Bacterial community in ancient permafrost alluvium at the Mammoth Mountain (Eastern Siberia)

Article *in* Gene · September 2017

DOI: 10.1016/j.gene.2017.09.021

9 authors, including:

A. V. Brouchkov
Lomonosov Moscow State University
36 PUBLICATIONS **269** CITATIONS
SEE PROFILE

Marsel Kabilov
Institute of Chemical Biology and Fundamental Medicine
130 PUBLICATIONS **321** CITATIONS
SEE PROFILE

Svetlana Filippova
Winogradsky Institute Of Microbiology
47 PUBLICATIONS **117** CITATIONS
SEE PROFILE

Victor V. Rogov
Lomonosov Moscow State University
35 PUBLICATIONS **106** CITATIONS
SEE PROFILE

Gene 636 (2017) 48–53

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

Bacterial community in ancient permafrost alluvium at the Mammoth Mountain (Eastern Siberia)

Anatoli Brouchkov[a,d], Marsel Kabilov[b], Svetlana Filippova[c], Olga Baturina[b], Victor Rogov[a], Valery Galchenko[c], Andrey Mulyukin[c], Oksana Fursova[a], Gennady Pogorelko[e,f,*]

[a] Lomonosov Moscow State University, Leninskie Gory, Moscow 119991, Russia
[b] Institute of Chemical Biology and Fundamental Medicine, SB RAS, Lavrentiev Avenue 8, Novosibirsk 630090, Russia
[c] Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Leninsky Avenue 33, bld. 2, Moscow 119071, Russia
[d] Tyumen State University, Volodarskogo 6, Tyumen 625003, Russia
[e] NI Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia
[f] Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Keywords:
Neogene
Permafrost
Bacteria
DNA
Microbial populations

ABSTRACT

Permanently frozen (approx. 3.5 Ma) alluvial Neogene sediments exposed in the Aldan river valley at the Mammoth Mountain (Eastern Siberia) are unique, ancient, and poorly studied permafrost environments. So far, the structure of the indigenous bacterial community has remained unknown. Use of 16S metagenomic analysis with total DNA isolation using DNA Spin Kit for Soil (MO-Bio) and QIAamp DNA Stool Mini Kit (Qiagen) has revealed the major and minor bacterial lineages in the permafrost alluvium sediments. In sum, 61 Operational Taxonomic Units (OTUs) with 31,239 reads (Qiagen kit) and 15,404 reads (Mo-Bio kit) could be assigned to the known taxa. Only three phyla, *Bacteroidetes*, *Proteobacteria* and *Firmicutes*, comprised > 5% of the OTUs abundance and accounted for 99% of the total reads. OTUs pertaining to the top families (*Chitinophagaceae*, *Caulobacteraceae*, *Sphingomonadaceae*, *Bradyrhizobiaceae*, *Halomonadaceae*) held > 90% of reads. The abundance of *Actinobacteria* was less (0.7%), whereas members of other phyla (*Deinococcus-Thermus*, *Cyanobacteria/Chloroplast*, *Fusobacteria*, and *Acidobacteria*) constituted a minor fraction of reads. The bacterial community in the studied ancient alluvium differs from other permafrost sediments, mainly by predominance of *Bacteroidetes* (> 52%). The diversity of this preserved bacterial community has the potential to cause effects unknown if prompted to thaw and spread with changing climate. Therefore, this study elicits further reason to study how reintroduction of these ancient bacteria could affect the surrounding ecosystem, including current bacterial species.

1. Introduction

Permafrost is defined as a lithosphere material (soil and sediment) that is permanently exposed to temperatures ≤ 0 °C and remains frozen for at least two consecutive years, normally hundreds and thousands of years. It covers about 26% of terrestrial soil ecosystems and can extend down to > 1500 m into the subsurface in the Northern Hemisphere (Williams and Smith, 1989; Steven et al., 2006).

Permafrost is regarded as the natural depository of extant microorganisms that have survived for up to millions of years (Friedmann, 1994; Vorobyova et al., 1997; Gilichinsky, 2002; Gilichinsky et al., 2007; Steven et al., 2008). Members of the major phyla (*Proteobacteria* and/or *Actinobacteria*) were found using culture-dependent and independent approaches in Alaskan, Canadian and Siberian permafrost of different ages and genesis (Shi et al., 1997; Vishnivetskaya et al., 2000, 2006; Katayama et al., 2007, 2009; Steven et al., 2008; Yergeau et al., 2010; Rivkina et al., 2015).

One of the oldest, most unique and poorly studied permafrost environments are permanently frozen alluvial Neogene sediments exposed in the Aldan river valley near the Mammoth Mountain in the Central Yakutia (Eastern Siberia). Intense cooling in the area began in the Late Pliocene (Tripati et al., 2008), and minimal temperature fluctuations did not affect the frozen state of these sediments (Lisiecki and Raymo, 2005; Demezhko and Golovanova, 2007). According to geological data, the age of Mammoth Mountain-associated alluvial sediment is estimated to be no < 3–3.5 Ma (Markov, 1973; Velichko and Isavea,

Abbreviations: OTU, Operational Taxonomic Unit; LOI, loss on ignition; SD, standard deviation; EDX, X-ray spectrometer

* Corresponding author at: NI Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia.
E-mail addresses: gennady@iastate.edu, gpogorelko@yandex.ru (G. Pogorelko).

http://dx.doi.org/10.1016/j.gene.2017.09.021
Received 2 March 2017; Received in revised form 7 September 2017; Accepted 11 September 2017
Available online 12 September 2017

1992). Little exploration of microorganisms in permafrost alluvium has been performed; hence, the structure of bacterial community remains unknown. However, it is likely that relatively poor diversity of cultured heterotrophic bacteria and a low proportion between the numbers of colony-forming units and the direct viable counts exists (Zhang et al., 2013).

