Note

Abundance of planktonic methane-oxidizing bacteria in a subtropical reservoir

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Abstract: Methane-oxidizing bacteria (MOB) are regarded as key players in aquatic ecosystems, which can mitigate greenhouse gas emissions to the atmosphere. Among phylogenetically diverse MOB, those of the phylum Proteobacteria have been regarded as major methane oxidizers in environments, and they are classified into two major groups, type I and type II. Another group of MOB, Candidatus Methyloirabilis oxyfera and its close relatives have also been detected in various environments, but their predominance over proteobacterial MOB has hardly ever been reported. Feitsui Reservoir (FTR) is a subtropical reservoir situated in Taiwan, where the predominance of M. oxyfera-like phylotypes in a planktonic MOB community was first reported. In this study, planktonic MOB of three types (M. oxyfera-like, type I and type II) were quantified with catalyzed reporter deposition-fluorescence in situ hybridization, to reveal seasonal variation and vertical distribution in the FTR. The MOB were enumerated for 161 samples obtained from 8 water depths on 23 sampling days over 16 months. The results obtained in this study will provide valuable basic data for a better understanding of MOB communities in the environment, giving insights into the global methane budget.

Key words: Candidatus Methyloirabilis oxyfera, methane-oxidizing bacteria, methane, catalyzed reporter deposition-fluorescence in situ hybridization, subtropical reservoir

Methane-oxidizing bacteria (MOB) oxidize methane, an important greenhouse gas that can affect the climate. With their unique physiology, MOB are regarded as key players in aquatic ecosystems and perform remarkable functions. They act as filters to reduce methane released into the air, and thus their activities can mitigate greenhouse gas emissions to the atmosphere. Additionally, MOB convert methane to cellular materials, resulting in reclamation of carbon released from organic matter degradation. At present, all MOB isolated in pure cultures belong to the phyla Proteobacteria or Verrucomicrobia. MOB of the phylum Verrucomicrobia are extremely acidophilic thermophiles, and their habitats are restricted to environments characterized by very low pH and high temperatures. On the other hand, MOB of the phylum Proteobacteria have been regarded as major methane oxidizers in many environments. There are two major groups of MOB in this phylum, type I and type II, belonging to the classes Gammaproteobacteria and Alphaproteobacteria respectively.

In addition to these, there is a phylogenetically distinct group of MOB that has not a single representative currently isolated. This group consists of Candidatus Methyloirabilis oxyfera and its close relatives. They are members of the candidate phylum NC10, and known to be dominant MOB in methane-fed enrichment cultures established under nitrite-reducing conditions (Shen et al. 2015a). For M. oxyfera, its ability to oxidize methane without an external supply of oxygen has been demonstrated (Ettwig et al. 2010). M. oxyfera-like bacteria have been detected in various types of environmental samples (reviewed in Shen et al. 2015b), but their proportion of the whole MOB community was not estimated in these studies. In fact, predominance of the M. oxyfera-like MOB over proteobacterial MOB has hardly ever been reported, except for in cultures designed specifically to enrich them. In a previous study performed in a subtropical reservoir, high relative abundance of the M. oxyfera-like phylotype within the bacterial community was observed in the deep waters of a subtropical reservoir that was characterized by anoxic conditions (Kojima et al. 2014). In the reservoir, Feitsui Reservoir (hereafter, FTR) in Taiwan, a close relative of M. oxy-
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`M. oxyfera` was the most frequently detected species-level phylotype among planktonic MOB detected using 16S rRNA gene sequences (Kojima et al. 2014). The reason for the dominance of this phylotype in the FTR is still unknown, but the unique MOB community in this reservoir provides an important opportunity to study the mechanisms determining community structure of MOB. In this study, the abundance of each type of MOB was analyzed to reveal their seasonal variations and vertical distributions in the FTR, to provide basic data for a better understanding of MOB—indispensable for obtaining a complete picture of the global methane budget.

The study site was the FTR, located in northern Taiwan. Its surface area is 10.2 km², and it can store 406×10⁶ m³ of water, when the water surface elevation is at 170 m above sea level. During the current study period, the elevation of the water surface ranged from 158 to 169 m above sea level, and the water depth at the sampling point was around 100 m. Water samples were collected with a 5-liter Go-Flo bottle (General Oceanics, Miami, FL, USA) at the same sampling point (121°34′E, 24°54′N) that was used in previous studies (Kojima et al. 2014, Itoh et al. 2015). The samples for catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) were taken from 6 depths (0, 10, 30, 50, 70, 90 m) during October 2012 to February 2014 and additional water samples were obtained from depths of 80 m and 100 m. The water samples were fixed with 2% (w/v) paraformaldehyde and were stored in a cooler box until filtration (within 6 hours). Three to five milliliter samples were passed through polycarbonate membrane filters (0.2 µm pore size) and the filters were stored at −30°C until further processing.

