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Research article

# The indicative effect of structures of archaeal communities at deep-water sediment cores on natural gas hydrate: A case study from Station 973-4 in the Southwest Taiwan Basin, South China Sea

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## Abstract

The formation and decomposition of submarine natural gas hydrate (hereinafter hydrate for short) are greatly influenced by methanogenesis and anaerobic methane oxidation of microorganisms, so it is necessary to conduct further analysis on the metabolic mechanism of archaeal communities and its indicative effect on hydrate reservoir formation. In this paper, the structures of archaeal communities at the cores of Station 973-4 in the Southwest Taiwan Basin of the South China Sea were studied by means of 16S rDNA molecular biological technologies, and then were compared with those at hydrate occurrence and non-occurrence zones in the Japan Sea and the Shenhu area of the South China Sea. It is shown that *Methanosarcina* is the predominant community at the cores from Station 973-4, with a percentage of 50% in the surface zone, 46.1% in the sulfate–methane transition zone (SMTZ) and 66.7% in the deep zone. *Methanomicrobiales* is the secondary predominant community, with a percentage of 28.3% in the surface zone, 30.7% in SMTZ and 11.1% in the deep zone. The percentage changing tendency of the above-mentioned communities are closely related with the variation of organic matter content and compositions during the early diagenetic process. It is concluded that the structures of archaeal communities are in accordance with the analysis results of bacteria, geochemistry and mineralogy, indicating the occurrence of hydrate at the bottom of the cores from Station 973-4. In addition, the structures of archaeal communities are characterized by diversity, so attention shall be paid to the synergy between geochemical parameters and geologic characteristics, so that the indicative effect on hydrate reservoir formation will be presented more accurately.

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**Keywords:** South China Sea; Southwest Taiwan Basin; Archaeal diversity; Japan Sea; Shenhu area; Natural gas hydrate; Methanogenesis; Anaerobic methane oxidation

The formation and decomposition of submarine natural gas hydrate (hereinafter hydrate for short) are greatly influenced by methanogenesis and Anaerobic Oxidation of Methane (AOM) of microorganisms [1,2]. The structures and indicative species of the microorganism communities can be used to study the hydrate reservoir formation, to which much attention has been paid by scientists in various countries. For instance,

Bidle et al. [3] found several types of sulfur metabolic bacteria, methanogenic bacteria (such as *Methanosarcinaceae*), methane-oxidizing bacteria (such as *Methylocaldum*) in Cascadia (Station ODP892B). Niemann et al. [4] found that there developed three major types of bacteria in the Haakon Mosby mud volcano: aerobic methane oxidizing bacteria *Methylococcales*, anaerobic methane oxidizing archaea ANME-2 and ANME-3; moreover, the diffusion of sulfate and free oxygen in porous fluid restrained the usability of electron acceptor of methane oxidizing bacteria, then limited its habitat scope. Based on the research results of Niemann et al. [4], Lösekann et al. [5], the 16S rRNA and fluorescence in situ hybridization

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technique were used to investigate the structures of the bacteria communities in the Haakon Mosby mud volcano. The search results of various scholars in various sea areas and habitats are useful for further understanding of the driving effect of the bacteria in the process of gas hydrate formation. However, it is necessary to conduct further studies on the metabolic mechanism of archaeal communities [6].

The South China Sea provides suitable conditions (e.g. source, temperature and pressure) for hydrate reservoir formation. Particularly, the passive continental margin in the northern South China Sea and the ocean basins and ocean trenches in the eastern South China Sea are the favorable areas for hydrate accumulation. After 2007, the Guangzhou Marine Geology Survey drilled and obtained hydrate samples again in the South China Sea in 2013, further promoting in-depth study on the mechanism of hydrate reservoir formation in the South China Sea by various scholars [7–12]. In the past few years, Zhang Yong et al. [13] have analyzed the archaeal diversity of surficial sediments in the Shenhu area of the northern South China Sea, and found that the major community of *Crenarchaeota* is C3, and the major *Euryarchaeota* is Marine Benthic Group (MBG)-D. Zhang Hao et al. [14] pointed out that *Crenarchaeota* is the major type in surficial sediments in Xisha Islands sea area, the main community is Marine Crenarchaeotic Group I (MGI), with apparently high diversity than that of the archaea. Shi Chunxiao et al. [15] found that *Proteobacteria* is the predominant community in the deposit cores near Jiulong methane reef in Dongsha Islands. Currently, though some progresses have been made in the surveys on the structures of archaeal communities in deposits in various sea areas of the South China Sea, the study on utilizing geomicrobiology to identify hydrate is still at its initial stage [16]. 16S rDNA molecular biological technologies were used to study the structures of archaeal communities at the cores of Station 973-4 (hereinafter 973-4 core for short) in the Southwest Taiwan Basin of the South China Sea. And those structures were compared with those in hydrate-occurrence and non-occurrence zones in the Japan Sea [17] and the Shenhu area [18] of the South China Sea so as to explore the indicative effect of archaeal communities on hydrate reservoir formation.

