

P017 Roles of nitrate and nitrite reductases in *Campylobacter jejuni*
Marc S. Pittman and David J. Kelly
*Department of Molecular Biology and Biotechnology, University
of Sheffield, Firth Court, Western bank, Sheffield S10 2TN, UK.*

The important food-borne pathogen *Campylobacter jejuni* has the capacity for periplasmic nitrate and nitrite reduction, as it contains both a Nap-type nitrate reductase and a Nrf-type cytochrome c nitrite reductase. The *nap* operon of *C. jejuni* does not contain *napC*, encoding the tetrahaem cytochrome c which acts as an electron donor to the NapAB complex in many bacteria. Instead, the iron-sulphur proteins NapG and NapH probably assume this role. Nitrate or nitrite dependent growth in *C. jejuni* could only be demonstrated under oxygen-limited, but not strictly anaerobic conditions. Mutants were constructed in *napA* and *nrfA*. While the *napA* mutant was completely devoid of benzyl viologen-linked nitrate reductase activity, was unable to produce nitrite from nitrate and was unable to grow under oxygen-limited conditions on nitrate, the *nrfA* mutant still exhibited about 20% of the viologen linked nitrite reductase activity of the parent strain, suggesting an additional nitrite reducing system is present in *C. jejuni*. NO electrode studies with the *nrfA* mutant have been used to assess the contribution of NrfA to NO detoxification. The *C. jejuni* NrfA contains a unique ligation at haem 1, with a CXXCH motif instead of CXXCK. Although the functional consequences of the K/H substitution are unknown at present, it is possible it could have important implications for the catalytic activity of the protein. We have therefore expressed NrfA from *C. jejuni* in *E. coli* for purification and characterisation to determine the properties of this unique enzyme.