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Bacterial and archaeal community structure of pan-Arctic Ocean sediments revealed by pyrosequencing

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Abstract

This study was to investigate bacterial and archaeal community structure of pan-Arctic Ocean sediments by pyrosequencing. In total, investigation of three marine sediments revealed 15 002 bacterial and 4 362 archaeal operational taxonomic units (OTUs) at the 97% similarity level. Analysis of community structure indicated that these three samples had high bacterial and archaeal diversity. The most relatively abundant bacterial group in Samples CC1 and R05 was Proteobacteria, while Firmicutes was dominant in Sample BL03. Thaumarchaeota was the most relatively abundant archaeal phylum in Samples CC1 and R05, and the relative abundance of Thaumarchaeota was almost as high as that of Euryarchaeota in Sample BL03. These two phyla accounted for nearly 100% of the archaeal OTUs. δ-Proteobacteria and γ-Proteobacteria were the two most relatively abundant classes at Proteobacterial class level, and their relative abundance was more than 60% in Samples CC1 and R05. There were also differences in the top 10 relatively abundant bacterial and archaeal OTUs among the three samples at the 97% similarity, and only 12 core bacterial OTUs were detected. Overall, this study indicated that there were distinct microbial communities and many unique OTUs in these three samples.

Key words: Arctic sediment, microbial community, diversity, pyrosequencing

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1 Introduction

The pan-Arctic region has various distinct habitats for microbes such as oceanic water, sea-ice, glacial ice, permafrost, tundra wetlands, subglacial soil, periglacial soil, and tundra soil (Reddy et al., 2009). Marine sediment represents one of the most complex microbial habitats on Earth. In these areas, microorganisms contribute to bulk biomass and play an important role in remineralization of organic matter (Ravenschlag et al., 2000; Tian et al., 2009). Microbial communities and their associated metabolic activities in marine sediment have profound impacts on global biogeochemical cycles (Jorgensen et al., 2012).

Marine sediment hosts the largest reservoir of organic carbon in the world and outnumbers any other environment with respect to microbial cell abundance (Whitman et al., 1998; Jorgensen et al., 2012). The heterogeneous nature of Arctic marine sediments implies that the microbial community structure might differ from geographical areas and depths of sediments throughout the pan-Arctic Ocean. The omnipresence of prokaryotic cells has recently been demonstrated (Jørgensen and Boetius, 2007; Roussel et al., 2008). In general, the microbial community in marine sediment appears to be dominated by a restricted number of bacterial and archaeal phyla (Jorgensen et al., 2012).

Compared with seawater, organic matter is 10^4–10^5 fold concentrated in sediments and is used as an energy source by microorganisms. Consequently, Arctic is regarded as a carbon sink (Trevors et al., 2010). Bacteria in sediment represent a major reservoir of genetic variability, similar to soil systems that show approximately 10^4 species per gram (Torsvik et al., 2002; Zeng et al., 2011). The density of prokaryotic cells in coastal and continental margin sediments is typically 10^8–10^9 cells/cm^3 in the top sediment layers and declines with the depth in a logarithmic fashion (Jorgensen et al., 2012).

Archaea are best known for their ability to thrive in extreme environments; however, recent molecular studies have shown that organisms from this prokaryotic domain of life are ubiquitous. Archaea play important roles in essential environmental processes such as the carbon and nitrogen cycles (Chaban et al., 2006; Albers and Pohlschröder, 2009). Despite their significant ecological roles, most archaeal community have been analyzed in hydrothermal sediments (Kormas et al., 2006; Wang et al., 2009; Nunoura et al., 2010) and hot springs (Segerer et al., 1993; Hetzer et al., 2007), while there have been only a few studies on the archaeal community of Arctic sediments based on traditional culture-independent approaches (Tian et al., 2009; Hamdan et al., 2013). It is now widely accepted that microbial community analysis should be culture-independent, and utilize molecular identification methods such as sequencing of 16S rRNA genes (Chun et al., 2010). Several studies on the bacterial diversity in Arctic marine sediments have been carried out using traditional culture-independent approaches such as PCR-DGGE and 16S rRNA libraries, and these studies have revealed higher diverse microbial communities than that characterized by conventional culture-dependent approaches (Li et al., 2009; Tian et al., 2009;

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Zeng et al., 2011). However, investigation of microbial community is insufficient due to the low throughput of traditional culture-independent approaches (Zeng et al., 2013). In recent years, pyrosequencing has been employed in various microbiological disciplines (Rothberg and Leamon, 2008; Jaenicke et al., 2011; Abbai et al., 2012; Li et al., 2013).

