Title:	To investigate the roll of a small RNA, GevB, in the regulation of D-galactonate metabolism in Escherichia coli
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Abstract:	Carbon source is one of the basic requirements for energy generation for all organisms, including bacteria. D-galactonate, an aldonic sugar acid, can be utilized as a carbon source by Escherichia coli, a common gram-negative bacterium. Classical mutagenesis and mapping studies performed in 1970's identified the genes involved in D-galactonate metabolism. The metabolism of D-galactonate is mediated by a pathway which comprises of a transporter and a set of metabolic enzymes, where the genes encoding the transporter and enzymes are organized in an operon known as dgo operon. In the dgo locus, sequence upstream of dgoR, the first gene of the operon, alone harbors the D-galactonate-inducible promoter that drives the expression of all the dgo genes. DgoR inhibits the expression of dgo operon by binding to two inverted repeats (IR) that overlap with the dgo promoter. D-galactonate binding induces a structural change in DgoR that derepresses the dgo promoter. Another study reported that dgo operon is also under positive regulation by cAMP-CRP. This suggests that D-galactonate metabolism is under complex regulation at a transcriptional level. In the current study, we have investigated post-transcriptional regulation on D-galactonate metabolism. A study carried out in 2019 described small RNA (sRNA) target prediction organizing tool (SPOT), which computationally predicts interactions between mRNAs and sRNAs. Interestingly, in the SPOT dataset, we came across few sRNAs which show interactions with dgo genes. GcvB, a sRNA that shows regulation on several carbon metabolic genes, was predicted to interact with dgoR and also with a few structural genes (dgoT and dgoK) involved in D-galactonate metabolism. The aim of this project was to investigate whether GcvB exerts any regulation during D-galactonate metabolism. For this, I made gcvB deletion strain to check whether absence of gcvB shows any growth phenotype in D-galactonate utilizing cells. Interestingly, we found that gcvB deletion strain grows faster as compared to WT BW25113 E. coli. Additionally, I cloned gcvB on a plasmid for its overexpression that can be used to assess whether this construct complements the growth phenotype in D-galactonate utilizing cells. Further, to investigate any transcriptional regulation of gcvB during D-galactonate metabolism, I prepared transcriptional reporter constructs of gcvB harboring its promoter fused with lacZ and Venus for β-galactosidase assay and fluorescence reporter assay, respectively.
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