

the orthogonal pair of  $O$  and  $E$  corresponds to a base of orthogonal metrics capturing the quality and excellence aspect of research performance and  $X$  then becomes a composite performance indicator for this<sup>2</sup>. Similarly, the orthogonal pair of STP and  $X$ /STP corresponds to a base of orthogonal metrics capturing the productivity aspect of research performance and  $X^2$ /STP then becomes a composite performance indicator for this. Figures 1–4 capture these as scatterplots on the respective pairs of parameter space for both countries. Some interesting results are noticed. The large HEIs in China are nearly five times bigger than their counterparts in India. However, India has a noticeable edge in quality/excellence and productivity. Both in China and India, two small institutions, the Chongqing University of Arts and Sciences (STP = 0.19) and the Gandhigram Rural Institute (STP = 0.19) perform extremely well on the size-independent performance indicators. In terms of size, the Indian Institute of Science, Bengaluru, and the Tsinghua and Peking Universities of China lead in their respective higher education systems.

The performance of leading HEIs in India and China has been compared using an end-to-end bibliometric performance analysis. Six carefully chosen primary and secondary bibliometric indicators summarize the chain of activity: From PCA it is established that the primary indicators are orthogonal and represent size-dependent quantity and a size-independent quality/productivity dimensions respectively. Two-dimensional maps can be used to visualize the results. Although the key Chinese institutions are considerably larger, the Indian counterparts have the edge in productivity and in maintaining excellence.

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## Distribution pattern of bacteria in the two geographic poles and Southern Ocean from the reported 16S rDNA sequences

Pratibha Gupta<sup>1</sup>, Harsh Kumar Agrawal<sup>1</sup> and Rajib Bandopadhyay<sup>1,2,\*</sup>

<sup>1</sup>Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi 835 215, India

<sup>2</sup>UGC-Centre of Advanced Study, Department of Botany, The University of Burdwan, Golapbag, Bardhaman 713 104, India

**16S rDNA bacterial sequences (913) from the Arctic Ocean, Southern Ocean and Antarctic Iceland were studied to understand the bacterial distribution pattern. Through phylogenetic study, it was observed that some bacteria were common in both the Arctic Ocean and Antarctic Iceland.  $\gamma$ -Proteobacteria occupied 77.7% of the total bacterial population in the Antarctic Iceland, whereas in the Southern Ocean it was 72.5% and in the Arctic Ocean it was 50.9%. GC (Guanine + Cytosine) content of the bacteria in the Arctic Ocean and Antarctic Iceland region was 54.4% and 53.8% respectively. Bacterial diversity was calculated using Shannon–Weiner index and was found to be highest in the Antarctic Iceland (1.6926).**

**Keywords:** Bacterial phylogeny, bioinformatic tools, geographic poles and oceans, microbial diversity.

ABOUT 75% of the Earth's surface is covered by oceans. There are major five oceans in the world, namely Pacific Ocean, Atlantic Ocean, Indian Ocean, Southern Ocean (earlier known as Antarctic Ocean) and Arctic Ocean. The Antarctic and Arctic regions are different from each other. The Arctic Ocean is surrounded by continents and there is only 10% of freshwater inflow, whereas there is no inflow of freshwater in the Southern Ocean<sup>1</sup>. The Southern Ocean surrounding the Antarctic continent is driven by a current system known as Antarctic Circumpolar Current (ACC), which is the strongest current system in the world oceans<sup>2</sup>. At the beginning of the 20th century, there was a concept 'everything is everywhere' implying that prokaryotic population genetics can never be broken by physical isolation, but by adaptation alone<sup>3</sup>. One of the major questions regarding biodiversity in the two geographic poles is whether marine bacterial species are the same at both the poles or not. According to endemism theory some microbes require special environment (hot springs, cryosphere and hyperhalophilic habitats) that are not commonly found throughout the globe<sup>4</sup> and thus they have restricted geographical range<sup>5</sup>. Studies till date have shown that members of the same genera occur

\*For correspondence. (e-mail: rajib\_bandopadhyay@yahoo.com)

at both the poles<sup>6</sup>, i.e. Arctic and Antarctic regions. To compare the microbial diversity in the two poles it is necessary to compare the 16S rDNA sequences of the bacteria, as the 16S rDNA region is expected to be least affected by horizontal gene transfer<sup>7,8</sup>. This conserved region also helps to design the PCR primers for various taxa at different taxonomic levels<sup>9</sup>.