Pyrosequencing has been used to enumerate and contrast marine microbial diversity in Arctic waters (Galand et al., 2009; Kirchman et al., 2010; Comeau et al., 2011; Bowman et al., 2012; Comeau et al., 2012; Zeng et al., 2013). However, there has been few investigation of the microbial community of Arctic sediments by pyrosequencing (Hamdan et al., 2013). In this study, the bacterial and archaeal community structures of three pan-Arctic Ocean sediments were investigated using the Roche 454 GS FLX Titanium platform, and the differences in microbial community among these samples were discussed.

2 Materials and methods

2.1 *Samples and chemical analysis*

Surface marine sediment (0–5 cm) was collected during the 5th Chinese National Arctic Expedition from July to September, 2012, and stored at –80°C until further analysis. Total nitrogen (TN), total carbon (TC) and total organic carbon (TOC) contents in the samples were analyzed using elemental analyser (Vario EL III, Elementar, Germany). The sampling dates, locations, depths and some physicochemical characters of samples were summarized in Table 1.

Table 1. Summary of Arctic marine sediments for microbial community structure analysis

Sample	Date	Location	Depth/m	TN/%	TOC/%	TC/%	Description
BL03	2012-07-12	53°58.934′N, 170°42.858′E	3 613	0.11	0.59	0.68	yellow clay
CC1	2012-07-18	67°28.615′N, 168°36.263′W	43.3	0.22	1.32	1.56	yellow-green silty clay
R05	2012-07-20	70°58.661′N, 168°46.135′W	36.9	0.12	0.95	1.07	yellow-green clayey silt

2.2 *DNA extraction, amplification and sequencing*

DNA was extracted from 1 g sediment using PowerMax Soil DNA isolation Kit (12988-10, MOBIO Laboratories, Inc, Carlsbad, CA) following the manufacturer's protocol.

The V1-V3 hypervariable regions of the bacterial 16S rRNA gene were amplified using the forward primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer 533R (5′-TTACCGCGGCTGCTGGCAC-3′) (Zeng et al., 2013). The partial archaeal 16S rRNA gene was amplified using the forward primer Arch341F (5′-ACGGGGYGCAGCAGGCGCGA-3′) and the reverse primer Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (Zheng et al., 2013). The target 16S rRNA gene fragments were pyrosequenced using the Roche 454 Genome Sequencer FLX Titanium platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China, as previously described (Wu et al., 2012).

2.3 *Filtering and removal of noise from amplicon sequence data*

Sequencing reads from different samples were separated by unique barcodes. The barcode, linker, and PCR primer sequences at both sides were then removed from the original sequencing reads. Next, the dataset was filtered and noise was removed using software Seqcln (http://sourceforge.net/projects/seqclean) and Mothur (http://www.mothur.org/wiki/Main_Page).

2.4 *Sequence analysis*

The raw sequence data were processed, and the reads were removed if at least one of the following criteria was met: (1) the average quality score was lower than 25, (2) reads were shorter than 200 bp, and (3) number of ambiguous bases was greater than 0.

The Mothur database (http://www.mothur.org/wiki/main_page) was used for taxonomic assignment of each pyrosequencing read. All sequences were classified from phylum to species according to the Mothur program using the default setting. If the similarity was below the cutoff point, the read was assigned to an unclassified group.

2.5 *Diversity and community structure analysis*

Sequences were aligned and compared with those available in the Bacterial SILVA database (http://www.arb-silva.de) using the Kmer Searching method (http://www.mothur.org/wiki/align.seqs) and representative sequences for shared OTUs were obtained. The Good's coverage, the index of community richness (Chao estimator and Ace estimator) and the index of community diversity (Shannon index and Simpson index) were also obtained using the method described at http://www.mothur.org/wiki.

2.6 *Nucleotide sequence accession numbers*

Bacterial and Archaeal 16S rRNA gene sequences derived from pyrosequencing have been deposited in GenBank under accession number SRP034720, SRP034817 and SRP034818.

3 Results

3.1 *Overview of pyrosequencing*

In this study, the bacterial and archaeal communities of three pan-Arctic Ocean sediments collected from different latitude were investigated. Pyrosequencing using the Roche 454 GS FLX Titanium system yielded a total of 79 124 valid bacterial sequences with an average length of 411 bp, and a total of 39 526 valid archaeal sequences with an average length of 362 bp. After the low quality reads were discarded, the final dataset contained 59 130 bacterial and 24 110 archaeal trimmed sequences in total. The number of different bacterial/archaeal sequences, namely OTUs, decreased substantially when placed into groups that share $\geqslant$97%, $\geqslant$95% and $\geqslant$90% similarity. At the 97% similarity level, a total of 3 640, 5 768, and 5 594 bacterial OTUs, and a total of 1 425, 1 165, and 1 772 archaeal OTUs were obtained from Samples BL03, CC1 and R05 respectively (Table 2).

