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A comparison of microbial communities in deep-sea polymetallic nodules and the surrounding sediments in the Pacific Ocean

Yue-Hong Wu[a,b,1], Li Liao[c,d,1], Chun-Sheng Wang[a,b], Wei-Lin Ma[b], Fan-Xu Meng[a,b], Min Wu[c], Xue-Wei Xu[a,b,*]

[a] Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration, 36 Baochubei Road, Hangzhou 310012, PR China
[b] Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China
[c] SOA Key Laboratory for Polar Science, Polar Research Institute of China, Shanghai 200136, PR China
[d] College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China

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ABSTRACT

Deep-sea polymetallic nodules, rich in metals such as Fe, Mn, and Ni, are potential resources for future exploitation. Early culturing and microscopy studies suggest that polymetallic nodules are at least partially biogenic. To understand the microbial communities in this environment, we compared microbial community composition and diversity inside nodules and in the surrounding sediments. Three sampling sites in the Pacific Ocean containing polymetallic nodules were used for culture-independent investigations of microbial diversity. A total of 1013 near full-length bacterial 16S rRNA gene sequences and 640 archaeal 16S rRNA gene sequences with ~650 bp from nodules and the surrounding sediments were analyzed. Bacteria showed higher diversity than archaea. Interestingly, sediments contained more diverse bacterial communities than nodules, while the opposite was detected for archaea. Bacterial communities tend to be mostly unique to sediments or nodules, with only 13.3% of sequences shared. The most abundant bacterial groups detected only in nodules were *Pseudoalteromonas* and *Alteromonas*, which were predicted to play a role in building matrix outside cells to induce or control mineralization. However, archaeal communities were mostly shared between sediments and nodules, including the most abundant OTU containing 290 sequences from marine group I Thaumarchaeota. PcoA analysis indicated that microhabitat (i.e., nodule or sediment) seemed to be a major factor influencing microbial community composition, rather than sampling locations or distances between locations.

1. Introduction

Deep-sea polymetallic nodules are potential sources of metals such as Fe, Mn, Ni, Cu and Co. They are heterogeneous concretions on the deep seafloor with semiconcentric layers of mainly iron and manganese hydroxides around a core of variable composition (e.g., volcanic debris, indurated clay or fish teeth) (Banakar et al., 2000; Murray and Renard, 1891). Economically valuable concentrations of Cu, Ni and Co in polymetallic nodules resulted in the 'Nodule Rush' in the 1970s, and are still of interest for future exploitation (Rona, 2002, 2003; Scott, 2011). Although deep-sea polymetallic nodules have been found in all oceans, only some areas have abundant nodule resources rich in elements of economic interest, these areas include the Clarion-Clipperton Fracture Zone (CCFZ) in the eastern Pacific Ocean, the Central East Indian Basin in the Indian Ocean, and seamounts in the middle of the Pacific Ocean (Banakar et al., 2000; Rona, 2002).

Our knowledge about the formation of deep-sea polymetallic nodules is very limited. Although they formed millions of years ago (Morgenstein and Felsher, 1971) and were first recovered in the 1870s, the mechanism of their formation has been studied only since the 1960s (Ehrlich, 1963). One hypothesis suggests that nodules form by metal oxide precipitation due to hydrogenetic and/or diagenetic processes (Banakar et al., 2000; Halbach et al., 1981); this idea considers only physicochemical reactions and ignores biogenic processes. The contribution of biogenic processes has not been widely accepted until very recently, although microorganisms have been shown to play a role in nodule formation for half a century (e.g., Ehrlich, 1968; Graham and Cooper, 1959). A number of studies have supported biogenesis of nodules from deep-sea or freshwater systems, including evidence from ultrastructures and mineral composition (Han et al., 1997; Kim et al., 2011), manganese-oxidizing/reducing bacteria cultured from nodules (e.g., Banakar et al., 2000; Stein et al., 2001), encrusting

* Corresponding author at: Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China.
Tel.: +86 571 81963208.
E-mail address: xuxw@sio.org.cn (X.-W. Xu).
[1] These authors contributed equally to this work.

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protozoans and microorganisms associated with nodules (e.g., Chen et al., 1997), and direct observation of microorganisms within nodules via microscopy (Burnett and Nealson, 1981; Han et al., 1997; Wang and Müller, 2009; Wang et al., 2009). Therefore, the importance of biogenic processes has been recognized.

