54. Responses of and interactions between nitrifying bacteria to environmental changes: a systems level approach

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Project Goals: The main focus of the project is to create predictive models of ammonia---oxidizing bacteria (AOB) and nitrite---oxidizing bacteria (NOB) that incorporate metabolism, the regulatory interactions that influence metabolism, and the signaling network for interaction with the environment. These models are derived from analysis of AOB and NOB cells grown in chemostats after the determination of their transcritptome, phsyiological responses, and changes in biochemistry. The models are providing tools and predictions for understanding the response of nitrifying organisms to changing environmental conditions, and likely contributions to climate change.

Nitrification is the microbially driven process that oxidizes ammoniacal---N (NH4+/NH3) into nitrate---N (NO3---) via the partially oxidized intermediate, nitrite (NO2). Phylogenetically specific types of beta---proteobacteria (AOB) and thaumarchaea (AOA) oxidize NH3 to NO2, and, sequentially several phylogenetically diverse bacterial genera oxidize NO2-to NO3 (NOB) (Fig. 1). Nitrifying microorganisms can also carry out denitrification under micro--- aerobic or anaerobic conditions producing NO and N2O, gases that can impact climate change. The project is characterizing the interactions between the two model nitrifying bacteria AOB Nitrosomonas europaea and the NOB Nitrobacter winogradskyi.

We have carried out transcriptome studies that showed ~30% of the genes of the AOB, N. europaea, are differentially expressed in steady-state chemostat co-culture with the NOB, N. winogradskyi, versus the transcriptome in single chemostat cultures under identical NH4+-limited conditions (1). The yield of AOB was higher in co-culture with the NOB, providing evidence that in NH4+-limited co-culture the AOB benefit more than did the NOB (1). In the case of N. winogradskyi, there was less evidence of transcriptome differences between co-culture and single culture (~11%). When N. winogradskyi was cultured singly however, in the presence of NO2 and excess

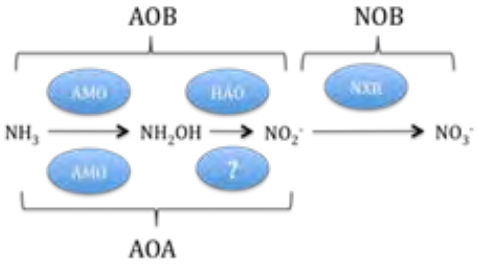

Fig. 1. The biological process of nitrification. Ammonia-oxidizing bacteria (AOB) Oxidize ammonia (NH_3) to nitrite (O_2) using ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Nitrite oxidizing Bacteria (NOB) Oxidize NO_2 To nitrate (NO_3) using nitrite oxidoreductase (NXR). Ammonia-oxidizing Archaea Oxidize NH_3 To NO_2-Using AMO by A mechanism that is not as yet established.

We have made progress in detecting evidence of bacterial cell---cell signaling or quorum sensing (QS) in the culture media of nitrifying bacteria, an understudied area. N. europaea and N. winogradskyi might interact via acyl-homoserine lactone (acyl-HSL) dependent QS. N. europaea showed a cell density-dependent recovery from starvation likely through acyl HSL auto inducer signals (3, 4). Using a broad-range acyl-HSL bioassay we detected evidence of acyl-HSL in N.europaea and N. winogradskyi grown singly and in co-culture in different amounts depending on the cell density of the cultures (Mellbye, manuscript in preparation).

We have established a stoichiometric model for energy metabolism in N. europaea and N. winogradskyi singly and in co-culture based upon the annotated N. europaea genome, and model building through the SEED. The results are in close agreement with our experimental data and with the literature. For example the N. europaea model predicted that growth under hypoxic conditions could be supported by the addition of pyruvate and nitrite, and that parameters that impact NOx production, include the ratio of CO2/HCO3- uptake rates, the O2 uptake rate, the proton (H+)-secretion rate, the NO2 secretion rate, and availability of potential exogenous electron donors for NO2 reduction (Chaplen, manuscript in preparation). We are attempting to refine the constraints models to better understand the factors impacting reductant allocation sinks (including NOx gases) in single and co-cultures.

References:

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