Indian J.Sci.Res. 20(1): 56-62, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

# BACTERIAL COMMUNITIES FROM NEAR GLACIER AND TRANSITION ZONES OF KONGSFJORDEN, ARCTIC: A METAGENOMIC STUDY

**ANJALI S. MOHAN[a], P. NUSAIRA[b], RESHMA RAJU[c], JAYESH PUTHUMANA[d], BHAVYA KACHIPRATH[e], SOLLY SOLOMON[f], G. JAYANATH[g] AND ROSAMMA PHILIP[h1]**

[abcefgh]Department of Marine Biology, Microbiology & Biochemistry, Cochin University of Science and Technology, Cochin, India
[d]National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Fine Arts Avenue, Kochi-16, Kerala, India
[f]Fishery Survey of India, Mormugao, Zonal Base, Vasco da Gama, Goa, India

## ABSTRACT

**Arctic region is of paramount importance due to its unique biodiversity and associated biogeochemical cycles. It encompasses more than 20,000 species widely distributed to various phyla. The present study is focused on some of the bacterial communities involved in the biogeochemical cycles in Kongsfjorden stretch of Arctic Ocean. Surface water samples were collected from near glacier and transition zones of Kongsfjorden, Arctic and sequential multistage pre-filtration of water samples were carried out to separate out the bacterial fraction followed by metagenomic DNA extraction. 16S rDNA amplification revealed the presence of ammonia oxidizing bacteria, methanogenic bacteria and archaea and a nested PCR approach exposed the occurrence of sulphate reducing bacteria and denitrifying bacteria. Representatives of *Desulfomaculum* sp., Desulphobulbaceae, *Desulphovibrio* sp., *Desulphobacter* sp., Desulphobacteriaceae and *Nitrosospira* sp. were detected from both near glacier and transition zones of Arctic. This study offered a profile of bacterial diversity responsible for modulating microbial driven processes such as sulphate reduction, ammonia oxidation, nitrogen fixation, etc. in Arctic region. Such fundamental studies are crucial for improving the knowledge with reference to community composition in polar marine regions which are currently bearing the brunt of climate change.**

**KEYWORDS:** Arctic; Kongsfjorden; Metagenomics; Marine Bacteria; Sulphate Reducing Bacteria.

Even though smaller in size, the role of microbial communities along the ecosystems is inevitable. Present status of our planet is a cumulative effect from various activities of early microbes which played crucial role in planetary as well as biological evolution. Based on the adaptability to various physico-chemical features, microbes endure and contribute themselves as mineralizers and recyclers of essential *nutrients* in biogeochemical cycles. Unique ability of marine microbes also facilitates smooth functioning of nutrient cycles which are unfeasible with other organisms. Moreover, metabolic capabilities of marine microbes can also be exploited for biotechnological applications such as manufacturing industrial energy products (Madhavan et al., 2017) and they also serve as rich source of novel bioactive compounds which can be used as pharmaceuticals. Microbial populations inhabited in extreme marine environments with high rate of adaptations are reported to play significant role in balancing the ecosystem (Zahran, 1997). Even though viruses are the abundant life forms, bacterial communities overrule almost all the metabolic and ecological pathways in polar ecosystem. Polar bacterial communities exhibit uniqueness over other microbial forms due to rare and diverse physiological and biochemical properties.

Nevertheless microbes are the most abundant biological entities in the biosphere, their discovery and analysis cannot be properly attained due to the confines in culture dependent techniques. Though marine microbial exploration is a thorny area of research, innovative ideas and emergence of new tools in genomics as well as metagenomics enhance prospects for exploring enormous diversity of marine microbial communities (Handelsman, 2004; Kennedy et al., 2010; Zielinska et al., 2017). To circumvent the difficulties of culture based techniques, metagenomic approach was enabled for the exposure of micro fauna from fjord environment. Monitoring of fjord bacterial community ensures us to study their ecological role in various biogeochemical cycles and in global climatic change.

