GENETIC HOMOGENOTIZATION

*Azotobacter vinelandii* contains as many as 40 to 80 copies of chromosomes in vegetative cells. This could decrease the possibility of obtaining mutants as very long segregation periods would be required. *Nif* mutants and some auxotrophic mutants of *A. vinelandii* have been isolated successfully. *A. vinelandii* probably has a very efficient recombination system.

*recA* and *recF* genes from *A. vinelandii* were selected as candidates for participating in this recombination process also referred to as “homogenotization” earlier. They were tested for the same and did not appear to participate in homogenotization function.

Mutant populations of *A. vinelandii* were generated using pUC4-KAPA and transposon miniTn10 (pNK862). A construct pRS3 (*nifHDK* mutant) was used to study homogenotization in these mutant populations.

The screening of KAPA mutants did not yield any mutant defective in homogenotization function. However, screening of miniTn10 population did yield some mutants, which appeared to be defective in homogenotization function. These mutants grew well in presence as well absence of a nitrogen source and they were also not sensitive to ultra-violet light.

Attempts were made to clone the element(s) involved in homogenotization function (disrupted by miniTn10) from *A. vinelandii* RS1. This construct was subjected to sequencing. The nucleotide sequence did not give a conclusive result.
**NIF GENE REGULATION**

The intervening sequence between *nifLA* promoter and the NtrC binding site exhibits anomalous electrophoretic mobility, suggesting intrinsic sequence induced curvature in DNA (Nichkawde, 1996). Additional protein(s) could influence this bending and therefore, transcription from *nifLA* promoter. The *in vivo* experiments carried out earlier (Nichkawde, 1996) in *hns*, *hupA* and *hupB* background suggested the involvement of H-NS and HU proteins in the transcriptional regulation of the *nifLA* promoter of *Klebsiella pneumoniae*.

In order to assess the role of these two proteins in *nifLA* regulation, H-NS and HU like proteins were purified from *Klebsiella pneumoniae*. These proteins were found to be heat stable like *E.coli* H-NS and HU proteins and they also cross-reacted with antibodies developed against *E.coli* H-NS and HU proteins. H-NS like protein from *Klebsiella pneumoniae* was also shown to bind preferentially to curved DNA.

Footprinting studies showed that both these proteins bound to upstream sequence in a somewhat nonspecific manner but primarily around the A and T stretches present in the sequence.

Mutations in these A and T stretches also referred to as ‘bent’ stretches were found to reduce H-NS binding to the upstream region whereas HU binding was not affected by these mutations.
HU protein was shown to cause a change in bending pattern of the DNA fragment comprising *nifLA* upstream sequence. H-NS did not show any effect on the intrinsic bending.

From the above observations, we can conclude that the DNA binding activities purified from *Klebsiella pneumoniae* could in fact be H-NS and HU proteins. The footprinting studies showed that these two proteins bound preferentially around the AT rich region in the upstream sequence. H-NS appeared to bind to the ‘bent’ stretches in the upstream sequence and mutations in the ‘bent’ stretches affect H-NS binding. HU on the other hand, affected the extent and pattern of bending. Further, the two proteins may influence each other in binding to the upstream sequence and thereby affect *nifLA* transcription differentially.