様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成 ３０ 年　 ６ 月 ２５ 日現在

機関番号：１２６０８
研究種目：挑戦的萌芽研究
研究期間：2015～2017
課題番号：１５Ｋ１４６０８

研究課題名（和文）Species interaction by direct electron transfer in syntrophic communities.

研究課題名（英文）Species interaction by direct electron transfer in syntrophic communities.

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研究成果の概要（和文）：本研究では、環境によって変化した微生物の研究に適用できる新型顕微鏡方式を示した。さらに、細胞外シトクロムと結合すると考えられる抗生物質を合成した。この物質は、新型顕微鏡と共に今後の研究に利用される。本研究では細胞外シトクロムを利用するような微生物を探索し、自然界での異種間電子移動の多様性と生態について知見を求め、ゲノム科学と地球化学的測定法に基づいて得られたこれらの知見を論文発表した。加えて、共同研究により陰極の電子移動できる新種有機物を発見した。最後に、メタン菌のC1代謝と嫌気的メタン酸化古細菌のエネルギーの保存方法についての知見をレビューし、まとめた。

研究成果の概要（英文）：We were able to demonstrate new microscopy methods which are applicable to studying environmentally derived microbes. In addition, we have now synthesized antibodies which are predicted to bind extracellular cytochromes. These will be used in future research, together with our new microscopy methods. During the project, we hunted for microbes which might be utilizing extracellular cytochromes to gain further insight into the diversity and ecology of interspecies electron transfer in nature, and we published our findings based on genomics and geochemical measurements. Additionally, through collaboration, we identified a new organism capable of cathodic electron transfer. Finally, we reviewed and integrated knowledge of C-1 metabolism in methanogens and ANME archaea with a focus on how energy conservation may occur in these organisms .

研究分野：Geomicrobiology

キーワード：electron transfer　methane oxidation　archaea　cytochrome　microscopy　antibody

１．研究開始当初の背景 Background of the Research at the Beginning of the Project

Anaerobic methane oxidation by archaea exerts significant control on the Earth's global carbon cycle. In 2015 and 2016, we demonstrated the possibility that direct interspecies electron transfer (DIET) was responsible for efficient metabolic coupling between methane oxidizing ANME archaea and partner sulfate reducing bacteria (McGlynn et al 2015 Nature, Scheller et al 2016 Science). A key prediction of those works is that a conductive protein layer surrounds ANME archaea and facilitates electron transfer to partner cells. This prediction has yet to be verified experimentally however.

２．研究の目的 Research Objectives

The objectives of this project were to further define our understanding of interspecies metabolic coupling by conducting light and electron microscopy studies of the microbial consortia responsible for methane oxidation, specifically with the goal of applying antibodies suitable for the detection of predicted proteins which exist on the outside of ANME cells.

３．研究の方法 Research Method

Antibody design was conducted *in silico* using predicted protein coding genes thought to bind heme and be exported to the cell exterior. Three separate antibodies were constructed against regions of extracellular multiheme cytochromes which are encoded by ANME genomes. These antibodies can be used as primary antibodies when applied to ANME cells, and secondary antibodies can be used for fluorescence or electron microscopy. Furthermore, light and electron microscopy techniques were advanced to realize the capability of pairing phylogenetic information of individual cells with fine scale, ultra-structural electron microscopy observables. Our work lays the foundation for detailed molecular studies of interspecies electron transfer using techniques that do not require traditional culturing. These culture independent techniques are predicted to be valuable for multiple disciplines where cellular interactions and symbioses together result in the observed microbial ecology. The basic methodology for this project was published in McGlynn et alia 2018 AEM.

４．研究成果 Research Achievements

We were able to demonstrate new microscopy methods which are applicable to studying environmentally derived microbes (Figure 1; McGlynn et al 2018 *AEM*). This work is critical for the advancement of this project because it allows multiple lines of information to be obtained on these uncultivated cells. Specifically, it is rather simple to quickly analyze experimental results with epifluorescence light microscopy, and it is routine to perform phylogenetic labling of cells with fluorescent probes. The problem with fluourescence techniques is that the spatial resolution of epifluorescence light microscopy is limited. Therefore, the ability to conduce transmission electron miscroscopy – which yields much more highly resolved images of cells – is desirable. The application of these methods resulted in new insights to the cells conducting anaerobic methane oxidation in cold seep environments, specifically, we found that ANME-2b contain a polyphosphate like material, whereas ANME-2a/2c do not. In addition, ANME-2b are much more variable in total biovolume and are larger than other cell types. We have now synthesized antibodies which are predicted to bind extracellular cytochromes. These will be used in future research, together with our new microscopy methods where we will be able to link phylogeny, protein expression, and ultrastructure.

During the project, we additionally hunted for microbes which might be utilizing extracellular cytochromes to gain further insight into the diversity and ecology of interspecies electron transfer in nature, and we published our findings based on genomics and geochemical measurements (Ward et al 2018 *Frontiers in Microbiology* & 2017 *Geobiology*). Additionally, through collaboration, we identified a new organism capable of cathodic electron transfer (Kawaichi et al 2018 *Frontiers in Microbiology*). Finally, we reviewed and integrated knowledge of C-1 metabolism in methanogens and ANME archaea with a focus on how energy conservation may occur in these organisms (McGlynn 2017 *Microbes and Environments*).

