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Stochastic and deterministic processes in the response of microbial to pressure gradients in deep-sea environments#

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ABSTRACT

Marine microorganisms play a vital role in the ocean's cycling of organic matter and nutrients. Here, we conducted a 150-day simulation experiment to investigate cold seep sedimentary microbial diversity under different pressure conditions (0.1–14 MPa) and the roles of various influencing factors. The results showed that the level of pressure affected the reaction rates of metabolic processes, especially those involved in the carbon cycle. The microbial diversity tended to decrease and then increase with increasing pressure. An environmental pressure of 7 MPa was the dividing line between stochastic and deterministic processes. The microbial community diversity was primarily influenced by sulfate ion (SO_4^{2-}), total organic carbon (TOC), and total inorganic carbon (TIC).

Keywords: microbial, species coexistence, incubation, different pressure conditions

NONMENCLATURE

Abbreviations	
AOM	Anaerobic Oxidation of Methane
SR	Sulfate Reduction
SRB	Sulfate-Reducing Bacteria

1. INTRODUCTION

Life on Earth exists in a wide range of environmental conditions, including extreme pressures, temperatures, pH, and salinity [1]. More than 90% of the Earth's microorganisms live in high-pressure environments, and these organisms are well adapted to such conditions in situ [2]. High hydrostatic pressure influences the physiology of organisms living in the deep ocean, and this acts predominantly on the conformation and supramolecular structures of biomolecular systems and thus on their functionality in the cells [3, 4]. For example, when the pressure in the environment changes, processes related to DNA replication and RNA translation are affected, which in turn affects the growth and reproduction of microorganisms [5, 6]. To date, the reactions of microorganisms under different pressures remain unclear, as less than 100 species have been isolated that are piezotolerant or piezophilic. Thus, the pressure response strategy of deep-sea microbial communities is a topic with significant knowledge gaps.

Microbial communities exposed to increasing pressure show compositional changes, and pressure can thus be a selective factor potentially influencing community composition. For example, when sediment was incubated for 240 days in a high-pressure bioreactor, the methane partial pressure influenced the growth of different subtypes of Anaerobic methanotrophic archaea (ANME) and Sulfate-Reducing Bacteria (SRB), i.e., at 10.1 MPa, only the ANME-2c and SEEP-SRB2 subtypes were enriched [7]. In a microbial community experiment, the deep-sea archaea population decreased threefold during 3 days of incubation, whereas the bacterial fraction doubled in size, and the dominance of the active ammonium-oxidizing bathypelagic Thaumarchaeota groups rapidly shifted. In short, indigenous microbial communities in deep waters express higher activities under pressurized conditions than the same communities incubated under atmospheric pressure. However, it is unclear how much pressure should be used to enrich endemic microorganisms (e. g., ANME), and the effect of pressure on microbial metabolic processes is similarly unknown.

Previous studies have primarily focused on the effects of pressure changes on the structure of microbial

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communities, with little attention being given to the mechanisms of pressure on microbial metabolism and community formation. In this study, based on previous research, we hypothesized that pressure gradients could shift microbial community dynamics and functions, drive community assembly processes, and alter patterns of species coexistence during incubation. To test this hypothesis, we used high-throughput sequencing of the 16S rRNA gene and metagenomics analysis to study sediments from the same site in the Haima cold seep area and used various pressures to incubate the microbial community present at the site. Our main goals were to (1) investigate the differences in the efficiency of microbial community utilization of chemical features under a pressure gradient, (2) determine the alteration of microbial community assembly and function under the pressure gradient, and (3) explore the effect of the pressure gradient on the microbial community assembly mechanisms and interaction patterns. Collectively, this study will extend our understanding of the effects of pressure gradients on the response strategies of deep-sea microorganisms and will provide a basis for the cultivation of deep-sea microorganisms.

