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　１８　日現在

機関番号：　８２７０６
研究種目：　研究活動スタート支援
研究期間：　2016～2017
課題番号：　１６Ｈ０７４８９

研究課題名（和文）Evaluating the Influence of Viruses on the Deep Biosphere Carbon Cycle

研究課題名（英文）Evaluating the Influence of Viruses on the Deep Biosphere Carbon Cycle

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,300,000円

研究成果の概要（和文）：IODP第370次航海にて採集された南海トラフ海底下200-1200mの海底堆積物のバイオマスは極めて少なく、従来の手法ではウイルスを発見できなかった。そこで新たな手法を開発したところ100倍以上のウイルスが検出された。これは従来考えられていたよりはるかに多くのウイルスが海底堆積物に存在することを示している。よりバイオマスの多いサンプルとして襟裳岬沖の海底下堆積物を入手し、TEM分析およびウイルスDNA抽出を実施中である。また下北半島沖の海底堆積物を種としたメタン生成バイオリアクター内で培養されたウイルスから、DNAの抽出及び配列決定に成功した。バイオインフォマティクス分析が進行中である。

研究成果の概要（英文）：Subseafloor sediment (200-1200m below seafloor) was collected during IODP Expedition 370 in the Nankai Trough. The sediment biomass was extremely low. Using traditional methods of virus extraction from sediment, it was not possible to find viruses. I developed a new method of virus extraction from sediment that improved extraction by more than 100x. This suggests that there are many more viruses in subseafloor sediment than previously known. In order to obtain higher biomass samples for analysis, subseafloor sediment from another site (offshore Cape Erimo) were obtained by drilling to 100mbsf during Chikyu Expedition 910. Cell extraction for TEM analysis and viral DNA extraction is in progress. An alternative approach was also taken using viruses cultivated from a methanogenic bioreactor which had been initially seeded with subseafloor sediment from offshore Shimokita during IODP Expedition 337. Viral DNA was extracted and sequenced. Bioinformatic analysis is in progress.

研究分野：　microbiology

キーワード：　subseafloor　virus　sediment　subsurface

１．研究開始当初の背景

Scientific drilling has revealed the existence of a large biosphere in the Earth's subsurface. Microbial communities in Earth's deep biosphere drive various biogeochemical cycles in the subseafloor and contain a significant portion (4.6×10^{15}g) of biomass on Earth (Kallmeyer et al 2012).

Viruses are the most abundant organisms on Earth and have been found in nearly every environment. Recently, scientific drilling expeditions also revealed an abundance of viruses within subseafloor sediments. In the deep subseafloor, viruses are as much as 225 times greater than bacteria (Engelhardt et al 2014).

Viruses are parasites of living cells and are dependent on host cells to reproduce. Viruses infect and kill cells, releasing nutrients back into the environment. Viruses kill as much as 20-40% of bacteria in the ocean each day (Suttle 2005), resulting in a significant flux of organic matter transferred from cells to pools of dissolved organic matter/particulate organic matter (DOM/POM). This process is recognized as globally important in marine biogeochemical cycling.

Many viruses also contain metabolic genes that enhance the host cell's metabolism (auxiliary metabolic genes). For example, genes for sulfur oxidation and photosynthesis have been found in viruses (Hurwitz et al 2016). During infection, auxiliary metabolic genes help the host obtain extra energy and nutrients for viral reproduction. This results in viral manipulation of biogeochemical cycles. Many microbial genomes derived from subsurface environments contain evidence of viral genes, suggesting that viral infection of microorganisms in the deep subsurface may be common.

Past research has shown that viruses are present in shallow subsurface sediments and increase in response to energy availability (Pan et al, 2014). Viruses have also been observed to be correlated with organic carbon in the subsurface (Pan et al, 2017). However, in the deep subsurface, energy and nutrients are much lower, so it is unknown how common viral infection and reproduction is. Therefore, the role of viruses in biogeochemical cycling within Earth's deep biosphere is not known. The existence of viruses poses fundamental questions for subsurface biogeochemistry: 1. "How much impact do viruses have on subsurface biogeochemical cycling?" and 2. "What are the mechanisms by which viruses influence key biogeochemical processes in the subsurface?"

２．研究の目的

Viruses are abundant in the deep subseafloor, but their role is unknown. I hypothesize that viruses influence the subseafloor carbon(C) cycle by killing bacteria and releasing organic C, and by providing bacteria with genes for C metabolism. In order to determine the impact of viruses on subseafloor C cycling, I propose to quantify the flux of C from subseafloor bacteria infected with viruses. I also propose to extract viral DNA/RNA from subseafloor sediments to find viral genes involved in key pathways in C cycling.

