EFFECT OF NTRC DNA-BINDING MUTATIONS ON NTR REGULATABLE PROMOTERS

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Klebsiella pneumoniae ntrC genes containing mutations corresponding to the DNA-binding motif of NtrC have been obtained by bisulphite mutagenesis and expressed from the lac promoter. The activity of the mutant proteins have been tested using lacZ transcriptional and translational fusions of several NtrC-regulated promoters. Results show that the effect of all mutations on promoters repressed by NtrC (ntrB, glnAp1) is quite severe, while some of the conservative point mutations retain activity when tested on activatable promoters (nifL, glnAp2). This suggests that tighter binding is required for repression than for activation. One of the NtrC mutants supresses the down phenotype exhibited by a double promoter mutation carrying two C→T substitutions in NtrC-binding sites located -148 and -169 bp upstream from the nifL start of transcription. This indicates that the helix-turn-helix motif is involved in recognition of upstream sequences (U.A.S.). The same NtrC mutant does not suppress the nifL mutations separately, suggesting a cooperative effect for the interaction with the two NtrC binding sites in which the promoter mutations are located.