## 1. Materials and methods

### 1.1. The archaeal diversity of 973-4 core in the SW Taiwan Basin

The South China Sea is the biggest marginal sea in China. The Southwest Taiwan Basin is located on the passive continental margin in the northern South China Sea, adjacent to the Dongsha Uplift of the Pearl River Mouth Basin. Its north is the Penghu Beigang Uplift, and its east boundary is the Quchi–Laonong Fault on west fringe of the Taiwan Central Mountains, striking in NE direction. Its tectonic evolution underwent early extension and later compression. The sea area of the Southwest Taiwan Basin has plentiful terrigenous organic matters, with a high deposition rate, wide bottom-simulating reflector (BSR). The analysis results of geochemical features

of carbonate rocks, pyrite and pore water in the deposits indicate that this sea area is the optimum region of hydrate occurrence in the South China Sea [7,9]. The Station 973-4 is situated near the Jiulong methane reef in the Southwest Taiwan Basin, with water depth of 1666 m, from which 13.95 m cores were obtained (Fig. 1). This core was cut according to the max sampling density, then to measure the structures of archaeal communities in various depths. The concrete methods and steps are described below.

- 1) Take the 1 g deposit sample, and use the Power Soil DNA Isolation Kit reagent box to extract DNA.
- 2) Take the DNA sample as the template to do PCR amplification. The primer is 355F/1068R.
- 3) Connect the amplification product to 19T-Vector, and then transfer it into the DH5 $\alpha$  cell to select blue-white plaques; select positive clones by bacterial colonies PCR, and then send the positive clones to Sangon Biotech (Shanghai) Corporation to do sequencing.
- 4) Compare the measured archaeal sequences on NCBI, and search for bacterial types with higher homology.

Based on the measured Sulfate–Methane Transition Zone (SMTZ) data (<900 cm) [9] and the analysis result of bacterial diversity, the 973-4 core was divided into three zones within 12 various depths [15]: 20–382 cm in surface zones, 552–796 cm in SMTZ, and 862–1196 cm in deep zones, then the structures of the bacteria communities were explored (Fig. 2a).

### 1.2. Analysis of archaeal diversity in hydrate occurrence and non-occurrence zones

The hydrate occurrence zones in the Japan Sea are located in Umitaka Spur (the SW Joetsu Basin), including three

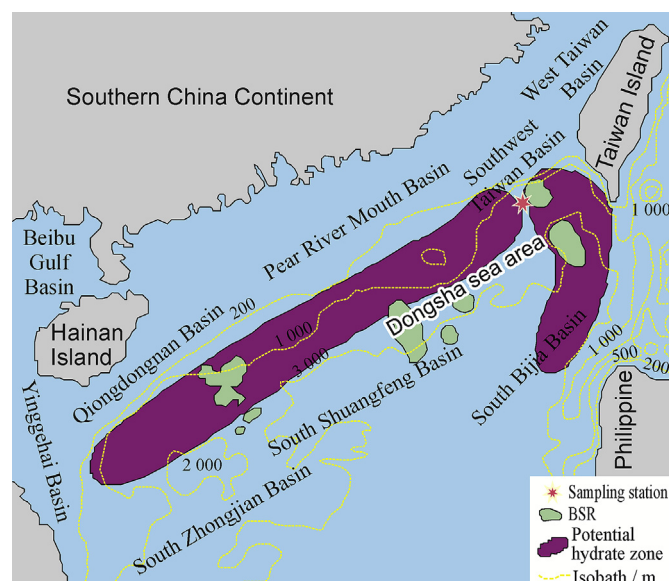


Fig. 1. Location of 973-4 core in the SW Taiwan Basin [15].

