Spatio-Temporal Monitoring and Ecological Significance of Retrievable Pelagic Heterotrophic Bacteria in Kongsfjorden, an Arctic Fjord

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Abstract Kongsfjorden is a glacial fjord in the Arctic that is influenced by both Atlantic and Arctic water masses. In the present report retrievable heterotrophic bacteria isolated from two distinct zones (outer and inner fjord) of Kongsfjorden was studied during summer to fall of 2012. 16S rRNA gene sequences of the retrievable heterotrophic bacteria corresponded to *γ-proteobacteria* (13 phylotypes), *α-proteobacteria* (3 phylotypes), *Bacteroidetes* (4 phylotypes) and *Actinobacteria* (2 phylotypes). The heterotrophic bacterial community structure was fundamentally different in different months which could be linked to changes in the water masses and/or phytoplankton bloom dynamics. It is hypothesized that monitoring the retrievable heterotrophic bacterial assemblage in the fjord would give valuable insights into the complex ecological role they play under extreme and dynamic conditions.

Keywords Kongsfjorden · Retrievable heterotrophic bacteria · Water · Arctic fjord

The polar regions by virtue of their extreme conditions promote the growth and establishment of niche-specific microbial flora. However, in contrast to the Southern Ocean that surrounds the Antarctic continent and receives virtually no freshwater inflow and terrigenous organic matter [1], the relatively land-locked Arctic Ocean receives terrestrial inflow from the surrounding lands and waters from the Pacific and Atlantic Oceans, and therefore the Arctic marine ecosystems are influenced both by polar and non-polar microbial communities [2]. Kongsfjorden is a polar fjord situated between 78°04'N–79°05'N and 11°03'–13°03'E on the west coast of Spitsbergen, Svalbard Archipelago and represents a border area between Atlantic and Arctic biogeographic zones. The microbial diversity in this fjord is strongly structured by the different physical factors that influence the fjord from both the ends [3]. An increased influx of Atlantic Water (AW) carrying terrestrial inflow into the Kongsfjorden would alter the species composition towards global, whereas the glacial input and distance from the coast would tend to make the inner part of the fjord more Arctic [3]. Therefore, in the present study, the retrievable heterotrophic bacterial communities of Kongsfjorden were categorized into non-polar and polar group to understand their abundance and distribution pattern. The pelagic retrievable heterotrophic bacteria in Kongsfjorden were monitored during June–October 2012. Observations on water mass composition and phytoplankton biomass were also made in order to understand the impact of the same on the occurrence and abundance of heterotrophic bacterial species.

Water samples were collected from 10 locations (Fig. 1) at various depths from 5 m to a maximum of 80 m in the month of June, July, August, September and October.

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Fig. 1 Location of sampling sites in Kongsfjorden, Ny-Ålesund in Svalbard, Arctic. The stations 2, 3, 3.1, 3.4 and 4 represent the outer fjord (filled circle) and stations 6, 7, 7.1, 7.4 and 8 represent the inner fjord (filled circle) (modified from Hop et al. [3])

Sampling was done following the observations with conductivity-temperature-depth (CTD) profiler (SBE 19 plus V2, Sea Bird Electronics, USA) equipped with a fluorescence sensor (Wet Labs, Philomath, USA). Water masses were delineated based on the classification by Cottier et al. [4]. The samples were processed and subjected to bacteriological analysis in the field laboratory without delay. Total cells in the water samples (10 ml each) collected from all the discrete depths were stained with 4', 6-di-amidino-2-phenylindole (DAPI) [5] before enumeration. For retrieval of heterotrophic bacteria, three replicates of water samples per depth were spread plated using 100 µl aliquot on pre cooled (4 °C) quarter strength (full strength: 40.25 g/L) Zobell Marine Agar (Himedia, Mumbai, India) and incubated at 4 °C for 1–2 weeks. Each morphotype were then subjected to 16S rRNA gene sequencing analysis. Total 51 bacterial isolates were sequenced. Bacterial genomic DNA was extracted and 16S rRNA gene was amplified using the universal bacterial 16S forward primer (27f) 5'-AGA GTT TGA TCM TGG CTC AG-3' and bacterial 16S reverse primer (1492r) 5'-GGT TAC CTT GTT ACG ACT T-3' [6]. The 16S rDNA sequence of the isolate (~1200 nt) was compared with type strains belonging to the same phylogenetic group obtained from