Complex microbial communities have been discovered in the sediments surrounding nodules. Several studies of microbial diversity in sediments from the CCFZ have been performed using culture-dependent and -independent approaches (Wang et al., 2010; 2009; Wu et al., 2008; Xu et al., 2005, 2007, 2009). However, previous studies were based on relatively small clone libraries constructed only from sediment samples. Little is known about microbial communities in nodules. The polymetallic nodules represent a relatively isolated microhabitat in the deep-sea environment, and may contain microbial communities different from those in the surrounding environment. However, no comparison has been made between the microbial communities inside nodules and in the immediately surrounding sediments. Since nodules are distributed broadly in oceans, it is important to investigate whether nodule microbial communities resemble one another across locations due to similar environmental conditions within nodules (e.g., high level of metals) or if they differ between locations. Hence, in this study we aimed to (1) investigate microbial diversity and community composition of polymetallic nodules and the surrounding sediments from the CCFZ, as well as from seamount regions in the middle of the Pacific Ocean; and (2) compare microbial communities inside nodules and in the surrounding sediments across sampling sites. To our knowledge, this is the first molecular comparison of microbial communities between nodules and their surrounding sediments from multiple sampling sites.

2. Methods

2.1. Site description and sample collection

Samples were collected from three different sites in the Pacific Ocean, the X1 seamount site and two sites (WS0902 and WS0904) in the CCFZ polymetallic nodule province. Abundant nodules occur at these sites, covering generally over 50% of the seafloor surface (Fig. 1). The three sites are geographically distant and represent two oceanic landforms. Sites WS0902 and WS0904 lie in the CCFZ abyssal plain in the eastern Pacific Ocean, while site X1 is located on the flank of X1 seamount, rising at least 3500 m above the deep-sea floor in the middle of the Pacific Ocean (Fig. 1). The distance between the CCFZ and X1 seamount is approximately 5300 km, while sites WS0902 and WS0904 are only 5 km apart in the CCFZ. Water depths at the three sites are similar (Table 1).

Deep-sea polymetallic nodules and sediments were collected from the three sites by using a TV-guided multicorer during RV *DA YANG YI HAO* cruise DY115-21. Three to four cores from one multicorer deployment were obtained at each site, and one core from each site was chosen for microbiological analysis, the remaining cores being used for other studies. All the following processes were carried out using aseptic technique. On board the top 5 cm of sediments (including embedded nodules) were moved from the core into sample collection cups. The sediments looked like brown clay with iron and manganese oxides (Table 1). Nodules, usually 3–6 cm in length with various morphologies, were picked out and gently washed to remove surface sediments. The nodules and the remaining sediments in the cups were then stored separately at −40 °C for further analyses.

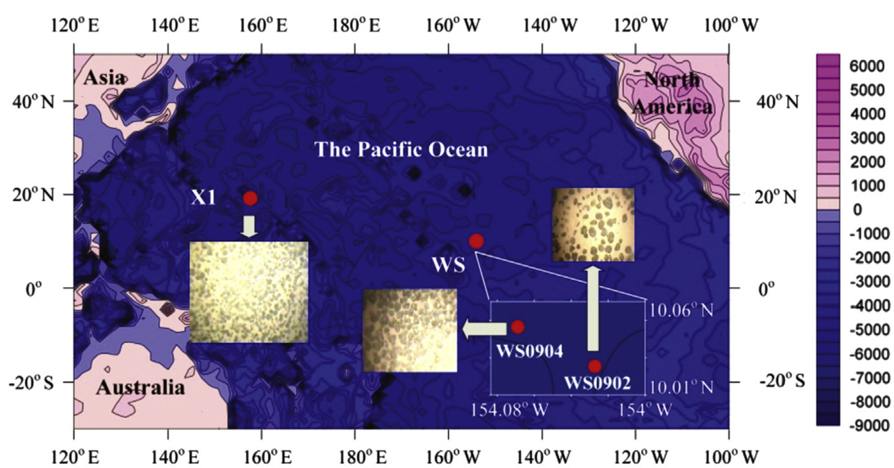

Fig. 1. Locations of three sampling sites X1, WS0902 and WS0904 (filled circles). The 3 insets show the polymetallic nodule abundance in the surface sediments.

Table 1
Description of deep-sea polymetallic nodules and sediments sampled at three sites from the Pacific Ocean.

Site	Latitude (°N)	Longtitude	Depth (mbsl)[a]	Sampling site description	Sample type (symbol)	Sample description
X1	19.20	157.60°E	5513	Seamount flank	Nodule (NA)	Abundant; mostly spherical or elliptical; 4–6 cm in diameter
					Sediment (SA)	Brown clay possibly due to coatings of iron and manganese oxides[b]
WS0904	10.05	154.07°W	5189	Abyssal plain in the CCFZ	Nodule (NB)	Abundant; Polymorphic
					Sediment (SB)	Brown clay possibly due to coatings of iron and manganese oxides
WS0902	10.03	154.03°W	5178	Abyssal plain in the CCFZ	Nodule (NC)	Fewer nodules than sites X1 and WS0904; multi-mophology
					Sediment (SC)	Brown clay possibly due to coatings of iron and manganese oxides

[a] Meters below sea level (mbsl).
[b] The brown color usually results from coatings of iron and manganese oxides, as suggested by analysis of similar sediments from a neighboring site (data unpublished). However, the concentrations of iron and manganese oxides in the neighboring sediments were much lower than those in the nodules.