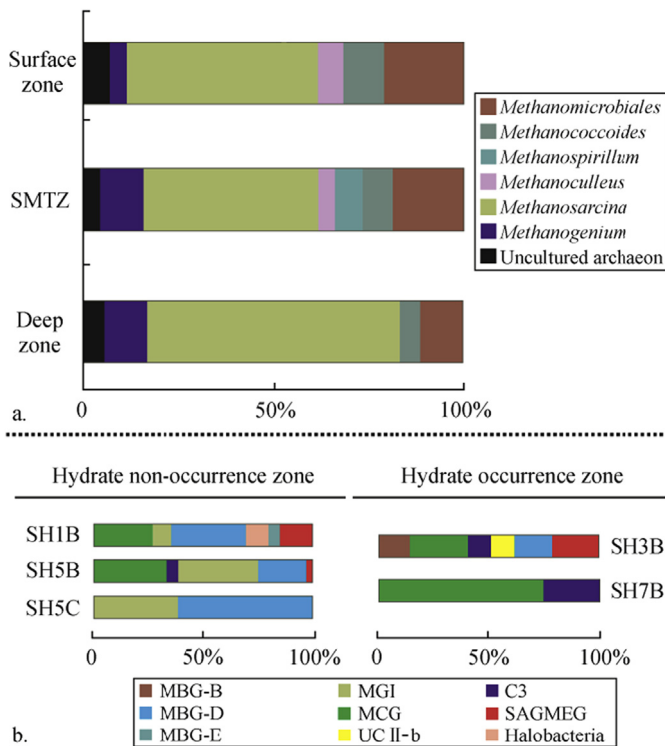


Fig. 2. Structures of archaeal communities in 973-4 core (a) and deposits [18] in the Shenhu area (b).

stations: MD3301 (37°27.59'N, 138°04.60'E), MD3304 (37°25.80'N, 138°00.35'E), and MD3296 (37°24.81'N, 138°00.80'E). The hydrate non-occurrence zones in the Japan Sea include three stations: MD3312 (37°32.09'N, 138°08.30'E) in Unnamed Ridge; MD3325G (38°16.52'N, 137°55.04'E) in Toyama Trough and MD3326G (40°31.32'N, 138°37.99'E) in West Tsugaru. The cores in the above six stations all cross SMTZ (<500 cm), reaching deep deposit layers. By sequencing, we obtained the structures of these archaeal communities [17].

All the deposit samples in the Shenhu area are surface layer samples (0–30 cm below seabed). Hereinto, the samples of the hydrate occurrence zones are from two stations (SH3B and SH7B), and the samples of the hydrate non-occurrence zones are from three stations (SH1B, SH5B and SH5C) [18]. By DNA extraction, PCR amplification and sequencing, we obtained the structures of archaeal communities (Fig. 2b).

## 2. Structure differences of archaeal communities in cores

### 2.1. Structures of archaeal communities in 973-4 core

The archaeal communities detected in the 973-4 core include *Methanomicrobiales*, *Methanococcoides*, *Methanospirillum*, *Methanoculleus*, *Methanosarcina*, and *Methanogenium* (Fig. 2a). Among them, *Methanosarcina* is the predominant community, accounting for the highest ratio in the deep zone (up to 66.7%); the surface zone is secondary in ratio (50%); and the SMTZ zone accounts for the lowest ratio

(46.1%). *Methanomicrobiales* is the secondary predominant community, 28.3% in the surface zone, 30.7% in SMTZ, and 11.1% in the deep zone. Uncultured archaeon also accounts for some ratios in the 973-4 core (Fig. 2a), but its metabolic mechanism is not clear. But the study on its metabolic process is beneficial for understanding the methane metabolic and ecologic features of this core.

### 2.2. Structure differences of archaeal communities in 973-4 core, hydrate occurrence and non-occurrence zones

In the hydrate occurrence zones in the Japan Sea, the developed archaeal communities include MBG-D, Deep-Sea Archaeal Group (DSAG, or MBG-B), South African Gold Mine Euryarchaeotic Group (SAGMEG), Deep-Sea Hydrothermal Vent Euryarchaeotic Group (DHVE)-6 and Ancient Archaeal Group (AAG). In the hydrate non-occurrence zones, there are SAG-MEG and DHVE-6 [17]. In the hydrate occurrence zones in the Shenhu area, the predominant archaeal community is Miscellaneous Crenarchaeotic Group (MCG), and the others include MBG-B, Uncultured Crenarchaea Group II b (UCII-b), SAGMEG and C3. In its hydrate non-occurrence zones, the predominant archaeal community is MBG-D, and the others include MGI, SAGMEG, C3 and Halobacteria [18] (Fig. 2b).

The archaeal communities detected in the 973-4 core, the deposits from the Japan Sea and the Shenhu area mainly belong to Crenarchaeota and Euryarchaeota. Among which, the Crenarchaeota include DSAG, MCG, MGI and C3, and the Euryarchaeota include MGB-D, DHVE-6, SAGMEG, Halobacteria, and *Methanomicrobiales*, *Methanococcoides*, *Methanospirillum*, *Methanoculleus*, *Methanosarcina* and *Methanogenium*. This shows that the diversity of the Crenarchaeota species is more than that of the Euryarchaeota species. The archaeal communities in the 973-4 core are all Crenarchaeota; the hydrate occurrence zones and non-occurrence zones in the Japan Sea and the Shenhu area include both Crenarchaeota and Euryarchaeota. However, though the 973-4 core is similar, its major species are obviously different.