Ribosomal Database Project (<http://rdp.cme.msu.edu/>) [7]. Phylogenetic trees were constructed using maximum likelihood and Neighbour-joining methods of tree making algorithms in MEGA Version 7 [8]. The 16S rRNA gene sequences of the strains were deposited with accession numbers LN624599–LN624631 and LK391677–LK391694 in the EMBL database. Percentage abundance of the representative species of each phylotype was calculated out of the total number of bacterial species retrieved during each month. Alpha diversity indices were calculated using software PAST 3.11 [9].

During June, warmer surface water (SW) (max temperature of 7 °C) was observed confined to the inner fjord. The Intermediate water (IW) and Transformed Atlantic water (TAW) reached till the surface in the central and outer fjord respectively, which co-occurred with the increase in the abundance of autotrophic biomass (Supplementary Figs. 1 and 2). Increased phytoplankton abundance therefore could be due to favorable advection processes functional during the formation of TAW [10]. During fall (September–October), the inner fjord experienced decline in the phytoplankton biomass (Supplementary Fig. 2) which could be due to strong increase in sediment load that could limit light penetration [11].

Total bacterial counts ranged from 10^7 to 10^8 cells L^{-1} in both outer and inner fjord (Supplementary Table 1). Earlier study by Jankowska et al. [12] conducted during summer 1999 has reported bacterioplankton abundance in the range of 10^8 – 10^9 cells L^{-1} in Kongsfjorden and adjacent Krossfjorden. Localized and transient increase in the abundance of phytoplankton could alter the levels of dissolved organic matter and thereby influence the abundance and activity of bacterial community [13]. Retrievable heterotrophic bacterial counts were in the range of 10^6 CFU L^{-1} from June to October. Previous studies from Kongsfjorden have reported the viable counts in the range of 10^5 – 10^6 CFU L^{-1} [14] with the low counts attributed to the culture-dependent approach using only one medium [15].

All the bacterial isolates recovered from the water samples collected in June, July, August, September and October were categorized into 15, 21, 19, 19 and 7 phylotypes respectively (Supplementary Table 2). Shannon diversity index was highest for July (2.49) and lowest for October (1.59) (Supplementary Table 3). Out of the total 22 distinct phylotypes, 13 belonged to the non-polar group and 9 to the polar group (Supplementary Figs. 3A and 3B). The bacterial isolates retrieved during the analyses belonged to four phyla, viz. the γ -proteobacteria, α -proteobacteria, Bacteroidetes and Actinobacteria. Out of the 22 phylotypes, 13 belonged to phylum γ -proteobacteria, 3 to α -proteobacteria, 4 to Bacteroidetes and 2 to Actinobacteria (Supplementary Table 2). 16S rRNA gene sequences corresponding to α -proteobacteria, Firmicutes and γ -proteobacteria have been earlier reported from freshwater as well as marine water samples of the Kongsfjorden [16]. In the current study, phylum Bacteroidetes and Actinobacteria were also retrieved along with α -proteobacteria and γ -proteobacteria. The predominance of Actinobacteria, Proteobacteria, and Bacteroidetes has already been reported in Arctic water, ice and sediments [14, 15, 17, 18]. γ -proteobacteria was the most dominant group from June to October (Supplementary Table 4). Bacteroidetes was the second most abundant phylum in the fjord water. Maximum retrieval of α -proteobacteria ($\sim 7\%$) was in August while they were not retrieved in October. Actinobacteria showed decrease in their population from June (22.6 %) to September (5.5 %) in the outer fjord (Supplementary Table 4). Decrease in population of Actinobacteria and α -proteobacteria was found to co-occur with decrease in the autotrophic biomass from June to October. Studies based on stable carbon isotope probes have demonstrated that members of α -proteobacteria and Actinobacteria can assimilate algal extracts quickly and have a broad range of hydrolases that can be expressed in immediate response to phytoplankton detritus availability [19].

