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Title:	To understand the regulation of D-galactonate metabolism in Escherichia coli under stress conditions
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Abstract:	<p>Bacteria use different types of carbon sources, including sugar acids, the oxidized derivatives of sugars, for their growth and virulence. Sugar acids are widely found in nature, including in plant cell walls and animal tissues and are produced as metabolic intermediates by various microbes. Escherichia coli can use a variety of sugar acids, i.e., hexonates, hexuronates, hexuronides, and aldarates, as carbon and energy source. D-galactonate, a hexonate sugar acid, is metabolized by E. coli by the products encoded by the dgo operon. The dgo operon consists of four structural genes, dgoK, dgoA, dgoD and dgoT, which transport D-galactonate inside the cytoplasm and convert it into D-glyceraldehyde 3-phosphate and pyruvate, which enters central metabolism. The dgo operon is negatively regulated by DgoR, product of the first gene of the dgo operon. DgoR binds to the promoter region of the operon and represses its transcription in the absence of D-galactonate. Since dgo promoter has recognition sequence for σS, our lab explored the role of σS in D-galactonate metabolism. Unpublished work from the lab has shown that σS negatively regulates the dgo operon. Since the levels of σS increase under a variety of stress conditions, the aim of my project was to understand the regulation of D-galactonate metabolism under stress conditions. The regulation of the dgo operon was investigated under pH and temperature stress using a chromosomal transcriptional reporter where the fluorescent Venus was expressed from the dgo promoter. But unfortunately, the fluorescence values of the promoterless Venus reporter itself fluctuates in response to different stresses. I, therefore, made another reporter construct where the lacZ reporter was placed under the transcriptional control of dgo promoter. The conditional-replication, integration, and modular (CRIM) plasmid pAH125 was used to clone the cis-acting element of the dgo operon upstream of lacZ. It was then integrated into the chromosome of the E. coli strain (BW25113) to create the transcriptional reporter construct. In future experiments, we will use this transcriptional reporter construct to investigate the regulation of the dgo operon under different stress conditions using β-galactosidase activity assay.</p>
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