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A global analysis on the distribution pattern of the bacteria coupling simultaneous methane oxidation to nitrite reduction

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ABSTRACT

The dominant bacterium *Candidatus* Methylomirabilis oxyfera (NC10 phylum) was first identified from an enrichment culture and subsequently from various other habitats. To date, the global distribution pattern of this bacterium has not been summarized. This study analyzed the distribution pattern and diversity of nitrite-dependent anaerobic methane oxidation (n-damo) bacteria by retrieving 2343 and 2555 16S rRNA gene and *pmoA* sequences from public databases, respectively. The phylogenetic trees showed that the distribution of this bacterium in marine notably distinct from other habitats, and the 16S rRNA gene sequences amplified from enrichment cultures established a separate phylogenetic group. The highest richness of their community was detected in marine habitat. Shannon-Wiener index showed that the diversity of them was higher in marine and coastal habitats without distinctive differences between them. In addition, a significant community difference was also found between the marine and freshwater habitats by calculating ANOSIM *p* values and shared OTUs. PCoA analysis shows that their community composition is habitat specific and shaped by salinity or anthropogenic influence. This analysis provides a synthesis on the global diversity and distribution pattern of this new bacterium with currently available information to date.

1. Introduction

Methane is an important greenhouse gas in the atmosphere (Gray, 2016) and assumed to be inert under anaerobic condition for a long time before the discovery of ANME, which can oxidize methane with different electron acceptors, including sulfate, nitrate and metals (Lu et al., 2016; Mulder et al., 1995; Schreiber et al., 2010). A new anoxic methane oxidation process was discovered by bacteria in the anoxic enrichment cultures under laboratory conditions (Ettwig et al., 2008; Raghoebarsing et al., 2006). The dominant bacterium in this culture was identified as a member of a new candidate phylum NC10, and named as *Candidatus* Methylomirabilis oxyfera (Ettwig et al., 2009; Hu et al., 2009). It could oxidize methane coupled with denitrification process under anoxic conditions, and to date, this so-called nitrite-dependent anaerobic methane oxidation (n-damo) bacterium is a unique microorganism that links carbon and nitrogen cycles in the environment. The metagenomic analysis on *M*. oxyfera shows that it could conduct both anoxic and aerobic metabolic pathways (Ettwig et al., 2010). *pmoCAB* operon, which codes the particulate methane monooxygenase (pMMO), is harbored in the 2.7 Mb circular single chromosome and leads the complete aerobic methane oxidation pathway. With the development of PCR primers specifically for n-damo bacterial 16S rRNA gene and α-subunit of pMMO gene (*pmoA*), n-damo bacteria have been successfully retrieved from different nature environments by molecular detection (Deutzmann and Schink, 2011; Ettwig et al., 2009; Luesken et al., 2011c; Chen et al., 2014, 2015).

At the beginning of the study on n-damo bacteria, bioreactor and enrichment cultures were the main focus since they were firstly discovered from enriched communities and higher abundance of them (Ettwig et al., 2009; Hu et al., 2009, 2011; Luesken et al., 2011a, 2011b; 2012; Wu et al., 2011a, 2011b). Recently, investigations on the diversity and distribution of n-damo bacteria have been conducted in different habitats, such as lakes, rivers, wetlands, coasts and marine environments. The presences of n-damo bacteria in the lacustrine ecosystem were detected from Lake Constance in German (Deutzmann and Schink, 2011; Deutzmann et al., 2011), Lake Biwa in Japan (Kojima et al., 2012) and Baiyangdian Lake, Chaohu Lake and Yunnan plateau lakes in China (Liu et al., 2015; Zhu et al., 2015). While the researches

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of fluvial n-damo bacteria were mainly conducted on the rivers located in China, like Qiantang River, Pearl River, and Shahe River (Shen et al., 2014; Zhu et al., 2015). In addition, wetland is confirmed to be another suitable habitat for n-damo bacteria grow (Hu et al., 2014; Shen et al., 2015a, 2015b; Wang et al., 2012; Chen et al., 2014, 2015; Zhou et al., 2014; Zhu et al., 2015). Among these studies, most communities were detected in freshwater lakes, rivers and wetlands except two saline lakes located in Qinghai-Tibetan. n-damo bacteria were also been retrieved from some non-freshwater environments, like estuary (Shen et al., 2014; Yan et al., 2015a,b) and coastal wetlands (Chen et al., 2015). However, marine n-damo bacteria were less investigated than other habitats (Chen et al., 2014; He et al., 2015; Shen et al., 2014).

Beside the geographic feature, environmental factors also influence bacterial community significantly. Salinity is a key factor that affects microorganism community by changing its diversity and distribution due to high salinity increases the osmotic potential and alters the ionic composition in the samples (Yan et al., 2015a,b). Many evidences are available from previous studies showing that salinity shapes the microbial community structure and affects microbial activity and biomass (Andronov et al., 2012; Batra and Manna, 2009; Rousk et al., 2011; Setia et al., 2011). Similarity, salinity strongly affects n-damo communities, especially in marine habitat (Chen et al., 2014). In addition, the sample type is another important factor dictating the bacterial community. As communities would be different in sediments, soils and aquifers resulting from different water content, biomass abundance and nutrient composition. It has been reported that denitrifying microbial community and methane oxidation bacteria are both influenced by water contents (Henckel et al., 1999; Stres et al., 2008). Also, the concentration of total carbon or nitrogen, organic matter, inorganic matter, ammonium and nitrate/nitrite are other parameters influencing the diversity of the n-damo community (Chen et al., 2015, 2014; Hatamoto et al., 2014; Liu et al., 2015; Shen et al., 2014; Wang et al., 2012; Yan et al., 2015a,b).

