57. Transcriptome profiling of *Nitrosomonas europaea* grown singly and in co-culture with *Nitrobacter winogradskyi*

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Project Goals: 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 will provide powerful tools for understanding responses of nitrifying organisms to different environmental conditions, and predicting how they will behave in response to changes in the environment.

Nitrification is the aerobic microbial process in which ammonia (NH_3) is oxidized to nitrate (NO_3^-). Nitrification can be carried out sequentially in two steps by the action of two groups of mostly chemolithoautotrophic bacteria. In the first step the AOB extract energy for growth from the oxidation of NH_3 to nitrite (NO_2^-). In the second step the NOB oxidize the NO_2^- produced by the AOB to NO_3^- to extract energy for growth. In aerobic ecosystems, these two groups of bacteria tend to work in concert and, in most situations NO_3^- accumulates, rather than NO_2^-.

In a first effort to establish whether there are interactions between AOB and NOB in the process of nitrification, *N. europaea* (AOB) and *N. winogradskyi* (NOB) were co---cultured with NH_4^+ as the sole growth substrate. The transcriptome profile of *N. europaea* grown singly and in co-culture with *N. winogradskyi* was examined and provided clues to the adaptations that might be contributing to differential growth of the co-culture compared to single culture.

Growth parameters and mRNA levels showed discernible differences between growing singly and in co-culture. Co-culture growth in a medium containing 60 mM NH_4^+ resulted in a cell density greater than that of the compounded single chemostat cultures when grown at equivalent 60 mM concentrations of NH_4^+ or NO_2^-, respectively. Oxygen substrate dependent consumption rates (NH_4^+ or NO_2^-) for each nitrifier allowed the estimation of the relative cell density contribution to the co-culture and suggested that the increase in cell density was due mainly to *N. europaea*.

The analysis of the transcriptome of *N. europaea* showed that the mRNA of 726 genes were at different levels between single culture and in co-culture. Compared to single culture, *N. europaea* in co-culture had 279 genes (38%) with higher mRNA levels and 447 genes (62%) at lower mRNA levels.

Examples of genes at lower mRNA levels (Table 1) included genes encoding for biosynthetic functions (flagella synthesis, amino acid synthesis, iron dependent metabolism, carbon fixation related), and genes involved in stress responses (nitrite reduction, glutathione synthesis, DNA repair DNA and oxidative stress). *N. europaea* apparently benefits from the interaction while in co-culture with *N. winogradskyi* compared to when grown singly. Transcriptome and physiological analyses of co-cultures are revealing interactions that go beyond nitrite acting as the growth substrate for NOB. The data is being used to construct predicting models.

Table 1: *N. europaea* mRNA fold changes of selected genes in co-culture.

Locus tag	Function	Gene name	Description	Fold: RNAseq
NE0202	Energy generation related	---	F0F1 ATP synthase	0.84
NE0207				1.28
NE1764	Electron transport chain	*nuo*	NADH dehydrogenase	1.64
NE1767				1.72
NE0102	Electron transport chain	*cytC552*	cytochrome 552	0.82
NE2377	Fe---S cluster	*bolA*	2Fe2S homeostasis	0.70
NE0582	Sulfur uptake	*sbp1*	sulfate/thiosulfate binding protein	0.78
NE0852	Sulfur reduction	*yvgQ*	sulfite reductase subunit beta	1.20
NE0448	NH_3 uptake	*amtB*	ammonia transporter	1.81
NE1919		*cbbQ*		-2.59
NE1920	Carbon metabolism	*cbbS*	RuBisCo	-1.69
NE1926	Carbon uptake	*cynT*	carbonic anhydrase	0.70
NE2149	Glycolate pathway	*cbbZ*	phosphoglycolate phosphatase	-0.40
NE0675	Glycolate pathway	*glcD*	glycolate oxidase subunit	-1.20
NE0589	Carbon fixation	*ppc*	phosphoenolpyruvate carboxylase	-1.20
NE1649	Fatty acid synthesis	*acpP*	acyl carrier protein	1.16
NE0925	Stress: N_2 inhibition	*nirK*	Nitrite reductase: cytochrome C	-9.93
NE1736	NO2--- pump	*nitT/tauT*	nitrate/taurine transport	-1.05
NE0308	NO2--- avoidance	*flgH*	flagellar basal body protein	-1.26
NE0870	Oxidative stress	*sodB*	superoxide dismutase	-0.80
NE0104	Glutathione biosynthesis	---	dihydroxy-aciddehydratase	-1.42
NE1669	Antioxidant	*coq7*	ubiquinone biosynthesis monooxygenase	-1.11
NE1638	Heavy metal pump	*czcA*	cobalt-zinc-cadmium efflux pump	-1.08
NE1721	Iron uptake	---	iron complex receptor	-1.24