P009 N-oxides sensing in *pseudomonas aeruginosa*: expression and characterization of dnr, a fnr-crp type transcriptional regulator

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Expression of denitrification genes is tightly controlled through a cascade of oxygen and N-oxides responsive regulators. The latter regulators belong to the FNR-CRP superfamily, but lack the cysteines coordinating the [4Fe-4S] centre. Among these *fnr* homologous, those from *Paracoccus denitrificans* (*nnr*) and *Pseudomonas* sp. (*dnr*) induce nitrite reductase (NIR) and nitric oxide reductase (NOR) genes in response to N-oxides suggesting a role as NO sensors *in vivo*.
We have cloned the *dnr* gene from *P. aeruginosa*, expressed it in *E. coli* and purified to the homogenity the DNR protein. The recombinant protein is produced in high yields (15 mg/lt), is soluble and stable as a dimer. DNR, compared to *E. coli* CRP, presents more accessible hydrophobic pockets as probed by ANS binding experiments ($K_{dDNR}=7\mu M$, $K_{dCRP}=600\mu M$).
The DNA binding activity of DNR under different oxygen tensions, and/or in the presence of NO donors and putative cofactors is being characterized *in vitro*. DNA-binding activity on the *nirS* promoter was obtained in air using a partially purified *P. aeruginosa* extract containing DNR. Current experiments involve crystallization of DNR to determine the 3-D structure and *in vivo* trans-activation of a *fnr*-like promoter, using a *lacZ* reporter system.