Indeed, how bacteria belonging to different phylogenetic groups could exist before freezing is an intriguing issue. On the other hand, due to particular genesis of these sediments (Markov, 1973) and contributions of different sources (such as relict plants as reported by Baranova et al., 1976), the microbial communities may differ from the other permafrost sediments. Next generation sequencing of 16S rRNA genes as a culture-independent approach is attractive due to the possibility of eliminating the clone library production step, generating numerous sequences per sample (Rothberg and Leamon, 2008; Caporaso et al., 2012), and especially to capture low-abundant microbial taxa in permafrost (Yang et al., 2012), soils, and deep sea water (Sogin et al., 2006; Roesch et al., 2007). One possible bias in microbial community profiling from read abundance data can be associated with nucleic acid extraction (Amend et al., 2010), and previous research recommended different protocols to isolate total DNA from a studied sample (Feinstein et al., 2009; Brooks et al., 2015).

The objective of this study was to characterize the bacterial community from Mammoth Mountain alluvial sediments using high-throughput sequencing of 16S rRNA gene amplicons of total genomic DNA isolated using two different kits.

2. Materials and methods

2.1. Sampling site

The sampling site was located at the ice-covered exposure (Fig. 1) of Mammoth Mountain (N62°56′ E134°0.1′) in the left bank of the Aldan river valley in Central Yakutia (Eastern Siberia). No specific permission was required for sampling, and no endangered or protected species were involved in this study. The mean annual temperature near the exposure surface is presently about − 4 °C. The exposure consisted of three sediment layers attributed to (1) Late Pleistocene (about 15 Ka–40 Ka-old Ice Complex); (2) Middle and Early Pleistocene (0.1–1 Ma old, frozen at the time of formation); (3) Neogene (frozen probably at the end of Neogene about 3–3.5 Ma ago) (Markov, 1973).

2.2. Collection of samples

Samples of alluvial Neogene sands were collected at 83-m altitude above the sea level (northern exposition) in a pit (about 100 × 100 cm) dug to a 1.5-m depth. The pit surface was sterilized under the flame of a gas burner. Contamination of the processed pit was controlled by spreading 2-mL suspensions of *Yarrowia lipolytica* Y-3603 tagged with red fluorescent protein (Yuzbasheva et al., 2011) (10^8 cells/mL) onto a 200 cm^2 surface and was not detected after plating and direct microscopy examinations.

Sterile DNA-free 50-mL Cellstar® tubes were opened near flame and immediately pushed into exposed frozen sediments (− 4 °C); the sampling procedure excluded any contact between exterior surfaces and sampling devices. These samples were allotted for DNA isolation. In addition, frozen samples were taken from the same pit with extreme precautions using metal bores, forceps, and scalpels sterilized with ethanol and flamed which were then used for physicochemical and microscopy analyses. All samples were transported in frozen state in a 36-L Coleman® cooler with saturated NaCl solution as a cooling agent and were stored at − 5 °C effectively mimicking natural conditions.

2.3. Analyses of sediments

Outer layers (2–3 cm) of stored frozen 4–5 kg samples were removed in the laboratory with sterile knifes in a class II laminar hood and only internal parts were used. Composite samples were prepared by mixing portions from three sediment samples. The grain-size distribution was determined using the common sieving technique. The water content was estimated after heating wet samples at 105 °C for 24 h. Loss on ignition (LOI) was measured as a difference before and after burning of dry samples at 430 °C for 3 h in a muffle furnace and served to estimate the organic carbon content (Schinner et al., 1996). The pH and Eh values of sediment suspensions (in 10 mM $CaCl_2$) were measured using a Thermo Scientific Orion Model 261S pH/mV/Temperature meter. Physicochemical analyses were performed in three independent sets, each in triplicates with calculations of the mean and SD using Excel software. Values of pH and Eh were measured in triplicates for 17 subsamples of the same sample.