2. MATERIAL AND METHODS

2.1 Source of biomass and cultivation conditions

In May 2022, the vessel Kexue (Institute of Oceanology, Chinese Academy of Sciences, China) used the remotely operated vehicle (ROV) Faxian to collect samples from the Haima cold Seep (16° 43′ N, 110° 28′ E) located in the South China Sea. The samples were cultured in laboratory-made high-pressure incubation bioreactors (HPBs), HPBs had a volume of 200mL and a diameter of 50mm. The sediment and medium were combined in a sterile bag in a ratio of 2:1, where the medium was prepared according to the methodology described by Laso-Pérez. [8], and then the 120 mL of the mixture was transferred to the HPB. A vacuum pump was used to create a vacuum inside the HPB. The entire process was conducted within a sterile anaerobic chamber. The pressures employed in the experiments were achieved using methane gas. The partial pressure of methane was employed to set the incubation differences pressures of 0.1, 3.5, 7, 10.5, and 14 MPa at the in situ temperature. Three replicates were conducted for each treatment group.

2.2 Geochemical analysis

The pH of the samples was measured using an HACH pH meter (HQ4300, Loveland, CO, USA). The total organic carbon (TOC) and total inorganic carbon (TIC) in the samples were measured using a TOC-L analyzer (TOC-L, Shimadzu, Kyoto, Japan). Anions in the samples were analyzed using ion chromatography (Aquion 1200, Waltham, MA, USA). The cations in the samples were analyzed using inductively coupled plasma mass spectrometry (Waltham, MA, USA).

2.3 Deoxyribonucleic acid extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from the samples using a Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). Amplification of total genomic DNA from the samples used the primers 349F (5′-GYGCASCAGKCGMGAAW-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) targeting the V3–V4 region of the 16S rRNA gene. The PCR amplification conditions were 94°C for 5 min, 94°C for 30 s, 55°C for 45 s, 72°C for 1 min with 30 cycles, and a final extension at 72°C for 10 min. After detection using 0.8% agarose gel electrophoresis, three PCR amplification products from the same sample were combined and sequenced using the Illumina Hiseq 2500 sequencing platform (Illumina, CA, USA).

2.4 Data processing and bioinformation analysis

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

3. RESULTS AND DISCUSSION

3.1 Effect of pressure on the chemical characteristics of the environment

Pressure differences affected chemical indices (SO_4^{2-}, TOC, and TIC) and cell numbers to different degrees. The concentrations of SO_4^{2-}, TOC, TIC, and metal ions and cell numbers under different pressures are shown in Fig. 1. Under incubation at different pressures, the rate of SO_4^{2-} utilization by microorganisms, which was maintained at 2.5–4.1 mg L-1·d, was unaffected by pressure. The consumption rate of TOC was higher at 0.1 MPa than under high-pressure conditions (1.6–5.5 times higher), and the pressure showed a positive correlation with the rate of TOC consumption (at high-pressure conditions) (Fig. 1a). Microorganisms transformed some of the metabolites of TOC into TIC, but the production of TIC was 3.6–4.4 times higher in the high-pressure environment than at 0.1 MPa

(Fig. 1a), indicating that microorganisms primarily used other carbon sources in the high-pressure environment, e.g., by oxidation of methane, to produce TIC.

Microorganisms use trace elements during growth and reproduction. Pressure affected the efficiency of Ni utilization in microorganisms (Fig. 1b) so that the synthesis efficiency at 0.1 MPa was 1.8–3.4 times that in a high-pressure environment, but the utilization efficiency of other trace elements was not significantly altered.

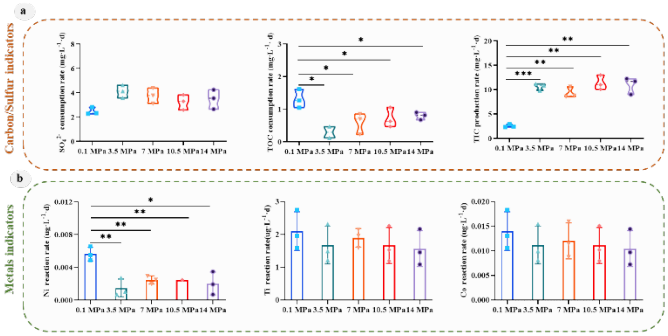

Fig. 1 Geochemical properties and cell numbers under different pressure conditions. (a) SO_4^{2-}, TOC, and TIC concentrations under different pressure conditions; (b) Ni, Ti, and Co concentrations under different pressure conditions

