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Systems level insights into alternate methane cycling modes in a freshwater lake via community transcriptomics, metabolomics and nanoSIMS analysis

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Executive Summary

The research conducted as part of this project contributes significantly to the understanding of the microbes and their activities involved in methane metabolism in freshwater lake sediments and in the environment in a more global sense. Significant new insights have been gained into the identity of the species that are most active in methane oxidation. New concepts have been developed based on the new data on how these organisms metabolize methane, impacting not only environmental microbiology but also biotechnology, including biotechnology of next generation biofuels. Novel approaches have been developed for studying functional microbial communities, via holistic approaches, such as metagenomics, metatrancriptomics and metabolite analysis. As a result, a novel outlook has been obtained at how such communities operate in nature. Understanding methane-oxidizing communities in lakes and other environments is of significant benefit to the public, in terms of methane emission mitigation and in terms of potential biotechnological applications.

Project Results

The actual accomplishments of this project are in line with the original goals and objectives, as proposed. One additional outcome is a novel concept of methane oxidation as a community function. This concept suggested some preliminary investigations that laid the groundwork for a new project now funded by the DOE.

Identification of actively transcribed pathways

In collaboration with the JGI, as part of the SCP program, we generated metagenomic sequence datasets representing communities responding to methane stimuli in a variety of conditions, as follows. Four were labeled with methane in oxic and microoxic conditions and in the absence or in the presence of nitrate. Four additional samples were obtained by sorting specifically fluorescently labeled cells, after methane stimulation. Two of the samples represented unamended native sediment communities. All the datasets were analyzed for the signatures of the active methane-utilizing species, identifying *Methylobacter* as the major active species, contrary to prior understanding. In addition to *Methylobacter*, prominent signatures were found for species belonging to *Methylophilaceae*, implicating them in methane metabolism, through cooperative behavior/crossfeeding. A strong signature was also detected for nitrate metabolism genes, corroborating a positive effect of nitrate on the methane oxidizing communities, thus connecting the methane cycle to the nitrogen cycle. Surprisingly, *Methylobacter* signatures were also strong in microoxic conditions, pinpointing their role in microoxic niches. This property, along with the fate of the carbon from labeled methane that we identified in a variety of non-methanotroph species, suggested a novel mode of metabolism involving community function. Indeed, experiments with model organisms provided further evidence for novel metabolism, obtaining, for the first time, experimental evidence of fermentation of methane. Transcriptomics and metatranscriptomics experiments provided validation for metabolic reconstruction of methane metabolism as carried out by bacterial consortia. Isolation of the major players in pure cultures and comparative genomic analysis validated the conclusions from the genomes. These new isolates will serve as model organisms for future research.

Identification of physiologically active pathways

We developed and implemented methods for detection and quantification of organic and amino acids, sugars and sugar phosphates that are signatures of alternative metabolic pathways participating in assimilation of carbon from methane, for both pure cultures of methanotrophs and for mixed communities (microcosms). We demonstrated that the ribulose monophosphate pathway was prevalent in the communities, supporting data from metagenomics and metatranscriptomics. Moreover, label-based tracing of specific intermediates in this cycle demonstrated that key methanotrophs such as *Methylobacter* predominantly utilize the most energy-efficient variant of assimilatory metabolism, involving reactions of glycolysis, which in turn allows for fermentative mode of methane metabolism. This is one of the key discoveries of this project.

Identification of activities of individual cells

NanoSIMS was employed to follow the fate of methane and CO2, to follow the fate of carbon in select methylotrophs and in communities stimulated by labeled methane, in order to refine the metabolic schemes employed by key methane oxidizers. NanoSIMS-based analyses were also utilized to evaluate cell-to-cell variation in carbon flux.

Additional experiments

We carried out additional experiments in order to test a hypothesis of methane metabolism in lake sediment as a community function. We followed dynamics of microcosms stimulated with methane and uncovered stable assemblages of methanotrophs with non-methanotrophs. We also observed that species selection in such microcosms is determined by environmental parameters such as oxygen tension and temperature. These experiments provided the necessary preliminary data for the follow up research, currently funded by the DOE. These data also validated our conclusions from the analysis of the metagenomes and metatranscriptomes.

Publications

1. Chistoserdova L (2014) Functional metagenomics of the nitrogen cycle in freshwater lakes with focus on methylotrophic bacteria. In: D.E. Marco, Ed. Metagenomics of the microbial nitrogen cycle: theory, methods and applications. Caister Academic Press, Norwich, UK, pp. 195-208.