Arctic Ocean is encircled by relatively large land masses and influenced by the influx from land as well as Pacific / Atlantic Oceans; hence the microbial flora depends on polar and non-polar entities (Galand, 2009). Atlantic waters contributing the terrestrial inflow revise the fjord environment to a great extent. Nevertheless, both glacial input and structure of fjord contributing the remoteness from the coast gradually keep the inner fjord more Arctic (Hop et al., 2002). The present study focused on Kongsfjorden, Arctic, one of the largest fjords of Svalbard Archipelago which is highly influenced by afflux and other anthropogenic actions, but less studied. This work strives to delineate the bacterial communities along

[1]**Corresponding Author**

the Kongsfjorden and thereby assessing their role in ecosystems.

## MATERIALS AND METHODS

### Sample Collection and Harvesting of Bacterial Communities

Surface seawater was collected from near glacier ($78^0$99'31"N; $12^0$3'00"E) and transition ($78^0$5'54"N; $11^0$51'24"E) zones of Kongsfjorden, Arctic, during Summer Arctic Expedition 2015 (NCAOR) (Fig. 1). 20 liters seawater was pre-filtered through filter membranes of various pore sizes (11µm, 1.45µm, 0.45µm & 0.22µm respectively) in a stainless steel filter holder (Millipore) pressured by a peristaltic pump (Solomon et al., 2016). Both 1.45µm and 0.22µm filter membranes with entrapped bacterial particles were used for extraction of bacterial metagenomic DNA.

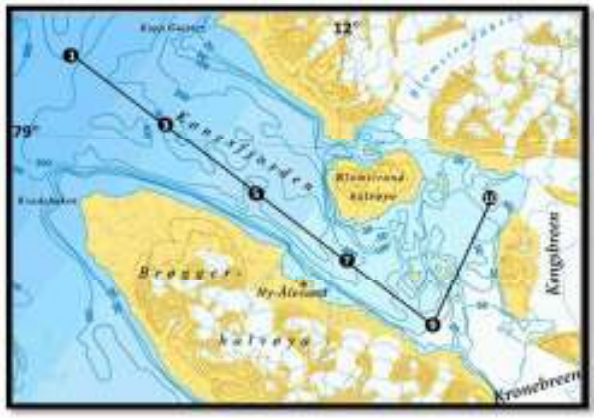


**Figure 1: Map indicating sampling sites from Kongsfjorden, Arctic**

### Metagenomic DNA Extraction

Bacterial fraction entrapped in 1.45µm and 0.22µm cellulose nitrate filter membranes were detached by treating the filter membranes with a mixture of 1.5ml extraction buffer and 1.5µl of Tween-20. Subsequently, 50ml centrifuge tubes with filter membranes were vortexed for 10 minutes to facilitate the detachment of bacterial cells. Extraction mixture with bacterial cells was then centrifuged at 12,000rpm for 15 minutes. DNA extraction was performed using modified Zhou et.al. (1996) protocol and the procedure consists suspending bacterial cells (pellet) in 500 µL extraction buffer (100mMTris-HCl, 100mM EDTA, (pH 8.0), 100mM sodium phosphate (pH 8.0) and 1.5 M NaCl). To the suspension, 10% CTAB, 20% SDS and proteinase K (20mg/ml) were added and incubated at 55°C for 2 hours. The resulting supernatant was mixed with an equal volume of chloroform: iso-amylalcohol (24: 1, vol/vol) after a high speed centrifugation. To the aqueous phase obtained after chloroform: iso-amylalcohol wash, 600µl iso-propanol was added and the mixture was left overnight at $4^0$C, followed by centrifugation (14,000 rpm, 30 min). Washing was carried out using70%ice-cold ethanol followed by 100% (vol/vol) ethanol rinse. Dried pellets were dissolved in TE buffer.

