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Phylogenetic diversity of bacteria in the Arctic Ocean sediments neighboring the Bering Strait

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ABSTRACT

To expand investigations and insights into the phylogenetic diversity of bacteria inhibiting seafloor biosphere, six Arctic Ocean sediments neighboring the Bering Strait were sampled and their bacterial diversities were investigated by pyrosequencing of 16S rRNA genes. A total of 157,454 trimed sequences were obtained, resulting in 9413 OTUs at the 97% sequence identity ($OTU_{3\%}$). This pyrosequencing allowed detection of higher than 85% of richness estimator Chao1 and Ace at the $OTU_{3\%}$ level. Higher coverage (≥0.97) and much less of rare types (singletons, only accounting for 24.5% of all $OTU_{3\%}$) indicated that this pyrosequencing recovered most of bacteria inhabiting these biospheres. At the phylum level, the high relative sequence abundance (42.0% to 63.3%) showed that Proteobacteria was the dominant member at all these sampling sites. At the class level, Deltaproteobacteria, Gammaproteobacteria, and Flavobacteriia composed the majority of bacterial communities, and the relative abundance of Cyanobacteria and Bacilli varied significantly among the six samples. At the genus level, abundant OTUs related with sulfate reduction, including *Desulfobulbus* and *Desulforhopalus*, were identified. Shared and unique OTUs analysis revealed that, at the $OTU_{3\%}$ level, 508 OTUs were shared by all the six samples, and the number of unique OTUs ranged from 98 (R02) to 195 (NB04). Principal coordinates analysis PCoA analysis revealed that samples C04 and NB04 had the similar communities and were distinct from the others. Canonical correspondence analysis (CCA) revealed that temperature was the most significant factors that correlated with the bacterial community composition. The differences in bacterial compositions and diversities indicate that the similar sediment habitats contain a large variation in microbial biodiversity.

1. Introduction

The Bering Strait, <60 m deep, is characterized by a strongly advective physical regime that consists of three water masses following around St. Lawrence Island north through Bering Strait. It acts as a shallow channel between the Pacific Ocean and the Arctic Ocean, and connects the Chukchi Sea to the north and the Bering Sea to the south [1–3]. As a shallow sea near continent, the sedimentary environment of the Bering Strait is more complex, and could be affected by water bodies, landmasses by coastal erosion, river discharge, and sea ice (iceberg) transport [4–5].

Seafloor offers a vast matrix of inorganic and organic solid surface with heterogeneous and complex organic polymers as substrates for bacterial growth. Marine benthic microbial communities are essential to global biomass, nutrient cycling, and biodiversity [6]. Recent observations have proved both cosmopolitanism (global occurrence, generalist) and provincialism (geographically localized occurrence, specialist) for microbes [7–9]. The patterns of beta-diversity in seafloor ecosystems showed unexpectedly biogeographical patterns of bacterial communities, which reflected not only physical, chemical and biological contrasts, but also biogeochemical interconnections between the pelagic and the benthic realms [6]. Previous studies also indicated that the origin of water overlying sediments shaped the benthic communities locally and globally, and that hydrography exerted important influence on the microbial community structure [10–11]. It also suggests that individual species responds to environmental heterogeneity, and therefore, certain local conditions may favor some species and not others [12]. Different parts of bacterial communities were potentially assembled by different mechanisms depending on intrinsic properties or traits. Species sorting, that is 'filtering by local environmental conditions', is important in structuring bacterial metacommunities [9].

Despite the recent findings, knowledge on the spatial distributions of seafloor life, highly relevant to the understanding of dispersal, habitat ranges and ecological processes, is still sparse [13–14]. The objectives of this study were accordingly 1) to describe the bacterial community in term of both richness and diversity, 2) to identify abundant bacterial types and significantly varied types, and 3) to find out bacterial types affiliated with both cosmopolitanism and provincialism.

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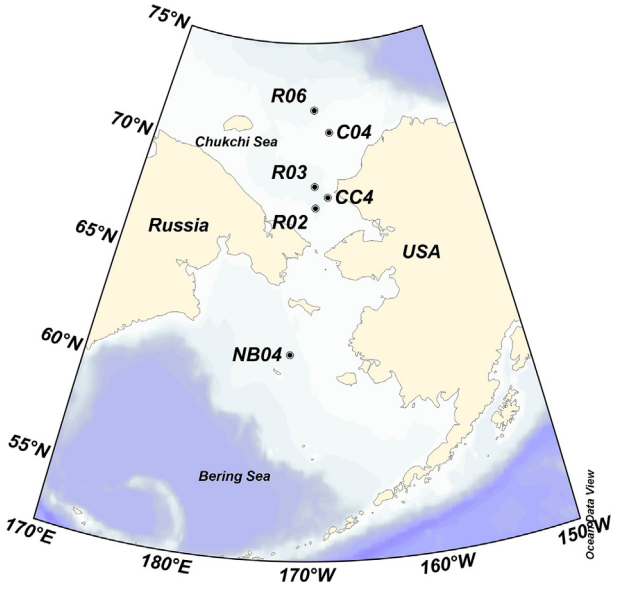

Fig. 1. Locations of sampling sites neighboring the Bering Strait (NB04 was in the Bering Sea, R02, CC4 and R03 were in the Bering Strait, C04 and R06 were in the Chukchi Sea).

