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Growth and species coexistence modes of Anaerobic Methane-Oxidizing Archaea in a high-pressure incubation bioreactor

Song Zhong[1,2], Jing-chun Feng[1,2,3*], Yongji Huang[2,3] and Si Zhang[2,3]

1Guangdong Provincial Key Laboratory of Water Quality Improvement and Ecological Restoration for Watersheds, Institute of Environmental and Ecological Engineering, Guangdong University of Technology, Guangzhou 510006, PR China

2Research Centre of Ecology &Environment for Coastal Area and Deep Sea, Guangdong University of Technology, Guangzhou, 510006, PR China

3Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, 511458, PR China

(*Corresponding Author: Jing-Chun Feng, fengjc@gdut.edu.cn)

ABSTRACT

High-pressure environments have a significant impact on anaerobic oxidation of methane (AOM), increase in anaerobic methanotrophic euryarchaeota (ANME) growth rate at higher methane pressures. This study investigated the effect of temperature on the anaerobic methane-oxidizing and sulfate-reducing (AOM-SR) activities by a highly enriched ANME-2c community (high-pressure environments). The ANME-2c-enriched biomass was incubated at different temperature for 75 days. The most favorable condition for AOM-SR activity in the studied communities was set as the in situ temperature (4°C), suggesting that the studied ANME-2c is well adapted to conditions similar to those of its origins. Moreover, the dispersal process of the species is promoted by 4°C, whereas the symbiotic network is decreased.

Keywords: Species coexistence, Anaerobic oxidation of methane, Temperature, ANME-2c.

NONMENCLATURE

Abbreviations	
AOM	Anaerobic oxidation of methane
SR	Sulfate reduction

1. INTRODUCTION

In the anoxic marine subsurface, large quantities of the potential greenhouse gas methane are formed by microbial and thermal degradation of organic matter [1]. However, the flow of methane from the sediments into the water column was decreased, which is mostly due to the effective barrier of methanotrophic microorganisms [2; 3]. It has been estimated that the AOM coupled with sulfate reduction might consume up to 90% of the methane emitted from the sediments and thus mediate the flux of methane from the seabed to the overlying water column in deep-sea cold seeps. AOM-SR is performed by anaerobic methanotrophic euryarchaeota (ANME) and sulfate-reducing bacteria (SRB) belonging to the Deltaproteobacteria [4]. The quantitatively most important sink is the coupling of methane oxidation to the reduction of sulfate according to the net reaction:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$$

ANME are widely distributed in marine habitats including Hydrate Ridge, the Black Sea, and Gulf of Mexico [5]. Dense ANME populations of >1010 cells cm−3 have been reported in these systems. With the exception of the unique ecosystem in the Black Sea, most of the studies indicate a dominance of ANME-2 or ANME-3 in near-surface sediments (top 10 cm). ANME-2 subgroups revealed different preferences for either ANME-2a or ANME-2c fields, indicating that different environmental conditions were selected for different ANME groups [6].

ANME-2 is the most prominent methanotrophic branch of marine cold gas seeps. The temperature at those locations usually ranges from 4 to 14°C. Although the factors responsible for ANME growth and distribution are not clearly understood, as temperature affects microbial enzyme activity [7]. At present, the microbial communities in the cold seeps related to the gas hydrate are not as yet fully understood, especially in the South China Sea. The study shows that incubation of

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the ANME-2a enrichment is the most active at 10 MPa of incubation pressure with saturated methane and 15°C, suggesting that the enriched ANME-2a community was adapted to almost the same pressure as at the in situ conditions of the biomass origin [4]. The majority of previous high pressure batch incubations and bioreactor-based studies have shown increased AOM-SR activities upon increasing the methane partial pressure. Hence, high-pressure environments have a significant impact on AOM. The aim of this study was to investigate the effect of the temperature on the AOM-SR activity of an enriched ANME-2c community. Prior to this study, researcher used enrichments of three marine AOM consortia growing at different temperature regimes [7]. The response of this enriched ANME communities to different environmental conditions, especially temperature, has not yet been studied, which is the major focus of this study. The response of this enriched ANME community to different environmental conditions, especially temperature, has not yet been studied, which is the major focus of this study.

2. MATERIAL AND METHODS

2.1 Source of biomass and cultivation conditions

Sediment was collected during an expedition in May 2022 at the Haima cold seep (16° 43′ N, 110° 28′ E) located in the South China Sea. The experiments were conducted in high pressure incubation bioreactors (HPB) in triplicate. The HPB had a volume of 200 mL and could handle up to 30 MPa pressure. The medium was prepared according to Rafael et al [8]. The temperature effect was investigated in there sets of triplicate HPB incubated at 4,15and 30°Cin the dark (10.5 MPa).