### Quality and Quantity Determination of Metagenomic DNA

Quality estimation of extracted DNA was performed by agarose gel electrophoresis and quantification was carried out using Qubit® dsDNA HS (High Sensitivity) Assay Kit in a Qubit® Fluorometer as per manufacturer's instructions.

### PCR Amplification and Screening of Amplicons with Group Specific Primers

For detecting the occurrence of ammonia oxidizing bacteria, methanogenic bacteria and archaea, PCR amplification with group specific primers was performed. A nested PCR approach was also employed in which at first the 16SrDNA genetic marker was PCR amplified using universal primers, (27F:5'-AGAGTTTGATCTGGCTCAG-3'; 1492R: 5'-TACGGYTACCTTGTTACGACTT-3'; Lane, 1991)which was followed by the screening of 16S rDNA amplicons with group specific primers to confirm the presence of sulphate reducing bacteria and denitrifying bacteria (Table 1).

### Sequencing and Phylogeny Analysis

PCR products were sequenced at M/s Scigenom, Cochin and sequences were used for phylogenetic tree construction using Neighbour-joining analysis for determining the evolutionary relationships among the taxa *via* NCBI BLAST, BioEdit and MEGA 5.2.

## RESULTS AND DISCUSSION

Concentration of extracted metagenomic DNA was 24.5µg/ml and 25.6µg/ml from the transition zone and near glacier zone respectively. Total DNA yield obtained from both stations was found to be 122.5µg/L and 128µg/L respectively. Gel images also substantiate that extracted DNA was of good quality (Fig. 2). PCR amplification of 16SrRNA also implies that extracted metagenomic DNA is highly free from various inhibitory compounds like humic acids (Fig. 3).

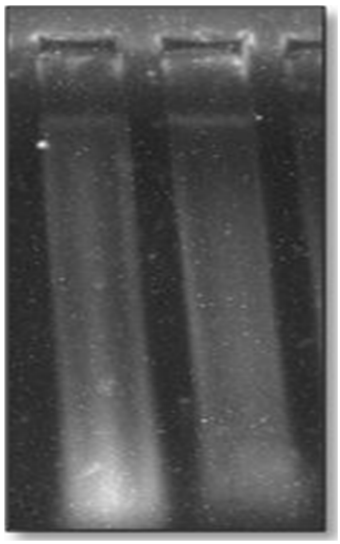

**Figure 2: Metagenomic DNA extracted from both transition (Lane 1) and near glacier (Lane 2) zones of Kongsfjorden**

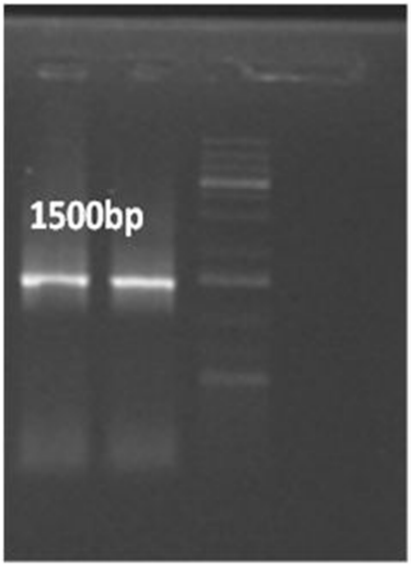


**Figure 3: Gel image of 16S PCR amplicons from both stations of Kongsfjorden, Arctic. (Lane 1- from transition zone, Lane 2 - from near glacier zone and Lane 3-1Kb ladder)**

Metagenomic DNA from both stations were screened with different groups of functional primers for nitrifying bacteria, sulphate reducing bacteria, ammonia oxidising bacteria, methanogenic bacteria, denitrifying bacteria and Archaea. Archaea, the extreme environmental components and methanogens, the anoxic methane producers were also detected from the near glacier zone (Fig. 4).

