XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

2 al 4 de agosto de 2017

San Miguel de Tucumán | ARGENTINA

SAMIGE

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La Comisión Organizadora Local agradece muy especialmente la colaboración, trabajo ypermanente disposición de Daniela Russo y Diana Vullo.

BB-014

CRY PROTEIN ANALYSIS WITH MOSQUITOCIDAL ACTIVITY AND ANTIMICROBIAL PEPTIDE SEARCH FOR THE CONTROL OF PATHOGENS VECTORIZED BY MOSQUITOES

M Florencia Gil[1 2], Rocio P Lopez[1 2], J Nicolás Lazarte[1 2], Marina Battaglia[1 2], Corina M Berón[1 2]

[1]*Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC-CONICET).* [2]*Fundación para Investigaciones Biológicas Aplicadas (FIBA).*

floor.mfg@hotmail.com

Bacillus thuringiensis is an entomopathogenic bacteria that produces a parasporal inclusion composed by Cry proteins, toxic against mosquitoes and other insects. When this crystal is ingested by insect larvae, is solubilized in alkaline gut environment and proteolytically cleaved by gut proteases. After binding to the specific receptors on the brush border membrane of the midgut epithelium, the activated toxin would lead to insect death. Recently a novel polycation peptide, BTM-P1 with antimicrobial (AM) activity, was described based on the amino acid sequence of domain I of some Cry protoxins. *Aedes aegypti* is the principal vector for Zika, chikungunya, yellow fever, and dengue worldwide and itis the main target in vectorial control program. Some *Culex* species are vector of some encephalitis viruses with relevance in public health. In this context, cry toxin studies result interesting as a putative alternative for biological control of *Aedes* and *Culex* mosquitoes. On the other hand, AM peptides can be used for the control of human pathogens vectorized by these insects. In this work we analyzed several Cry proteins isolated from a native strain with mosquitocidal activity against *Ae. aegypti*, *Aedes* (*Ochlerotatus*) *albifasciatus*, *Culex pipiens* and *Culex apicinus*. The isolated toxins were identified as Cry4-like1, Cry4-like 2, Cry19-like1, Cry19-like2 and Cry24Ca by phylogenetic analysis. We performed the alignment by ClustalW in MEGA Software between all known Cry proteins with mosquitocidal activity and the ones isolated from the native strain and we constructed a tree using the statistical method UPGMA. Then, we analyzed structural differences among native Cry proteins plus other toxins near the native ones according to the previous phylogenetic tree. We focused on: i) number of a-helix, ii) large of b-sheets, iii) loops similarity and iv) conserved motifs of functional importance. Cry native toxins show the typical domains (I, II and III) present in Cry mosquitocidal proteins. Structural analysis revealed that a motif located in a5 is conserved in all native Cry; this motif is involved in oligomerization which is necessary for pore formation. Moreover, specific residue located in a4-a5 loop is highly conserved among all native Cry and it is involved in lipid membrane interaction. In this context, we hypothesize that native Cry have mosquitocidal activity and Cry4-like1 and Cry4-like2 have at least the same efficiency that Cry4Aa and Cry4Ba. Furthermore, Cry proteins will be cloned and expressed in an heterologous system and the toxic activity will be measured by bioassays against mosquito larvae. *In silico* analysis shows that domain I of all native Cry toxins have hydrophobic regions, which could be used as templates for the generation of putative AM peptides. In conclusion, native Cry toxins are a promising option as biological agents for mosquito control or for the control of pathogens vectorized by them.

BB-015

NATIVE BACTERIOPHAGES INFECTING *Bacillus thuringiensis:* MORPHOLOGY AND THERMOSTABILITY

Flavia V Loto[1], Sofía M Diaz[2], Mario D Baigori[1 2], Licia M Pera[1]

[1]*Laboratorio de Morfogénesis y Fermentaciones (PROIMI - CONICET).* [2]*Universidad Nacional de Tucumán. San Miguel de Tucumán, Argentina.*

flavialoto722@hotmail.com

The production of bioinsecticides based on *Bacillus thuringiensis* (*Bt*) is sensitive to bacteriophage infection. As lytic bacteriophages, they could act directly on the bacteria cells and/or bacteria could have prophages integrated to the genome or as a plasmid. Consequently, the isolation, characterization and identification of the viruses infecting entomopathogenic strains are crucial for improvement the industrial process. *Bt* RT (EF638795.1) is a native strain isolated from an indigenous *Spodoptera frugiperda* (*Sf*) larva, and its efficacy against different pests including *Sf* has been proven. In this work, we proposed the characterization of two environmental bacteriophages (M3 and M4) active against *Bt* RT. Those viruses were previously isolated from soil samples from Tucumán. Thermostability of M3 and M4 was evaluated as follow: Single lysis plaques of M3 and M4 phages were propagated in LB cultures of *Bt* RT. Suspensions were filtered (0,22 µm) and titrated by the Double Agar Overlay Plaque Assay. Thermal assays were carried out at 45, 50, 55, 60, 65, 70 and 75°C during 30 min. Samples were taken every 10 min. Later, 3 µl of each suspension were dropped onto a Bt RT lawn and incubated at 30°C ON. As a result, after 30 min of treatment at 60°C both bacteriophage suspensions were completely inactivated. For Transmission Electron Microscopy, a drop of purified bacteriophage was negatively stained with 1% uranyl acetate on formvar-coated copper grids and photographed on a Zaiss EM109 microscope (Oberkochen, Germany). This study showed tailed bacteriophages. So, according to the morphological analysis, both bacteriophages belonging to the order Caudovirales. In this order, M3 belong to the *Siphoviridae*family based on the observed non-contractile and flexible tail (length= 293 nm; width= 9nm) with a capsid width= 58.6 nm and a capsid height= 55 nm). M4 showed a morphology similar to the *Myoviridae* family with the peculiar contractile tail (length= 66 nm; width= 18 nm) and with an isometric head with a diameter of ~66 nm. In general, both phages exhibited similar behavior regarding temperature. The results obtained will allow us to design more effective control procedures to avoid contamination in fermentations with loss of the product which may be partial or total depending on the type of bacteriophage. In additionthe isolated bacteriophages could be also used for the phagotyping of this bacterial species. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).