3.2 Microbial community composition in the high-pressure incubation bioreactors at different pressure

The structure and function of microbial communities varied under different pressures (Fig. 2). The sequencing depth for all experimental samples was sufficient, with a Good's coverage index of over 99% (Fig. 2a), indicating that all species in the samples were covered. Alpha diversity was used to evaluate the differences in species richness and diversity of the microbial communities in each sample. Microbial diversity varied under different pressure conditions after incubation. With increasing pressure, the microbial diversity tended to decrease and then increase, and minimum values were observed at 7 MPa (Fig. 2a). The number of unique ASVs for the microbial community showed a decreasing and then increasing trend according to the flower plot analysis. The number of shared ASVs was highest at 0.1 MPa (814) and lowest at 7 MPa (141) (Fig. 2b). Non-metric multidimensional scaling analysis (Fig. 2b) were used to visualize the overall variability of community composition, and the results indicated that the archaeal communities varied with pressure.

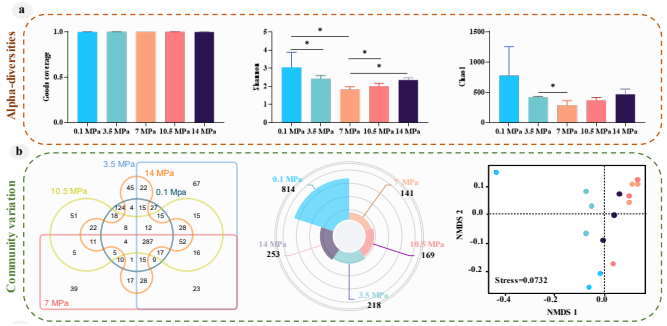

Fig. 2 Compositions of bacterial communities. (a) Microbial α-diversity. (b) Graphs of Veen and NMDS under different pressure conditions

3.3 Process of microbial community construction under different pressure conditions

The NTIs were all greater than 0, indicating that the archaeal communities in the samples were structurally aggregated. Correlation analyses via NTI and pressure showed that the phylogeny of archaea varied with the pressure (Fig. 3a). The βNTI was calculated to reveal the contributions of stochastic and deterministic processes to microbial community assembly under different pressures. The βNTI was correlated with the pressures, indicating that pressure was an important factor influencing community assembly (Fig. 3a). When the pressure was less than 7 MPa, deterministic processes dominated community formation. However, when the pressure was greater than or equal to 7 MPa, stochastic processes dominated community formation (Fig. 3a).

Microbial co-occurrence network was constructed to assess the co-occurrence of species at different pressures (Fig. 3b). The networks exhibited stronger interactions at 7 MPa than at other pressures. However, there was a negative correlation between pressure and the clustering coefficient. The corresponding node-level and network-level topological features are shown in Fig. 3c.

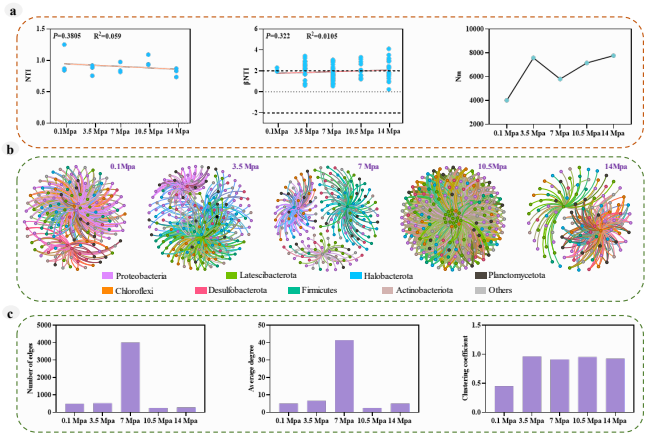

Fig.3 Community assembly mechanisms and co-occurrence patterns of archaea. (a) Community assembly mechanisms. (b) The nodes are colored based on the phylum level of archaea. (c) Network topological features at each pressure level

3.4 Microbial community composition in the high-pressure incubation bioreactors at different pressure

The Average Variation Degree (AVD) index was employed to evaluate the effect of pressure on microbial community stability. Compared with 0.1 MPa, higher pressures significantly increased community stability (Fig. 4a). Meanwhile, the AVD index was positively correlated with ASV richness ($P < 0.001$) (Fig. 4a). In addition, pressure did not affect the ecological niche width of microorganisms (Fig. 4a).

