A liquid chromatography-coupled tandem mass spectrometry method for quantitation of cyclic di-guanosine monophosphate

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Cyclic di-guanosine monophosphate (c-di-GMP) represents an important ubiquitous second messenger in bacteria. It controls the transition between a sessile and a motile lifestyle of bacteria and, hence, affects the formation of biofilms which are highly resistant to antimicrobial treatment. c-di-GMP is synthesized by di-guanylate cyclases (DGCs) and degraded by specific phosphodiesterases (PDEs), two highly abundant protein families in bacteria. We have established a robust and highly sensitive high performance liquid chromatography-coupled tandem mass spectrometry (HPLC-MS/MS) based method for the quantitation of c-di-GMP and investigated various method performance parameters such as limit of detection (LOD), lower limit of quantitation (LLOQ), linearity, accuracy, recovery and analyte stability. As a proof of principle we used this method to accurately measure the activity of the prototype DGC PleD* from Caulobacter crescentus in vitro. In addition the methodology was successfully applied to determine in vivo levels of c-di-GMP in bacterial extracts of E. coli at different stages of bacterial growth. This demonstrates that our method is suitable for the sensitive and specific quantitation of c-di-GMP in bacterial cell extracts.
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