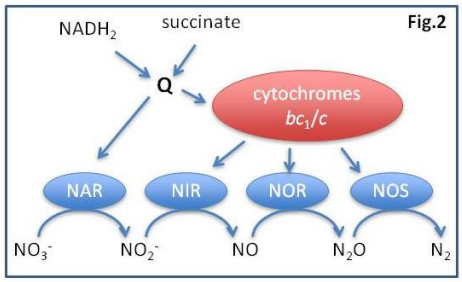
Project 1. Biochemistry - from genome to denitrification proteome.
Leader: Richardson (UEA)



Aim: To characterize functional enzymes (kinetics under physiological conditions) and transcriptome in model organisms providing basic information for modelling.

Background: *Paracoccus denitrificans (Pd)* is a type strain for denitrification research, a point of reference and a source of novel discoveries and hypotheses regarding fundamental aspects of the regulatory biology of denitrification. Recent examples are impaired expression of N_2O reductase by Cu-deficiency [1] and low pH [2].

Partner 2 is now uniquely placed to address the regulatory networks behind these discoveries after establishing functional genomics in *Pd* and routinely combining microbial physiology, biochemistry and transcriptomics. The functional genomics data will be used to inform mathematical modelling in P3. Studies will focus on *Pd* and another paradigm denitrifier, *Achromobacter xylosoxidans (Ax)*.

The key difference between the two species is that NIR (Fig 2) in *Pd* is a heme iron-dependent enzyme (NirS), whereas in *Ax* this step is catalysed by Cu nitrite reductase (NirK). Both organisms use copper containing NOS (also called N2OR) to reduce N_2O to N_2 and both groups of denitrifying bacteria (NirK and NirS-types) are widespread in the environment.
Methodology: Biochemistry and bioenergetics of denitrification at the cellular and sub-level of electron transfer networks. This project will have a central role in the training network, since techniques developed for enzymology and bioenergetics of *Pd* in continuous culture are transferable to other organisms.

Tasks and collaborations with other partners:

1. Systems biology analysis of *Pd* and *Ax* biochemistry, including evaluation of enzyme characteristics under different physiological conditions, refinement and enrichment of the phenotypic characteristics **(UMB).**
2. Contribution to modelling (iterative modelling and refined experiments) **(UMB, VUA, TUD)**.

Skills: ESR will learn continuous culture methodologies to study bioenergetics of denitrification, methods for quantification and qualitative analyses of functional enzymes, and interface microbial growth analyses with data collected from proteomics, transcriptomics and metabolomics analyses and mathematical models.

Individual research plan:

ESR2 Biochemistry - from genome to denitrification proteome

[1] Richardson D, Felgate H, Watmough N, Thomson A, Baggs E (2009) Mitigating release of the potent greenhouse gas N(2)O from the nitrogen cycle - could enzymic regulation hold the key? Trends Biotechnol. 2009, 27(7):388-97.

[2] Liu B, Mørkved P, Frostegard A, Bakken LR (2010) Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. *FEMS Microbiol Ecol* 72:407-417.