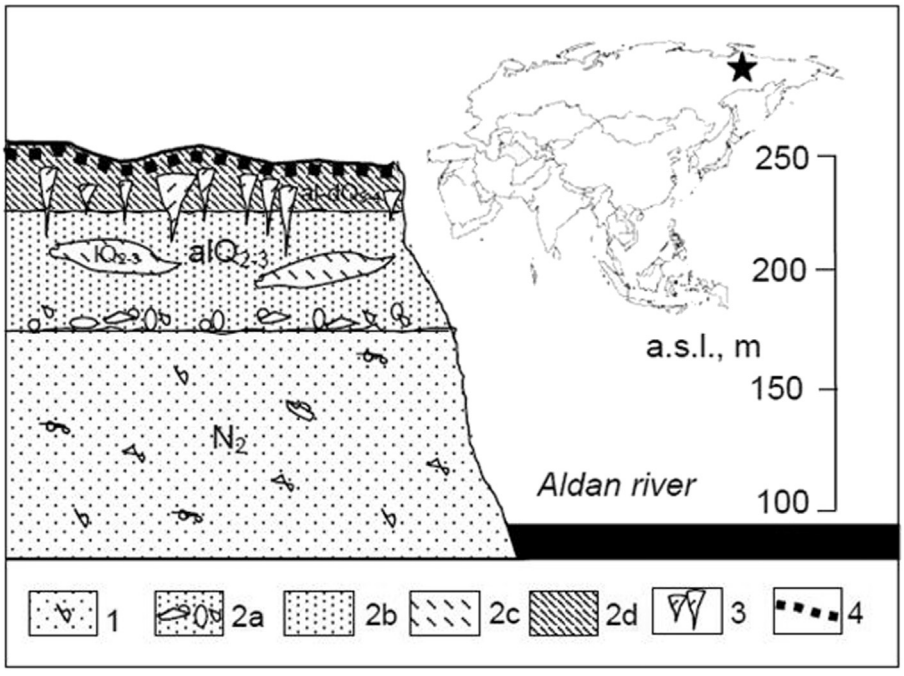

Fig. 1. Scheme showing the sampling site at the Mammoth Mountain exposure. Designations: (1) Neogene (N_2) alluvial sediments used in this study; (2) Pleistocene sediments: (2a) pebbles in the ferrous sands, (2b) sands, (2c) lacustrine silt, (2d) silt; (3) ice wedge; (4) active layer.

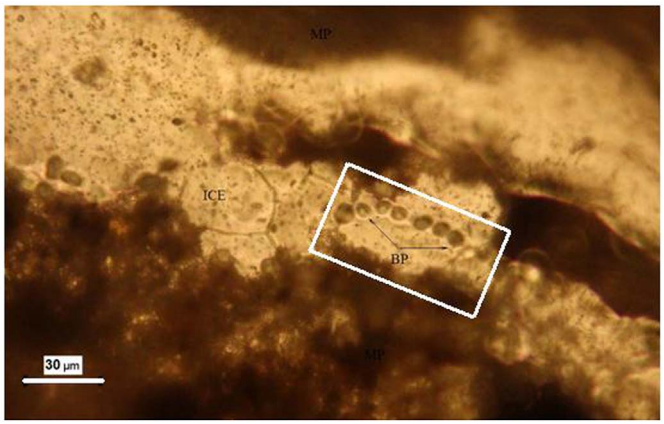

Fig. 2. Images of the studied alluvial sediments as viewed by light microscopy. Biomorphic particles observed within a white frame.

2.4. Light microscopy

Morphology and distribution of particles in permafrost alluvium were studied by light and electron microscopy of freeze fractures prepared as described (Rogov and Kurchatova, 2013). Unthawed samples were broken at < 0° and the fracture surface was coated with Formvar. After thawing, samples were examined under a light Reichert Microstar IV microscope. Representative images of 30–40 micrographs are shown in Fig. 2.

2.5. 16S rRNA metagenomic sequencing

All reagents used for DNA extraction and further analyses were molecular biology grade. Solutions and buffers were DNase-free and sterilized. All procedures were conducted in class I hoods.

Total community DNA was extracted and analyzed independently from 0.25 g of sample material with a DNA Spin Kit for Soil (MO-Bio) and a QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturers' protocols. In these experiments, three equivalent subsamples from a single original sediment sample were isolated by pushing in 50-mL sterile vials. Thus, three biological replicates were used for this study. Each biological replicate represents 40–50 g of sediment taken from different sides of the original 4–5 kg sample. The three biological replicates were then independently mixed until homogenous and 0.25 g of the material was used in each protocol mentioned above to generate average abundance of the content.

The V3-V4 region of the 16S rRNA genes was amplified with the primer pair 343F (5′-CTCCTACGGRRSGCAGCAG-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) combined with Illumina adapter sequences, a pad and a linker of two bases, as well as barcodes on the primers (Caporaso et al., 2012). PCR amplification was performed in 50 μL reaction mixtures containing 0.7 U Phusion Hot Start II High-Fidelity and 1 × Phusion GC buffer (Thermo Fisher Scientific), 0.2 μM of each forward and reverse primers, 10 ng template DNA, 2.3 mM $MgCl_2$ (Sigma-Aldrich) and 0.2 mM of each dNTP (Life Technologies). Thermal cycling conditions were as follows: initial denaturation at 98 °C for 1 min, followed by 30 cycles of 98 °C for 15 s, 62 °C for 15 s, and 72 °C for 15 s, with final extension at 72 °C for 10 min.

A total of 200 ng PCR product from each sample was pooled together and purified using a MinElute Gel Extraction Kit (Qiagen). Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina) and the protocol described previously (Caporaso et al., 2011, 2012). Sample denaturation was performed by mixing 4.5 μL of combined PCR products (4 nM) and 4.5 μL 0.2 M NaOH. Denatured DNA was diluted to 14 pM and 510 μL was mixed with 90 μL of 14 pM Phix library. μLSample mixtures totaling 600 μL, together with customized sequencing primers for forward, reverse, and index reads, were loaded into the corresponding wells on the reagent cartridge of a Miseq 500 cycles kit and run on Miseq for 2 × 250 bp paired-ends sequencing (Illumina) at the SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia). The 16S read data were deposited in the NCBI database under accession SRP075638.

2.6. Bioinformatic analysis

Raw sequences were analyzed with UPARSE pipeline (Edgar, 2013) using USEARCH v8.1.1861. The UPARSE pipeline included merging of paired reads; read quality filtering; length trimming; merging of identical reads (dereplication); discarding singleton reads; removing chimeras and OTU clustering using the UPARSE-OTU algorithm. The OTU sequences were assigned a taxonomy using the RDP classifier 2.11 (Wang et al., 2007) and searched against the NCBI 16S database using BLASTN (Altschul et al., 1990). Community structure analyses were based on the phylum, class and genus taxonomy levels.