3.2 *Analysis of community diversity*

The richness and diversity indexes of the microbial community structure at the 97% similarity level were summarized in Table 3. The higher Good's coverage of 0.88 (bacteria) and 0.90 (archaea) suggested that the number of unique sequence types sampled from these libraries almost approached the total number of unique sequences (Chelius and Triplett, 2001). The high Shannon index and low Simpson index indicated high bacterial and archaeal diversity in these sediments. As a whole, the overall bacterial richness was notably higher than the archaeal richness.

Table 2. Summary of pyrosequencing results

	BL03		CC1		R05	
	Bacteria	Archaea	Bacteria	Archaea	Bacteria	Archaea
Valid sequences	17 071	9 615	36 255	13 872	25 798	16 039
Numbers of bases/bp	7 945 995	4 524 990	13 664 699	4 555 485	10 879 733	5 237 575
Average length/bp	466	471	377	328	421	327
Trimed sequences	14 340	6 927	24 769	7 826	20 021	9 357
⩾97% clusters per sample	3 640	1 425	5 768	1 165	5 594	1 772
⩾95% clusters per sample	2 371	1 056	4 206	868	4 306	1 341
⩾90% clusters per sample	1 612	625	2 609	520	2 741	765

Table 3. Diversity indexes of microbial community structure (97% similarity level)

Sample	Domain	OTU	Coverage	Ace	Chao	Shannon	Simpson
BL03	Bacteria	3 640	0.88	6 914	5 473	6.01	0.046
	Archaea	1 425	0.90	3 334	2 426	6.24	0.004
CC1	Bacteria	5 768	0.81	26 073	14 355	7.00	0.005
	Archaea	1 165	0.88	3 510	2 421	4.75	0.065
R05	Bacteria	5 594	0.76	25 836	14 717	6.96	0.014
	Archaea	1 772	0.85	7 520	4 270	5.59	0.018

The bacterial richness of Sample BL03 was lower than that of Samples CC1 and R05, probably due to the lower content of TN, TOC and TC in Sample BL03 (Table 1). There was a positive correlations between bacterial richness and a total of TN, TOC and TC contents among the three samples we tested (R^2=0.70). However, there were no significant differences in archaeal richness among the three samples tested. These results further confirm that marine sediment is a complex microbial habitat with high microbial diversity that contributes to bulk biomass.

3.3 *Taxonomic composition*

All bacterial and archaeal sequences were classified from phylum to species according to the Mothur program (http://www.mothur.org/wiki/classify.seqs). The trimmed bacterial sequences were assigned to 24, 20 and 23 phyla or groups from Samples BL03, CC1 and R05 respectively. However, only three archaeal phyla and one unclassified archaea were assigned in each sample.

Although the same taxa were generally present in all samples, the relative abundance varied substantially among different microbial communities. The ten most relatively abundant bacterial phyla of each sample were shown as Fig. 1. Although the composition of the ten most relatively abundant phyla seemed similar, their relative abundances were remarkably different. Compared with Sample BL03, Samples CC1 and R05 shared a more similar bacterial community structure at the phylum level. Proteobacteria was most relatively abundant in Samples CC1 and R05, and the sequences belonging to this phylum accounted for more than 60% of the bacterial sequences. However, Firmicutes was the most relatively abundant phylum in Sample BL03, and sequences affiliated with this phylum accounted for more than 40% of the bacterial sequences.

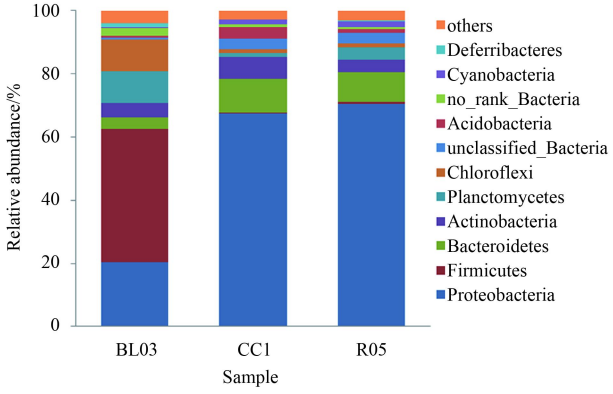

Fig. 1. Taxonomic distributions of the ten most relatively abundant bacterial phyla in three Arctic sediments. Relative read abundance of different bacterial phyla within the different communities.

The three samples contained three archaeal phyla and one unclassified archaea. Thaumarchaeota and Euryarchaeota were the dominant phyla, and the sequences belonging to these two phyla accounted for almost 100% of the total archaeal sequences (Fig. 2). Compared with Euryarchaeota, the sequences belonging to Thaumarchaeota were more abundant, and the relative abundance was nearly 80% in Samples CC1 and R05.