## 3. Discussion

### 3.1. The indication of structures of archaeal communities in 973-4 core to hydrate

In the 973-4 core, *Methanosarcina* is the predominant community. Except for the methanogens bacteria in methyl-using type [19], the other bacteria species *Methanosarcinales*/ANME can consume methane, representing methanotrophs, which is similar to the archaeon in the deposits of lakes, oil reservoir beds and seas [20–23]. *Methanomicrobiales* is the secondary predominant archaeal community, representing CO<sub>2</sub> type methanogens [24–26]. In the surface zone and SMTZ zone of the 973-4 core, there is *Methanoculleus*, being methanogens [19], which was ever

detected in the Nankai Trough, the Japan Sea [27]. In the 973-4 core, the *Methanosarcina* ratio firstly decreases with the depth increase, and then gradually increases; however, *Methanomicrobiales* firstly increases and then decreases. This indicates that from the surface zone to the deep zone, microorganisms use different substrates to produce methane, i.e.,  $\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ ,  $2\text{CH}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2$ . Different reaction substrates are closely related to the organic matter content and composition in the early diagenetic process of marine sediments [28]. Based on the research results of the bacteria community structures in the 973-4 core [15], a conclusion can be drawn that in the 552–800 cm interval, *Methanosarcinales*/ANME and *Desulfuromonadales* bacteria [15] together drive the AOM reaction [1,6]:  $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$ , which led to a sharp decline of the sulfate content [9] in pore water in this depth interval, and morphology of the framboid pyrite [8] in the 973-4 core (Fig. 3). The archaeal community structure in the 973-4 core shows that the 973-4 core was located in the setting mainly with methyl-using type and  $\text{CO}_2$ -using type methanogens, and there were anaerobic oxidation of methane.

Previous research results indicate that the sulfate–methane interface in the 973-4 core is shallower (about 900 cm) [9]. Observed by scanning electron microscope, the pyrite in the deposits of the area is mainly framboid, with octahedral crystallite morphology and cubic pyrite crystal in local zones [8] (Fig. 3). All of these evidences show that methane flux in the lower zone of the 973-4 core is bigger. Moreover, comparative analysis shows that the 973-4 core [15] and both the hydrate-occurrence zones in the Japan Sea [17] have the assemblage feature of *Proteobacteria* and *Chloroflexi*, which is apparently different from the bacteria community structures in the hydrate non-occurrence zones in the Japan Sea. The

comparison result of the bacteria community structures between the 973-4 core, the hydrate-occurrence and non-occurrence zones in the Shenhu area [18] is also similar to the above. A comprehensive analysis shows that the structures of archaeal communities are in accordance with the analysis results of bacteria, geochemistry and mineralogy, indicating the occurrence of hydrate at the bottom of the 973-4 core.

### 3.2. Difference analysis of structures of archaeal communities in various sea areas

The archaea detected in the deposits of the Japan Sea [17] and the Shenhu area [18] were found in other sea areas and stations. For example, the DSAG, DHVE-6 and AAG were found in deep-sea hot spring environments [29,30]. SAGMEG was found in HS-PC500 core from the Shenhu area [31], in the surface deposits from Station 1227 of the ODP 201 Voyage [32], and in the gold mine water in South Africa [33]. Some researchers found that MBG-D developed in the deposits from some stations [34] in the Atlantic Ocean [34] and the Qiongdongnan sea area of the South China Sea [35]. Jiao Lu et al. [31] and Inagaki et al. [32] found MCG in the deposits from the north slope of the South China Sea and the equatorial area of the eastern Pacific Ocean, respectively. Just because of these widely developed archaea in the hydrate-occurrence zones and non-occurrence zones, Jiao Lu et al. [31] pointed out that we should not just use the existence of one type of archaea to indicate the hydrate occurrence, such as SAGMEG and DSAG. Hence, while studying the application of geomicroorganisms in exploring submarine resources, except for geologic and geochemical conditions, we must consider the ecological niche of the biogeochemical reaction driven by microorganisms, and use the microbial community composition to indicate hydrates.

