POSTER - PROTEINS AND PROTEOMICS

LIC_11920 IS A PROTEIN CONTAINING A FUNCTIONAL PILZ DOMAIN

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Cyclic di-GMP (c-di-GMP) is a bacterial second messenger that regulates various important biological bacterial processes. This molecule is synthesized by enzymes known as diguanylate cyclases (DGCs) that contains GGDEF domain, and this molecule is degraded by phosphodiesterases (PDEs) known as EALs or HD-GYPs. The genome of Leptospira interrogans, one of the species that causes human leptospirosis, express important proteins that bind

to c-di-GMP. The protein expressed by the locus_tag LIC_11920 (protein LIC_11920) of L. interrogans has a DUF1577 domain in its N-terminal domain and a truncated PilZ domain in its C-terminal domain. Bioinformatics analyses showed that LIC_11920 maintains the conserved residues to c-di-GMP binding. Therefore, the objective of this work is to show by structural and functional methods that PilZ of LIC_11920 still has its capacity to bind c-di-GMP. For this, LIC_11920 was cloned, fused to a 6×His tag in its N-terminal portion, in the pET-28a(+) expression vector. The gene was expressed in E. coli BL21(DE3)-RIL cells and purified using chromatograph techniques. We first evaluate the ability of this protein to dimerize by SEC-MALS (Size Exclusion Chromatography – Multiple Angle Light Scattering) assays to determine its experimental molecular mass. To test the affinity of the protein for c-di-GMP, we performed a more robust approach by nuclear magnetic resonance (NMR). As a result, we can state that LIC_11920 is a monomeric protein in solution, and analyses of 1H-15N (TROSY) HSQC spectra revealed significant chemical N-H shifts in LIC_11920 when the protein is in the presence of c-di-GMP. These results indicate that the truncate PilZ domain of LIC_11920 remain its ability to bind this cyclic dinucleotide.