Among the non-polar phylotypes, *Halomonas titanicae* was the dominant γ -proteobacteria in the outer fjord with

maximum abundance in October (25.8 %) (Supplementary Figs. 4A and 4B). The type strain of this species has been isolated from the samples of rusticles collected from the RMS Titanic wreck site in the Atlantic Ocean [20]. Recent study from the North Atlantic Ocean showed that distinct water bodies host different bacterial populations which may serve as biological markers for oceanic provinces [21]. The increased population of *H. titanicae*, therefore, could be due to the increased intrusion of Atlantic water into the Kongsfjorden during October. In the outer fjord, members of the phylum Actinobacteria (*Rhodococcus yunnanensis* and *Rhodococcus fascians*) constituted $\sim 11\%$ of the non-polar population in June. Increased autotrophic biomass in June at the outer fjord could promote the growth of *R. fascians* which is a well-known phytopathogen. A study by Mergaert et al. [22] showed that a large proportion of facultative and psychrotrophic strains isolated from Arctic and Antarctic seawaters has been grouped into the *R. fascians* cluster. Similar distribution was observed for *Maribacter stanieri*, the lone representative of non-polar Bacteroidetes. This phylotype was pre-dominantly isolated from the phytoplankton dominated niche in the outer fjord from July to September with maximum population observed in August (8.8 %). The type strain of this species was isolated from the green alga *Ulva fenestrata* collected from the Sea of Japan [23]. Analyses of phytoplankton pigments from the same stations have shown higher concentration of Chl *b* (marker pigment for *Chlorophyceae*) in the outer fjord (8.09 mg m^{-2}) as compared to inner fjord (3.04 mg m^{-2}) during August (Supplementary Table 5). This observation supports the report of its association with green algae which could be the reason for its higher abundance in the outer fjord. The non-polar α -proteobacteria was represented by *Erythrobacter*, *Sulfitobacter* and *Sphingopyxis* with lower population density as compared to the members of γ -proteobacteria, Bacteroidetes and Actinobacteria. Retrieval of such known oligotrophic strains [24, 25] indicates the high level of metabolic plasticity exhibited by bacteria in highly dynamic fjord conditions.

A number of studies have reported the distribution patterns where bipolar distribution of bacteria appears to be common [26–28]. Polar isolates retrieved in this study were classified under the phyla γ -proteobacteria and Bacteroidetes. γ -proteobacteria was represented by 6 phylotypes under the genera *Pseudomonas*, *Pseudoalteromonas*, *Shewanella*, *Glaciecola* and *Alteromonas* while Bacteroidetes was represented by 3 phylotypes under the genera *Algoriphagus*, *Leeuwenhoekella* and *Aequorivita* (Supplementary Figs. 4C and 4D). Many genera belonging to γ -proteobacteria (*Alteromonas*, *Pseudoalteromonas* and *Glaciecola*) are common inhabitants of the marine part of the biosphere and have very diverse habitats [29]. These bacteria play an important role in marine environment

owing to their abundance and high metabolic activities. Pseudoalteromonads are highly capable of surviving in nutrient-poor marine environment by adjustment of their biochemical pathways and production of a wide variety of metabolites including biologically active compounds and enzymes [30, 31]. *Leeuwenhoekiella aequorea* constituted the major fraction of polar *Bacteroidetes* (Supplementary Figs. 4C and 4D). The other two *Flavobacteria* retrieved in this study, viz. *Algoriphagus ratkovskyi* and *Aequorivita antarctica* were originally isolated from sea ice and under-ice sea water respectively, from Antarctica [32, 33]. Maximum abundance of *Aequorivita antarctica* in the inner fjord in August coincides with high autotrophic biomass (Supplementary Fig. 2). Cultivation and molecular studies suggest that *Flavobacteria* can colonize living algae, absorbing nutrient exudates produced during photosynthesis [34–36]. Over half of the organic matter formed by photosynthetic primary production is broken down by bacteria [37] and it has been demonstrated that *Flavobacteria* are the major decomposers of high-molecular-mass organic matter in sea water [38].

In conclusion, the heterotrophic bacterial community structure was fundamentally different in different months which could be linked to changes in the water masses and/or phytoplankton bloom dynamics. Thus, assessment of the retrievable heterotrophic bacterial assemblage in the Arctic fjord on long term may give valuable insights into the complex ecological role they play under extreme and dynamic conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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