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

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Project Goals: In nitrification, ammonia oxidation and nitrite oxidation are usually coupled; however it is not known to what extent ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) interact with each other. Two model organisms, *Nitrosomonas europaea* (AOB) and *Nitrobacter winogradskyi* (NOB) are being used to test the hypothesis that AOB and NOB interact with each other, affecting their growth rate, cell yield, and protein and gene expression, and that these effects are distinct from the individually cultured bacteria. Their genome inventories and physiological and gene expression responses to environmental changes, singly and in co-cultures, will be used to construct genome-scale metabolic pathway models. We will create predictive models of AOB and NOB that incorporate metabolism, the regulatory interactions that influence metabolism, and the signaling networks for interaction with the environment. The models will help predict how AOB and NOB will behave in response to changes in the environment.

This project started October 2011. During this time we established the protocols to culture *N. europaea* and *N. winograskyi*, singly and in coculture. The cells are being grown in chemostats and in pH controlled batch fermentors.

A viable chemostat with a coculture of *N. europaea* and *N. winograskyi* was obtained within a month and has been successfully maintained over four months under constant conditions. The hydraulic retention time of 8 days was established and cells were harvested for analysis, allowing for recovery between samplings to minimize variation. From these coculture samples total RNA was isolated and is being used to determine the whole-genome transcript levels. The batch cultures and cocultures were grown in similar medium and at a constant pH. The cells in the batch cultures were harvested at late logarithmic phase. Similarly, total RNA was isolated and is being used to determine the whole-genome gene expression levels.

We are using RNA-Seq to quantify gene expression levels, to detect all messages (including small RNAs) and to avoid cross-hybridization artifacts. An optimized protocol to discriminate the transcriptomes of *N. europaea* and *N. winogradskyi* cocultures is being applied. The computational and statistical methods allow discriminating of the gene expression levels of the two model organisms (Table 1).

Table 1: The large majority of RNA-Seq reads can be uniquely assigned in the genomes of *N. europaea* and *N. winogradskyi*.*

CDS	Possible 25mers	Unique to itself	% to itself	Unique to other	% to other
N. europaea	2,345,948	2,224,679	94.83%	2,224,640	94.83%
N. winogradskyi	2,785,084	2,638,289	94.73%	2,638,250	94.73%

*Unique 25mer sequences from either the concatenated coding sequences (CDSs) per genome were identified. Unique is defined as a sequence found only once (unambiguous). We attempted to align the unique 25mers against the concatenated coding sequences (CDSs) or genome of the other species with no allowance for mismatches. We selected a 25mer for the comparison since most errors in the ~36-bases reads of RNA-Seq are at either end. The reads are trimmed prior to the alignments.

We designed a method to monitor cell viability of *N. europaea* and *N. winogradskyi* while in coculture using allylthiourea (ATU). ATU is a selective inhibitor of ammonia oxidation but not of nitrite oxidation. In this method the ammonia- and nitrite-dependent oxygen uptake are monitored to assess the overall cell health and abundance of each population. The method also allows the monitoring of AOB and NOB activities in coculture through growth, presenting an advantage over monitoring nitrite or nitrate accumulations solely. With this method the relative cell abundances of *N. europaea* and *N. winogradskyi* during coculture growth can be determined within 10 % accuracy.

We anticipate that analysis of transcriptomes, growth rates, and metabolites will provide insights to the responses of cells when in co-cultures. We anticipate that changes in gene expression will reflect how cells are sensing and responding to the environmental changes we impose on the co-cultures. The metabolic models will provide a basis to predict and analyze responses of the cell types in co-culture.

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