce probe release. These results would indicate that the organization of FENG in the membrane environment could leads to the formation of ion channels.

Palabras clave: specific cyclic lipopeptides – membrane target – antibacterial