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**Title:** Characterization of DNA Binding Site and Oligomerization Status of DgoR: A Negative Regulator of D-galactonate Metabolism in Escherichia coli

**Authors:** Sangwan, A. (/jspui/browse?type=author&value=Sangwan%2C+A.)

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**Abstract:** Escherichia coli, an enteric bacteria, is able to utilize certain sugar acids as carbon source for colonization of mammalian gut, one of its natural habitats. Dgalactonate, a hexonate sugar acid, is an intermediate of D-galactose metabolism of gut bacteria such as Stenotrophomonas maltophilia. Utilization of Dgalactonate as a carbon source by E. coli was studied in 1970's using classical mutagenesis and mapping studies where it was proposed that D-galactonate is metabolized via a pathway involving a putative transporter and three enzymes organised in a putative dgo operon. Further, the first gene of the operon, dgoR, was proposed to encode a negative regulator of dgo genes. Computational studies have classified DgoR into the GntR family of transcriptional regulators having a characteristic N-terminal DNA binding domain and a C-terminal ligand binding domain. Although DgoR has been suggested to be a repressor of dgo genes, detailed characterization of its repressor activity has not been performed. The present study aims to investigate the DNA binding property of DgoR. Previous experiments in our lab suggested that DgoR binds to the sequences upstream of the dgo operon. Also, bioinformatics analysis of dgo cis-element predicted three inverted repeats that could serve as binding sites for DgoR. In this work, mutagenesis of predicted DgoR binding sites followed by in vitro gel retardation assay and in vivo fluorescence reporter assay revealed the importance of two inverted repeats in binding of DgoR to the dgo cis-element. Oligomeric status of DgoR was confirmed by the dominant negative phenotype of DNA binding defective mutants of DgoR. Unpublished work from our lab reveals that D-galactonate is the ligand that binds to DgoR leading to de-repression of dgo genes. However, in depth quantitative study of this interaction has not been performed. For this purpose, the ligand binding domain of DgoR was purified.

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