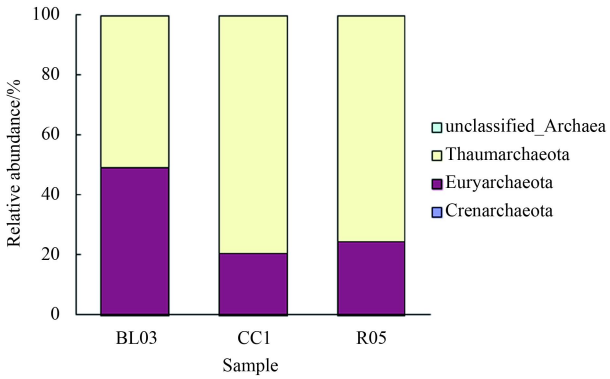

Fig. 2. Taxonomic distributions of archaeal phyla in three Arctic sediments. Relative read abundance of different archaeal phyla within the different communities.

3.4 *Taxonomic distributions of Proteobacterial and archaeal class*

Proteobacteria was the most relatively abundant bacterial phylum among the three samples investigated. All Proteobacterial classes except ζ-Proteobacteria (Zetaproteobacteria) existed in each of these samples, and their taxonomic distributions also ex-

hibited differences (Fig. 3). δ-Proteobacteria and γ-Proteobacteria were the two most abundant classes among the three samples, the relative abundance ranged from 9.3% (BL03) to 47.4% (R05) and from 5.3% (BL03) to 31.6% (CC1) respectively.

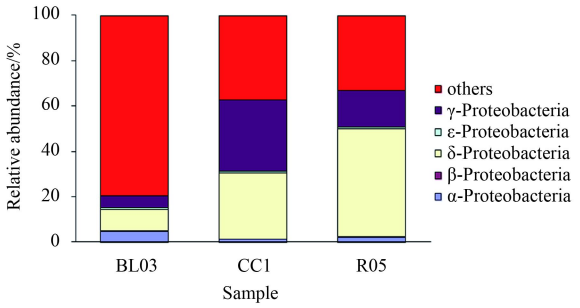

Fig. 3. Relative abundance of different proteobacteria classes within different communities. Others represent all classes except those belonging to Proteobacteria.

There was also similar taxonomic composition of archaeal class between Sample CC1 and Sample R05. The sequences affiliated with no_rank_Thaumarchaeota were dominant among the three samples, and it was up to 80% in Sample CC1. Halobacteria, a class belonging to Euryarchaeota, was the major class following no_rank_ Thaumarchaeota in all these three samples investigated (Fig. 4).

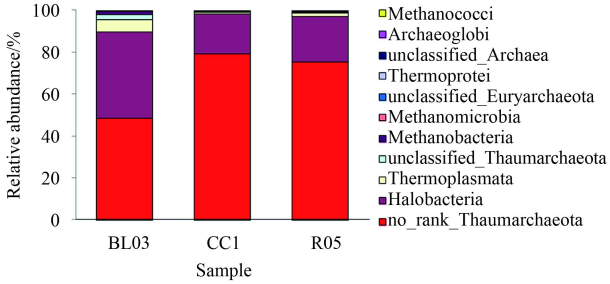

Fig. 4. Relative abundance of different archaeal classes within different communities.

3.5 *Ten most abundant bacterial and archaeal OTUs at the 97% similarity level*

The ten most abundant bacterial OTUs at the 97% similarity level were summarized to further understand the important bacteria in pan-Arctic sediments (Table 4). There were distinct differences among the ten most abundant OTUs in the three samples. The most abundant OTU in Sample BL03 was affiliated with *Lactococcus piscium*. The high relative abundance of 32.7% implied that this species was the dominant species in Sample BL03. Conversely, the relative abundance of the most abundant OTU in Samples CC1 and R05 accounted for only 5.8% and 10.9%, respectively, indicating that there was no obvious dominant species among these samples.

There was obvious dominant archaeal OTUs in all three samples, the relative abundance of sequences affiliated with Group_C3_uncultured_crenarchaeote was up to 66.1% in Sample CC1. The abundant OTU in Sample R05 were affiliated with Group_C3_uncultured_crenarchaeote, Candidatus_ Nitrosopumilus_unidentified_archaeon and Deep_Sea_Hydrothermal_Vent_Gp_6_ marine_metagenome, with a total abundance of up to 80.4%. Even in Sample BL03, the relative abundance of OTU affiliated with Deep_Sea_Hydrothermal_Vent_Gp_6_uncultured_ archaeon was also up to 20.6% (Table 5). These findings indicated that archaeal diversity was lower than bacterial diversity, with the relative abundance of the top ten OTUs being more than 90% in Samples CC1 and R05, while that of bacteria was only about 50%.

