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SIRIRAT FA-AROONSAWAT : NUCLEOTIDE SEQUENCE AND  
TRANSCRIPTION INITIATION ANALYSIS OF NON-*E.COLI*-LIKE PROMOTER  
OF *SYNECHOCOCCUS* PCC7942. THESIS ADVISORS: WIPA  
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Cyanobacteria have been used as hosts for expression of heterologous genes. However, expression levels of heterologous genes in cyanobacteria are lower than those in *E.coli*. The low expression is probably due to the low activity of promoters that control the expression of the genes. The use of endogenous strong promoter might improve heterologous gene expression.

In this thesis, the cyanobacterium *Synechococcus* PCC7942 was used as a model system for studying cyanobacterial promoters. The previously constructed plasmid pKG-E14, which contained the 1.6 kb of E14 promoter-active fragment upstream of the promoterless  $\beta$ -glucuronidase (*GUS*) gene in pKG plasmid, was characterized. *GUS* activity of *Synechococcus* harbouring pKG-E14 was  $313.79 \pm 19.27$  nmole MU/min/mg protien. In order to locate the promoter region, deletion derivatives of the E14 fragment were constructed. The resulting plasmids pKG-E14D1, pKG-E14D2, pKG-E14D3 and pKG-E14D4 were evaluated by measuring the *GUS* activities. The results revealed that the *GUS* activity of *Synechococcus* harbouring pKG-E14D2 was as high as pKG-E14. The transcription initiation sites of E14-*GUS* and E14D2-*GUS* transcripts were determined using 5'RACE-PCR method. The inferred promoters upstream of the transcription initiation sites were identified. The inferred -10 and -35 promoter regions of E14-*GUS* major transcripts were TAgcAT and TcGATc, respectively. The inferred promoter -10 region of E14D2-*GUS* major transcripts was not detected whereas the -35 region was TcGAgc. The inferred -10 and -35 promoter regions of E14D2 minor transcripts were cAgAAg and gcGACc, respectively. The -10 and -35 regions of E14 and E14D2 promoters were conformed to low frequency nucleotides of *E.coli*  $\sigma^{70}$  promoter consensus. This results agreed well with the fact that promoter active fragment E14 and E14D2 could function in *Synechococcus* but not in *E.coli*. Deletion analysis indicated that distance enhancing element upstream of the inferred promoter of E14D2 might play an important role in *GUS* expression.

No significant similarity to nucleotide sequence of E14 was detected in the database and thus the E14 and E14D2 promoters probably belong to an unknown gene.