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Dense microbial community on a ferromanganese nodule from the ultra-oligotrophic South Pacific Gyre: Implications for biogeochemical cycles

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ABSTRACT

During Integrated Ocean Drilling Program (IODP) Expedition 329, a deep-sea ferromanganese nodule and surrounding sediment were collected from the South Pacific Gyre, the most oligotrophic oceanic environment on earth. Using a combination of cryo-sectioning and fluorescence-based cell counting techniques, we determined that the microbial cell density at the very surface of the nodule was $\sim 10^8$ cells cm^{-3}, three orders of magnitude higher than that in the surrounding sediment. Analysis of bacterial and archaeal 16S rRNA gene sequences (~1400 bp) indicated that the taxonomic composition of the nodule-associated community differed markedly from that of the sediment-associated community. Members of Marine Group I (MGI) Thaumarchaeota are potentially crucial for sustaining the high cell density because both ammonia and Cu were available on the nodule surface, making it suitable for ammonia-oxidizing chemolithoautotrophy mediated by copper enzymes. Combined cryo-sectioning and synchrotron analysis of the nodule surface revealed both hexagonal birnessite resembling δ-MnO_2 and triclinic birnessite, minerals characteristic of biogenic oxide and its secondary product, respectively. Regardless of these possible biogenic features, only one gene sequence exhibited some similarity to previously identified manganese-oxidizing bacteria. On the other hand, MGI Thaumarchaeota were assumed as potential candidate of manganese oxidizers because they have multi-copper oxidase that is utilized by most known manganese oxidizers. Therefore, this archaeal group is considered to play a significant ecological role as a primary producer in biogeochemical elemental cycles in the ultra-oligotrophic abyssal plain.

1. Introduction

Ferromanganese nodules (also known as manganese nodules or polymetallic nodules) contain various metals, including Ni, Cu, and rare earth elements. Together with ferromanganese crusts, ferromanganese nodules were recently reevaluated as potential metal resources (Hein et al., 2013). Ferromanganese nodules are usually found on the abyssal seafloor, where the sedimentation rate is low, and they are formed very slowly (several to hundreds of mm myr^{-1}) via the concentric accumulation of iron and manganese oxides around nuclei. Due to the stability of dissolved manganese, surface catalysis of oxides and/or bacterial oxidation is necessary for ferromanganese nodule formation under general seawater conditions (Glasby, 2006).

Although inorganic processes are often emphasized with respect to nodule formation, biogenic Mn(II) oxidation is considered to be crucial because it is generally up to five orders of magnitude faster than inorganic oxidation (Tebo et al., 2004). The potential contribution of benthic organisms in nodule formation was examined after it was reported that organic material is present in some ferromanganese nodules (Graham, 1959). In early studies, protozoa were considered to be potential contributors; in particular, agglutinated foraminifera were thought to play a crucial role in nodule formation because their shells are comprised of agglutinated fine particles (Graham and Cooper, 1959; Greenslate, 1974).

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However, such protozoa cover only a few percent of the nodule surface (Veillette et al., 2007), and their quantitative significance is thus questionable.

In contrast, bacteria are now thought to contribute to nodule formation. The pioneering study of Ehrlich (1963) examined ferromanganese nodule formation through incubation experiments in the laboratory. The results of these experiments suggested that nodules are formed via enzymatic manganese oxidation by bacteria. The presence of microbial life in ferromanganese nodules was also demonstrated through culture-dependent isolation (Ehrlich et al., 1972), electron microscopy (LaRock and Ehrlich, 1975; Wu et al., 2013), and culture-independent molecular (DNA) analyses (Blöthe et al., 2015; Tully and Heidelberg, 2013; Wu et al., 2013). Due to the potential involvement of microbes in their formation and their laminated to columnar fabric, ferromanganese nodules may be similar to stromatolite, which is defined as a laminated benthic microbial deposit.

Although evidence indicating that microbes live in association with ferromanganese nodules is accumulating, how they contribute to and/or benefit from nodule formation is poorly understood, primarily due to the difficulty of investigating spatial relationships between microorganisms, minerals, and elements in nodules. For example, scanning electron microscopy (SEM) is frequently utilized to observe microorganisms in ferromanganese nodules, and the elemental composition of targeted spots can be also analyzed by the combination of energy-dispersion spectroscopy. However, identifying an object as a microbial cell in SEM depends heavily on the object's shape and size, and uncertainty may remain regarding an object's biogenic origin. DNA analysis, on the other hand, enables the elucidation of microbial community composition and metabolism. However, DNA must be extracted from bulk samples, which precludes investigations of the spatial distribution of microorganisms, minerals, and elements.