3.6 *Abundant versus rare bacterial OTUs*

The Venn diagram shows the number of OTUs unique to a single sample or shared by two or three samples. Abundant or core bacterial OTUs at the 97% similarity level were compared with rare and unique OTUs among the three samples (Fig. 5a). Only 12 core bacterial OTUs were detected in the three samples, most of which were affiliated with Proteobacteria. The vast majority of OTUs were singletons. For example, they accounted for 98.95% of total bacterial OTUs in Sample BL03. Sample BL03 only shared 25 and 37 OTUs with Samples CC1 and R05, respectively; however, Sample CC1 shared 1401 OTUs with Sample R05, which implied that there were similar bacterial communities in these two samples. The dendrogram also indicated that Samples CC1 and R05 had similar bacterial communities (Fig. 5b).

4 Discussion

High-throughput pyrosequencing, a newly-developed powerful tool for studying microbial communities, has been used to investigate marine bacterial communities in the polar region (Bowman et al., 2012; Zeng et al., 2013; Hamdan et al., 2013). Compared with the previous studies on bacterial diversity by conventional culture-independent approaches (Tian et al., 2009; Zeng et al., 2011), higher diversity was detected by pyrosequencing, although both approaches revealed a similar composition of the major bacterial groups. The bacterial diversity based on the 16S rRNA clone library showed that sequences of sediment from the deep Sta. DBS1 fell into 15 phyla (Zeng et al., 2011), which were also detected in our study; however, our study showed that more than 20 bacterial phyla were presented in the three samples investigated. The newly-detected bacterial phyla included Aquificae, Armatimonadetes, Spirochaetes and Gemmatimonadetes. Only nine bacterial phyla were identified from 463 clones from the 16S rRNA clone library of four surface sediment samples (0–5 cm) collected from the Pacific Arctic Ocean (Li et al., 2009). The bacterial community richness, Chao estimator and ACE estimator, were more than 20 000; meanwhile, there were more than 5 000 OTUs at the level of 97% similarity. However, a previous study based on a 16S rRNA library showed a species richness of only about 250 (Zeng et al., 2011).

As a whole, due to the similar depth, TN, TC and TOC contents, there were similar bacterial and archaeal communities in Samples CC1 and R05. Taxonomic distributions of the ten most abundant phyla of bacteria (Fig. 1) and archaea (Fig. 2) in the three samples indicated Samples CC1 and R05 had similar microbial community structure. Furthermore, there were much more shared OTUs between Sample CC1 and Sample R05 (Fig. 5a); meanwhile, the dendrogram also showed there were similar bacterial communities in these two samples (Fig. 5b).

Proteobacteria were dominant in Samples CC1 and R05, and it was similar to the previous studies in the Arctic sediment by conventional culture-independent approaches (Tian et al., 2009; Zeng et al., 2011). However, the relative abundance of Proteobacteria class was different from previous studies (Tian et al., 2009). It was notably that only ζ-Proteobacteria of six Proteobacterial classes was not detected in our study, which was consistent with

Table 4. Ten most abundant bacterial OTUs at the 97% similarity

Sample	Top 10 species	Relative abundance/%
BL03	*Lactococcus_piscium*	32.70
	*Dehalococcoides*_sp._enrichment_culture_clone_ATV1-1	5.62
	MSBL9_uncultured_Planctomyces_sp.	2.72
	MSBL9_uncultured_bacterium	2.11
	Sva0081_sediment_group_metagenome_sequence	1.91
	*Desulfovibrio*_sp._Mic1c01	1.34
	Desulfuromonadales_bacterium_Tc37	1.27
	*Thermodesulfobium_narugens*e	1.26
	Planctomycetaceae_uncultured_marine_metagenome	1.25
	Rhodospirillaceae_uncultured_marine_metagenome	1.15
	others	48.67
CC1	*Acidiferrobacter*_uncultured_marine_bacterium	5.76
	unclassified_Marinicella	4.85
	Marinicella_uncultured_sediment_bacterium	4.62
	Sva1033_uncultured_bacterium	4.13
	unclassified_Sinobacteraceae	4.07
	unclassified_Flavobacteriaceae	3.60
	unclassified_Proteobacteria	3.55
	unclassified_Bacteria	3.31
	uncultured_delta_proteobacterium_Sva1041	3.13
	unclassified_Sandaracinaceae	3.09
	others	59.89
R05	Sva1033_uncultured_bacterium	10.93
	uncultured_delta_proteobacterium_Sva1041	6.05
	unclassified_Desulfuromonadales	5.85
	*Desulforhopalus*_uncultured_delta_proteobacterium	5.70
	unclassified_Sinobacteraceae	3.83
	unclassified_Bacteria	3.38
	unclassified_Proteobacteria	3.21
	unclassified_Sandaracinaceae	2.63
	unclassified_Marinicella	2.36
	uncultured_delta_proteobacterium_Sva0103	2.19
	others	53.87

the investigation of Hamdan et al. (2013). Firmicutes was the dominant phyla in Sample BL03, which was also consistent with the results of Hamdan et al. (2013) showing that Firmicutes and Chloroflexi were the dominant phyla in Arctic sediments.