2.2 Geochemical analysis

The total organic carbon (TOC) and dissolved inorganic carbon (DIC) were analyzed using a TOC-L analyser (TOC-L, Shimadzu, Japan). Analysis procedures for the above equipment followed the manufacturer's instructions.

2.3 Deoxyribonucleic acid extraction, PCR amplification, and sequencing

The microbial DNAs in sediment and bottom seawater were extracted using a power soil DNA extraction kit following the manufacturer's instructions. The DNA was subjected to quality inspection using a NanoDrop 2000 spectrophotometer (Thermo, ND 2000c, USA). Thirty nanograms of qualified genomic DNA of each sample was used for PCR amplification targeting the V3–V4 region of the 16S rRNA gene with primer pairs 349F (5′-GYGCASCAGKCGMGAAW-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) (Takahashi et al., 2014). Eligible libraries were sequenced based on insert size using a HiSeq platform (Illumina, Hiseq2500, USA).

2.4 Data processing and bioinformation analysis

The data were analyzed by one-way analysis of variance (ANOVA) for physical and chemical indicators in the environment. SPSS Statistics 25 was used for the analyses. Microbiological data analyses were performed in R software (version 4.0.5).

3. RESULTS AND DISCUSSION

3.1 Effect of temperature on AOM-SR activity

An increase in sulfate reduction was observed in all incubations at different temperatures, where the rate of sulfate reduction was significantly higher at 4°Cthan at 30°C, the maximum estimated sulfate consumption rate was 12.58mg/L·d (Fig. 1a). In contrast, TOC had the lowest reaction rate at 4°C (minimum 0.11 mg/L·d), which was significantly lower than at 15°C and 30°C (Fig. 1b). Thus, it indicated that the process of sulfate reduction mainly relied on anaerobic oxidation of methane, rather than on the reduction of organic matter. Moreover, the pH during the reaction at 4°C was significantly lower than other temperature conditions, which indicated that the pH of the solution was not significantly changed by the anaerobic oxidation of methane (Fig. 1c). The microbial production of TIC was not affected by temperature (Fig. 1d).

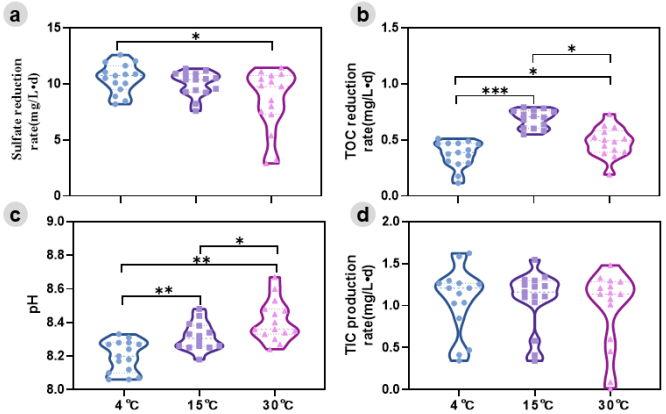

Fig. 1 Geochemical characterization of processes incubating ANME-2c at different temperatures.

3.2 Archaea community composition in the HPB incubation at different temperatures

The microbial α-diversities of the 45 samples are shown in Fig. 2a. The α-diversities of the microbial communities increased with increasing temperatures, which was reflected the observed Sobs, Chao1, Ace, and Shannon indices ($P < 0.05$). This indicated that temperature was the key factor influencing the diversities of community structure. Principal coordinate analysis (PCoA) used to visualize the overall variability of community compositions showed that the archaea communities varied with temperature (Fig. 2b), archaea communities are highly influenced by temperature. The similarity of the samples gradually decreased with the increase of temperature. The relative abundances of microbial taxa were analyzed at the phylum level (Fig. 2c). Halobacterota abundance was significantly higher in the 4°C than in the 30°C. Therefore, it was shown that 4°Cwere more appropriate for the growth of phylum, at this point, the structure of the community appeared to be simplified.

