

Investigation of Diversity of Microbial Metal Transporters in Methane Seep Ecosystems using Metagenomics

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Motivation: methane, microbes and metals

-Methane seep ecosystems are significant sources of methane, a greenhouse gas

-Metagenomic sequencing provides insight into genes that are important in the consumption of methane in methane seep ecosystems

-The symbiotic relationship between sulfate reducing bacteria (SRB) and anaerobic methanotrophic archaea (ANME) mediates methane consumption

-The anaerobic oxidation of methane (AOM) is a metal-expensive process, but little is known about the uptake of metals in methane seep ecosystems

Metagenomics

Metagenome Sequencing (Previous Work)

- 1) Fixed sediment sample collected from Eel River Basin on cruise AT-15-11
- 2) Magnetic Bead Capture of ANME-2c aggregates (Perthaler et al 2008)
- 3) DNA Extraction & Sequencing
 - a. BC2- GS-20
 - b. BC3- GS-FLX
 - c. BC4- Titanium

Metagenome Analysis (This Study)

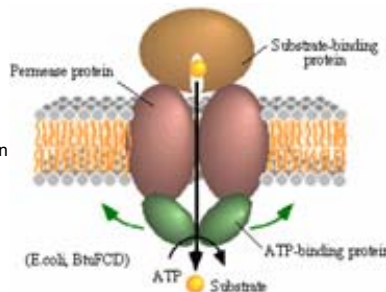
- 1) Imported sequences into MEGAN (MEtaGenome Analyzer)
- 2) Organized genes by pathway with KEGG analyzer
- 3) Catalogued top BLAST hit for each read for each ABC transporter gene
- 4) Catalogued hits by taxonomic order

ABC Transporters

-Transmembrane proteins that use ATP to transport substrates (including essential nutrients) into the cell

-One of the largest of all protein families (Klein & Lewinson 2011)

-Our study focuses on the diversity of microbial transition metal ABC transporters in the BC2, BC3 and BC4 Eel River metagenomes



Example of a bacterial ABC transporter, BtuFCD

Conclusions

-The majority of the BLAST hits for cobalt/nickel, molybdenum, and zinc ABC transporters were *Methano*- orders (likely ANME), although sulfate reducers were also found

-The entirety of the BLAST hits for tungstate ABC transporters were sulfate reducers

-The majority of the BLAST hits for iron complex ABC transporters were sulfate reducers

-The majority of the BLAST hits for heme and peptide/nickel ABC transporters were neither sulfate-reducers nor *Methano*- orders, although sulfate-reducers and *Methano*- order archaea were found

-The majority of the BLAST hits for iron (III) transporters were microbes that were neither sulfate reducers nor *Methano*- orders, although sulfate reducers were found

Overall, these results suggest that all metals examined (cobalt, molybdenum, zinc, tungsten, iron, nickel) can be transported by sulfate-reducers in Eel River Basin methane seep ecosystems

