Characterization of HpdR – a 3-hydroxipropionic acid responsive transcription activator protein in Pseudomonas denitrificans

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LysR-type protein is common class of transcriptional regulatory proteins in prokaryotes. It has been known to regulate gene of interest at the transcriptional level and auto-regulate their own synthesis [1]. In Pseudomonas denitrificans, HpdR is a putative LysR-type transcriptional regulator protein which regulates the expression of hpdH gene involving 3-hydroxypropionic acid (3-HP) degradation pathways under the control of 3-HP inducible promoter. The role of HpdR has been clarified via the HpdR deletion and complementation in minimal medium with or without 3-HP as an inducer. To elucidate the regulatory mechanism of HpdR on the transcription of hpdH, the intergenic region between hpdR and hpdH was analyzed via nucleotide sequence to find out the operator sites for protein binding [2]. It was shown to have two pseudo-palindromic sequences, each corresponding to putative operator site responsible for HpdR binding in the form of dimer. Recombinant strain E. coli BL21 (DE3) overexpressing HpdR was developed to purify HpdR with co-expression of pTf16 chaperone. The molecular weight of HpdR showed 34.4 kDa through the deduced amino acid sequence. The soluble expression of HpdR protein was verified by SDS-PAGE and Western blot analysis. To determine the conformation of HpdR protein as dimer form for binding to operator sites, Native PAGE is being carried out. Electrophoretic mobility shift assay (EMSA) is being performed to assess binding affinities between operator regions and HpdR protein in the presence and absence of 3-HP. The significant changes in the conformation of HpdR protein in crystallography and the binding affinities of HpdR with operator regions are being investigated.