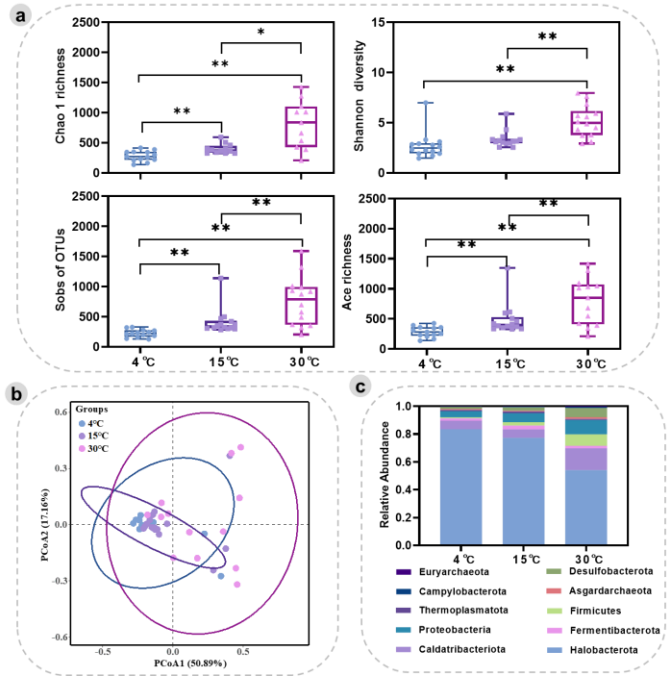

Fig. 2 Compositions of bacterial communities. (a) Microbial α-diversity ($P < 0.05$; multiple comparison with ANOVA tests). (b) Principal coordinate analysis showing differences in community composition with temperature. (c) Relative abundance of community compositions at the phylum level with temperature.

In order to further characterize relative abundance sizes of methane metabolized species in different temperatures, according to the data presented in Fig. 3, the influence of temperature on different types of ANME was observed in the ternary plots, ANME-2c was the main component at 4°C, with less presence of ANME-1, while ANME-1 significantly increased at 30°C. Furthermore, we discovered through random forest analysis that the main functions at 4°Cwere hydrocarbon, sulfur compound degradation, this suggested that strong anaerobic oxidation of methane in this environment, consumed large quantities of methane.

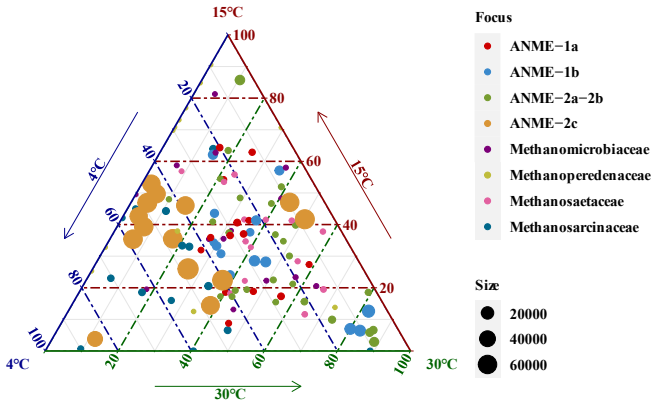

Fig. 3 Relative abundance sizes of methane metabolized species in different temperatures.

Microbial co-occurrence networks were constructed to evaluate species coexistence at different temperatures (Fig. 4a). The co-occurrence networks exhibited more microbial nodes and interconnections at 30°C than at other temperatures, the results indicated that temperature variations led to more intricate interconnections and a complex network structure among archaea OTUs. Furthermore, we observed significantly higher average clustering coefficient and average degree of archaea at 30°Cthan other temperatures, while modularity was lower than others. In order to clarify the potential influence of stochastic processes on the assembly process of archaea communities, we used a neutral community model (Fig. 4b), and the results showed that stochastic processes were important for the assembly process of archaea communities at different temperatures. Additionally, species dispersal process was enhanced by low temperature.

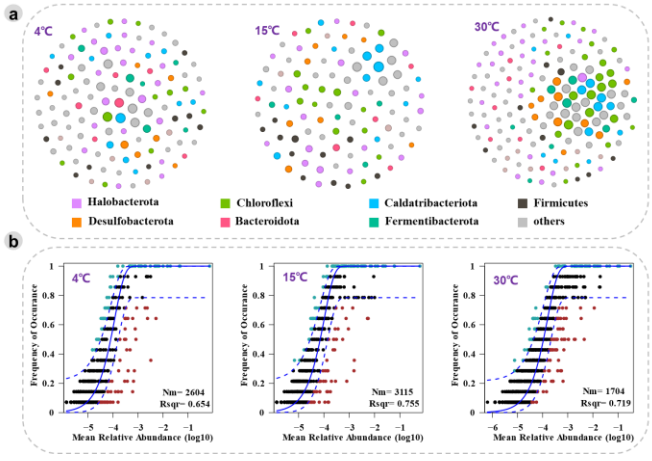

Fig. 4 Community assemblage mechanisms and co-occurrence patterns of archaea along a temperature dimension. (a)Microbial co-occurrence networks across temperature. The nodes are colored based at the phylum level for archaea. (b) Neutral community model of archaea along a temperature dimension. A connection indicates a strong (Spearman's ρ > 0.9) and significant (p < 0.01) correlation. The size of each node is proportional to the degree of OTUs

