FEMS Microbiology Ecology 54 (2005) 141–155

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

Variability in microbial community and venting chemistry in a sediment-hosted backarc hydrothermal system: Impacts of subseafloor phase-separation

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Received 8 December 2004; received in revised form 10 March 2005; accepted 16 March 2005

First published online 28 April 2005

Abstract

Phase-separation and -segregation (boiling/distillation of subseafloor hydrothermal fluids) represent the primary mechanisms causing intra-field variations in vent fluid compositions. To determine whether this geochemical process affects the formation of microbial communities, we examined the microbial communities at three different vent sites located within a few tens meters of one another. In addition to chimney structures, colonization devices capturing subseafloor communities entrained by the vent fluids were studied, using culture-dependent and -independent methods. Microbiological analyses demonstrated the occurrence of distinctive microbial communities in each of the hydrothermal niches. Within a chimney structure, there was a transition from a mixed community of mesophiles and thermophiles in the exterior parts to thermophiles in the interior. Beside the transition within a chimney structure, intra-field variations in microbial communities in vent fluids were apparent. Geochemical analysis demonstrated that different vent fluids have distinctive end-member compositions as a consequence of subseafloor phase-separation and -segregation, which were designated gas-depleted, normal and gas-enriched fluids. In comparison to gas-depleted and normal fluids, gas-enriched fluids harbored more abundant chemolithoautotrophs with gaseous component-dependent energy metabolism, such as hydrogenotrophic methanogenesis. Subseafloor phase-separation and -segregation may play a key role in supplying energy and carbon sources to vent-associated chemolithoautotrophs and subvent microbial communities.

Keywords: Backarc hydrothermal system; Phase-separation; Methanogen; Chemolithoautotroph; Culturability; *Epsilonproteobacteria*

1. Introduction

Deep-sea hydrothermal systems provide physically and geochemically diverse habitats for mesophilic to hyperthermophilic prokaryotes [1]. Recent researches expanded the microbial habitat to subseafloor environments of the mid-ocean ridge hydrothermal systems [2–4]. Microbial communities in a variety of hydrothermal habitats are composed of physiologically and phylogenetically diverse microorganisms [1,5–9]. To address the relationships of the microbial community structures

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0168-6496/$22.00
doi:10.1016/j.femsec.2005.03.007

to the environmental conditions, microbial communities occurring within the chimney structures have been intensively studied by the combined use of quantitative cultivation and culture-independent molecular techniques [10,11]. These works provided new insights into zonation and diversity of microbial communities associated with steep physical and geochemical gradients within the chimney structure. However, they mainly focused on *Archaea* or thermophiles. Thus, it is still unclear how the physical and geochemical parameters are associated with in situ ecophysiological functions of the overall microbial community.

In deep-sea hydrothermal systems, indigenous microbial primary production is achieved by chemolithoautotrophs utilizing H_2, reduced sulfur compounds and CO_2 [1,12]. The energy and carbon sources are provided from magma degassing and/or from the reaction between seawater and rocks at high temperatures [13]. Even in a single hydrothermal field, the amount of energy and carbon sources in the venting fluids significantly varies from vent to vent [13]. This intra-field variation of the fluids is attributed to subseafloor phase-separation and -segregation (different extents of mixing of the vapor and liquid phase) [14]. We hypothesized that this geochemical process might have a considerable impact on the formation of the subvent microbial community and on the supply of energy and carbon sources to chemolithoautotrophs at the seafloor. Among the chemolithoautotrophs occurring in deep-sea hydrothermal environments, members of the *Aquificales*, *Epsilonproteobacteria* and *Methanococcales* have been predominantly detected in various deep-sea hydrothermal fields [4,7,9,15–19]. Although very few cultures of the *Aquificales* and *Epsilonproteobacteria* were reported, a number of isolates within these groups has been recently obtained and characterized as hydrogen- and/or sulfur-oxidizing chemolithoautotrophs [20–29]. Based on their metabolic characteristics, less-selective media were designed here for the quantification of the chemolithoautotrophs with versatile energy metabolism.

We investigated the chimney structures, vent fluids and colonization devices obtained from a deep-sea hydrothermal system, the Iheya North field in the Mid-Okinawa Trough. In this hydrothermal field, seven large hydrothermal mounds having hydrothermal venting or diffusing are localized in North-South direction. The hydrothermal activity center is a large sulfide mound called North Big Chimney (NBC) (approximately 30 m high) [30]. The temperature of the vent fluid is highest within NBC and generally decreases with distance from NBC. Together with the shift in the fluid temperature, variation of the Cl end-member concentration in the different vent fluids is noted. This is explained by an increasing input of vapor-phase into the hydrothermal fluid due to the subseafloor phase-separation [Chiba, H., Ishibashi, J., Kataoka, S., Umeki, Y., Kouzuma, F., Nakayama, N. and Tsunogai, U. (2002) Eos Trans. AGU Fall Meet. Suppl. 83(47), abstr. V72A-1295]. In an effort to understand how physical and geochemical parameters controlled by phase-separation and -segregation are associated with the formation of microbial communities, we performed culture-dependent and -independent analyses on the microbial communities at three vent sites in this hydrothermal system. Our results show the occurrence of distinctive microbial communities in different vent sites.

2. Materials and methods

2.1. Study site

Seven large hydrothermal mounds having vents and diffusing flows, including North Big Chimney (NBC), Central Big Chimney (CBC) and Event 18 (E18), were discovered in the Iheya North field in 1995 (reviewed in [31]). The hydrothermal activity occurs in a backarc volcanic ridge in a continental margin and is hosted by thick (several hundreds meters to several kilometers) organic-rich sediments. As a consequence of the interaction between the sediments and hot fluid, geochemical features of vent fluids in the Iheya North are high alkalinity and high concentrations of NH_4^+, carbon dioxide, hydrogen sulfide and methane [32].

2.2. Sample collection and subsampling

All samples used in this study were obtained by means of manned submersible *Shinkai 2000* in the cruise of April–May 2002. Bulks of chimney structures (~100 g) were obtained from the main vent orifices at the summits of NBC and CBC. Each chimney sample was aseptically subsampled into three sections on board as previously described [11]. Each subsample was further divided and subjected to nucleic acid extraction, microscopic observation and liquid serial dilution culture. For liquid serial dilution culture experiments, the subsamples of the chimneys (~10 g) were slurried with sterile MJ synthetic seawater (~30 ml) [33] containing 0.05% (wt/vol) of Na_2S under N_2 atmosphere, and stored at 4 °C in the dark until they were used. For microscopic observation, the subsamples (~5 g) were fixed with sterile MJ synthetic seawater (~30 ml) containing 3.7% (wt/vol) of formaldehyde. The remaining subsamples were stored at −80 °C for nucleic acid extraction.

In addition to analyzing naturally occurring chimney structures, three in situ colonization systems (ISCSs) [26] were directly deployed into vent orifices in the cruise of June 2000 and recovered in April–May 2002 (Table 1). During both sampling cruises, the maximum temperatures of the vent fluids at NBC and CBC were stable, being 311 and 247 °C, respectively. The temperature of