Cloning, Expression and Purification of Bacterial Dyguanilate Cyclases for Structural Studies and Inhibitor Design

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The diguanylate cyclases (DGCs) are modular proteins containing the GGDEF domain, responsible for the biosynthesis of bis-(3,5)-cyclic dimeric guanosine monophosphate (c-di-GMP) from two GTP molecules. It has been shown that c-di-GMP functions as an intracellular bacterial signal molecule controlling the phenotypic transition of microbes from a free-swimming, planktonic form to an immobilized, self-contained community called biofilm. It has been estimated that up to 80% of chronic infections can be attributed to biofilms, often escaping treatment with traditional antibiotics. Given the absence of c-di-GMP in eukaryotic cells, c-di-GMP biosynthesis might be an attractive target for the development of novel therapeutics against bacterial infections. Thus, in this work we developed expression systems for several GGDEF-containing proteins from different organisms aiming structural characterization and rational inhibitors design. Selected proteins from Xanthomonas citri (XAC0614 and XAC2482), Pseudomonas aeruginosa (PA14_43930, PA14_16500) and Escherichia coli (YdeH) were amplified from the respective genomic DNA and cloned into the expression vector pSMT3, which produces a fusion protein with a 6xHis_SUMO tag. Initial tests presented high expression levels of all cloned proteins. Large-scale production was carried out for YdeH and the optimized expression/purification protocol resulted in a 5-fold increase of protein yield compared to the previously reported in the literature. Activity tests employing reverse-phase chromatography and fluorescence of thiazole orange confirmed the DGC function of YdeH. Crystallization trials and inhibitor screening are currently in progress. With this study we expect to contribute for the understanding of biofilm formation at molecular level and to develop tools to interfere in this process.

Word Keys: Biofilm, GGDEF domain, c-di-GMP
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