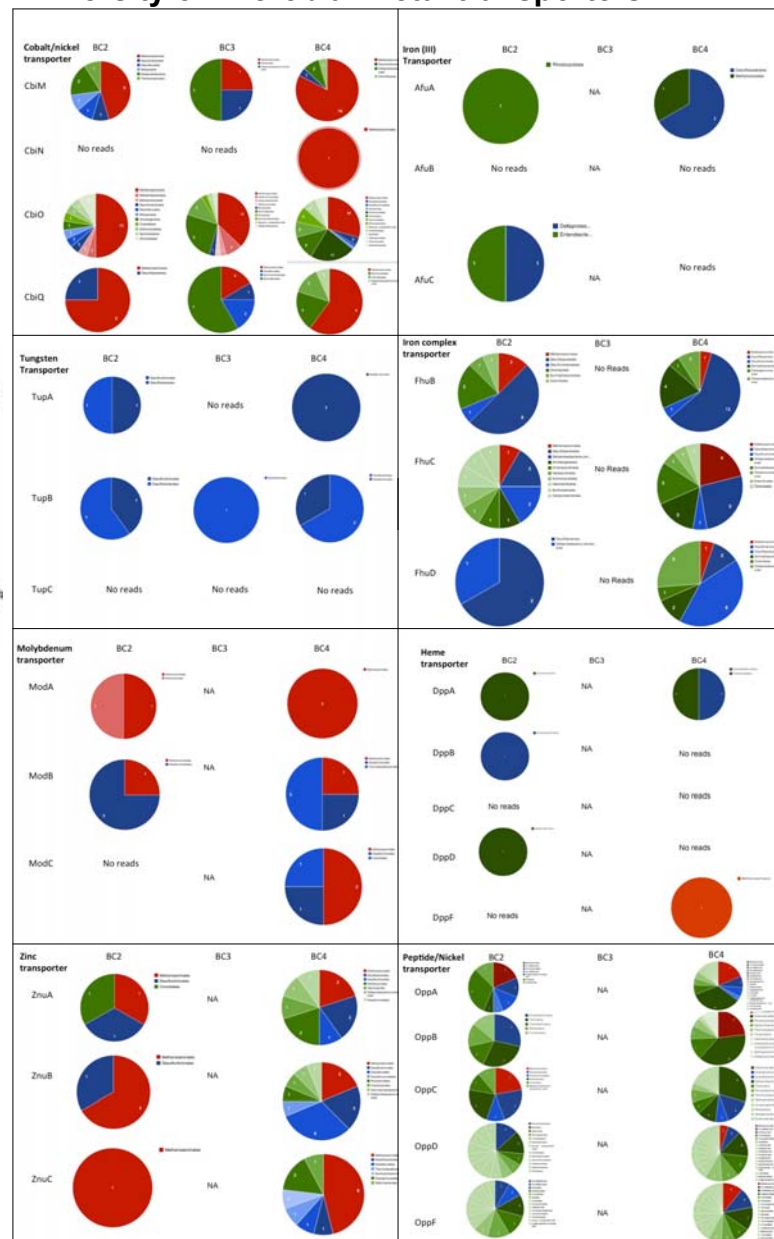
Furthermore, the results suggest that all metals examined except tungsten and iron(III) can be transported by ANME in Eel River Basin methane seep ecosystems

References

-Perthaler, A., Dekas, A. E., Brown, C. T., Goffredi, S. K., Embaye, T., Orphan, V. J. 2008. Diverse syntrophic partnerships from deep-sea methane vents revealed by direct capture and metagenomics. *PNAS*, 105, 7052-7057

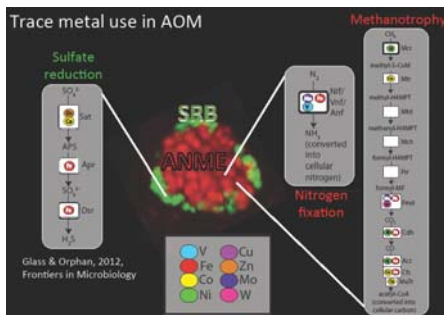
-Klein, J. S., Lewinson, O. 2011. Bacterial ATP-driven transporters of transition metals: physiological roles, mechanisms of action, and roles in bacterial virulence. *Metallomics*, 3, 1098-1108

Diversity of microbial metal transporters



Color Legend

Methanogens/ANME **Sulfate Reducers** **Others**



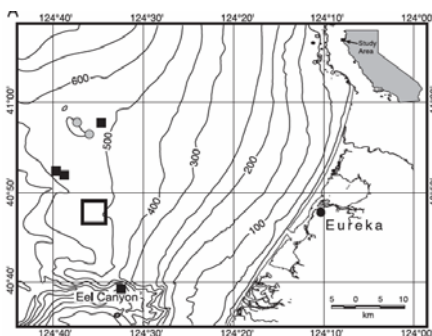
Methane Seep Ecosystems

-Massive deposits of methane hydrate on and beneath the sea floor

-Dominant microbes are consortia of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria

-Samples used in this study originate from sediment collected on a scientific cruise to the methane seeps of the Eel River Basin, offshore Northern California

Eel River Basin



(Orphan et al 2004)