Notably, n-damo bacteria have been detected in various natural ecosystems. However, all the previous molecular studies on this type of bacteria were focused on the individual source of a single habitat or sample type except only one research on geographical distribution of them in Chinese wetland ecosystems (Zhu et al., 2015). Therefore, the overall understanding of global ecological pattern of this microorganism still remains unclear, especially between the different habitats. In addition, how ecological factors shaped the community assembly of them on a global scale and whether the phylogenic relationship corresponds with habitat or other scales are still unanswered questions. To further advance our understanding and knowledge, meta-analyses is a useful method that can explain the phylogeographical clues on microbial community as used on bacteria (Lozupone and Knight, 2007), archaea (Auguet et al., 2010) and denitrifiers (Cao et al., 2013; Sonthiphand et al., 2014).

In the current study, we investigated the global nitrite-dependent methane oxidizing bacterial distribution pattern and diversity, and delineated the environmental factors influencing the bacterial community. n-damo bacterial 16S rRNA gene and *pmoA* sequences from public sources and gene repositories were gathered and analyzed by phylogenetic and multivariate statistical approach. Phylogenetic trees based on both 16S rRNA and *pmoA* gene were constructed for different environments, including lakes, rivers, wetlands, paddy soils, reedbeds, reservoirs, coasts, estuary, peatlands, ground waters, bioreactors, WWTPs, marine and deep sea. These environments were classified into six habitat groups: lake, river, wetland, marine, coast and enrichment. Alpha-diversity and beta-diversity of n-damo bacteria across these habitats were indicated by Chao1 value, Shannon-Wiener index and ANOSIM's R value, shared OTU and PCoA analysis. The influence of salinity and sample type on phylogeny and distribution were identified among all factors in this study. This study is an attempt to provide a better understanding of the prevalence of n-damo bacterial diversity and distribution based on specific 16S rRNA and *pmoA* gene across different habitats and key variables impacting their community patterns.

2. Materials and methods

2.1. Data set construction

All nitrite-dependent anaerobic methane oxidizing bacterial 16S rRNA gene and *pmoA* nucleotide sequences surveyed from both published literature and GenBank Database were extracted on February 2017. All retrieved sequences matched the following criteria: (i) high-quality data without ambiguities, meaning they are identified as the genes belong to n-damo bacteria; (ii) sequences no less than 300 bp since shorter sequences may manipulate statistic or phylogenetic consequences; (iii) most of the identified PCR products were recovered by the same primers to cover the same gene region. The main primers for 16S rRNA gene was qp1F and qp2R, while *pmoA* were mostly amplified using nested PCR with the first step primer set of A189F and cmo682, followed by a second step primer set of cmo182 and cmo568. To screen n-damo bacteria-related sequences, collected database was searched by BLAST against known n-damo species. We homogenized the variations in methodologies and sampling efforts among different studies by clustering sequences at 95% threshold value. After removing non-n-damo and low-quality sequences, two globally distributed n-damo bacterial databases with 2343 16S rRNA sequences from 64 clone libraries and 2555 *pmoA* sequences from 66 clone libraries were assembled for further analysis (Table S1).

The different clone libraries were classified based on the information obtained from published literature or inferred from the described details (sample type, location and description) presented in GenBank annotations of unpublished studies. Sequences retrieved from individual site were treated as a single clone library, even if they were taken from one mixed ecological area in the same study. After considering all the characteristics of the environmental geochemistry, 6 habitat groups (river, coast, marine, wetland, lake and enrichment), 3 sample types (sediment, soil, aquifer) and 3 different levels of salinity (saline/ocean, mixture/coast and freshwater) were assigned to these data.

2.2. Community phylogenetic construction

All defined nucleotide sequences were checked for quality using a Perl script that determines the chimeras and the number of base-calling errors in each sequence. Software MAFFT (Katoh and Standley, 2013) was applied to align all the sequences in the database. Too divergent regions and poorly aligned positions were trimmed using the Gbloks (Castresana, 2000) and DNA sequences less than 300 bp were also removed after trimming, resulting in the fragments with 411 bp and 389 bp length of conserved region corresponded for 16S rRNA gene and *pmoA* for the further analysis. Representative OTU sequences were defined by software Mothur (Schloss et al., 2009) with 97% cutoff from the database. A total of 356 and 362 OTU sequences of 16S rRNA gene and *pmoA* across all data were used to generate the phylogenetic inference. Phylogenetic trees were constructed with CIPRES science gateway and estimated by maximum likelihood criterion with RAxML-HPC BlackBox (Miller et al., 2010). Nodes supported for trees were determined using fast bootstrapping option with 1000 bootstrap replicates, and the output images were visualized and modified by iTOL.

2.3. Diversity analysis

Based on the classification of the 6 habitat groups mentioned before, the α-diversity indices Shannon-Wiener, rarefaction and richness indicator Chao1 for each group were calculated by Mothur program (Chao, 1984; “Measuring Biological Diversity,” 2013). Boxplot was used to present the value of Shannon-Wiener indices. The similarities