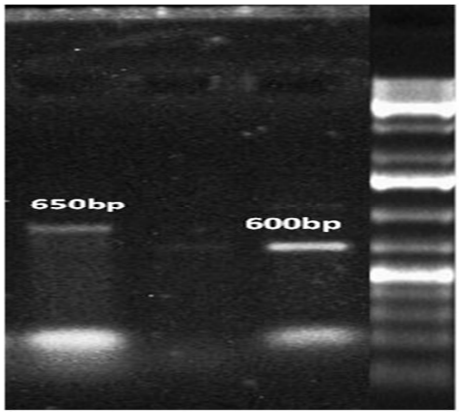


**Figure 4: Gel image showing the presence of Archaea and Methanogen from near glacier region. (Lane 1- Archaea (650bp), Lane 3- Methanogen (600bp) and Lane 4- 1Kb ladder)**

Ammonia oxidizing bacteria belonging to the γ-Proteobacteria showed their prevalence in both fjord environments. Autotrophic ammonia-oxidising bacteria (AOB) which produce the key functional enzyme, ammonia monooxygenase (AMO) have historically been thought solely responsible for most ammonia oxidation, the rate limiting step in nitrification, in terrestrial and aquatic ecosystems (Banning et al., 2015). Ammonia oxidizing bacteria converts the most reduced form of nitrogen i.e., ammonia to nitrate ($NO_3$). *Nitrosomonas* sp., the dominant ammonia oxidizing bacterial group was detected from the near glacier zone while *Nitrosospira* sp. was detected from both stations (Fig. 5A & 5B). *Nitrospira* like groups already detected from the central Arctic region (Beman, 2006) implies their effect on monitoring the global biogeochemical cycling in the frosty environment.

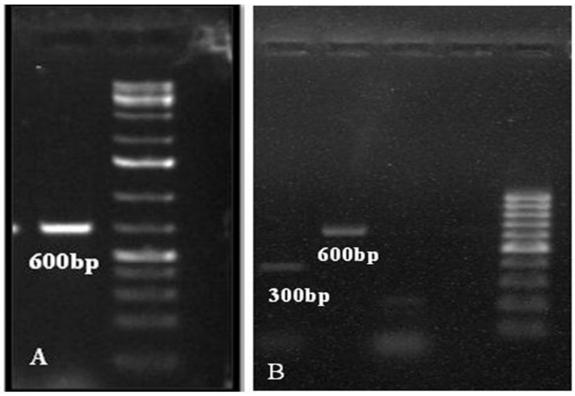


**Figure 5: Gel images showing the occurrence of ammonia oxidizing bacteria from transition zone and near glacier region. 5A) Lane 1- *Nitrosospira* sp (600bp), Lane 2- 1Kb ladder; 5B) Lane 1 -**

***Nitrosomonas sp.*** **(300bp), Lane 2-** ***Nitrosospira*** **sp (600bp) and Lane 5 - 1Kb ladder.**

Community composition was analysed using a nested PCR approach for the detection of sulphate reducing bacterial groups. Sulphate reducing bacteria of the genus *Desulfomaculam* (DEM), *Desulfobacter* (DSB); family Desulfobulbaceae (DSBB) and Desulphobacteriaceae (DSR) and order Desulfovibrionales (DSV) were detected from transition zone of Kongsfjorden, Arctic (Fig. 6A to 6D). *Desulfomaculam*, *Desulfovibrio, Desulfobacter* and members from Desulfobacteriaceae were identified from the near glacier zone (Fig. 6E & 6F). Sulphate reducing bacterial groups obtained energy by oxidizing organic compounds or hydrogen ($H_2$) and reducing sulphate ($SO_4^{2-}$) to hydrogen sulphide ($H_2S$). Sulphate reducing bacteria also play momentous roles in the anaerobic oxidation of methane. At similar declined temperatures, psychrophilic sulfate reducers exhibit comparable elevation in metabolic rates than their mesophilic counterparts as a physiological adaptation to the permanently cold Arctic environment (Knoblauch, 1999) which elucidates the successful functioning of nitrogen cycle in polar environment.

