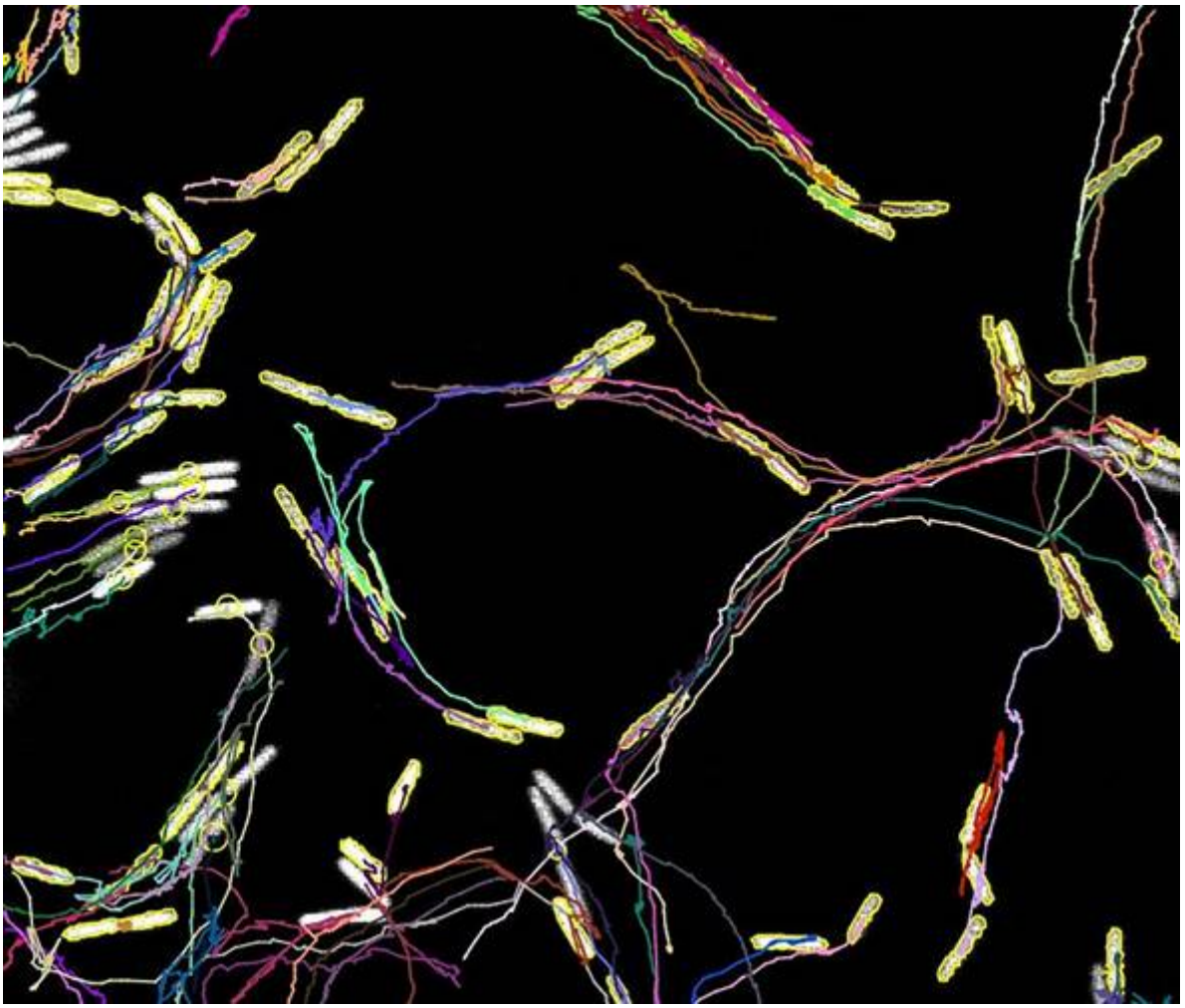
Sociedad Argentina de Microbiología General

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.



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CJO-1 AND CIM-2, NOVEL ENVIRONMENTAL MEMBRANE-BOUND METALLO- ?-LACTAMASES WITH IMPAIRED ACTIVITY AGAINST CEPHALOSPORINS

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The emergence of bacteria resistant to antibiotics threatens the extraordinary health benefits that humanity has achieved since the advent of these life-saving drugs. Carbapenems are the latest generation of ?-lactam antibiotics and, as such, they are currently employed as last-resort drugs in intensive care units. Metallo-?-lactamases (MBLs) represent one class of ?-lactamases, that have become relevant due to their ability to hydrolyze carbapenems. Among MBLs, the New Delhi Metallo-?-lactamase (NDM-1) has shown so far, the fastest and largest geographical spread, involving more than 86 countries. Since its discovery, NDM-1 and its variants have represented the only group of MBLs experimentally characterized as lipoproteins. A recent work reported the presence of a new MBL from a *Chryseobacterium* indologenes isolate that, as NDM-1, has also proven to be a membrane-bound enzyme. *Chryseobacterium* spp. are intrinsically resistant gram-negative bacteria widely distributed in natural environments, such as water, soils, rhizospheres, plants, frogs, chicken, fish, and raw milk that may constitute a reservoir of uncharacterized MBLs. With the aim of

studying novel *Chryseobacterium* MBLs we performed a bioinformatics search using NCBI data bases; we analize the subcelular localization of two novel putative MBL proteins through western blot assays; we examined their capacity of confering resistance through MIC assays; we purify them and studied their in vitro lactamase activity through spectrophotometry and their structure through protein crystallography and Molecular Dinamic Simulations. As results, we report an analysis of the extensiviness of B1 ?-lactamases containing lipoboxes in different *Chryseobacterium* spp. We found that, in addition to CIM-1, there are other 70 B1 MBLs containing lipoboxes. We also report the biochemical and structural characterization of two of them: one MBL from *Chryseobacterium* indologenes named CIM-2 and a closely related MBL from *Chryseobacterium joostei*, named CJO-1. Both enzymes proved to be membrane-anchored and able to confer resistance to a broad spectrum of ?-lactam antibiotics. When we tested them in vitro they displayed an impaired activity towards several ?-lactams. In particular, they showed a selectivity against some cephalosporins that can be explained by the presence of a positively charged residue in the flexible active site loop L3. Positively charged residues in equivalent positions has been described for clinically important variants of VIM and IMP MBLs. Overall, these results provide a general information on the structure-function relationship of the B1 subclass and reveal that Chryseobacterium is an environmental reservoir of membrane-bound MBLs that could potentially be transferred to human pathogens species.

Palabras clave: Antimicrobial Resistance, carbapenemases, NDM-1, Chryseobacterium, enzyme structure