There were more TN and TOC in Samples CC1 and R05 (Table 1), which might have been due to the rich nutrient input by the Pacific waters flowing northward through the Bering Strait and more organic materials settling in the sediments (Cooper et al., 1997), and the rich nutrients from the mainland and increased nutrients sedimented from the surface water. Correspondingly, these two samples had higher bacterial richness than Sample BL03. Furthermore, due to the similar depth and physicochemical characters (Table 1), Samples CC1 and R05 had similar microbial community.

The diversity of archaea generally appears to be much lower than that of bacteria, and marine archaea could be 5–10 times less diverse than bacteria (Galand et al., 2009). Thaumarchaeota, the dominant archaeal phylum among the three samples investigated, is a phylum of Archaea that was proposed in 2008 after the genome of *Cenarchaeum symbiosum* was sequenced and found to differ significantly from other members of the hyperthermophilic phylum Crenarchaeota (Brochier-Armanet et al., 2008). This recently proposed phylum may represent the deepest branching lineage in the archaeal phylogeny emerging before the divergence between Euryarchaeota and Crenarchaeota (Muller et al., 2010). All organisms of this lineage identified to date are chemolithoautotrophic ammonia-oxidizers that may play important roles in biogeochemical cycles, such as the nitrogen cycle and the carbon cycle. This novel phylum comprises all known archaeal ammonia oxidizers as well as several clusters of environmental sequences representing microorganisms with unknown energy metabolism (Pester et al., 2011). The dominance of ammonia-oxidizing Thaumarchaeota among the three samples tested indicates that this archaeal phylum might play an important role in the nitrogen and carbon cycles (Wang et al., 2009).

According to the definition of Galand et al. (2009), abundant phylotypes are those representing grater than 1% within a sample, while rare phylotypes have an abundance less than 0.01% within a sample. Sequences affiliated with *Lactococcus piscium* accounted for 32.7% of total bacterial sequences in Sample Bl03; however, this sample also comprised a higher number bacterial OTUs (206) containing only one trimmed sequence, which only accounted for 1.46% of the total bacterial trimmed sequences. *L. piscium* was firstly isolated from salmonid fish and proposed as a novel strain by Williams et al. (1990). *L. piscium* was shown to grow in a variety of modified atmosphere packaged (MAP) meat products, including broiler, pork, turkey, and minced meat made of beef and pork, where they belonged to the

Table 5. Ten most abundant archaeal OTUs at the 97% similarity

Sample	Top 10 OTU	Relative abundance/%
BL03	Deep_Sea_Hydrothermal_Vent_Gp_6_uncultured_archaeon	20.63
	Marine_Benthic_Group_B_uncultured_archaeon	18.04
	Deep_Sea_Euryarchaeotic_Group_uncultured_archaeon	6.68
	Marine_Benthic_Group_A_uncultured_archaeon	6.36
	Marine_Group_I_uncultured_sediment_archaeon	4.42
	Deep_Sea_Hydrothermal_Vent_Gp_6_uncultured_crenarchaeote	3.49
	Candidatus_Nitrosopumilus_uncultured_sediment_archaeon	3.16
	Deep_Sea_Hydrothermal_Vent_Gp_6_uncultured_euryarchaeote	3.14
	Marine_Group_I_uncultured_marine_group_1_crenarchaeote	2.33
	Miscellaneous_Euryarchaeotic_Group_uncultured_archaeon	2.25
	others	29.5
CC1	Group_C3_uncultured_crenarchaeote	66.07
	Deep_Sea_Hydrothermal_Vent_Gp_6_marine_metagenome	16.97
	Candidatus_Nitrosopumilus_unidentified_archaeon	2.42
	uncultured_Desulfurococcus_sp.	1.43
	Miscellaneous_Crenarchaeotic_Group_uncultured_crenarchaeote	1.25
	Marine_Benthic_Group_B_unidentified_archaeon	0.58
	Marine_Benthic_Group_D_and_DHVEG-1_unidentified_archaeon	0.53
	Marine_Group_I_unidentified_archaeon	0.40
	Methanobacteriaceae_archaeon_RMAS	0.25
	Candidatus_Parvarchaeum_unidentified_archaeon	0.20
	others	8.90
R05	Group_C3_uncultured_crenarchaeote	30.35
	Candidatus_Nitrosopumilus_unidentified_archaeon	29.91
	Deep_Sea_Hydrothermal_Vent_Gp_6_marine_metagenome	20.13
	Marine_Group_I_unidentified_archaeon	5.47
	Candidatus_*Nitrosopumilus*_uncultured_bacterium	2.70
	Candidatus_*Nitrosopumilus*_sp._NM25	1.50
	uncultured_*Desulfurococcus*_sp.	1.00
	VC2.1_Arc6_uncultured_crenarchaeote	0.79
	*Methanococcoides*_sp._MO-MCD	0.54
	Miscellaneous_Crenarchaeotic_Group_uncultured_crenarchaeote	0.50
	others	7.11