The clustering analysis results of the microbial communities in the hydrate-occurrence zones and non-occurrence zones in the Japan Sea and the Shenhu area indicate that the occurrence or non-occurrence of hydrates is the key factor for deciding the structures of archaeal communities [17,18]. The structure of archaeal community of the 973-4 core is apparently different from those in the hydrate-occurrence zones and non-occurrence zones in the Japan Sea and the Shenhu area of the South China Sea. One possible reason may lie in the experimental method. Several studies were based on the 16S rRNA molecular biology technique. They lack a DNA extraction method with high efficiency and no deviation [36], or suitable primers for amplifying microorganisms in deep-sea deposits [37], which may lead to the microorganism diversity. Another interpretation is that the structures of archaeal communities in deposits of various sea areas and various stations have their own specialties. For instance, in the same type of hydrate-occurrence zones, the predominant community in the deposits of Cascadia exterior sea area is DSAG [32], but that in the Shenhu area is MCG (Fig. 2b). This may be the synergy between the geochemical features of the deposits and the possibilities of hydrate occurrence [18]. Finally, there is a disjointed relationship between the understanding of the

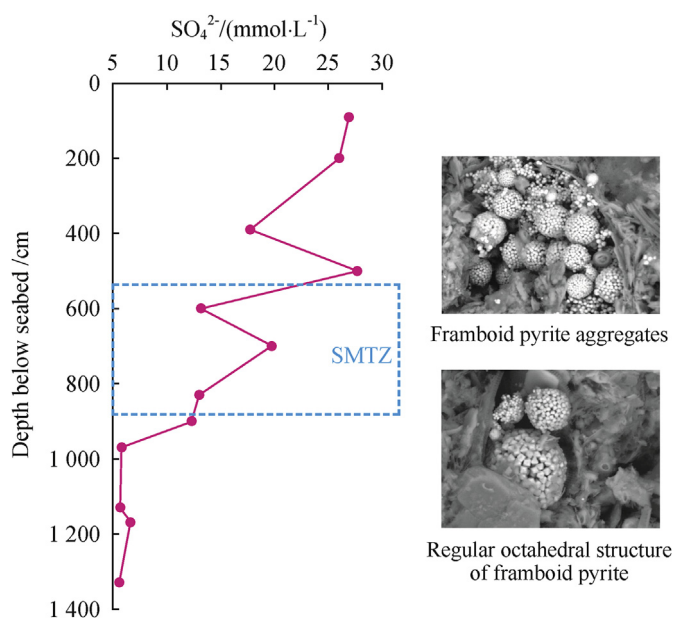


Fig. 3. SMTZ scope and pyrite morphology of the 973-4 core [8,9].

methane metabolism and the results of the investigation of marine sediments. Parkes et al. [38] and Fry et al. [39] utilized 16S rRNA technique to detect methanogen in methanogenic environment, but failed. Biddle et al. [40] found that in deep methanogenic environment, the relative abundances of the known methanogen and the methane-generating gene are lower. This means that the known methanogen may be just a small sub-set of the methanogen species in deep deposits, and there may be new methanogenic archaea, such as DSAG or ANME-1 [19,39]. For methane oxidation, Beal et al. [41] found methane oxidation process that can use high valence ferrous and manganese ions as the electron acceptors in marine ecosystems, i.e., the methane oxidation coupling the reduction of  $\text{Fe}^{3+}$  and  $\text{Mn}^{4+}$ , respectively. Milucka et al. [42] found that the zero valence sulfur ( $\text{S}_0$ ) is a critical intermediate product of AOM process, and ANME can directly drive the coupling reaction between AOM and sulfate reduction. Haroon et al. [43] pointed out that as a unique biological lineage, “*Methanoperedenaceae*” plays an important role in connecting the global carbon cycle and the nitrogen cycle in anaerobic environment. These research results mean that there is still controversy about the reaction mechanism of microorganisms driving methane consumption [6].

#### 4. Conclusions

- 1) All the archaea in the 973-4 core are Euryarchaeota. *Methanosarcina* is the predominant community, and *Methanomicrobiales* is the secondary predominant community. The percentage changing tendency of the above-mentioned communities is closely related to the variation of organic matter content and compositions during the early diagenetic process. The structures of archaeal communities are in accordance with the analysis results of bacteria, geochemistry, indicating the occurrence of hydrates at the bottom of the 973-4 core.
- 2) The indicative effect of the combination of archaeal communities on hydrate reservoir formation is more effective than that of single archaea.
- 3) The structure differences of archaeal communities in the 973-4 core, the Japan Sea and the Shenhu area reflect the specialties of the structures of archaeal communities in various sea areas and layers. The structures of archaeal communities have fine indicative effect on hydrate reservoir formation. It is necessary to integrate geochemical parameters and geologic characteristics to conduct a comprehensive analysis to improve its accuracy.

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