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Principal Investigator: Daniel James Arp 541-737-1294
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Institution: Oregon State University
Title: Metabolomic Functional Analysis of Bacterial Genomes
SC Division: SC-23.2
Program Manager: Daniel W. Drell 301-903-4742
Research Areas:

Recent results to date

I. Endogenous glycogen and sucrose in the metabolism and survival of *Nitrosomonas europaea*

Nitrosomonas europaea is an obligate chemolithotroph that grows using NH₃ and CO₂, but has genes for glycogen and sucrose metabolism. We tested whether these genes have a role in its carbon metabolism and in adaptation to starvation and osmotic stress. Glycogen was detected in *N. europaea* by EM and biochemical methods. Equimolar amounts of glucose and fructose were produced from sucrose in *N. europaea* lysates treated with invertase. *N. europaea* grown in medium supplemented with higher levels of C (carbonate) and NH₃ (energy) accumulated more glycogen. Glycogen was at lower levels in starved cells than in non-starved cells. *N. europaea* is capable to produce glycogen, likely for energy or carbon storage. Sucrose content was higher in cells grown in medium with added NaCl and KCl, likely for protection during osmotic stress.

Null mutants in the genes for sucrose-P synthase/synthase (NE1213/NE1214) and for glycogen synthase (NE2264) were produced. The NE1213/NE1214 mutant (defective in sucrose synthesis/breakdown) grew poorly and clumped extensively in NaCl-containing medium, suggesting a role for NE1213/NE1214 in osmotic protection. Stationary-phase inoculant from the NE2264 mutant (defective in glycogen synthesis) recovered slightly slower and attained lower final cell densities than wild type inoculant.

II. Fur in *Nitrosomonas europaea*

We also studied iron homeostasis in *N. europaea*. The gene for the ferric uptake regulator (*fur*) of *N. europaea* was inactivated. Compared to the wild type, the *fur* mutant grew similarly, contained higher levels of intracellular iron but lower levels of heme C, and over expressed proteins possibly involved in iron homeostasis.

Papers and other products delivered:

Wei X, Sayavedra-Soto LA, Arp DJ. Characterization of the ferrioxamine uptake system of *Nitrosomonas europaea*. *Microbiology*. 2008 Jan;154(Pt 1):139-47.

New notes:

Neeraja Vajrala, Luis Sayavedra-Soto and Dan Arp. Fur in the metabolism of Fe in *Nitrosomonas europaea*. 2008. Manuscript in Preparation. Results of this work are part of a poster presented in the International Meeting in Boston ASM 2008.

Summary:

Bioinformatics suggests that *Nitrosomonas europaea* has ~90 genes dedicated to Fe acquisition.

A ferric uptake regulator (fur gene, NE0616), homologous to *E. coli* fur, was identified in the *N.europaea* genome. A consensus nucleotide sequence of the Fur binding motifs (Fur boxes) in *N.europaea* was deduced using HMMER package version 2.3.2.

The deduced consensus sequence for the Fur box sites in the genome of *N.europaea* was shown to be functional by the Fur Titration Assay (FURTA). Positive FURTA results were obtained in Fur boxes of 2 outer membrane receptors (Genes NE1205, NE1540) and of gene NE0616, showing that the Fur box nucleotide sequence consensus is valid.

We tested further functionality of the Fur box in gene NE0616, and concomitantly, whether gene NE0616 indeed regulates other Fe acquisition genes. We inserted a Kanamycin cassette in the Fur box upstream of gene NE0616 to produce the mutant strain *N. europaea furbox::kan*.

The mutant did not transcribe NE0616 gene and over-expressed proteins with molecular weights similar to Fe-siderophore outer membrane receptors. This negative regulation of expression of Fe acquisition systems by Fur in response to iron availability is similar to that documented for other species such as *E. coli* and *P. aeruginosa*.

Analysis of the cellular Fe contents by ICP-AES showed that *N. europaea furbox::Kan* mutant grown in Fe-replete (10 μ M) conditions had 1.5 fold more Fe than wild-type grown in similar conditions. Consistently *N. europaea furbox::Kan* mutant was more sensitive than the wild-type to increasing concentrations of Fe in the growth media. Growth was inhibited likely to Fe toxicity and consequent oxidative damage to the cells. Together these suggest that NE0616 plays a key role in regulation of iron-repressed *N. europaea* genes and likely is fur in *N. europaea*.

Manuscripts:

ER63781-1: Microbiology. 2007 Dec;153(Pt 12):3963-72.

Characterization of the ferrioxamine uptake system of *Nitrosomonas europaea*.

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The chemolithoautotroph *Nitrosomonas europaea* has two genes predicted to encode outer-membrane (OM) ferrioxamine transporters. Expression of the ferrioxamine uptake system required induction, as shown by the shorter lag phase in ferrioxamine-containing cultures when ferrioxamine-exposed cells were used as an inoculum. The two OM ferrioxamine siderophore transporters encoded by *foxA*(1) (NE1097) and *foxA*(2) (NE1088) were produced only in cells grown in Fe-limited ferrioxamine-containing medium. The inactivation of *foxA*(1), singly or in combination with *foxA*(2), prevented growth in Fe-limited medium containing excess desferrioxamine (DFX). The *foxA*(2)-disrupted single mutant grew poorly in the regular Fe-limited (0.2 microM) medium with 10 microM DFX, but grew well when the Fe level was raised to 1.0 microM with 10 microM DFX. For efficient acquisition of Fe-loaded ferrioxamine, *N. europaea* needs both ferrioxamine transporters *FoxA*(1) and *FoxA*(2). *FoxA*(1) probably regulates its own production, and it controls the production of *FoxA*(2) as well.

ER63781-2: Arch Microbiol. 2006 Aug;186(2):107-18. Epub 2006 Jun 21.

Iron nutrition and physiological responses to iron stress in *Nitrosomonas europaea*.

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Nitrosomonas europaea, as an ammonia-oxidizing bacterium, has a high Fe requirement and has 90 genes dedicated to Fe acquisition. Under Fe-limiting conditions (0.2 microM Fe), *N. europaea* was able to assimilate up to 70% of the available Fe in the medium even though it is unable to produce siderophores. Addition of exogenous siderophores to Fe-limited medium increased growth (final cell mass). Fe-limited cells had lower heme and cellular Fe contents, reduced membrane layers, and lower NH₃- and NH₂OH-dependent O₂ consumption activities than Fe-replete cells. Fe acquisition-related proteins, such as a number of

TonB-dependent Fe-siderophore receptors for ferrichrome and enterobactin and diffusion protein OmpC, were expressed to higher levels under Fe limitation, providing biochemical evidence for adaptation of *N. europaea* to Fe-limited conditions.