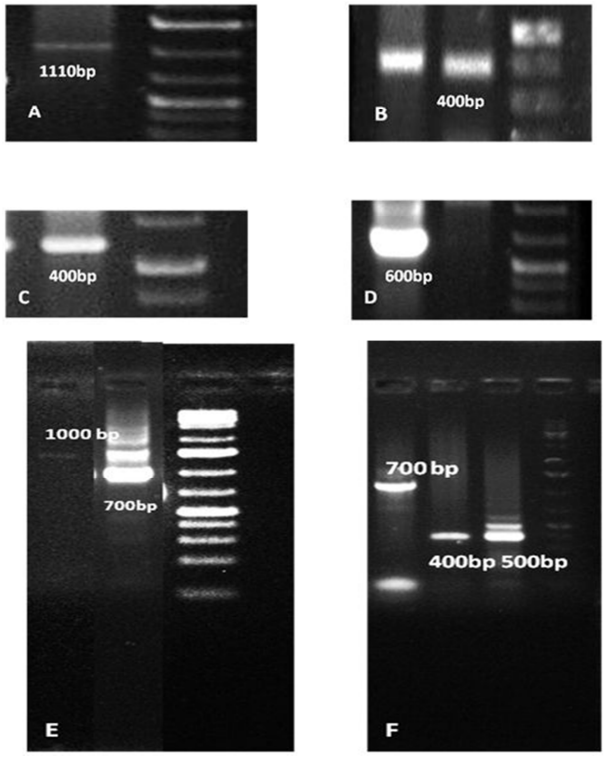


**Figure 6: Agarose gel images showing the presence of sulphate reducing bacteria from transition zone (6A to 6D) and from near glacier region (6E & 6F). 6A)** ***Desulfomaculum*** **sp.; 6B)** ***Desulphobacter*** **sp.; 6C) Desulphobacteriaceae; 6D)** ***Desulphovibrio*** **sp.; 6E) Lane 1-** ***Desulphovibrio*** **sp. (1000bp), Lane 2-** ***Desulfobacter*** **sp. (700bp), Lane 3 - 1kb ladder; 6F) Lane 1 -** ***Desulfobacter*** **sp. (700 bp), Lane 2 and 3 - Desulfobacteriaceae (400 and 500 bp), Lane 4 - 1 Kb ladder.**

Occurrence of denitrifying bacteria was confirmed from both stations using the unique nitrous oxide reductase (nosZ) gene, the key gene for the detection of denitrifiers from environmental samples. From the transition and near glacier zones, nosZ genes with an amplicon size of 700bp were detected (Fig. 7A & 7B). Phylogenetic analysis implied that nosZ gene in the metagenomic DNA exhibit assemblage with partial nosZ genes from uncultured forest soil bacterium which indicates the significance of metagenomics from extreme environments where culturable microbes are considerably less (Fig. 8).

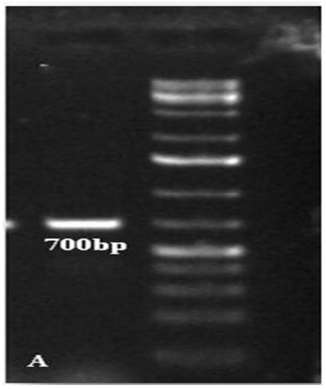


**Figure 7A: Gel images showing presence of denitrifying bacteria (nosZ) from transition zone; Lane 1- nosZ gene (700bp) Lane 2- 1Kb ladder**

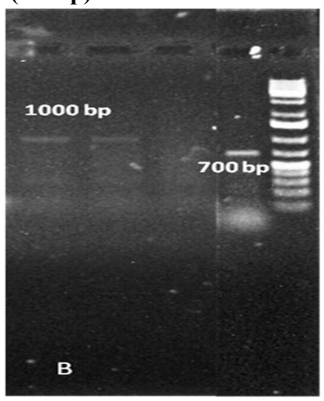


**Figure 7B: Denitrifying bacteria (nirS and nosZ) detected from near glacier region; Lane 1- nirS gene (1000bp) , Lane 4- nosZ gene (700bp) and Lane 5 - 1Kb ladder.**