OTUs reads were aligned with the MUSCLE v.3.8.31 (Edgar, 2013) and phylogenetic analysis was performed using the maximum likelihood phylogeny tool in CLC GW 8.5 (1000 bootstrap pseudoreplicates and GTR + G + T evolutionary model). Rarefaction curves of observed OTUs were obtained using USEARCH.

3. Results

3.1. Properties of alluvial Neogene sediments at Mammoth Mountain

Alluvial sediments with the bulk density of 1.7 g cm^{-3} and porosity values of 45% were composed mainly from coarse sand fraction (0.2–2 mm, 84%) with pale quartz and feldspar as major minerals (Table 1). The pH values of sediment suspensions (in 0.1 M $CaCl_2$) after thawing varied from 7.08 to 8.90; and the Eh was in a range + 355 mV–+406 mV (17 measurements). Light microscopy of the sediment fractures demonstrated the cryogenic structure with crystals and thin streaks of ice with embedded biomorphic particles (Fig. 2).

3.2. General profile of bacterial community

DNA was extracted from the same sample using a DNA Spin Kit for Soil (MO-Bio) or QIAamp DNA Stool Mini Kit (Qiagen) with yields of

Table 1
Physical and chemical properties of alluvial Neogene sediments (composite sample).

Major minerals	Quartz, feldspar
Grain size distribution particle size	
1–2 mm	3%
0.5–1 mm	20%
0.25–0.5 mm	61%
0.1–0.25 mm	10%
< 0.1 mm	6%
Density	
ρ	1.72 g cm^{-3}
ρ_d	1.39 g cm^{-3}
ρ_s	2.51 g cm^{-3}
Porosity	45%
Water content	24%
Loss on ignition[a]	3.6%
Organic carbon[a]	1.6–2%
Total salinity[a]	< 0.05%
Content[a]	
NO_3	< 20 mg kg^{-1} dw
NO_2	< 10 mg kg^{-1} dw
Total P	< 20 mg kg^{-1} dw
pH of suspension (1:1 in 0.01 M $CaCl_2$)	7.08–8.90
Eh	+ 355–+406 mV

Table shows the mean values; SD ± 5% are not indicated.
[a] Data were taken from a different study (Zhang et al., 2013).

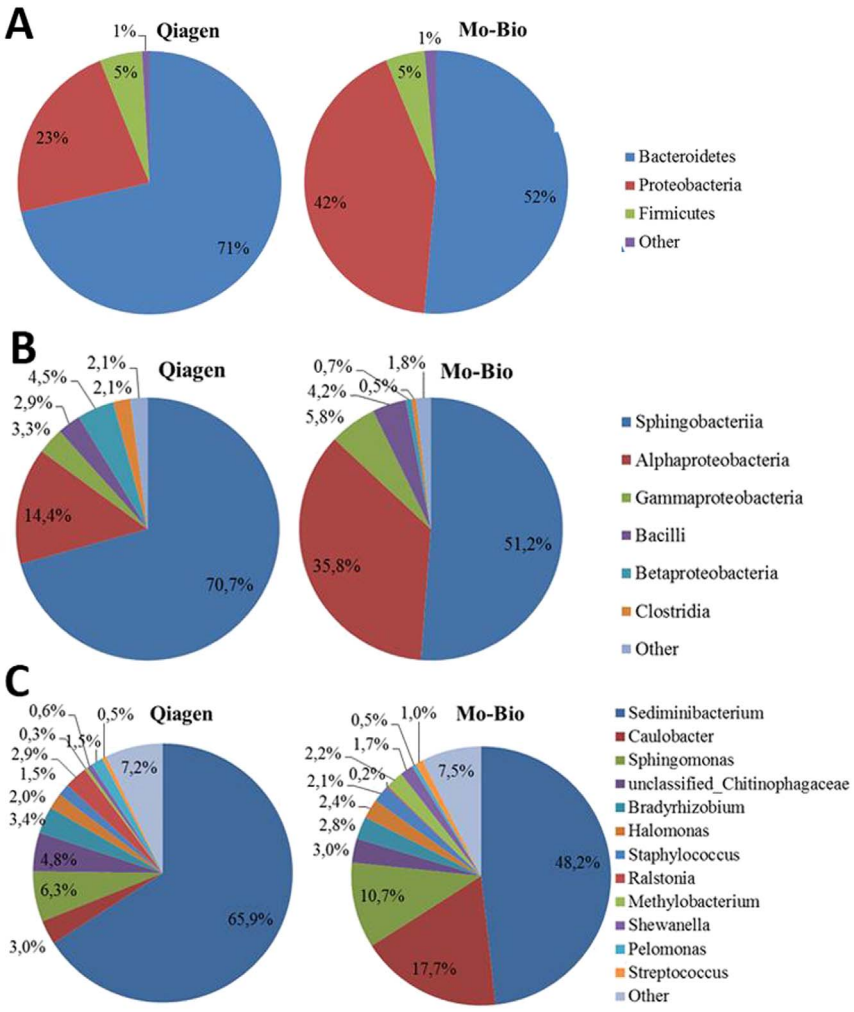

Fig. 3. Composition of the bacterial community from 3 to 3.5 Ma permafrost alluvial sediments under Mammoth Mountain (Eastern Siberia). Relative read abundance of different bacterial phyla (A), classes (B), and genera (C).

