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Characterization of Enterotoxigenic Escherichia coli CS6 Promoter

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Abstract : CS6 is the prevalent CF in our region and deciphering its molecular regulators would play a pivotal role in reducing the burden of ETEC pathogenesis. In prokaryotes, most of the genes are under the control of one operon and the promoter present upstream of the gene regulates the transcription of that gene. Here the promoter of CS6 was characterized by computational method and further analyzed by β-galactosidase assay and sequencing. Promoter constructs and deletions were prepared as required to analyze promoter activity. The effect of different additives on the CS6 promoter was analysed by the β-galactosidase assay. Bioinformatics analysis done by Softberry/BPROM predicted fur, lrp, and crp boxes, -10 and -35 region upstream of the CS6 gene. The promoter construction in no promoter plasmid pTL61T showed that region -573 to +1 is actually the promoter region as predicted. Sequential deletion of the region upstream of CS6 revealed that promoter activity remains the same when -573bp to -350bp is deleted. But after the deletion of the upstream region -350 bp to -255bp, promoter expression decreases drastically to 26%. Further deletion also decreases promoter activity up to a little range. So the region -355bp to -255bp holds the promoter sequence for the CS6 gene. Additives like iron, NaCl, etc., modulate promoter activity in a dose-dependent manner. From the promoter analysis, it can be said that the minimum region lies between -254 and +1. Important region(s) lies between -350 bp to -255 bp upstream in the promoter, which might have important elements needed to control CS6 gene expression.

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