Hundreds of marine bacterial genome sequences have been made available in the NCBI database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), which provides an opportunity to study evolutionary genomic diversity. Phylogenetic tree can be used for assigning taxonomy to community sequence data<sup>10</sup>, inferring co-speciation gene prediction<sup>11</sup>, identifying ecological trends<sup>12</sup>, etc.

The present study shows the distribution pattern of bacterial species at the two different poles and Southern Ocean using bioinformatics tools such as MEGA 5.2, MolQuest, etc. For the study 392 16S rDNA bacterial sequences from the Arctic region and 492 16S rDNA bacterial sequences from the Antarctic region were downloaded from the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Also, 29 bacteria were isolated using culture-dependent method from the Southern Ocean water sample amplified using 16S rDNA primers and sequenced using the Sanger method. These 29 sequences have been submitted to NCBI by the authors. All the 913 bacterial sequences were analysed using MolQuest for sequence similarity and MEGA 5.2 was used to calculate the GC (Guanine + Cytosine) content. The sequences were analysed with a Classifier program of Ribosomal Database Project (RDP) and classified according to phylum, class, order, family and genus.

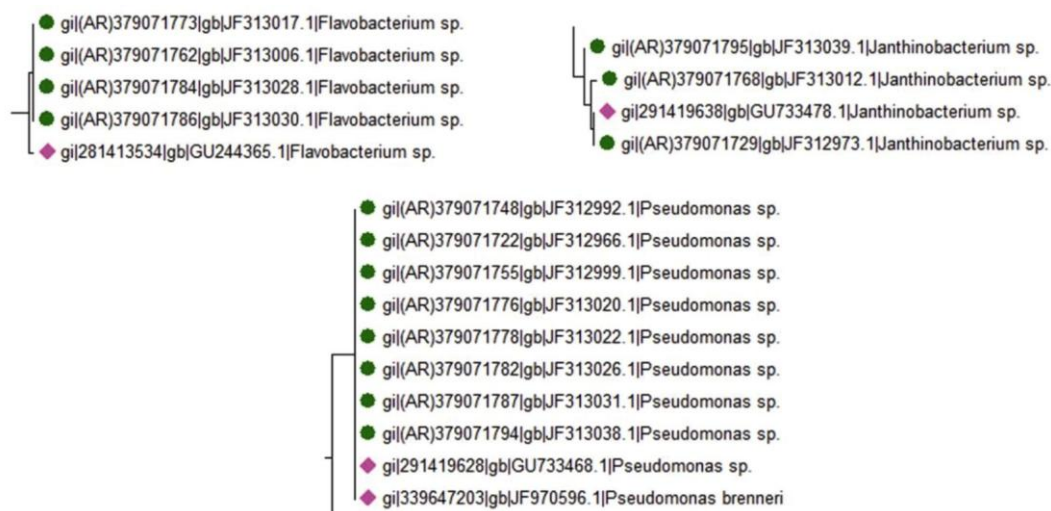
The 29 bacteria were isolated using culture-dependent method in marine agar medium (peptone 5 g/l, yeast extract 3 g/l and volume made up with sea water). The bacterial DNA was amplified using universal 16S rDNA

primer pairs (forward 8f (5'-GAGTTTGATCATGGCTC-AG-3') and reverse 1459r (5'-CTACGGCTACCTTGT-TACG-3') primers) followed by sequencing. GC content of the bacterial sequences (Arctic Ocean, Antarctic Iceland and Southern Ocean) was calculated using MEGA 5.2 (ref. 13). MolQuest was used for determining the similarity percentage between the sequences. Phylogenetic tree was constructed using the Neighbour-joining method having Kimura 2-parameter with 1000 bootstrap value. Bacteria were classified using the RDP classifier (<http://rdp.cme.msu.edu/classifier>). Diversity index of the Arctic Ocean, Southern Ocean and Antarctic Iceland bacteria was calculated using Shannon-Weiner diversity index ( $H'$ ) as

