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Dissecting the regulation of the three tandem promoters of the *Escherichia coli* kps gene cluster at chromosomal level

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There are three tandem promoters (PR1-1, PR1-2 and PR1-3) within the PR1 regulatory region of *Escherichia coli* kps capsule gene cluster. In this study, we dissect out the regulation of the PR1 promoters in the chromosome and examine the effect of global regulators known to regulate transcription of the kps gene cluster such as IHF, HNS and slyA. Several constructs were made in UTI89 and UTIP1lacZ strains with mutations in the -10 of the selected promoter using gene doctoring followed by K1 phage sensitivity assay and FACS analysis in UTI89 strain and measuring the transcriptional response using chromosomal lacZ reporter fusion assay in UIP1lacZ strain. The mutation of PR1-2 UTIP1lacZ reduced β-galactosidase activity by 50% indicating that PR1-2 is a functional promoter. In contrast mutation of PR1-1 abolished transcription from the whole PR1 promoter indicating that both PR1-2 and PR1-3 are dependent on PR1-1. These data were mirrored by K1 capsule production as detected by sensitivity to K1-specific bacteriophage and FACS analysis. Mutation of PR1-2 reduced the capsule production by half whereas mutation of PR1-1 hindered capsule formation. Moreover, three different mutations in ihf, hns and slyA were made in those promoter constructs and showed a reduction in the β-galactosidase activity in PR1-2 mutant, where it has PR1-1 acing, indicating that these regulators may act in PR1-1 specifically. Overall, these results demonstrate the importance of PR1-1 promoter in the PR1 regulatory region and the complex interplay between multiple promoters. Going forward I intend to study the individual role of each promoter during infection of particular cell line.

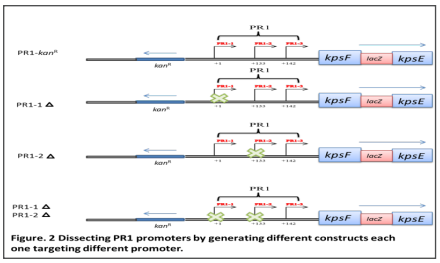

Figure. 2 Dissecting PR1 promoters by generating different constructs each one targeting different promoter.

Biography

Esraa Aldawood is pursuing PhD in Molecular Microbiology at the University of Manchester, Uk

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