A Mantel test and RDA based on ASV data were performed to explain how the geochemical variables influenced the archaeal community composition along the pressure gradient (Fig. 4b). We found that SO_4^{2-}, TOC, TIC, and Co were the main environmental factors influencing the community structure. The explained ratio of RDA reached 90.59%.

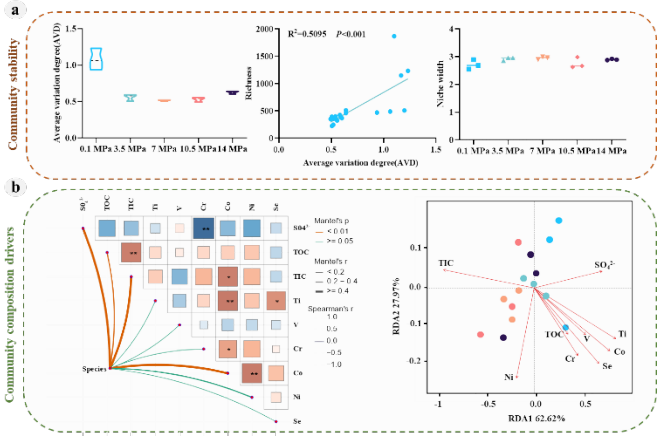

Fig.4 Environmental drivers of archaeal communities under different pressures. (a) AVD and niche width of archaeal communities. (b) Mantel test and RDA correlating geochemical variables and archaeal communities.

The methane-oxidizing activity of microorganisms was noticeably decreased by HPB incubation at 20 and 30 MPa when the methane partial pressure was maintained at 10 MPa [9]. The main reason for this was the low solubility of methane at 0.1 MPa and the extremely low affinity of the AOM process for methane. HPB has been applied in AOM studies, resulting in high AOM activity [10]. In the present study, we found a similar pattern in that the consumption rate of TOC was higher (1.6–5.5 times higher) at 0.1 MPa than under high-pressure conditions, and the pressure showed a certain positive correlation with the rate of TOC consumption under high-pressure conditions. However, the production of TIC was 3.6–4.4 times higher in the high-pressure environment than at 0.1 MPa (Fig. 1a).

High pressure inhibits cellular processes and the formation of macromolecular structures, resulting in positive changes in volume. For example, many microorganisms become filamentous when incubated at pressures below those that prevent cell growth; E. coli FtsZ rings are largely absent at high pressures but rapidly form at 0.1 MPa. Isolated microorganisms are often able to grow more efficiently under high hydrostatic pressure than under atmospheric pressure; piezophiles have optimal growth rates at pressures greater than 1 atm or 0.1 MPa [11]. In this study, we found that microbial diversity varied under different pressure conditions after incubation. With increasing pressure, the microbial diversity tended to decrease and then increase (Fig. 2a); the microbial community showed a decreasing and then an increasing trend (Fig. 2b).

Microbial community assembly processes are controlled by selective and neutral processes at different geographical scales. Regularity of assembly patterns is not consistently observed among different microorganisms and habitats [12, 13]. In contrast, under different pressure conditions, the main driving factors of community assembly were, in order, homogeneous selection (0.1 and 3.5 MPa), undominated (7 MPa), and homogenizing dispersal (10.5 and 14 MPa) (Fig. 3a).

The stability of the microbiome has been attributed to species diversity, and biodiversity has a positive effect on the stability of the microbiome [14]. Species loss usually leads to impaired ecosystem function [15, 16]. Compared with 0.1 MPa, higher pressure significantly increased community stability, and the community remained stable at higher pressures (Fig. 4a). Meanwhile, the AVD index was positively correlated with ASV richness (Fig. 4a). Mantel tests showed that SO_4^{2-},

TOC, TIC, and Co significantly affected community diversity.

4. CONCLUSIONS

In short, this study investigated the environmental heterogeneity of microbial communities under different pressures, the mechanisms driving community diversity, and the patterns of species coexistence. The results provide new insights into the adaptation strategies of microorganisms in deep-sea sediments under different pressures and provide technical and theoretical support for subsequent application of microbial culture. The level of pressure affected the reaction rates of metabolic processes, especially for the carbon cycle. With increasing pressure, the microbial community diversity tended to decrease and then increase. An environmental pressure of 7 MPa was the dividing line between stochastic and deterministic processes. SO_4^{2-}, TOC, TIC, and Co had significant effects on community diversity.

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