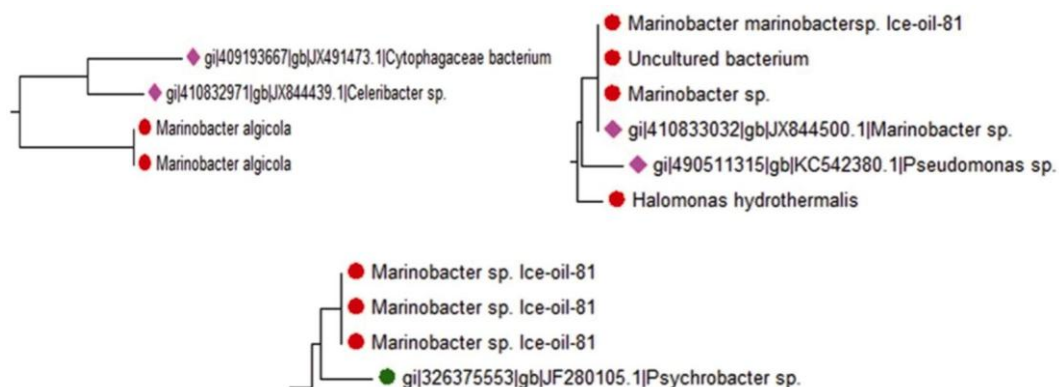
$$H' = -\sum_{i=1}^R P_i \ln P_i.$$

The proportion of species  $i$  relative to the total number of species ( $P_i$ ) and  $\ln P_i$  is the natural logarithm of the proportion of species.

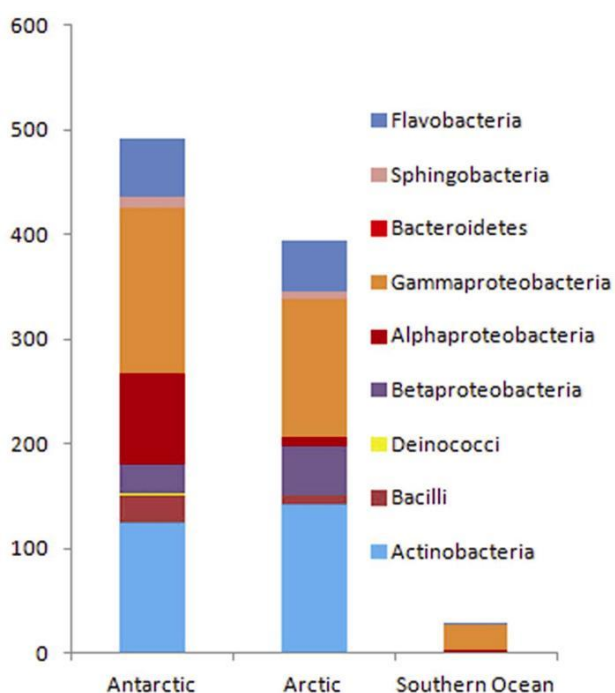
It is difficult to distinguish the bacteria on the basis of their morphology. Despite the recognized importance of marine microorganisms, very little is known about their magnitude and diversity in nature<sup>14</sup>. An increasing molecular technique (such as sequencing, metagenomics, etc.) helps in the study of community structure within the bacterioplankton and phytoplankton<sup>15</sup>. The 29 bacteria were isolated on the basis of their morphology. The bacterial DNA was isolated, amplified and sequenced using 16S rDNA primer and the sequence was submitted to NCBI (accession nos KF019643 to KF019671). In the present study, phylogenetic tree of total 913 bacterial sequences from the Arctic Ocean, Southern Ocean and Antarctic Iceland was studied using MEGA 5.2 having



**Figure 1.** Bacteria common in both the Arctic Ocean (green colour) and Antarctic Iceland (pink colour).



**Figure 2.** Bacteria that are common in the Arctic Ocean (green colour), Southern Ocean (red colour) and Antarctic Iceland (pink colour).



