

Effects of surface interaction on c-di-GMP regulation in *Clostridioides difficile*

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Clostridioides difficile is a bacterial pathogen that causes antibiotic-associated intestinal disease.² The signaling molecule cyclic diguanylate monophosphate (c-di-GMP) regulates various physiological changes such as biofilm formation, motility, and cell differentiation in many bacterial species.¹ In *C. difficile*, c-di-GMP regulates motility, colonization and virulence.⁵ In many bacteria, an increase in c-di-GMP levels is associated with the transition from a motile to a sessile lifestyle.¹ In the presence of an abiotic surface, bacteria more likely organize themselves in a protective manner through biofilm formation and become sessile.⁴ With these relationships in mind, we wanted to test if there was a difference in c-di-GMP levels between *C. difficile* growing in a liquid culture and cells on a solid surface. We hypothesize that being on a surface will lead to higher intracellular c-di-GMP levels. We used a plasmid where transcription of a red fluorescent reporter, *mCherry*, is regulated by an upstream riboswitch that allows for transcription of *mCherry* under high c-di-GMP levels. With this plasmid, we quantified the percentage of the bacterial population that fluoresced red, indicative of an increase in c-di-GMP levels, at different time points. In this study, we found that the percentage of fluorescent cells on a solid surface closely resembles those in a liquid medium within a 24-hour period, suggesting that the levels of c-di-GMP remained similar in both conditions. Further work is needed to understand at which timepoint the percentage of the population exhibiting a difference in c-di-GMP dependent regulation would differ following movement to a surface.

Sources:

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