150 and 438 ng DNA per each 0.25 g mixed homogenous sediment subsample, respectively. After extraction, DNA was quantified with the NanoDrop 200c (ThermoFisher Scientific) by analyzing absorbance of UV–visible light. All three biological replicates produced comparably similar results. A total of 31,239 (Qiagen kit) and 15,404 (Mo-Bio kit) 16S rRNA gene sequences (400–435 bp) were assigned to 61 OTUs (Supplementary data, Table S1). Only three phyla comprised a > 5% OTUs abundance: *Bacteroidetes* (71% and 52%, Qiagen and Mo-Bio kits), *Proteobacteria* (23% and 42%), and *Firmicutes* (5%) and jointly covered 99% of reads. The use of two DNA isolation kits caused no variations in the order of predominance at the phylum level: *Bacteroidetes* > *Proteobacteria* > *Firmucutes* (Fig. 3A). Rarefaction curves reached asymptotic values, thus indicating sufficient read coverage for identification of taxa constituting the studied bacteria (Supplementary data, Fig. S1). Differences in OTU contributions for the two DNA isolation procedures (Qiagen and Mo-Bio kits) were prominent for *Bacteroidetes* and *Proteobacteria*, but not for *Firmicutes*.

3.3. Major bacteria classes, families and genera

In total, members of 6 classes held 98% of the total reads and the top 11 families (and genera) shared a > 90% contribution to the bacterial community from ancient permafrost alluvium (Fig. 3 B, C). Representatives of the class *Sphingobacteriia* dominated within *Bacteroidetes* and sustained 70.7% (Qiagen kit) and 51% (Mo-Bio kit) of the total reads (Fig. 3). Major contributors were members of the family *Chitinophagaceae* and the genus *Sediminibacterium* (65.9% and 48.2% for the two kits, respectively) and an unclassified group (Fig. 3C) that possessed 92.5% similarity to the genus *Niabella* (Table S1).

The second abundant lineage *Proteobacteria* was dominated by *Alphaproteobacteria* (35.8% and 14.4%) followed by *Gammaproteobacteria* (3.3% and 5.8%) and *Betaproteobacteria* (4.5% and 0.7%). Major groups within *Alphaproteobacteria* with > 1% abundance represented the following families and genera: *Caulobacteraceae* (the genus *Caulobacter*, the mean contribution 11.4%), *Sphingomonadaceae* (*Sphingomonas*, 8.5%), *Bradyrhizobiaceae* (*Bradyrhizobium*, 3.1%), and *Methylobacteriaceae* (*Methylobacterium*, 2.2%). Among *Gammaproteobacteria*, the most abundant were members of *Halomonadaceae* and *Shewanellaceae* and especially the genera *Halomonas* (2.2%) and *Shewanella* (1.2%). The *Betaproteobacteria* class was mainly represented by the families *Burkholderiaceae* and the genus *Ralstonia* (1.5%) and *Comamonadaceae*, the genus *Pelomonas* 1.1%. Dominant sub-divisions within *Firmicutes* were the class *Bacilli* (especially *Staphylococcaceae*, *Streptococcaceae*, and *Lactobacillaceae*); members of the genera *Staphylococcus* and *Streptococcus* contributed with means of 1.8% and 0.75% of the total reads, respectively (Fig. 3C).

Overall, variations of DNA extraction procedures produced no substantial interferences in the qualitative profile, except for the reliable detection of *Burkholderiaceae* with the Qiagen kit (Table S1). However, differences in numerical contributions from most top divisions (especially, *Sediminibacterium*, *Caulobacter*, and *Sphingomonas*) were significant for two groups of data obtained using the two kits.

3.4. Minor groups

Remaining phyla contributed roughly 1% to the total reads.

Actinobacteria shared approximately 0.7%, among which members of the *Micrococcaceae* and *Brevibacteriaceae* families (closely related to *Arthrobacter* and *Brevibacterium* genera) were detected by sequencing after DNA isolation with both kits. Representatives of the other lineages (*Deinococcus-Thermus*, *Cyanobacteria/Chloroplast*, *Fusobacteria*, and *Acidobacteria*) were rare and detected using only one of DNA isolation procedures, and some of them were assigned to unclassified groups (Table S1). On the whole, the bacterial community from the studied permafrost sediments is composed from members of both major and minor groups with varying relatedness to the known taxa (Fig. S2).

4. Discussion

16S metagenomic analysis allowed us to find bacterial lineages in ancient permafrost alluvium, which were not captured by culture-dependent approach, except for some members of *Actinobacteria* (Zhang et al., 2013). Moreover, variations of DNA isolation procedures (two kits for the same sample) were useful mainly to detect some minor groups, although caused no changes in the compositional profile for major taxa at least. Taken together, the obtained data show some specific features of the bacterial component in permafrost alluvium.

Strikingly, *Bacteroidetes* constitute the most abundant fraction in the ancient permafrost alluvial sediments under study. Members of this lineage occur but do not dominate over *Proteobacteria* in various unfrozen alluvial aquifers and sediments (Medihala et al., 2012; Yergeau et al., 2012; Gibbons et al., 2014; Missimer et al., 2014; Handley et al., 2015; Li et al., 2015), active and permafrost soils (Roesch et al., 2007; Yang et al., 2012), and are rather rare in some permafrost sediments (Yergeau et al., 2010; Rivkina et al., 2015). Second, low abundance of *Actinobacteria* in the studied 3–3.5 Ma permafrost alluvium contrasts permafrost soils and lake sediments (Vishnivetskaya et al., 2006; Yang et al., 2012; Rivkina et al., 2015), as well as deep ground ice (Steven et al., 2008; Yergeau et al., 2010) but is common in unfrozen alluvial water and sediments (Medihala et al., 2012; Amalfitano et al., 2014; Missimer et al., 2014). The significant proportion of *Proteobacteria* as in this study and even their predominance is common, as demonstrated in the above-cited works.