CARD-FISH was performed by following a protocol previously described (Pernthaler et al. 2002) with some modifications. The filters were coated with 0.1% (w/v) low-melting-point agarose, and the collected cells were permeabilized with lysozyme solution (consisting of 0.05 M EDTA, 0.1 M Tris-HCl 10 mg ml⁻¹ lysozyme) for 1 hour at 37°C. The specific probe DBACT-1027 (Raghoebarsing et al. 2006) was used to detect the `M. oxyfera`-like organisms. The probes Mγ84, Mγ705 (both specific for type I), and Mα450 (for type II) were used to detect the proteobacterial MOB (Eller et al. 2001). The probes were labeled with horseradish peroxidase. The filters were cut into small pieces and soaked with 315 µl of hybridization buffer containing 0.5 mg ml⁻¹ of each probe. The composition of the hybridization buffer was as follows: 900 mM NaCl, 20 mM Tris-HCl, 10% (w/v) dextran sulfate (average molecular weight >500,000), 0.05% (v/v) TritonX100, and 20–40% (v/v, depending on the probes) formamide. The formamide concentrations were 20% for Mγ84 and Mγ705, 30% for Mα450, and 40% for DBACT-1027. The hybridization was performed for 15 h at 37°C with mild agitation. After hybridization, the filters were washed with washing buffer (20 mM Tris-HCl, 5 mM EDTA, 0.01% SDS) for 15 min at 37°C. Signal amplification was performed with Alexa Fluor 488 tyramide at 37°C for 45 min in the dark. The filters were mounted on glass slides with an anti-fading reagent (Citifluor (Citifluor Ltd, London, UK): Vectashield (Vector Laboratories, Burlingame, CA, USA)=4 : 1] containing 1 µg ml⁻¹ of 4′,6-diamidino-2-phenylindole (DAPI). The hybridized cells were counted under a fluorescence microscope (ZEISS AX10, Carl Zeiss, Jena, Germany).

The cells positive for the MOB-specific probes were enumerated for 161 water samples obtained from 8 depths on 23 sampling days. The seasonal changes in depth-related distributions of the cells hybridized with the probes are visualized in Fig. 1. The cells of type I MOB and the `M. oxyfera`-like phylotype mainly occurred in the deep layers (Fig. 1a, 1b), but the cells of type II MOB were observed at all water depths (Fig. 1c). In the FTR, methane accumulates in an anoxic zone (90 m and greater depths) during period of stratification, but much of it is oxidized in the water column (Itoh et al. 2015). Considering the vertical profile of methane characterized by low concentrations in the upper layers of water, methane con-
sumption around 90 m may play an important role in the control of methane emissions from the FTR. In the present study, it was shown that the numerically dominant MOB in the deep layers were close relatives of ‘M. oxyfera’ and type I MOB (Fig. 1).

In the interpretation of the counts obtained by CARD-FISH, the coverage and specificity of the probes must always be taken into account. The probes DBACT-1027 and M\textsubscript{705} perfectly match all 16S rRNA sequences of targeted MOB, detected in the clone libraries of the water from FTR (Kojima et al. 2014). On the other hand, probes M\textsubscript{705} and M\textsubscript{705} have mismatches to some type I MOB detected in the FTR, and these MOB might have been missed with the probes. Therefore, the actual abundance of type I MOB might be greater than the estimates obtained by these probes. It has also been shown that the diversity of planktonic type I MOB in FTR is greater than those of the other two groups and extends to include several genera (Kojima et al. 2014). Cells stained with the probes for type I MOB would include those of some species belonging to different genera, which may have different ecological characteristics.

This study succeeded in quantifying planktonic MOB in FTR and characterizing their distribution. There is still very limited information about ‘M. oxyfera’-like MOB in various environments, and further quantitative studies are needed to reveal the ecology of these recently discovered methane oxidizers. In particular, the application of CARD-FISH will be useful for unveiling their ecological characteristics and will enhance our understanding of methane dynamics. In previous studies, the abundances of ‘M. oxyfera’-like bacteria in the environment were estimated mainly by qPCR (Shen et al. 2015b). However, it has been demonstrated that this method is not suitable for absolute quantification of ‘M. oxyfera’ (Ettwig et al. 2009), and CARD-FISH has been regarded as a superior quantification method to qPCR because of its lower detection limits and higher accuracy (Coskuner et al. 2005, Burns & Valdivia 2008).

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