ESR 1 (WP1) Pawel Lycus (pawel.lycus@nmbu.no), Norwegian University of Life Sciences, Norway

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Project: "Phenomics - from enzymes to cellular dynamics"

Pawel Lycus´ research will be part of Work Package 1, "Biochemistry", which comprises basic research on the biochemistry of selected microorganisms growing in pure cultures, addressing how the impact of environmental factors such as O_2, nitrate and nitrite levels, Cu availability and pH affect regulation of the different steps involved in denitrification. A large part of this research will be done using the well-studied model organisms *Paracoccus denitrification* and *Achromobacter xylosoxidans*, to provide parameters for a mathematical model that predicts the organisms' phenotypes under given conditions. In addition, phenotypic characterisations will be performed of a wider range of denitrifying and ammonia oxidizing organisms, obtained from existing culture collections as well as by our own isolation efforts.

Specific tasks:

- To perform an isolation program aiming at a collection of pure culture bacteria covering a wide taxonomic diversity, including full-fledged and partial denitrifiers, as well as those performing DNRA (dissimilatory nitrate reduction to ammonia) or only nitrate reduction. The soils used will be two "model soils" of contrasting pH, extensively studied by our research group.

- To analyse the gas kinetics and transcription patterns of a selected number of organisms from the collection, complemented with bacteria obtained from other collections. Responses to various environmental factors such as O_2, carbon substrate, nitrate/nitrite and pH levels will be investigated. Special emphasis will be on the comparison of NirK vs NirS carrying organisms, with respect to their control of NO and N_2O production (working hypothesis: NirS carrying organisms are typically NO-homeostatic at nM concentrations whereas NirK carrying organisms have less strict control of the NO concentrations).

- To investigate the fate of the denitrification proteome during oxic conditions: The denitrification proteome appears to be relatively persistent during oxic growth, and preliminary experiments have demonstrated gradually changing denitrification phenotypes through several generations of oxic growth due to dilution of the denitrification enzymes. Analyses of the denitrification kinetics (when exposing these cells to anoxia) suggested a polar localization of NIR and possibly other denitrification enzymes, leading to assymetric distribution of the denitrification reductasese. To verify this, a fusion of NirS with either FbFP or sfGFP in *P. denitrificans* will be constructed. Thus the fraction of NirS positive cells can be monitored through generations of oxic growth, and the localization of NirS can be determined by high resolution confocal fluorescence microscopy. If we achieve proof of concept, we will select a set of well-known model organisms and investigate whether asymmetrical distribution of N-oxide reductases is a general character. The work will be performed in collaboration with Partner 2 (UEA).

- In addition to the research outlined above, ESR1 will participate in the development of a top-down mathematical model of denitrification in *P. denitrificans*, and two other, denitrifiers that will be selected based on results from experiments described above. The mathematical modelling will be done in collaboration with, and during secondment to, Partner 3 (VU).