2. Kalyuzhnaya MG, Yang S, Rozova ON, Smalley NE, Clubb J, Lamb A Gowda GA, Raftery D, Fu Y, Bringel F, Vuilleumier S, Beck DA, Trotsenko YA, Khmelenina VN, Lidstrom ME (2013) Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. Nat Commun 4: 2785.

3. Chistoserdova L, Kalyuzhnaya MG, Lidstrom ME (2013) Cycling single-carbon compounds: from omics to novel concepts. Microbe 8:395-400.

4. Beck DA, Kalyuzhnaya MG, Malfatti S, Tringe SG, Glavina del Rio T, Ivanova N, Lidstrom ME, Chistoserdova L (2013) A metagenomic insight into freshwater

methane-utilizing communities and evidence for cooperation between the *Methylococcaceae* and the *Methylophilaceae.* PeerJ 1:e23.

5. Chistoserdova L, Lidstrom ME (2013) Aerobic methylotrophic prokaryotes. The Prokaryotes, Fourth edition. Rosenberg E, DeLong EF. Thompson F, Lory S, Stackebrandt E, Eds, Springer, pp. 267-285.

6. Matsen JB, Yang S, Stein L, Beck DA, Kalyuzhnaya MG (2013) Global molecular analyses of methane metabolism in methanotrophic Alphaproteobacterium, *Methylosinus trichosporium* OB3b. Part I. Trasnscriptomic study. Frontiers Chem Microbiol 4:40.

7. Yang S, Matsen JB, Konopka M, Green-Saxena A, Clubb J, Sadilek M, Orphan VJ, Beck DA, Kalyuzhnaya MG (2013) Global molecular analyses of methane metabolism in methanotrophic Alphaproteobacterium, *Methylosinus trichosporium* OB3b. Part II. Metabolomics and ^{13}C-labeling study. Frontiers Chem Microbiol 4:70.

8. Yang S, Nadeau JS, Humston-Fulmer EM, Hoggard JC, Lidstrom ME, Synovec RE (2012) Gas chromatography-mass spectrometry with chemometric analysis for determining ^{12}C and ^{13}C labeled contributions in metabolomics and ^{13}C flux analysis. J Chromatogr A 1240:156-64.

9. Kalyuzhnaya MG, Beck DA, Chistosedova L (2011) Functional metagenomics of methylotrophs. Meth Enzymol 495: 81-98.

10. Yang S, Synovec RE, Kalyuzhnaya MG, Lidstrom ME (2011) Development of a comprehensive ion exchange solid phase extraction protocol coupled with liquid chromatography mass spectrometry to analyze central carbon metabolites in lake sediment microcosms. J Sep Sci 34: 3597-3605.

11. Svenning MM, Hestnes AG, Wartiainen I, Stein LY, Klotz MG, Kalyuzhnaya MG, Spang A, Bringel F, Vuilleumier S, Lajus A, Médigue C, Bruce DC, Cheng JF, Goodwin L, Ivanova N, Han J, Han CS, Hauser L, Held B, Land ML, Lapidus A, Lucas S, Nolan M, Pitluck S, Woyke T (2011) Genome sequence of the Arctic methanotroph *Methylobacter tundripaludum* SV96. J Bacteriol 193: 6418-6419.

12. Stein LY, Bringel F, Dispirito AA, Han S, Jetten MS, Kalyuzhnaya MG, Kits KD, Klotz MG, Op den Camp HJ, Semrau JD, Vuilleumier S, Bruce DC, Cheng JF, Davenport KW, Goodwin L, Han S, Hauser L, Lajus A, Land ML, Lapidus A, Lucas S, Médigue C, Pitluck S, Woyke T (2011) Genome sequence of the methanotrophic Alphaproteobacterium, *Methylocystis* sp. Rockwell (ATCC 49242). J Bacteriol 193: 2668-2669.

13. Chistoserdova L (2011) High-resolution metagenomics: assessing specific functional types in complex microbial communities. In: F.J. de Bruijn, Ed. Handbook of Molecular Microbial Ecology I: Metagenomics and complementary approaches. Wiley, pp. 225-233.