３．研究の方法

(1) To test this, the flux of C from infected bacteria in the subseafloor will be quantified by determining the rate of viral infection from subseafloor sediments. Cells from deep subseafloor sediments will examined by transmission electron microscopy (TEM) to determine how many cells are infected with viruses. By factoring the average mass of C per cell, the flux of C from infected bacteria can be determined.

In order to identify infected viral cells, cells must first be extracted from sediment. Cells will be detached from sediment according to previously described methods (Morono et al 2013). Sediment slurry will be diluted with a solution of 2.5% NaCl solution, detergent mix and methanol. The slurry will be shaken and sonicated. The mixture will be layered onto a multi-layer high density cushion solution of Nycodenz/polytungstate and centrifuged. Cells in the supernatant will be removed and transferred to a new vial.

(2) Viruses will be extracted from subseafloor sediment using previously published methods (Yanagawa et al 2014, Engelhardt et al 2014). DNA/RNA will be extracted and sequenced from the extracted viruses. Biogeochemically important

metabolic genes will be identified in the viral DNA/RNA. Particular focus will be placed on genes for methanogenesis and sulfate reduction, which are especially important metabolisms that influence the deep subseafloor C cycle.

4．研究成果

(1) Sediment samples were collected during IODP Expedition 370 from Site C0023 in the Nankai Trough, off of Muroto Peninsula. Sediment samples ranged from 200mbsf to 1200mbsf. The sediment biomass was extraordinarily low. The number of cells that could be extracted from the sediment was approximately 10^4 cells/cc from 200-300mbsf but less than 10^3 cells/cc in deeper regions (Heuer et al 2017), too low for meaningful TEM analysis of viral infected cells. Using traditional methods (Yanagawa et al 2014, Engelhardt et al 2014) of virus extraction from the sediment, viruses could not be found.

(2) Because traditional methods of virus extraction from sediment were insufficient for deep sediments collected from the Nankai Trough, a new method had to be developed. Based on a previously published method that was used to improve the separation of cells from subseafloor sediment (Morono et al 2013), I developed a novel method for the separation of viruses.

The Nycodenz method substantially increases the recovery of viruses in a variety of subseafloor sediments including clay-rich and low porosity samples. Sediment samples from the Nankai Trough (IODP Expedition 370) were spiked with a known concentration of viruses and extracted using the Nycodenz method. The efficiency of virus recovery was compared with methods used in previously published subseafloor virus studies (Yanagawa et al 2014, Engelhardt et al 2014). By using the Nycodenz method, virus extraction efficiency is less variable with sediment lithology compared to conventional methods, allowing more accurate comparisons between samples of differing lithology. Virus recovery was improved by as much as 100 times (Fig 1).

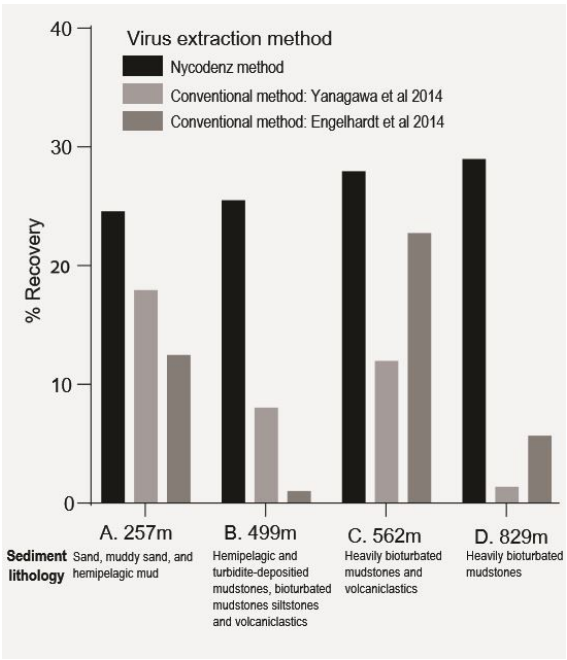

Fig 1. Recovery of spiked viruses from IODP Exp 370 samples comparing the new Nycodenz method with older methods

Previously published virus counts from subseafloor sediment core samples collected offshore Shimokita (Yanagawa et al 2014) and South Pacific Gyre (Engelhardt et al 2014) were re-examined using the Nycodenz method (Fig 2). The Nycodenz method resulted in several orders of magnitude greater virus abundance compared to published figures. This suggests that viral abundances in sediments are greatly underestimated in previously published literature.

