GC.06 - A Family of T6SS Antibacterial Effectors related to L,D-transpeptidases Targets the Peptidoglycan
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Type VI secretion systems (T6SSs) are contractile nanomachines widely used by bacteria to intoxicate competitors. *Salmonella* Typhimurium encodes a T6SS within the *Salmonella* pathogenicity island 6 (SPI-6) that is used during competition against species of the gut microbiota. Characterize a new SPI-6 T6SS antibacterial effector containing DUF2778 (STM14_0336) and its cognate immunity protein containing DUF2195 (STM14_0335). Bioinformatic analyzes of the SPI-6 T6SS cluster revealed a putative effector and immunity protein pair. Effector was cloned with or without N-terminal PelB periplasmic localization sequence and its toxicity was analyzed by microscopy. Searches for DUF2778 homologs at NCBI nr database established its evolutionary relationship. Effector was expressed as recombinant protein for enzymatic assays using muropeptides as substrates and analyzed by RP-HPLC coupled to MS. STM14_0336, renamed Tlde1 (T6SS L,D-transpeptidase effector 1), was toxic in target-cell periplasm. Its toxicity was neutralized by co-expression with immunity protein Tldi1 (T6SS L,D-transpeptidase immunity 1) (STM14_0335). Time-lapse microscopy revealed that intoxicated cells displayed altered cell division, swelling and lysis, indicating cell wall damage. Bioinformatics analysis showed that DUF2778-containing proteins comprise a superfamily evolutionarily related to L,D-transpeptidases that further divided into three families (Tlde1a, Tlde1b, Tlde1c). Point mutations on conserved His121 and Cys131 residues eliminated toxicity. Co-incubation of purified Tlde1 and peptidoglycan tetrapeptides showed that Tlde1 displays L,D-carboxypeptidase activity,cleaving GM-tetrapeptides between *m*DAP[3] and D-Ala[4]. Results suggest that Tlde1 promotes depletion of acceptor GM-tetrapeptides, thus preventing formation of new crosslinks and weakening the peptidoglycan mesh structure. Tlde1 comprise a new family of antibacterial effectors with L,D-carboxypeptidase activity evolutionarily related to L,D-transpeptidases. DUF2778 superfamily is widespread in Proteobacteria.
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GC.07 - A New Synthetic Antimicrobial Peptide Bioinspired from a Plant Protein is Active against Staphylococcus saprophyticus biofilms
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Bacterial resistance is the cause of overwhelming number of deaths, a public health problem. One of the causes of bacterial infections, mainly in the urinary tract, is *Staphylococcus saprophyticus*. Furthermore, *S. saprophyticus* biofilms are naturally resistant against the available antimicrobial therapies. Antimicrobial peptides (AMP) are short-chain amino acid molecules with a broad spectrum of activity. Considering the urgent need for discovery of compounds with antibiofilm activity, we investigated antibiofilm properties of a new AMP, designed from the *Inga laurina* trypsin inhibitor (ILTI) amino acid sequence, named KWI-19. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of KWI-19 against *S. saprophyticus* ATCC 49453 was evaluated according to CLSI Protocol, using a KWI-19 solution prepared in a sterile 0.9% NaCl solution and serially diluted (from 10 to 0.02 μM). The positive control was carried out with vancomycin (from 87 to 0.17 μM), the negative control was prepared with MH broth and bacterial suspension. The effects of KWI-19 on the inhibition of *S. saprophyticus* ATCC 49453 biofilm formation and the eradication of mature biofilm were also evaluated. The biofilm viability was quantified as a percentage of the total number of viable colony-forming units (CFU). KWI-19 presented MIC and MBC of 1.25 and 2.5 μM, respectively, while vancomycin presented MIC and MBC of 0.68 μM and 1.36 μM, respectively. The peptide inhibited 40.6% and 46.6% of the biofilm formation at 1.25 and 12.5 μM, respectively. KWI-19 eradicated 48% and 57.9% of 24-h mature biofilms at 1.25 and 12.5 μM, respectively. KWI-19 inhibited the biofilm formation better than vancomycin at MIC, suggesting the potential use of AMP to control biofilm infections. Therefore, KWI-19 showed antibiofilm potential to control *S. saprophyticus*. The potential of KWI-19 to control infections through *in vivo* models can be investigated in the future. **Keywords:** peptide, antibacterial, antibiofilm. **Supported by:** FUNDECT, FINEP, CNPq and CAPES