FUNCTIONAL ORGANISATION OF THE nif GENES CLUSTER
OF KLEBSIELLA PNEUMONIAE.

Jean HOUMARD, Claudine ELMERICH, Lionel SIBOLD, Didier BOGUSZ, Daniel KAHN,
Nicole CHARPIN and Régis BIGAULT
Unité de Physiologie Cellulaire, Département de Biochimie et Génétique Microbiologique
Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex, France.

In order to study the functional organisation of the nif genes of K. pneumoniae, genetic and biochemical properties of both chromosomal and plasmid point mutants, prophage Mu insertion mutants and deletion mutants were examined.

Genetic analysis was performed with diploids by recombination or complementation using recA recipient strains. A fine structure map of the nif cluster was established.

Analysis of \(^{14}C\) pulse-labelled proteins synthesized by either Nif\(^+\) or Nif\(^-\) strains was achieved by comparison of two-dimensional gel autoradiograms. In vitro complementation tests for nitrogenase activity, using crude extracts of different mutants, were done either with purified nitrogenase components or by mixing crude extracts from different mutants. In addition, we looked for CRM with specific antisera obtained against Kp\(_1\) or Kp\(_2\). We thus were able: 1) to characterize some of the nif genes products, 2) to underline the negative pleiotropic effect of nif\(^+\) A mutations on the expression of the other nif genes, 3) to identify Nif\(^-\) cryptic mutants which exhibit nitrogenase activity \(\text{in vitro}\). Finally, both genetic and biochemical analysis of polar mutants lead to the conclusion that the 14 nif genes are organised into 8 independent transcription units.