TITLE: CLONING, EXPRESSION AND PRELIMINARY CHARACTERIZATION OF VapBC-4 TOXIN-ANTITOXIN MODULE FROM *LEPTOSPIRA INTERROGANS* SEROVAR COPENHAGENI

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ABSTRACT

Leptospira interrogans is one of the causative agents of human leptospirosis. Toxin-Antitoxin (TA) systems are largely distributed among bacteria, encoding a stable toxin and an unstable antitoxin. Its physiological function is the reversible cessation of cellular growth under stress conditions, but their general function remains unknown. TA modules have been related to bacterial infection by playing a role on virulence and bacterial persistence. VapBC is the major TA family, grouped due to toxin PIN domain homology, which is thought to act as ribonuclease. The analysis of *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 genome allowed the prediction of four VapBC TA modules. VapBC-3 was previously characterized. The aim of this study was to clone, express and preliminarily characterize leptospiral VapBC-4 TA module and evaluate the toxic component presence on other *Leptospira* spp. The *vapB*, *vapC* and *vapBC* genes fragments were amplified from *Leptospira* genomic DNA by PCR, cloned into pGEM-T Easy and subcloned into the expression vector pET28a. VapBC-4 module components were successfully cloned in both vectors, which were validated by restriction analysis using *Nco* I and *Xho* I and DNA sequencing. Protein expression was carried out in *E. coli* BL21(DE3) in LB medium, induced by IPTG and analysed by SDS-PAGE. Expression of VapB and VapC were confirmed by Western blotting using anti-6X His tag antibodies. Currently, we are working on optimizing the expression of VapB and C-4 and purification on Ni^{+2}-Sepharose and SP-Sepharose. The toxic effect of VapC-4 on *E. coli* was evaluated through growth kinetic analysis. Data obtained showed that cells expressing VapC-4 had a retarded growth, when compared to strains expressing VapB-4 and the module VapBC-4, suggesting that VapC-4 has a toxic effect on *E. coli* cells, which corroborates with TA characteristics described in the literature. We have also investigated whether the *vapC-4* is present in different *Leptospira* spp through bioinformatic analysis using TADB and BLASTp tools, from which we inquired that this toxin is the second most widely distributed among *Leptospira* ssp. Works around the world have identified how this toxins impacts in several metabolic processes, mainly in bacterial adaptation and defense. TAs functional studies are useful not only to understand bacterial physiology but also develop new anti-microbial treatments, highlighting the necessity of identification and characterization of new TAs.

Keywords: Toxin-antitoxin, VapBC, *Leptospira*.

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