TITLE: CHARACTERIZATION OF A NOVEL T6SS ANTIBACTERIAL EFFECTOR/IMMUNITY PAIR OF *SALMONELLA* TYPHIMURIUM

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ABSTRACT:
Salmonella is a gram-negative bacteria that cause diseases ranging from gastroenteritis to typhoid fever. *Salmonella* are acquired by ingestion of contaminated food and water. After reaching the gut, *Salmonella* must face a first line of defense composed by the indigenous microbiota. Competition between microorganisms for nutrients/space determines which species will emerge and dominate or be eradicated. Bacteria use a series of mechanisms to kill competitors, which can be mediated by diffusible factors secreted into the medium or by factors transferred directly into target cells in a contact-dependent manner. The type VI secretion system (T6SS) is a dynamic contractile organelle enabling the injection of proteinaceous effectors into target cells in a contact-dependent manner. *S.* Typhimurium encodes a T6SS within the *Salmonella* pathogenicity island 6 (SPI-6). In this work, we identified a new pair of effector/immunity protein (Tae5^{TM}/Tai5^{TM}) encoded within SPI-6 T6SS. Tae5^{TM}/Tai5^{TM} are repressed by the histone-like nucleoid structuring protein (H-NS) under standard culture conditions. To evaluate the toxicity of Tae5^{TM} upon expression in *E. coli* and to establish in which cellular compartment Tae5^{TM} exerts its effect, we cloned the full-length protein under control of the P_{BAD} promoter both with and without an N-terminal signal sequence. We also cloned Tai5^{TM} immunity protein under the control of P_{TAC} promoter. Results showed that Tae5^{TM} is toxic only when directed to the periplasm, and co-expression with Tai5^{TM} could neutralize Tae5^{TM} toxicity. We performed time-lapse microscopy to evaluate growth and morphology of individual *E. coli* cells expressing a periplasmic version of Tae5^{TM} and observed that bacteria underwent abnormal cell division, elongation, swelling and finally lysis. Bioinformatic analyses revealed that Tae5^{TM} display similarity with transpeptidases, suggesting Tae5^{TM} targets the peptidoglycan layer. We are currently performing point mutations in conserved putative catalytic residues to access loss of function/toxicity. Tae5^{TM} was expressed as recombinant protein and we are performing enzymatic degradation assays with purified peptidoglycan. Tae5^{TM} has no similarity to known T6SSs effector and may represent a novel family of antibacterial toxins. This work expands our knowledge about the bacterial arsenal used in competitions and provides molecular insight into the mechanism by which *Salmonella* overcomes the microbiota during infection.

KEYWORDS: *Salmonella*; bacterial competition; microbiota, type VI secretion system; T6SS, effector; toxin.

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