**Figure 8: Phylogenetic tree of nosZ genes from different species of denitrifiers**

**(**The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary distances were computed using the Maximum Composite Likelihood method)

**Table 1: List of primers used for screening of functional groups of bacteria**

| Sl. No. | Category | Primer Name | Sequence (5' --- 3') | Bacteria (Functional groups)/ Target gene |
|---|---|---|---|---|
| 1 | Archaea | Arch 21F<br>Arch 958 R | TTC CGG TTG ATC CYG CCGGA<br>YCCGGCGTTGAMTCCAATT | Archaea |
| 2 | Methanogenic bacteria | 146F<br>1324R | GGSATAACCYCGGGAAAC<br>GCGAGTTACAGCCCWCRA | Methanogens |
| 3 | Sulphate reducing Bacteria | DEM 116F<br>DEM 1164R | GTAACGCGTGGATAACCT<br>CCTTCCTCCGTTTTGTCA | *Desulphovibrio* sp. |
| 4 | | DSBB 280F<br>DSBB 1297R | CGATGGTTAGCGGGTCTG<br>AGACTCCAATCCGGACTGA | Desulphobulbaceae |
| 5 | | DSV 682F<br>DSV 1402R | GGTGTAGGAGTGAAATCCG<br>CTTTCGTGGTGTGACGGG | *Desulphovibrio* sp. |
| 6 | | DSB 127F<br>DSB 1273R | GATAATCTGCCTTCAAGCCTGG<br>CYYYYYGCRRAGTCGSTGCCCT | *Desulphobacter* sp. |
| 7 | | DSR 1F<br>DSR 4R | ACSCACTGGAAGCACG<br>GTGTAGCAGTTACCGCA | Desulphobacteriaceae |
| 8 | Denitrifying Bacteria | nosZ F<br>nosZ R | CGYTGTTCMTCGACAGCCAG<br>CATGTGCAGNGCRTGGCAGAA | Nitrous oxide reductase gene |
| | | nirS1f<br>nirS6r | CCTYATGGCCGCCRCART<br>CGTTGAACTTRCCGGT | Nitrite reductase gene |
| 9 | Ammonia oxidising bacteria | Ns 85F<br>Ns 1009R | TCGCCACCAGGGTTGCCCCC<br>GCACACCCACCTCTCAGCGG | *Nitrosospira* sp. |

Nitrite reductase gene (nirS) of denitrifying bacteria was detected from near glacier zone of Kongsfjorden. Role of these α-proteobacteria are crucial in marine coastal sediments, that they successfully remove 40 to 50% of external inputs of dissolved inorganic nitrogen by means of denitrification (Seitzinger, 1990) which in turn causes unbalanced nitrogen content in ocean. The resultant product of denitrification dangerously leads to accumulation of different oxides of nitrogen which in turn causes serious impacts on global warming and thereby contributes to ozone layer depletion (Conard, 1996). Consequently, existence of denitrifiers is significant to a wide extent and should be well monitored.

Co-existence of ammonia oxidising bacteria along with denitrifiers in the fjord well explains that the two microbial components regulate the nitrogen cycling all along the fjord effectively, which in turn functionally regulates the nitrogen input and output to the fjord ecosystem. On the other extent, the methanogens and sulphate reducing bacteria would lead to the generation of methane and hydrogen sulphide in the system.

## CONCLUSION

The 16S PCR based screening of metagenomic DNA depict the existence of archaea, methanogens, nitrifiers, denitrifiers and sulphate reducing bacterial communities in Kongsfjorden, Arctic. From the current study, it is evident that bacterial communities resided in the fjord environment plays crucial role in the uninterrupted manoeuvre of bio-geochemical cycling and thereby regulating the global climatic changes.