2. Materials and methods

2.1. Study sites and sampling

Six Arctic Ocean sediment samples, from a longitude transect (approximate 170°W) with latitude ranged from 61°N to 77°N, were collected during the 6th Chinese National Arctic Expedition from July 24 to July 31, 2014 (Fig. 1). The water depth of the six sampling sites was similar (45 m to 57 m), one station was located in the Bering Sea, three stations were located in the Bering Strait, and the other two were located in the Chukchi Sea. The sediment samples were stored at −80 °C until further analysis. The sampling dates, locations, depths and some physicochemical characters of the samples were summarized in Table 1.

2.2. DNA extraction, amplification and pyrosequencing of 16S rRNA genes

Total DNA was extracted from 1 g sediment using PowerMax Soil DNA isolation Kit (12988-10, MoBio Laboratories, Inc., Carlsbad, CA) following the manufacturer's manual. Amplicons of hypervariable V1-V3 regions of the bacterial 16S rRNA gene were amplified using primers 27F (5′-AGAGTTTGATCCT GGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) fused with the adapters and tags [15–16]. PCR reactions were performed using TransGen AP221-02 (TransStart Fastpfu DNA polymerase), and the PCR conditions were as following: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR amplicons were purified using AxyPrepDNA purification kits (Axygen), and concentrations were measured by QuantiFluor™-ST (Promega). The purified amplicons were mixed in equimolecular amounts and subjected to pyrosequencing using Roche Genome Sequencer FLX by Majorbio Bio-Pharm Technology Co, Ltd., Shanghai, China as previously described [17].

2.3. Filtering and analyzing of pyrosequencing data

The pyrosequencing reads from the different samples were separated by unique barcode, and then, the barcode, linker and PCR primer sequences were removed. Raw sequences were processed using the Qiime software (version 1.17, http://qiime.org), and then the trimed sequences were obtained [18]. Operational taxonomy units (OTUs) were clustered by Usearch (version 7.1, http://drive5.com/uparse) with the similarity cutoff of 97%. The Good's coverage, community richness (Chao1 and Ace estimator) and community diversity (Shannon and Simpson index) were obtained by software Mothur (version v.1.30.1, http://www.mothur.org/wiki/Schloss_SOP# Alpha_ diversity) [19].

2.4. Taxonomic and phylogenetic analysis

The most abundant sequence of each OTU was selected as the representative sequence, and its taxonomic classification from phylum to genus was achieved using Silva (Release119 http://www.arb-silva.de) [20].

2.5. Comparison of bacterial communities

To compare the community structure from different samples, the community similarity among samples investigated was determined by using the Weighted-UniFrac principal coordinates analyses (PCoA) [21]. The relationships between the bacterial communities and the geochemical factors were analyzed using Canonical Correlation Analysis (CCA) with the software Canoco (version 5, Microcomputer Power, USA) [22].

2.6. Availability of pyrosequencing dataset

The bacterial 16S rRNA gene sequence datasets derived from the pyrosequencing have been deposited in GenBank under accession number SRR2595010-SRR2595015 (Table 2).

3. Results

3.1. Overview of pyrosequencing

Briefly, the pyrosequencing yielded a total of 157,454 trimed sequences with an average length of 477 bp after the low quality reads

Table 1
Geographical locations, environmental and physicochemical characteristics of the samples.

Sample	Sampling time	Location	Depth (m)	Temperature (°C)[a]	pH	TON (%)	TOC (%)
NB04	2014-07-24	171°33′19″W 61°12′02″N	57	−1.14	7.39	0.117	0.403
R02	2014-07-28	169°01′18″W 67°40′52″N	50	2.41	7.27	0.222	1.076
CC4	2014-07-29	167°31′56″W 68°07′48″N	49.4	2.92	7.50	0.123	0.599
R03	2014-07-29	169°03′12″W 68°37′23″N	53.7	2.50	7.39	0.179	0.937
C04	2014-07-31	166°59′37″W 71°00′48″N	45.3	0.59	7.14	0.261	1.522
R06	2014-07-31	168°57′28″W 72°00′36″N	51.4	1.35	7.06	0.276	1.345

[a] The temperature of bottom seawater.

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