TITLE: ACTIVITY OF THE *SALMONELLA BONGORI* SPI-22 T6SS AND CHARACTERIZATION OF A FAMILY OF EFFECTORS

AUTHORS: HESPANHOL, J.T[1]; LIMACHE, D.E.S[1]; NICASTRO, G.G[1]; CHAGAS-SANTOS, G[1]; LLONTOP, E[2]; SOUZA, R.F. [1]; BAYER-SANTOS, E[1].

INSTITUTION: [1]INSTITUTO DE CIENCIAS BIOMEDICAS, UNIVERSIDADE DE SÃO PAULO, SP - BRAZIL); [2]INSTITUTO DE QUIMICA, UNIVERSIDADE DE SÃO PAULO, SP - BRAZIL).

ABSTRACT:

The type VI secretion system (T6SS) is a contractile apparatus capable of delivering effectors into several target cells. The T6SS is composed of 13 components that assemble into 3 complexes: trans-membrane complex, baseplate, and tail. A conformational change in the T6SS baseplate trigger the contraction of a cytoplasmic sheath, expelling a spear-like structure to puncture target cell membranes. The spear is composed of Hcp (hemolysin co-regulated protein) hexamers capped with a trimer of VgrG (valine-glycine repeat protein G) proteins and a PAAR (proline-alanine-alanine arginine) protein tip. Cargo effector proteins associate through non-covalent interactions with these structural components, while specialized effectors are presented as additional C-terminal domains fused to Hcp, VgrG, or PAAR proteins. *Salmonella bongori* encodes a T6SS within the SPI-22 (*Salmonella* pathogenicity island 22) and its target activity was unknown. We analyzed the function of the SPI-22 T6SS and characterized a family of effectors secreted by this system. Interbacterial competition assays using *S. bongori* wild-type and T6SS-mutant strains as attacker against *Escherichia coli* as prey were performed. Results showed that more prey cells were recovered when incubated with the T6SS-mutant, indicating that the SPI-22 T6SS has antibacterial activity. Bioinformatic searches for putative effectors in *S. bongori* NCTC 12419 genome identified several candidates. Proteins containing an N-terminal PAAR domain and a C-terminal nuclease domain (named Nuc1, Nuc2, Nuc3 and Nuc4) were chosen for further characterization. The putative nucleases were encoded next to several genes containing domains of unknown function (DUFs), which resembled putative immunity proteins (Imm1.1, Imm1.2, Imm2.1, Imm2.2, Imm3 and Imm4). The nucleases and their respective immunity proteins were cloned in compatible plasmids and co-transformed into *E. coli*. Nuc1 and Nuc4 were not toxic to *E. coli,* but Nuc2 and Nuc3 reduced bacterial growth. Co-expression of Nuc2 with Imm2.1, and Nuc3 with Imm3, was able to neutralize the toxic effect. Bioinformatic analyzes showed that the effectors belong to the PD(D/E)xK superfamily of phosphodiesterase, which target nucleic acids. Point mutations in conserved aspartic acid (D) residues abrogated toxicity. We are currently investigating the molecular mechanism of these antibacterial toxins. We believe that these results will increase knowledge on bacterial antagonistic mechanisms.

Keywords: *Salmonella,* T6SS, antibacterial toxins, effectors, nucleases.

Development agency: FAPESP (São Paulo Research Foundation).