Members of most bacterial families and genera in the studied community from nutritionally poor permafrost alluvial sediments are widespread in terrestrial and aquatic habitats. Thus, closest relatives of almost all *Bacteroidetes* in the studied sample pertain to the genus *Sediminibacterium* firstly described by Qu and Yuan (2008) who isolated the type strain *S. salmoneum* from sediment of a eutrophic reservoir. Other species (*S. ginsengisoli* and *S. goheungense*) were found in soils and freshwater (Kim et al., 2013; Kang et al., 2014). Representatives of the genus *Caulobacter* inhabit oligotrophic environments (Abraham et al., 1999) and of the genus *Sphingomonas* are ubiquitous in a variety of aqueous and terrestrial habitats (Balkwill et al., 2006). The occurrence of *Bradyrhizobium* nitrogen-fixing plant root symbionts (Jordan, 1982) and *Staphylococcus* is not surprising since plant and animal remnants are present in studied sediments; staphylococci were also found among the isolates from some permafrost environments (Steven et al., 2008). It is noteworthy that even halophiles of the genus *Halomonas* (Dobson and Franzmann, 1996) persisted in permafrost alluvium. These bacteria likely entered before freezing from neighboring saline environments currently and probably previously widespread in this region, taking into account the prevalence of evaporation over precipitation. As a result of freezing, the salinity of unfrozen water microenvironments could increase and be suitable for halophilic bacteria.

Preliminary16S metagenomic analysis of the bacterial community in Pleistocene ice veins from the upper horizons at the same site as the studied ancient permafrost alluvium have demonstrated the predominance of *Proteobacteria* (> 80%) and the occurrence of *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (2–3% abundances). *Gammaproteobacteria*, the most abundant lineage in the ice veins, was represented mainly by the genera *Marinobacter* > *Methylophaga* > *Pseudomonas* > *Halomonas* (unpublished results). Hence, bacterial communities in ancient permafrost alluvium and ice veins even at the same sampling site differ in general profile. Further studies are necessary to gain insight into a more complete bacterial community structure in the entire Neogene alluvium and outlier horizons, with the use of many independent samples.

The conditions in permafrost alluvial Neogene sediments are unlikely to support active growth of many bacteria. In this light, an issue on the survival mechanisms becomes intriguing, especially for non-spore-forming bacteria that are predominant in the studied sediments and a variety of natural habitats. Specifically, little is known about the adaptation mechanisms adopted by bacteria belonging to *Sediminibacterium* genus; a recent study has shown a flexible adaptation of closely related isolates to withstand stresses (Ayarza et al., 2015). It is quite plausible that most bacteria in ancient permafrost alluvium exist or even survive as uncultivable (or yet-to-be-cultivated) and viable non-culturable cells (Vartoukian et al., 2010; Puspita et al., 2012), common in natural habitats (Roszak and Colwell, 1987; Amann et al., 1995). Specifically, readily cultivable non-spore-forming bacteria entering a non-culturable state in response to nutrient starvation, temperature downshift, and other stresses is well characterized for numerous species (Roszak and Colwell, 1987; Kaprelyants et al., 1993; Oliver, 2010; Puspita et al., 2012; Li et al., 2014 and references therein). Moreover, non-spore-forming bacteria are able to produce dormant cyst-like cells intended for long-term survival, which were found in some ancient permafrost and unfrozen environments using direct electron microscopy (Mulyukin et al., 2003; Soina et al., 2004; Suzina et al., 2004). Specific procedures are often required for growth recovery in cultured and permafrost populations containing cyst-like cells (Mulyukin et al., 2009; Kriazhevskikh et al., 2012) and certain approaches are recommended to improve PCR-based detection of dormant cystous cells and spores (Mulyukin et al., 2013). Further studies are needed to clarify in what state microbial cells persist in permafrost alluvium and similar environments.

Hence, ancient permafrost Neogene alluvial sediments represent an environment with a particular compositional profile of bacterial community where diverse microorganisms survive for long periods of time. The consequences of future reintroduction of preserved bacterial communities in permafrost such as that in this study to the surrounding ecosystem are unknown. Further studies would benefit the understanding of how thaw and flow of these materials will affect present-day organisms in this environment.

Funding

This work was supported by the Russian Foundation for Basic Research (grants 16-05-00296, 14-04-01645) and the Presidium of Russian Academy of Sciences (program 44 “Fundamental research oriented to development of Russian Federation Arctic area”).

Conflict of interest

The authors have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2017.09.021.

