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Project: " Biochemistry - from genome to denitrification proteome"

This project will address the following questions:

- The mechanism of NosC and NosR in regulating expression of the $N_2O$ reductase NosZ. It is known that these proteins are transcriptionally regulated by environmental copper availability [Sullivan *et al.* (2013) *Proceedings of the National Academy of Sciences USA* 110: 19926-31] and play a role in activating 'maximal' expression of $N_2O$ reduction by NosZ. However, we do not know whether NosC or NosR proteins directly bind copper or how the signal is transduced from the cytoplasmic membrane to initiate transcription. Neither NosC nor NosR contain recognisable DNA-binding domains, so there are likely components of this regulatory cascade that are still unidentified. We will clone NosR and NosC and express them in different expression systems (either in *Escherichia coli* or in *Paracoccus denitrificans*), in order to study their biochemical characteristics. In addition, we will perform transcriptomic analysis of the previously generated *nosC* and *nosR* mutant strains to reveal regulatory targets and possible protein partners.

- How the copper required for $N_2O$ reductase activity is chaperoned around the cell and delivered to the NosZ holoenzyme. A previous transcriptomic analysis performed by Sullivan and co-workers revealed a gene cluster encoding the Pden_4445, 4444, and 4443 proteins that play a pivotal role in anaerobic Cu-metabolism. We have predicted that the proteins are involved in either insertion or maintenance of the Cu-centres of NosZ. We will study the properties and function of the putative Cu-binding proteins 4444 and 4443 and the role of the product of Pden_4445. He will express these proteins that have already been cloned into pLMB509 and, afterward, apply different chromatographic techniques to purifying them. In addition, to detect and characterize protein-protein interactions *in vivo,* a bacterial two-hybrid system will be produced.

- Identify the DNA-binding protein(s) that control gene expression according to cellular Cu concentrations. We will use the mobilizable multipurpose cloning vector pK18*mobsacB* to generate defined deletions in the chromosome of *P. denitrificans*. By using this technique we will knock-out the putative DNA binding proteins and characterise their denitrification phenotype in batch and continuous cultures.

- To verify if this regulatory mechanism hold for other denitrifying bacterial species. For example, *Achromobacter xylosoxidans* is another model denitrifying organism to be studied. In contrast to *P. denitrificans* NIR that contains a heme iron-dependent enzyme (NirS), the *A. xylosoxidans* NIR is a copper containing-nitrite reductase (NirK) system, which may compete with NosZ for Cu when intracellular levels are low.

- Using a transcriptomics approach, we will study which other environmental signals are integrated into regulation of *nosZ*.