**Figure 3.** Abundance of bacteria in the Antarctic, Arctic and Southern Ocean.

Kimura 2-parameter with 1000 bootstrap value (see [Supplementary Information, Figure S1 online](#)). Some of the bacteria were present both in the Arctic Ocean and Antarctic Iceland, e.g. *Pseudomonas sp.*, *Flavobacterium sp.* and *Janthinobacterium sp.* (Figure 1), whereas some were present in the Arctic Ocean, Southern Ocean as well as in Antarctic Iceland (Figure 2). Using this result, it may be hypothesized that during continental drift, some of the bacteria were separated in two different poles and they survived in the respective conditions. It also proves the theory ‘everything is everywhere’, that is, there is no limitation in dispersal and environmental selection will decide the survival of organisms<sup>5,16,17</sup>. Most of the bacte-

rial phyla are widely distributed among different habitats, and it has been demonstrated that, the difference of bacteria may be due to salinity<sup>18</sup>.

It was observed that different species of the same bacterial genus in the Arctic Ocean, e.g. *Pseudomonas sp.*, *Cellulomonas sp.*, *Cryobacterium sp.*, *Paenibacillus sp.*, *Hafnia sp.*, *Flavobacterium sp.*, *Janthinobacterium sp.*, *Arthrobacter sp.*, *Formosa sp.*, *Pseudoalteromonas sp.*, *Moritella sp.*, *Psychrobacter sp.*, *Oxalobacteraceae bacterium*, *Pedobacter sp.*, *Leifsonia sp.*, *Demequina lutea*, *Shewanella sp.*, *Colwellia sp.*, *Streptomyces sp.*, *Mycobacterium sp.* and *Vibrio sp.* were closely related. Also, in Antarctic Iceland some of the bacteria were closely related.

Five out of 29 bacteria, isolated from the Southern Ocean were closely related to those from the Arctic Ocean, viz. *Marinobacter sp. Ice-oil-81* was close to *Psychrobacter sp.* Uncultured bacterium was close to *Arthrobacter sp.* (see [Supplementary Information, Figure S1 online](#)) from the Arctic region.

Most of the bacteria that were reported from the Southern Ocean can be classified as uncultured bacteria. The average GC content of the isolated bacteria from the Arctic Ocean and Southern Ocean was 54.5% and 54.8% respectively, whereas in Antarctic Iceland the GC content was found to be 53.8%. Genes located in low-GC region show evidence of shorter intron and less biased codon usage relative to the higher-GC region<sup>19</sup>.

Bacteria isolated from the Antarctic ice core region were reported to be mostly halophilic and psychrotolerant in nature<sup>20</sup>. According to similarity search using Mol-Quest software, most of the bacterial species e.g. *Janthinobacterium sp.*, *Shewanella sp.*, *Flavobacterium sp.* and *Pseudomonas sp.* were common in both the Arctic Ocean and Antarctic Iceland and their percentage similarity was 99.6%, 94.2%, 88.5% and 87.6% respectively (Table 1). Similarly percentage identity among the bacteria from the Southern Ocean and Arctic Ocean was also calculated. It was observed that the DNA sequence of *Marinobacter sp.*