References

Abraham, W.R., Strömpl, C., Meyer, H., et al., 1999. Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. Int. J. Syst. Bacteriol. 49, 1053–1073.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Amalfitano, S., Del Bon, A., Zoppini, A., et al., 2014. Groundwater geochemistry and microbial community structure in the aquifer transition from volcanic to alluvial areas. Water Res. 65, 384–394.
Amann, R., Ludwig, W., Schleifer, K.H., 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. 59, 143–169.
Amend, A.S., Seifert, K.A., Bruns, T.D., 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? Mol. Ecol. 19, 5555–5565.
Ayarza, J.M., Mazzella, M.A., Erijman, L., 2015. Expression of stress-related proteins in *Sediminibacterium* sp. growing under planktonic conditions. J. Basic Microbiol. 55, 1134–1140.
Balkwill, D.L., Fredrickson, J.K., Romine, M.F., 2006. *Sphingomonas* and related genera. In: Dworkin, M. (Ed.), The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. Springer-Verlag, New York, pp. 605–629.
Baranova, U.P., Il'inskaya, I.A., Nikitin, V.P., et al., 1976. Miocene of the Mammoth Mountain. In: Works of Geological Institute of Russian Academy of Sciences. Nauka, Moscow (in Russian).
Brooks, J.P., Edwards, D.J., Harwich, M.D., et al., 2015. The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies. BMC Microbiol. 15, 66. http://dx.doi.org/10.1186/s12866-015-0351-6.
Caporaso, J.G., Lauber, C.L., Walters, W.A., et al., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108, 4516–4522.
Caporaso, J.G., Lauber, C.L., Walters, W.A., et al., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6, 1621–1624.
Demezhko, D.Y., Golovanova, I.V., 2007. Climatic changes in the Urals over the past millennium — an analysis of geothermal and meteorological data. Clim. Past 3, 237–242.
Dobson, S.J., Franzmann, P.D., 1996. Unification of the genera *Deleya* (Baumann et al. 1983), *Halomonas* (Vreeland et al. 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family *Halomonadaceae*. Int. J. Syst. Bacteriol. 46, 550–558.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.
Feinstein, L.M., Sul, W.J., Blackwood, C.B., 2009. Assessment of bias associated with incomplete extraction of microbial DNA from soil. Appl. Environ. Microbiol. 75, 5428–5433.
Friedmann, E.I., 1994. Permafrost as microbial habitat. In: Gilichinsky, D. (Ed.), Viable Microorganisms in Permafrost. Russian Academy of Sciences, Pushchino, Russia, pp. 21–26.
Gibbons, S.M., Jones, E., Bearquiver, A., et al., 2014. Human and environmental impacts on river sediment microbial communities. PLoS One 9, e97435. http://dx.doi.org/10.1371/journal.pone.0097435.
Gilichinsky, D., 2002. Permafrost as a microbial habitat. In: Bitton, G. (Ed.), Encyclopedia of Environmental Microbiology. Wiley, New York, pp. 932–956.
Gilichinsky, D.A., Wilson, G.S., Friedmann, E.I., et al., 2007. Microbial populations in Antarctic permafrost: biodiversity, state, age, and implication for astrobiology. Astrobiology 7, 275–311.
Handley, K.M., Wrighton, K.C., Miller, C.S., et al., 2015. Disturbed subsurface microbial communities follow equivalent trajectories despite different structural starting points. Environ. Microbiol. 17, 622–636.
Jordan, D.C., 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growig, root nodule bacteria from leguminous plants. Inter. J. Syst. Bacteriol. 32, 136–139.
Kang, H., Kim, H., Lee, B.-I., et al., 2014. *Sediminibacterium goheungense* sp. nov., isolated from a freshwater reservoir. Int. J. Syst. Evol. Microbiol. 64, 1328–1333.
Kaprelyants, A.S., Gottschal, J.C., Kell, D.B., 1993. Dormancy in non-sporulating bacteria. FEMS Microbiol. Rev. 104, 271–286.
Katayama, T., Tanaka, M., Moriizumi, J., et al., 2007. Phylogenetic analysis of bacteria preserved in a permafrost ice wedge for 25,000 years. Appl. Environ. Microbiol. 73, 2360–2363.
Katayama, T., Kato, T., Tanaka, M., et al., 2009. *Glaciibacter superstes* gen. nov., sp. nov., a novel member of the family *Microbacteriaceae* isolated from a permafrost ice wedge. Int. J. Syst. Evol. Microbiol. 59, 482–486.
Kim, Y.J., Nguyen, N.L., Weon, H.Y., et al., 2013. *Sediminibacterium ginsengisoli* sp. nov., isolated from soil of a ginseng field, and emended descriptions of the genus *Sediminibacterium* and of *Sediminibacterium salmoneum*. Int. J. Syst. Evol. Microbiol. 63, 905–912.
Kriazhevskikh, N.A., Demkina, E.V., Manucharova, N.A., et al., 2012. Reactivation of dormant and nonculturable bacterial forms from paleosols and subsoil permafrost. Microbiology (RU) 81, 435–445.
Li, L., Mendis, N., Trigui, H., et al., 2014. The importance of the viable but non-culturable state in human bacterial pathogens. Front. Microbiol. http://dx.doi.org/10.3389/fmicb.2014.00258.
Li, P., Wang, Y., Dai, X., et al., 2015. Microbial community in high arsenic shallow groundwater aquifers in Hetao basin of Inner Mongolia, China. PLoS One 10, e0125844. http://dx.doi.org/10.1371/journal.pone.0125844.
Lisiecki, L.E., Raymo, M.E., 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic $\delta^{18}O$ records. Paleoceanography 20http://dx.doi.org/10.1029/2004PA001071. PA1003.
Markov, K.K., 1973. Cross-section of the Newest Sediments. Moscow University Press, Moscow, Russia (in Russian).
Medihala, P.G., Lawrence, J.R., Swerhone, G.D.W., et al., 2012. Spatial variation in microbial community structure, richness, and diversity in an alluvial aquifer. Can. J. Microbiol. 58, 1135–1151.