To overcome the technological drawbacks associated with previously applied methods, this study employed cryo-sectioning in order to retain the spatial distribution of microbial cells and minerals (Shiraishi et al., 2008). Microorganisms were identified by staining of nucleic acids with a fluorescent dye, whereas minerals were identified by synchrotron analysis. These data were complemented by analysis of bulk DNA from the ferromanganese nodule and surrounding sediment, using a newly established DNA extraction method. These techniques, together with conventional X-ray analysis, were used to examine a ferromanganese nodule collected from the seafloor of the ultra-oligotrophic South Pacific Gyre during Integrated Ocean Drilling Program (IODP) Expedition 329, where microbial cell abundance is 3 to 4 orders of magnitude lower than that of previously explored subseafloor sediments (D'Hondt et al., 2009, 2015). This low microbial background provided an exceptional opportunity to examine the microbial community specific to ferromanganese nodules.

2. Materials and methods

2.1. Sampling site

The ferromanganese nodule and surrounding sediment (composed mainly of brownish pelagic clay) investigated in this study were collected from the seafloor of the South Pacific Gyre during IODP Expedition 329 (October to December, 2010). In this region, the subtropical anticyclonic gyre prevents nutrients being supplied to its center, depressing primary production of the surface ocean to the lowest level in the world (Fig. 1; D'Hondt et al., 2009). The resultant extremely low organic carbon burial rate leads to low microbial cell abundance and aerobic respiration in the sediment, with oxygen persisting deeper to the basaltic basement (D'Hondt et al., 2015). The sedimentation rate of this region is also very low,

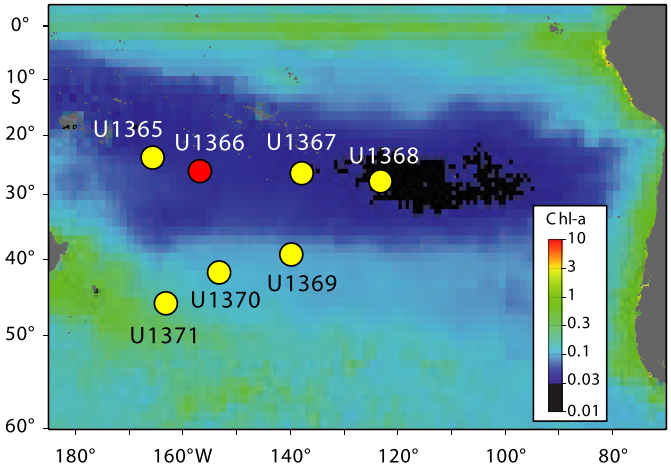

Fig. 1. Sampling sites of IODP Expedition 329 (U1365–U1371; yellow and red circles) and surface ocean concentration of chlorophyll-a ($mg\,m^{-3}$; after D'Hondt et al., 2011). The sampling site of this study (U1366) is indicated by a red circle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which is reflective of the large distance from the major continents (D'Hondt et al., 2009), and is favorable for the formation of ferromanganese nodules. Indeed, the nodule density on the seafloor of this area is among the highest in the world (Glasby, 2006; Glasby et al., 1980).

During IODP Expedition 329, ferromanganese nodules were intermittently observed in sediment cores from four of the seven sampling sites (U1365, U1366, U1369, and U1370) (Fig. 1). Relatively large ferromanganese nodules were recovered from the topmost part of two holes at U1366 (holes D and F; Fig. 2A). The nodules were considered to have been originally located on the seafloor, and hole D was selected for the sample in this study (sample ID: U1366D1H1 0/10). U1366 is relatively close to the central part of the gyre (156°53W, 26°3S, water depth 5130 m) and is almost identical to sampling site "SPG2" described by D'Hondt et al. (2009), Marcus et al. (2015), and Tully and Heidelberg (2013). A growth rate of ~4 $mm\,myr^{-1}$ was reported for ferromanganese nodules at this site (Marcus et al., 2015).

2.2. Sample collection and preparation

Sediment core samples were obtained during Expedition 329 using an advanced piston corer (APC). Core samples were transported to the refrigerator on the hold deck of the drilling vessel *JOIDES Resolution* soon after recovery and stored at 7–10 °C. In regular IODP operations, recovered cores are split into archive and working halves, whereas in this expedition, whole round sampling was adopted for several cores in order to minimize microbial contamination. This was the case for the sample examined in this study, and the topmost 10 cm of the hole D core was obtained on the ship without splitting. This sample contained pelagic clay and a ferromanganese nodule of about 8 cm in diameter. The side portion of this nodule was scraped away by the APC because the inner diameter of its core liner was 6.5 cm.

Subsampling was conducted on the ship 4 days after the recovery. First, the nodule was roughly separated from the surrounding sediment, and small portions of the remaining upper nodule surface were carefully collected using a sterile chisel and hammer. These subsamples were fixed using Tris-buffered saline (TBS) containing 3.7% formaldehyde for 2 days, after which the solution was replaced with 50% ethanol in TBS and the sample was stored at 4 °C until further processing. The remaining major portion of the nodule and the surrounding sediment were stored at −80 °C.