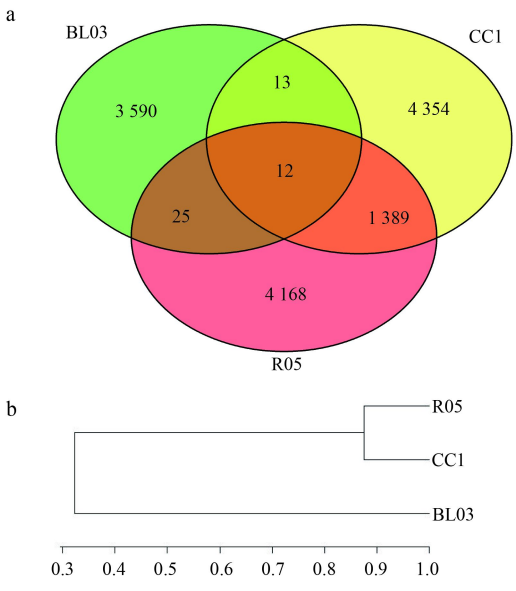

Fig. 5. Comparison of the bacterial community in three samples. a. Venn diagram showing the unique and shared OTUs at the 97% similarity level and b. dendrogram implying the similarity of microbial communities in Samples CC1 and R05.

predominating microbiota (Rahkila et al., 2012). The reason that why so many sequences were affiliated with *L. piscium* in BL03 was unclear and much more sediments near this location should be pyrosequenced.

The low coverage obtained even with pyrosequencing and the fact that the rarefaction curve did not level off indicate that more data should be obtained using this approach to better understand the complex microbial communities in marine sediments. In addition, due to the heterogeneous natures of marine sediments, large-scale pyrosequencing should be carried out, which might lead to intriguing observations that encourage future analysis.

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References

Abbai N S, Govender A, Shaik R, et al. 2012. Pyrosequence analysis of unamplified and whole genome amplified DNA from hydrocarbon-contaminated groundwater. Mol Biotechnol, 50(1): 39–48

Albers S V, Pohlschröder M. 2009. Diversity of archaeal type IV pilin-like structures. Extremophiles, 13(3): 403–410

Bowman J S, Rasmussen S, Blom N, et al. 2012. Microbial community structure of Arctic multiyear sea ice and surface seawater by