Missimer, T.M., Hoppe-Jones, C., Jadoon, K.Z., et al., 2014. Hydrogeology, water quality, and microbial assessment of a coastal alluvial aquifer in western Saudi Arabia: potential use of coastal wadi aquifers for desalination water supplies. Hydrogeol. J. 22, 1921–1934.
Mulyukin, A.L., Soina, V.S., Demkina, E.V., et al., 2003. Formation of resting cells by non-spore-forming microorganisms as strategy of long-term survival in the environment. Proc. SPIE 4939, 208–218.
Mulyukin, A.L., Demkina, E., Kryazhevskikh, N.A., et al., 2009. Dormant forms of *Micrococcus luteus* and *Arthrobacter globiformis* not platable on standard media. Microbiology (RU) 78, 407–418.
Mulyukin, A.L., Suzina, N.E., El-Registan, G.I., et al., 2013. Effective PCR detection of vegetative and dormant bacterial cells due to a unified method for preparation of template DNA encased within cell envelopes. Microbiology (RU) 82, 295–305.
Oliver, J.D., 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol. Rev. 34, 415–425.
Puspita, I.D., Kamagata, Y., Tanaka, M., et al., 2012. Are uncultivated bacteria really uncultivable? Microbes Environ. 27, 356–366.
Qu, J.H., Yuan, H.L., 2008. *Sediminibacterium salmoneum* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic reservoir. Int. J. Syst. Evol. Microbiol. 58, 2191–2194.
Rivkina, E., Petrovskaya, L., Vishnivetskaya, T., et al., 2015. Metagenomic analyses of the late Pleistocene permafrost – additional tools for reconstruction of environmental conditions. Biogeosci. Discuss. 12, 12091–12119.
Roesch, L.F.W., Fulthorpe, R.R., Riva, A., et al., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1, 283–290.
Rogov, V.V., Kurchatova, A.N., 2013. Method of Manufacturing Replica for Analyses of Microstructure of Frozen Rocks in Scanning Electron Microscope. Patent RU 2 528 256 C1. http://russianpatents.com.
Roszak, D.B., Colwell, R.R., 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. 51, 365–379.
Rothberg, J.M., Leamon, J.H., 2008. The development and impact of 454 sequencing. Nat. Biotechnol. 26, 1117–1124.
Schinner, F., Öhlinger, R., Kandeler, E., et al., 1996. Methods in Soil Biology. Springer Lab Manual, Berlin/Heidelberg.
Shi, T., Reeves, R.H., Gilichinsky, D.A., et al., 1997. Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. Microb. Ecol. 33, 169–179.
Sogin, M.L., Morrison, H.G., Huber, J.A., et al., 2006. Microbial diversity in the deep sea and the underexplored rare biosphere. Proc. Natl. Acad. Sci. U. S. A. 103, 12115–12120.
Soina, V.S., Mulyukin, A.L., Demkina, E.V., et al., 2004. The structure of resting bacterial populations in soil and subsoil permafrost. Astrobiology 4, 345–358.
Steven, B., Léveillé, R., Pollard, W.H., et al., 2006. Microbial ecology and biodiversity in permafrost. Extremophiles 10, 259–267.
Steven, B., Pollard, W.H., Greer, C.W., et al., 2008. Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. Environ. Microbiol. 10, 3388–3403.
Suzina, N.E., Mulyukin, A.L., Kozlova, A.N., et al., 2004. Ultrastructure of resting cells of some non-spore-forming bacteria. Microbiology (RU) 73, 435–447.
Tripati, A.K., Eagle, R.A., Morton, A., et al., 2008. Evidence for glaciation in the Northern Hemisphere back to 44 Ma from ice-rafted debris in the Greenland Sea. Earth Planet. Sci. Lett. 265, 112–122.
Vartoukian, S.R., Palmer, R.M., Wade, W.G., 2010. Strategies for culture of 'unculturable' bacteria. FEMS Microbiol. Lett. 309, 1–7.
Velichko, A.A., Isavea, L.L., 1992. Landscape types during the Last Glacial Maximum. In: Frenzel, B., Pecsi, B., Velichko, A.A. (Eds.), Atlas of Palaeoclimates and Palaeoenvironments of the Northern Hemisphere. INQUA/Hungarian Academy of Sciences, Budapest.
Vishnivetskaya, T., Kathariou, S., McGrath, J., et al., 2000. Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. Extremophiles 4, 165–173.
Vishnivetskaya, T.A., Petrova, M.A., Urbance, J., et al., 2006. Bacterial community in ancient Siberian permafrost as characterized by culture and culture-independent methods. Astrobiology 6, 400–414.
Vorobyova, E., Soina, V., Gorlenko, M., et al., 1997. The deep cold biosphere: facts and hypothesis. FEMS Microbiol. Rev. 20, 277–290.
Wang, Q., Garrity, G.M., Tiedje, J.M., et al., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.
Williams, P.J., Smith, M.W., 1989. The Frozen Earth. Fundamentals of Geocryology. Cambridge University Press, Cambridge.
Yang, S., Wen, X., Jin, H., et al., 2012. Pyrosequencing investigation into the bacterial community in permafrost soils along the China-Russia crude oil pipeline (CRCOP). PLoS One. http://dx.doi.org/10.1371/journal.pone.0052730.
Yergeau, E., Hogues, H., Whyte, L.H., Greer, C.W., et al., 2010. The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J 4, 1206–1214.
Yergeau, E., Lawrence, J.R., Sanschagrin, S., et al., 2012. Next-generation sequencing of microbial communities in the Athabasca river and its tributaries in relation to oil sands mining activities. Appl. Environ. Microbiol. 78, 7626–7637.
Yuzbasheva, E.Y., Yuzbashev, T.V., Laptev, I.A., et al., 2011. Efficient cell surface display of Lip2 lipase using C-domains of glycosylphosphatidylinositol-anchored cell wall proteins of *Yarrowia lipolytica*. Appl. Microbiol. Biotechnol. 91, 645–654.
Zhang, D.-C., Brouchkov, A., Griva, G., et al., 2013. Isolation and characterization of bacteria from ancient Siberian permafrost sediment. Biology 2, 85–106.