### Authors' Contributions

JP performed sample acquisition; RP conceived and designed the experiment; RR and NP performed the experiments; ASM wrote the manuscript. BK, JG, SS and RP participated in the discussion of the manuscript; All authors were involved in the revision of the draft manuscript and have approved the final manuscript.

### Conflict of Interest

The authors declare that they have no conflict of interest.

## ACKNOWLEDGEMENT

Authors are grateful to ESSO-NCAOR, Goa for providing sampling facilities. Authors are also thankful to Dept. of Marine biology, Microbiology and Biochemistry and National Centre for Aquatic Animal Health (NCAAH), Cochin University of Science and Technology for providing necessary facilities for this study. Thanks also extended to the authorities of 27th Swadeshi Science Congress for providing an opportunity to publish this work.

## REFERENCES

Banning N.C., Maccarone L.D., Fisk L.M. and Murphy D. V., 2015. Ammonia-oxidising bacteria not archaea dominate nitrification activity in semi-arid agricultural soil. Sci. Rep, **5**:11146 | DOI: 10.1038/srep11146.

Beman J.M. and Francis C.A., 2006. Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hyper nutrified subtropical estuary: Bahia del Tobari, Mexico. Appl. Environ. Microbiol., **72**(12): 7767-7777.

Conrad R., 1996. Soil microorganisms as controllers of atmospheric trace gases ($H_2$, CO, $CH_4$, OCS, $N_2O$, and NO). Microbiol Rev., **60**:609–640.

Galand P.E., Casamayor E.O., Kirchmann D.L. and Lovejoy C., 2009. Ecology of the rare microbial biosphere of the Arctic Ocean. Proc. Natl. Acad. Sci., **106**:22427–22432.

Handelsman J., 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev., **68**(4):669-685.

Hop H., Pearson T., Hegseth E.N., Kovacs K.M., Wiencke C., Kwasniewski S. and Lydersen C., 2002. The marine ecosystem of Kongsfjorden, Svalbard. Polar Res., **21**(1): 167-208.

Kennedy J., Flemer B., Jackson S.A., Lejon D.P., Morrissey J.P., O'gara F. and Dobson A.D., 2010. Marine metagenomics: new tools for the study and exploitation of marine microbial metabolism. Mar. Drugs, **8**:608-628.

Knoblauch C., Sahm K. and Jorgensen B.B., 1999. Psychrophilic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigusoceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofabagelida* gen. nov., sp. nov., *Desulfotaleapsychrophila* gen. nov., sp. nov.and*Desulfotaleaarctica* sp. nov. Int. J. Syst. Evol. Microbiol., **49**: 1631-1643.

Lane D.J., 1991. Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York: 115.

Madhavan A., Sindhu R., Parameswaran B., Sukumaran R. K. and Pandey A., 2017. Metagenome analysis: a powerful tool for enzyme bioprospecting. Biotechnol. Appl. Biochem., **183**(2): 636-651.

Seitzinger S.P., 1990. "Denitrification in aquatic sediments." Denitrification in soil and sediment. Springer. Boston, MA, pp.301-322.

Solomon S., Kachiprath B., Jayanath G., Sajeevan T. P., Singh I.B. and Rosamma P., 2016. High-quality metagenomic DNA from marine sediment samples for genomic studies through a preprocessing approach. 3Biotech, **6**(2): 160.

Zahran H.H., 1997. Diversity, adaptation and activity of the bacterial flora in saline environments. Biol. Fertil. Soils, **25**:211-223.

Zhou J., Bruns M.A. and Tiedje J.M., 1996. DNA recovery from soils of diverse composition. Appl. Environ Microbiol., **62**: 316-322.

Zielinska S., Kidawa D., Stempniewicz L., Los M. and Los J.M., 2017. Environmental DNA as a valuable and unique source of information about ecological networks in Arctic terrestrial ecosystems. Environ Rev., **999**:1-10.