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733-1 Leptospira interrogans presents genes that encode proteins with potentially functional PilZ domains

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Resumo:

Leptospira interrogans is a pathogenic bacterium that causes leptospirosis in humans or animals. Analyses of the *L. interrogans* genome showed that there is some locus_tag encoding PilZ domains, whose expression has already been confirmed by proteomics and probably are constitutively expressed and may play an important role in spirochete biology. The PilZ domain proteins have the potential to bind to the second messenger cyclic diguanylate monophosphate (c-di-GMP), a key regulator of various cellular processes, including biofilm formation, motility, and virulence factors. The aim of this study is to investigate, through structural and biochemical methods, whether PilZ domains retain their ability to bind c-di-GMP. Specifically, we focus on the proteins expressed by the locus_tags LIC_11920, LIC_10049, LIC_12723, LIC_12994, and LIC_14002, which contain a truncated PilZ domain in their C-terminal region and a DUF1577 domain in their N-terminal region. Bioinformatics analyses have revealed that these genes maintain conserved residues required for c-di-GMP binding. To facilitate our investigations, all the aforementioned locus_tags were cloned with a 6×His tag fused to their N-terminal portions in the pET-28a(+) expression vector. At present, only LIC_11920 has been successfully expressed in *E. coli BL21(DE3)-RIL* cells and purified using chromatographic techniques. The other locus_tags are currently undergoing sequencing and will subsequently be expressed in *E. coli* expression strains. We employed Size Exclusion Chromatography – Multiple Angle Light Scattering (SEC-MALS) to assess LIC_11920's ability to dimerize and determine its experimental molecular mass. Additionally, we employed nuclear magnetic resonance (NMR) and Isothermal Titration Calorimetry (ITC) to perform a comprehensive analysis of the protein's affinity for c-di-GMP. Our results thus far indicate that LIC_11920 is a monomeric protein in solution. Analyses of 1H-15N (TROSY) HSQC spectra have revealed significant chemical shifts in LIC_11920 when the protein is in the presence of c-di-GMP. These findings suggest that the truncated PilZ domain retains its ability to bind this cyclic dinucleotide. Consequently, we anticipate that the other cloned proteins will exhibit similar characteristics. In conclusion, this study provides valuable insights into the binding capability of truncated PilZ domains for c-di-GMP in *Leptospira interrogans*. Understanding the functional role of these proteins will enhance our comprehension of spirochete biology and potentially uncover new targets for combating leptospirosis.

Palavras-chave:

c-di-GMP, PilZ, Leptospira interrogans

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