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MUDCHAREE JULOTOK : CHARACTERIZATION OF *E.COLI*-LIKE PROMOTER OF *SYNECHOCOCCUS* PCC7942 IN CYANOBACTERIA AND *E.COLI*. THESIS ADVISORS : WIPA CHUNGJATUPORNCHAI, Ph.D., SAKOL PANYIM, Ph.D. 119 P. ISBN 974-661-999-3

Cyanobacteria have been used as the alternative hosts to express heterologous genes. However, the level of heterologous gene expression in cyanobacteria is very low when compared with that in *E.coli*. A possibility to improve the gene expression is to use an endogenous strong promoter, but the knowledge of cyanobacterial promoters is still limited.

In order to find out what is the promoter sequence of cyanobacteria, *Synechococcus* PCC7942 was used as a model system in this study. Plasmid pKG-D21 harbouring a *Synechococcus* chromosomal DNA fragment, designated D21, located upstream of the promoterless β-glucuronidase (*GUS*) gene, was characterized. Using GUS as a reporter, the 148-bp D21 fragment was able to function as promoter in both *E.coli* and *Synechococcus*. The optimal GUS activities of *E.coli* and *Synechococcus* harbouring pKG-D21 plasmid were 33.00 and 48.20 nmole MU/min/mg protein, respectively.

To locate the inferred promoter sequences in the D21 fragment, transcription start sites were determined. Transcription start sites of D21-GUS transcripts in *E.coli* were identified using primer extension method. The results revealed that there were two major transcription start sites at G and T which were located at nucleotide 70 and 71 of the D21 fragment, respectively. The inferred –35 and –10 regions were TTAATG and TAAGCT, respectively. The promoter sequence of D21 in *E.coli* was conformed to the *E.coli* σ^{70} promoter consensus. In *Synechococcus*, the transcription start sites were determined using 5'RACE-PCR method. The major and minor transcription start sites were at G and A which were located at nucleotide 139 and 133 of the D21 fragment, respectively. The inferred –10 region was TACCAA which was conformed to that of *E.coli* σ^{70} promoter consensus. However, no –35 region was detected. The results indicated that the promoter sequences of D21 fragment in *E.coli* and *Synechococcus* were different.

No significant similarity to nucleotide sequence of D21 was detected in the database and thus the D21 promoter probably belongs to an unknown gene.