Oral Presentations

1. August 2014. Methane metabolism in lake sediments: a community perspective.

15th International Symposium on Microbial Ecology, Seoul, South Korea (Chistoserdova L)

2. May 2014. Methane Cycle: a fresh look at an old story. 114th ASM General Meeting, Boston, MA (Kalyuzhnaya MG)
3. April 2014. Chasing sustainability: a return to methane biocatalysis. Scripps Institution of Oceanography, San Diego, CA (Kalyuzhnaya MG)
4. March 2014. Chasing Sustainability: a return to methane biocatalysis. San Diego State University, San Diego, CA (Kalyuzhnaya MG)
5. February 2014. Methane metabolism in lake sediments: a community perspective through microcosm manipulations. 2014 Genomic science contractor-grantee meeting XII. Arlington, VA (Chistoserdova L)
6. December 2013. Methanotrophy revisited: methane assimilation in Gammaproteobacteria. West Coast Bacterial Physiologists Meeting, Asilomar, CA (Kalyuzhnaya MG)
7. December 2013. Fermentation of methane: longstanding concepts, paradoxes and new lessons. University of Calgary, Calgary, Canada (Kalyuzhnaya MG)
8. September 2013. Methanotrophy revisited: from natural gas biocatalysis to greenhouse gas mitigation. University of Georgia, Athens, GA, (Lidstrom ME, Lars Lungdahl Distinguished Lecturer)
9. September 2013. Methanotrophy revisited: how bacteria grow on natural gas. Department of Microbiology, University of Washington, Seattle, WA (Lidstrom ME)
10. July 2013. Methane metabolism - from systems biology to biocatalysis Applied and Environmental Microbiology GRC (Kalyuzhnaya MG)
11. July 2013. Using metagenomics for understanding functionality of complex microbial communities. International Conference on High-throughput Sequencing in Metagenomics. Novosibirsk, Russia (Chistoserdova L)
12. July 2013. How to plan, execute and gain new knowledge from a metagenomic project: notes from analysis of past projects. International Workshop on High-throughput Sequencing in Metagenomics. Novosibirsk, Russia (Chistoserdova L)
13. June 2013. Fermentation of methane: new lessons from Nature. Joint BioEnergy Institute, Berkeley, CA (Kalyuzhnaya MG)
14. May 2013. Methane cycling by methanotrophs. ASM General Meeting, Denver, CO (Lidstrom ME)
15. April 2013. A fresh look at an old methane story. University of California San Diego, San Diego, CA (Kalyuzhnaya MG)

16. December 2012. What we know and what we do not know about methylotrophs sharing a lake sediment habitat. West Coast Microbial Physiology Meeting. Asilomar, CA (Chistoserdova L)

17. November 2012. Moving forward methane-based biocatalysis. NW-ASM, Seattle, WA (Kalyuzhnaya MG)

18. August 2012. Global molecular analysis of methane oxidation: from a single cell to an ecosystem. Molecular Basis of Microbial One-Carbon Metabolism GRC, Lewiston, ME (Kalyuzhnaya MG)

19. June 2012. Assessing the structure and function of lake sediment microbial communities using functional metagenomics. Gordon Research Conference Environmental Sciences: Water. Holderness, NH (Chistoserdova L)

20. June 2012. Who's cycling one-carbon compounds in the environment: from single cells to populations. ASM General Meeting, San Francisco, CA (Lidstrom ME)

21. May 2011. Global Molecular Analyses of Methane Oxidation: from Model Microbes to Natural Ecosystems. 111th general Meeting ASM New Orleans, LA (Kalyuzhnaya MG)

22. November 2010. Methylotrophy, revisited: new insights from the -omics approaches. Oregon State University, Corvallis, OR (Chistoserdova L)

23. August 2010. From meta-omics approaches to single organism physiology. Gordon Research Conference Molecular Basis of Microbial One-Carbon Metabolism. Lewiston, ME (Chistoserdova L)

24. March 2010. Overview of the Lake Washington meta-omics project. University of Washington Interdepartmental Workshop on Transcriptomics. Seattle, WA (Chistoserdova L)

Other products

Databases

Ten metagenomic datasets are available through the IMG/M interface (https://img.jgi.doe.gov/cgi-bin/m/main.cgi)
Sixty genomic datasets are available through the IMG interface (https://img.jgi.doe.gov/cgi-bin/w/main.cgi)

Physical collections

Over 200 methylotroph isolates have been preserved as part of the laboratory collection, available on request.