454 sequencing of the 16S RNA gene. ISMEJ, 6(1): 11–20
Brochier-Armanet C, Boussau B, Gribaldo S, et al. 2008. Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nature Reviews Microbiology, 6(3): 245–252
Chaban B, Ng S Y M, Jarrel K F. 2006. Archaeal habitats-from the extreme to the ordinary. Can J Microbiol, 52(2): 73–116
Chelius M K, Triplett E W. 2001. The diversity of archaea and bacteria in association with the roots of Zea mays L. Microb Ecol, 41(3): 252–263
Chun J, Kim K Y, Lee J H, et al. 2010. The analysis of oral microbial communities of wild-type and toll-like receptor 2-deficient mice using a 454 GS FLX Titanium pyrosequencer. BMC Microbiol, 10(1): 101
Comeau A M, Harding T, Galand P E, et al. 2012. Vertical distribution of microbial communities in a perennially stratified Arctic lake with saline, anoxic bottom waters. Sci Rep, 2: 604
Comeau A M, Li W K W, Tremblay J É, et al. 2011. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PLoS One, 6(11): e27492
Cooper L W, Whitledge T E, Grebmeier J M, et al. 1997. The nutrient, salinity, and stable oxygen isotope composition of Bering and Chukchi Seas waters in and near the Bering Strait. J Geophys Res, 102(C6): 12563–12573
Galand P E, Casamayor E O, Kirchman D L, et al. 2009. Ecology of the rare microbial biosphere of the Arctic Ocean. Proc Natl Acad Sci USA, 106(52): 22427–22432
Hamdan L J, Coffin R B, Sikaroodi M, et al. 2013. Ocean currents shape the microbiome of Arctic marine sediments. ISME J, 7(4): 685–696
Hetzer A, Morgan H W, McDonald I R, et al. 2007. Microbial life in Champagne Pool, a geothermal spring in Waiotapu, New Zealand. Extremophiles, 11(4): 605–614
Jaenicke S, Ander C, Bekel T, et al. 2011. Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing. PLoS One, 6(1): e14519
Jørgensen B B, Boetius A. 2007. Feast and famine—microbial life in the deep-sea bed. Nat Rev Microbiol, 5(10): 770–781
Jorgensen S L, Hannisdal B, Lanzén A, et al. 2012. Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. Proc Natl Acad Sci USA, 109(42): E2846–E2855
Kirchman D L, Cottrell M T, Lovejoy C. 2010. The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. Environ Microbiol, 12(5): 1132–1143
Kormas K A, Tivey M K, Von Damm K, et al. 2006. Bacterial and archaeal phylotypes associated with distinct mineralogical layers of a white smoker spire from a deep-sea hydrothermal vent site (9°N, East Pacific Rise). Environ Microbiol, 8(5): 909–920
Li An, Chu Ya'nan, Wang Xumin, et al. 2013. A pyrosequencing-based metagenomic study of methane-producing microbial community in solid-state biogas reactor. Biotechnol Biof, 6(1): 3
Li Huirong, Yu Yong, Luo Wei, et al. 2009. Bacterial diversity in surface sediments from the Pacific Arctic Ocean. Extremophiles, 13(2): 233–246
Muller F, Brissac T, Le Bris N, et al. 2010. First description of giant *Archaea* (*Thaumarchaeota*) associated with putative bacterial ectosymbionts in a sulfidic marine habitat. Environ Microbiol, 12(8): 2371–2383
Nunoura T, Oida H, Nakaseama M, et al. 2010. Archaeal diversity and distribution along thermal and geochemical gradients in hydrothermal sediments at the Yonaguni Knoll IV hydrothermal field in the southern Okinawa Trough. Appl Environ Microbiol, 76(4): 1198–1211
Pester M, Schleper C, Wagner M. 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Curr Opin Microbiol, 14(3): 300–306
Rahkila R, Nieminen T, Johansson P, et al. 2012. Characterization and evaluation of the spoilage potential of *Lactococcus piscium* isolates from modified atmosphere packaged meat. Int J Food Microbiol, 156(1): 50–59
Ravenschlag K, Sahm K, Knoblauch C, et al. 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. Appl Environ Microbiol, 66(8): 3592–3602
Reddy P V V, Rao S S S N, Pratibha M S, et al. 2009. Bacterial diversity and bioprospecting for cold-active enzymes from culturable bacteria associated with sediment from a melt water stream of Midtre Lovénbreen glacier, an Arctic glacier. Research in Microbiology, 160(8): 538–546
Rothberg J M, Leamon J H. 2008. The development and impact of 454 sequencing. Nat Biotechnol, 26(10): 1117–1124
Roussel E G, Bonavita M A C, Querellou J, et al. 2008. Extending the sub-sea-floor biosphere. Science, 320(5879): 1046
Segerer A H, Burggraf S, Fiala G, et al. 1993. Life in hot springs and hydrothermal vents. Origins Life Evol Biosphere, 23(1): 77–90
Tian Fei, Yu Yong, Chen Bo, et al. 2009. Bacterial, archaeal and eukaryotic diversity in Arctic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. Polar Biol, 32(1): 93–103
Torsvik V, Øvreås L, Thingstad T F. 2002. Prokaryotic diversity—magnitude, dynamics, and controlling factors. Science, 296(5570): 1064–1066
Trevors J T, Kevan P G, Tam L. 2010. Microbial diversity across a Canadian sub-Arctic, isostatically rebounding, soil transect. Polar Sci, 4(1): 81–91
Wang Shufang, Xiao Xiang, Jiang Lijing, et al. 2009. Diversity and abundance of ammonia-oxidizing archaea in hydrothermal vent chimneys of the Juan De Fuca Ridge. Appl Environ Microbiol, 75(12): 4216–4220
Whitman W B, Coleman D C, Wiebe W J. 1998. Prokaryotes: the unseen majority. Proc Natl Acad Sci USA, 95(12): 6578–6583
Williams A M, Fryer J L, Collins M D. 1990. *Lactococcus piscium* sp. nov. a new *Lactococcus* species from salmonid fish. FEMS Microbiol Lett, 68(1-2): 109–113
Wu Shangong, Wang Guitang, Angert E R, et al. 2012. Composition, diversity, and origin of the bacterial community in grass carp intestine. PLoS One, 7(2): e30440
Zeng Yinxin, Zou Yang, Chen Bo, et al. 2011. Phylogenetic diversity of sediment bacteria in the northern Bering Sea. Polar Biol, 34(6): 907–919
Zeng Yinxin, Zhang Fang, He Jianfeng, et al. 2013. Bacterioplankton community structure in the Arctic waters as revealed by pyrosequencing of 16S rRNA genes. Antonie van Leeuwenhoek, 103(6): 1309–1319
Zheng Xiong, Su Yinglong, Li Xiang, et al. 2013. Pyrosequencing reveals the key microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids production. Environ Sci Technol, 47(9): 4262–4268