The stability of the microbial community was evaluated by the average variation degree, we found that the stability of the ecosystem increased by 4°C incubation environment (Fig. 4a). To evaluate the contribution of species richness to the stability of the archaea community, the relationship between AVD values and archaea species richness were examined (Fig. 4b, c, d), the AVD values were negatively correlated with species richness (4°C).

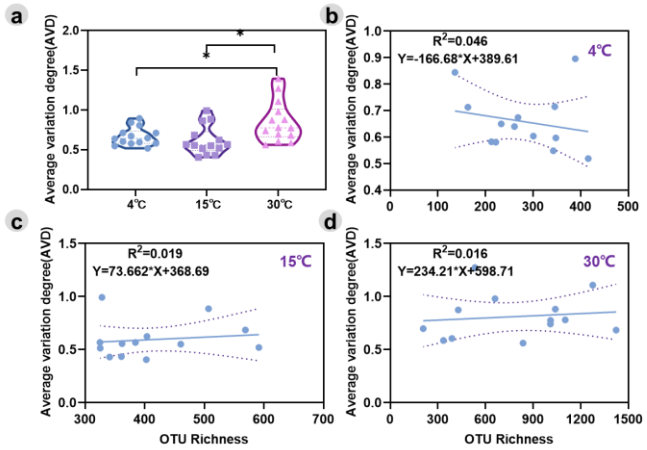

Fig. 5 Archaea OTU richness and AVD of re-assembled archaea communities. (a) AVD across temperature. (b), (c), (d) The relationship between archaea OTU richness and AVD across temperature.

ANME have been retrieved from environments with a wide range of temperatures, from -2 to 100°Cin different marine locations [9, 10]. ANME-2 is a prominent phylogenetic archaea group at most of marine cold gas seeps and some sulfate–methane transition zones where the temperature is 4–14 °C. Although the activity and growth of each ANME type at various temperatures have not yet been detailed, this study showed that ANME-2c was more active at temperature lower than 15°C. Similar to our study, bioreactor enrichments of ANME-2 with mud volcano sediment showed the maximum AOM rate when the bioreactor was operated at 4°C, rather than at 30°C [11]. This result was consistent with the previous research showing that a steady increment in the AOM rate from 4 to 20°C, which subsequently decreased upon further temperature elevations. This result was consistent with the previous research showing that a steady increment in the AOM rate from 4 to 20°C, which subsequently decreased upon further temperature elevations [12].

Co-occurrence networks further revealed variable microbial interactions across temperature gradients. Microbial communities had the tightest interconnections at 30°C. These results suggested that species coexist more frequently at 30°Cthan at other temperature. Previous research showed that microorganisms form close associations with each other in stable environments. Consequently, simple networks were formed in low temperature due to extreme environmental factors. In co-occurrence networks, nodes with interconnectivity with others are considered “hubs” of microbial communities. These hubs drive the evolution of microbial community compositions and function. In this study, the most abundant species, Halobacterota were the most important “hub” species in the co-occurrence network, which played an important role in holding the network together. Neutral community model results showed that stochastic processes were important for the assembly process of archaea communities at different temperatures, and that the species dispersal process is enhanced by low temperature.

Temperature is a highly relevant disturbance owing to its importance for biological processes and its great variability across space and time [13]. The AVD values were negatively correlated with species richness (4°C). That is, biodiversity can increase overall ecosystem stability when biodiversity is low, and decrease it when biodiversity is high, or the opposite. Linking the ecosystem multifunctionality concept and ecosystem stability can transform the perceived effects of diversity on ecological stability and may help to translate this science into policy-relevant information.

4. CONCLUSIONS

AOM activity and changes in microbial community composition of a highly enriched ANME-2c community were assessed at different temperatures. The highest AOM-SR activity was achieved in the incubation at 4°C, i.e., similar to the in situ conditions where the biomass was sampled as well as in the bioreactor enrichment prior to this experiment. The archaea community composition shifted during the 75-day incubations at different temperature, the species dispersal process is enhanced by low temperature.

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