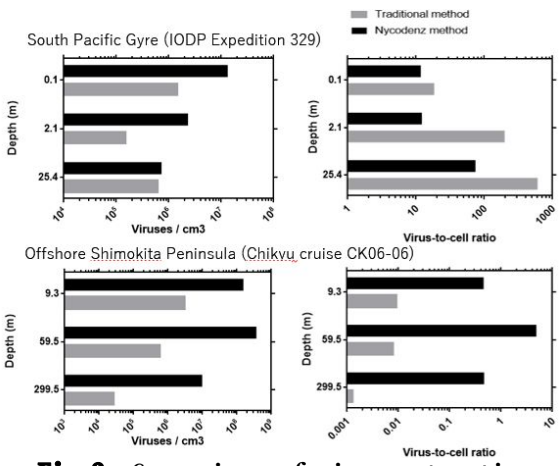

Fig 2. Comparison of virus extraction methods on previously published IODP Exp 329 and Chikyu Exp CK06-06 samples.

(3) Using the new virus extraction method, viruses from the Nankai Trough samples were enumerated (Fig 3). DNA extraction from these samples are in progress.

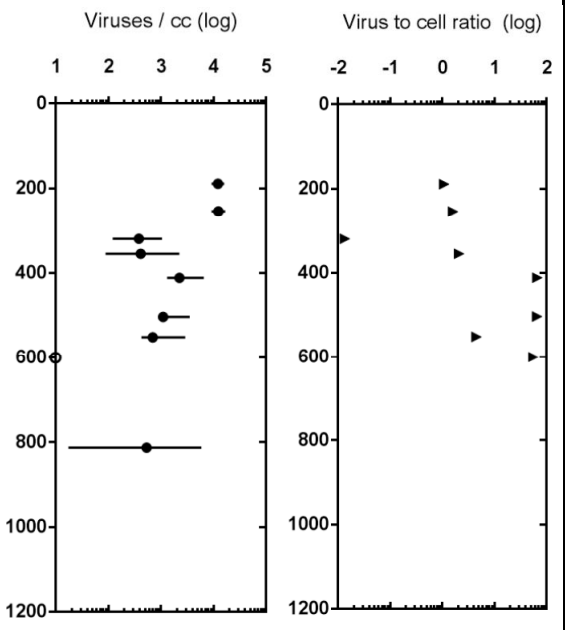

Fig 3. Preliminary virus counts from IODP Exp 370 samples.

(4) Because viruses from subsurface sediment collected from the Nankai Trough are so low in biomass, an alternative approach was taken to collect enough sufficient viral DNA/RNA for genetic analysis. Viruses were collected from a methanogenic downflow hanging sponge reactor which had been initially seeded with subseafloor sediment from offshore Shimokita collected during IODP Expedition 337 (Inagaki et al 2015, Imachi et al 2011). Viruses were collected from effluent for a period of 1 month (approximately 6L of effluent total) (Fig 4). Viruses were collected by the iron flocculation method onto filters (John et al 2011). Viral DNA and RNA was then extracted using the QIAamp MinElute Virus Spin Kit (Qiagen) without the RNA carrier. RNA was not detected, and 15ng dsDNA were extracted. The DNA was used to create libraries for sequencing using KAPA Hyper and Swift Accel-NGS 1S Plus kits. The DNA was then sequenced on the Illumina MiSeq platform. Bioinformatic analyses of the viral DNA is progress using the iVirus pipeline (Bolduc et al 2017).

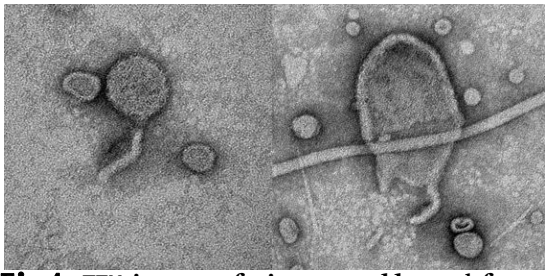

Fig 4. TEM images of viruses collected from a metanogenic bioreactor seeded by deep subsurface coalbed sediment.

(5) In order to obtain higher biomass samples for analysis, subseafloor sediment from another site (offshore Cape Erimo) were obtained by drilling to 100mbsf during Chikyu Expedition 910. Preliminary enumeration indicates viral abundances of 10^9-10^7 viruses/cc. Cell extraction for TEM analysis and viral DNA extraction is in progress.

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６．研究組織

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