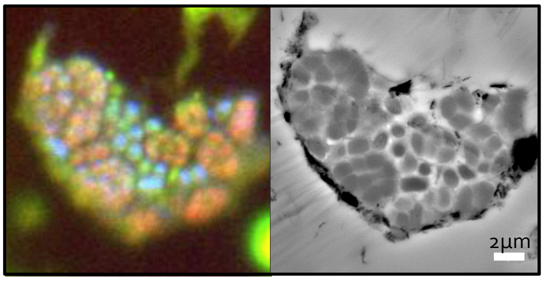

Figure 1. Demonstration of fluorescence in situ hybridization (left) with transmission electron microscopy on the same cells (right).

５．主な発表論文等 Main Research Products
（研究代表者、研究分担者及び連携研究者には下線）(The name of PI in the author list should be underscored.)

〔雑誌論文〕Journal Articles（計　5　件）

1) Shawn E McGlynn, Grayson L Chadwick, Ariel O'Neill, Mason Mackey, Andrea Thor, Thomas J Deerinck, Mark H Ellisman, Victoria J Orphan. Subgroup characteristics of marine methane-oxidizing ANME-2 archaea and their syntrophic partners revealed by integrated multimodal analytical microscopy. *Appl Environ Microbiol 84:e00399-18. https://doi.org/10.1128/AEM.00399-18.* 2018. (Spotlight Article of significant interest) (peer reviewed)

2) Lewis M Ward, James Hemp, Patrick M Shih, Shawn E McGlynn, Woodward W Fischer, Evolution of Phototrophy in the Chloroflexi Phylum Driven by Horizontal Gene Transfer. *Frontiers in Microbiology* 9, 260, 2018. (peer reviewed)

3) Satoshi Kawaichi, Tetsuya Yamada, Akio Umezawa, Satoshi Kawaichi, Shawn E McGlynn, Takehiro Suzuki, Naoshi Dohme, Takashi Yoshida, Sako Yoshihiko, Nobuhiro Matsushita, Kazuhito Hashimoto, Ryuhei Nakamura, Anodic and Cathodic Extracellular Electron Transfer by the Filamentous Bacterium Ardenticatena maritima 110S, *Frontiers in Microbiology* 9, 68, 2018. (peer reviewed)

4) Lewis M. Ward, Airi Idei, Takeshi Kakegawa, Woodward W. Fischer, Shawn E. McGlynn, Microbial diversity and iron oxidation at Okuoku-hachikurou Onsen, a Japanese hot spring analog of Precambrian iron formation, *Geobiology. 15:817-835 2017 (Cover article)* (peer reviewed)

5) Shawn E. McGlynn. Energy Metabolism during Anaerobic Methane Oxidation in ANME Archaea. *Microbes and Environments*, 32 (1), 5-13, 2017. doi:10.1264/jsme2.ME16166 (peer reviewed)

〔学会発表〕Presentations（計　5　件）(Number of presentations:)

1) 2017 Shawn E. McGlynn (Invited Speaker): KIOST International Seminar. Direct interspecies electron transfer as a mechanism of syntrophic coupling between ANME archaea and partner bacteria: current hypotheses and bioenergetic consequences. December 4, Busan Korea.

2) 2017 Shawn E. McGlynn (Invited Speaker): Japan Geoscience Union Lunch Seminar, Microbial Individuals and their roles in Biogeochemistry. May 20, Chiba, Japan

3) 2016 Shawn E. McGlynn (Invited Speaker): Japanese Society for Microbial Ecology Conference. Direct interspecies electron transfer as a mechanism of syntrophic coupling between methane oxidizing archaea and sulfate reducing bacteria. October 24, Yokosuka Japan.

4) 2016 Shawn E. McGlynn (Invited Speaker): New stable isotope techniques and applications to early Earth and life studies. ELSI. Analyzing microbial individuals and populations with SIMS: culture dependent and independent approaches. June 25, Tokyo Tech, Japan

5) 2016 Shawn E. McGlynn (Invited Speaker): Seoul National University Solar Fuel Workshop. Enzymology of anaerobic methane oxidation. May 11, Seoul, Korea

〔図書〕Books（計　　件）(Number of books:)

None

〔産業財産権〕Industrial Property Rights

None

○出願状況 Pending（計　　件）(Number of：)

名称 Name of the Industrial Property Rights：
発明者 Inventor(s)：
権利者 Rights Holders：
種類 Type (Patent, Utility Model, etc.)：
番号 Number：
出願年月日 Year Applied：
国内外の別 Japan/Foreign：

None

○取得状況 Granted（計　　件）

名称 Name of the Industrial Property Rights：
発明者 Inventor(s)：
権利者 Rights Holders：
種類 Type (Patent, Utility Model, etc.)：
番号 Number：
取得年月日 Year Granted：
国内外の別 Japan/Foreign：

〔その他〕Remarks
ホームページ等
Website URL, etc.

６．研究組織 Research Organization
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研究者番号：

(3)連携研究者 Co-Investigator(s)'s Name
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