DOE-DE-SC0006654: From genomes to metabolomes: Understanding mechanisms of symbiosis and cell-cell signaling using the archaeal system *Ignicoccus-Nanoarchaeum,*

Final Report

The main objective of this project was to use symbiotic Nanoarchaeaota, a group of thermophilic Archaea that are obligate symbionts/parasites on other Archaea, to develop an integrated multi-omic approach to study inter-species interactions as well as to understand fundamental mechanism that enable such relationships. As part of this grant we have achieved a number of important milestone on both technical and scientific levels.

On the technical side, we developed immunofluorescence labeling and tracking methods to follow Nanoarchaeota in cultures and in environmental samples, we applied such methods in conjunction with flow cytometry to quantify and isolate uncultured representatives from the environment and characterized them by single cell genomics. On the proteomics side, we developed a more efficient and sensitive method to recover and semi-quantitatively measure membrane proteins, while achieving high total cellular proteome coverage (70-80% of the predicted proteome). Metabolomic analyses used complementary NMR and LC/GC mass spectrometry and led to the identification of novel lipids in these organisms as well as quantification of some of the major metabolites. Importantly, using several informatics approaches we were also able to integrate the transcriptomic, proteomic and metabolomic datasets, revealing aspects of the interspecies interaction that were not evident in the single omic analyses (manuscript in review).

On the science side we determined that N. equitans and I. hospitalis are metabolically coupled and that N. equitans is strictly dependent on its host both for metabolic precursors and energetic needs. The actual mechanism by which small molecules move across the cell membrane remains unknown. The Ignicoccus host responds to the metabolic and energetic burned by upregulating of key primary metabolism steps and ATP synthesis. The two species have co-evolved, aspect that we determined by comparative genomics with other species of Ignicoccus (manuscript in preparation) and by characterizing other similar Nanoarchaeota systems. Using a single cell genomics approach we characterized the first terrestrial geothermal Nanoarchaeota system, from Yellowstone National Park. That nanoarchaeon uses a different host, a species of Sulfolobales, and comparative genomics with N. equitans-Ignicoccus allowed us to come up with an evolutionary model for the evolution of this group of organisms across marine and terrestrial ecosystems. Based on metabolic inferences we were also able to isolate in culture the first such terrestrial nanoarchaeal system, also from Yellowstone, which involves a species of Acidilobus. The novel nanoarchaeal system was characterized using proteomics and it helped us better understand the metabolic capabilities of these organisms as well as how co-evolution shapes the genomes of interacting species. It was also one of the very few cases in which prior genomic data was used to successfully design an approach to culture an organism, which remains the gold standard in microbiology research. As a better understanding of interspecies interaction requires multiple model systems, we have pursued identification and

genomic characterization or isolation of additional nanoarchaeal systems from geographically and geochemically distinct environments. Two additional nanoarchaeal systems are presently being characterized from hot springs in Yellowstone and Iceland and will be the subject to future publications.

Education

A. Graduate Students:

Michelle Tigges (Montana State University)
Timothy Hamerly (Montana State University)

B. Summer students and interns

Bernard Belisle (University of Tennessee)
Samantha VanCleave (University of Tennessee)
Carolyn Swift (University of Tennessee)
Marziah Hashimi (Montana State University)
Amanda Fuchs (Montana State University)
Rose Sullivan Springetti (Montana State University)
Ken Kwah (Montana State University)

C. Postdocs

Louie Wurch, PhD (University of Tennessee)
Brian Tripet, PhD (Montana State University)
Richard Giannone (Oak Ridge National Lab)

Publications

1. Podar M, Makarova KS, Graham DE, Wolf YI, Koonin EV, Reysenbach AL. (2013) Insights into archaeal evolution and symbiosis from the genomes of a nanoarchaeon and its inferred crenarchaeal host from Obsidian Pool, Yellowstone National Park. Biol Direct. 8:9. PMID: 23607440 PMCID: PMC3655853 DOI: 10.1186/1745-6150-8-9
2. Hamerly T, Tripet BP, Tigges M, Giannone RG, Wurch L, Hettich RL, Podar M, Copié V, Bothner B (2015) Untargeted metabolomics studies employing NMR and LC–MS reveal metabolic coupling between Nanoarcheum equitans and its archaeal host Ignicoccus hospitalis Metabolomics. 11(4):895-907. PMID: 26273237 PMCID: PMC4529127 DOI: 10.1007/s11306-014-0747-6.
3. Hamerly T, Tripet B, Wurch L, Hettich RL, Podar M, Bothner B and Copié V (2015). Characterization of Fatty Acids in Crenarchaeota by GC-MS and NMR. *Archaea.* PMID: 26880868 PMCID: PMC4736080 DOI:10.1155/2015/472726
4. Giannone RJ, Wurch LL, Podar M, Hettich RL. (2015) Rescuing Those Left Behind: Recovering and Characterizing Underdigested Membrane and Hydrophobic Proteins To Enhance Proteome Measurement Depth. *Anal Chem*. 87:7720-8. PMID: 26110888 DOI: 10.1021/acs.analchem.5b01187

5. Giannone RJ, Wurch LL, Heimerl T, Martin S, Yang Z, Huber H, Rachel R, Hettich RL, Podar M. (2015) Life on the edge: functional genomic response of Ignicoccus hospitalis to the presence of Nanoarchaeum equitans. *ISME J.* 9:101-114. PMID: 25012904 PMCID: PMC4274422 DOI: 10.1038/ismej.2014.112
6. Wurch L, Giannone RJ, Belisle BS, Swift C, Utturkar S, Hettich RL, Reysenbach AL, Podar M. (2016). Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment. *Nature Communications*. 7:12115. PMID: 27378076 PMCID: PMC4935971 DOI: 10.1038/ncomms12115
7. Rawle R, Hamerly T, Tripet B, Giannone R, Wurch L, Hettich, R, Podar M, Copié V; Bothner B (2016) Integrated Omics Analysis Provides Insight to the Ignicoccus hospitalis—Nanoarchaeum equitans Association. (Submitted, In review)