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SAOVAROS SVASTI : STUDIES ON EXPRESSION OF GIANT CATFISH (*PANGASIANODON GIGAS*) GROWTH HORMONE GENE IN *ESCHERICHIA COLI* AND CYANOBACTERIA. THESIS ADVISOR : SAKOL PANYIM, Ph.D. PRAPON WILAIRAT, Ph.D. AMARET BHUMIRATANA, Ph.D. WIPA CHUNGJATUPORNCHAI, Ph.D. 149 p. ISBN 974-661-095-3

Giant catfish (*Pangasianodon gigas*) is the biggest freshwater fish in the world. It has been shown that administration of recombinant growth hormone can stimulate growth of various fishes. A supplement of gcGH hormone in feed may benefit fish cultures. In order to obtain expression of giant catfish growth hormone (gcGH) in cyanobacteria *Synechococcus* sp. PCC7942, the giant catfish growth hormone gene (*gcGH*) expression shuttle vector was constructed in *Escherichia coli*. The *gcGH* gene was put under the control of the lambda phage P_R promoter. However, the resulting recombinant *E. coli* produced comparatively low amount of the gcGH protein. The mRNA secondary structure analysis predicts that there are two hairpin-loops: one at the Shine-Dalgarno (SD) sequence region with ΔG = -7.9 kcal/mol, the other at the initiation codon region with ΔG = -4.2 kcal/mol. Several site specific mutations were introduced into stem-loops at the Shine-Dalgarno (SD) sequence region and the initiation codon region to disrupt the secondary structures. Completely destabilizing the two stem-loops hence creating a new stem-loop increased the *gcGH* expression one hundred and five fold (pGHSDST) and increased the *gcGH* steady-state mRNA eleven-fold (pUC303-GHSDST).

For *gcGH* expression in cyanobacterium *Synechococcus* sp. PCC7942, the gene was put under control of the λP_R promoter, its own promoter E8 and its 154 bp $3'$ deletion fragment. The gcGH protein expression could not be detected by Coomassie-blue staining and Western blot analysis, even though both hairpin-loop structures were disrupted (pUC303-GHSDST). However the *gcGH* gene could be transcribed in all *Synechococcus* sp. PCC7942 transformants as *gcGH* mRNA could be detected by Northern blot and slot blot analysis. The *gcGH* steady-state mRNA in *Synechococcus* sp. PCC7942 harboring plasmid pE8-GH was about ten-fold lower than other transformants. The *gcGH* mRNA secondary structure at the SD sequence region of pE8-GH had structure energy of ΔG = -8.4 kcal/mol, which was higher than its derivative as well as that under control of λP_R promoter. Stem-loop structure with ΔG = -8.4 kcal/mol may be the threshold for the significant translation initiation in the cyanobacterium. For better understanding of gene expression in cyanobacteria, the effect of mRNA secondary structure on translation initiation should be further studied.