Sociedad Argentina de Microbiología General

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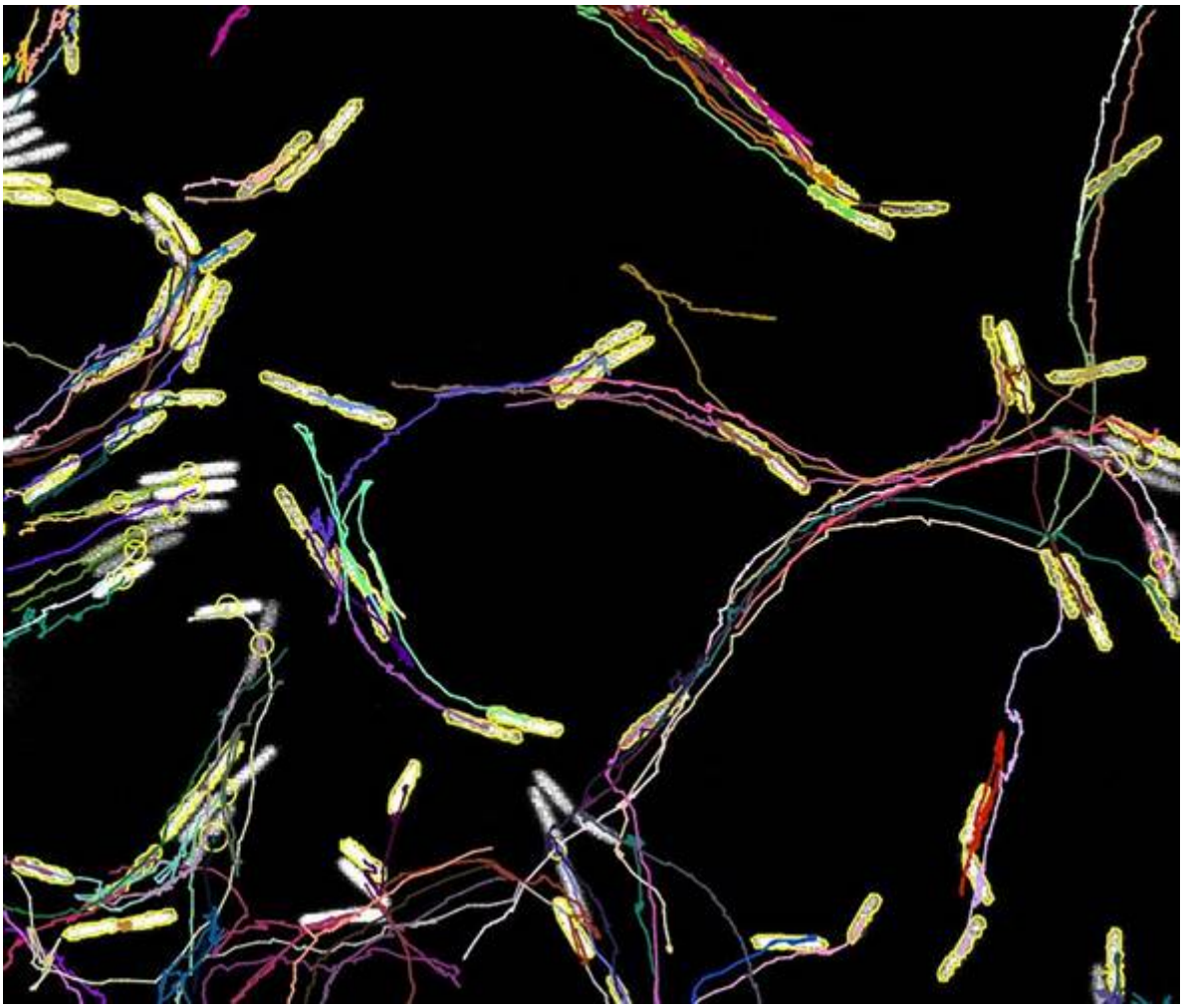

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

LRA-13: BIFUNCTIONAL ?-LACTAMASE IDENTIFIED IN AN ALASKAN SOIL METAGENOME — PHENOTYPIC AND BIOCHEMICAL CHARACTERIZATION.

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Through functional metagenomics, various genes encoding ?-lactamases from the four Ambler’s molecular classes were recovered from Alaskan soil samples and named LRA (for "?-Lactam Resistance Alaskan soil"). Among them, the blaLRA-13 gene stands out, as it encodes a 609-amino-acid protein that exhibits two well-differentiated regions, or "modules": the C-terminal sequence aligns with class C ?-lactamases (therefore, called LRA-13C), and the N-terminal one with class D ?-lactamases (LRA-13D). This work aims to study the behavior of clones expressing the whole LRA-13 protein and its sub-modules separately, and also to evaluate the kinetic parameters of the purified LRA-13C variant. The blaLRA-13full, blaLRA-13C, and blaLRA-13D genes synthesized in pUC57-kan were transformed into *Escherichia coli* DH5?. The MIC for ?-lactams was determined by the microdilution method, following the CLSI guidelines. The blaLRA-13C gene was subcloned into pET-28a, expressed in *E. coli* BL21(DE3) by IPTG induction, and the enzyme was purified by affinity chromatography. The main kinetic parameters were obtained by steady-state UV/VIS spectrophotometry against ?-lactam antibiotics. In silico molecular models were obtained using AlphaFold and visualized with PyMol. LRA-13C and LRA-13D share only 48% and 36% amino acid identity with AmpC from P. aeruginosa and OXA-1, respectively. Despite the low percentage of amino acid similarity with clinically relevant variants, in silico models show a high degree of structural similarity and the presence of conserved residues at the active site (Figure). Only LRA-13D confers resistance to ampicillin and ampicillin/sulbactam (MIC 32 and 16 ?g/ml, respectively), while clones producing both LRA-13C and LRA-13full are susceptible to all tested antibiotics. This suggests that the complete LRA-13 protein may have one of its active sites inaccessible due to structural hindrance or blockage. For the LRA-13C-producing clone, the susceptibility profile is compatible with a basal expression AmpC. However, the kinetic behavior of LRA-

13C shows high activity against nitrocefin and cephalothin (kcat/Km = 6 and 3.5 ?M-1 s-1, respectively), being 8–10 times higher than for ampicillin (0.7 ?M-1 s-1). The hydrolytic efficiency for cephalothin is 4,500 and 23,000 times greater than for ceftriaxone and imipenem, respectively. Overall, this work provides the first evidence of a chimeric ?-lactamase, with two independent modules belonging to different molecular Ambler classes (class C and D) that possess (at least one of them) hydrolytic activity against some ?-lactams, at levels similar to other variants expressed by human pathogens. These results reinforce the hypothesis of the environmental origin of many clinically important ?-lactamases and suggest the occurrence of hybrid genes that, along with the accumulation of mutations that modify the activity spectrum, represent an unprecedented pathway for the generation of ?-lactamase-encoding genes.

Palabras clave: Metagenomics - Environmental Resistome - ?-lactamase Evolution