**Table 1.** Percentage similarity between bacteria from the Arctic Ocean and Antarctic Iceland

Bacteria from Antarctic Iceland	Bacteria from the Arctic Ocean	Identity (%)
<i>Pseudomonadaceae</i> bacterium	<i>Formosa</i> sp.	44.3
<i>Sulfitobacter</i> sp.	<i>Sphingomonas</i> sp.	34.0
<i>Oxalobacteraceae</i> bacterium	<i>Variovorax</i> sp.	48.6
<i>Arthrobacter</i> sp.	<i>Bacillus</i> sp.	46.1
<i>Actinomycetales</i> bacterium	<i>Cellulomonas</i> sp.	39.9
<i>Pseudomonadaceae</i> bacterium	<i>Stenotrophomonas</i> sp.	67.7
<i>Marixanthomonas</i> sp.	<i>Cellulomonas</i> sp.	43.6
<i>Oxalobacteraceae</i> bacterium	<i>Pseudomonas</i> sp.	36.1
<i>Cryobacterium</i> sp.	<i>Flavobacteriaceae</i> bacterium	66.6
<i>Acinetobacter</i> sp.	<i>Pedobacter</i> sp.	40.2
<i>Erythrobacter</i> sp.	<i>Arthrobacter</i> sp.	44.3
<i>Arthrobacter sulfonivorans</i>	<i>Spirosoma spitsbergense</i>	60.5
<i>Acinetobacter</i> sp.	<i>Paenibacillus</i> sp.	69.3
<i>Actinomycetales</i> bacterium	<i>Aeromicrobium</i> sp.	96.0
<i>Flavobacteriaceae</i> bacterium	<i>Sphingobacteriaceae</i> bacterium	42.1
<i>Arenibacter</i> sp.	<i>Vitellibacter</i> sp.	26.2
<i>Sulfitobacter</i> sp.	<i>Arthrobacter</i> sp.	42.5
<i>Croceibacter</i> sp.	<i>Olleya</i> sp.	31.7
<i>Actinomycetales</i> bacterium	<i>Arthrobacter</i> sp.	93.1
<i>Shewanella livingstonensis</i>	<i>Shewanella</i> sp.	97.4
<i>Brevibacillus</i> sp.	<i>Janthinobacterium</i> sp.	74.8
<i>Roseobacter</i> sp.	<i>Rhodiferax</i> sp.	66.0
<i>Roseobacter</i> sp.	<i>Bacteroidetes</i> bacterium	71.6
<i>Bacillus cereus</i>	<i>Pseudomonas</i> sp.	38.0
<i>Oceanicola</i> sp.	<i>Arthrobacter</i> sp.	30.0
<i>Pseudomonadaceae</i> bacterium	<i>Alcanivorax</i> sp.	47.5
<i>Aurantimonas</i> sp.	<i>Sphingomonas</i> sp.	78.5
<i>Actinobacteridae</i> bacterium	<i>Formosa</i> sp.	30.8
<i>Roseobacter</i> sp.	<i>Bacillus</i> sp.	67.7
<i>Glaciecola punicea</i>	<i>Acinetobacter</i> sp.	16.1
<i>Arthrobacter</i> sp.	<i>Hafnia</i> sp.	75.5
<i>Flavobacteriaceae</i> bacterium	<i>Polaribacter</i> sp.	68.3
<i>Stenotrophomonas</i> sp.	<i>Pseudomonas</i> sp.	83.0
<i>Roseobacter</i> sp.	<i>Bacillus</i> sp.	43.0
<i>Oxalobacteraceae</i> bacterium	<i>Arthrobacter</i> sp.	69.8
<i>Phaeobacter</i> sp.	<i>Alpha proteobacterium</i>	24.8
<i>Arthrobacter phenanthrenivorans</i>	<i>Psychrobacter</i> sp.	65.2
<i>Pseudomonas</i> sp.	<i>Roseobacter</i> sp.	74.7
<i>Celeribacter</i> sp.	<i>Pseudomonas</i> sp.	14.3
<i>Psychrobacter</i> sp.	<i>Colwellia</i> sp.	84.1
<i>Roseobacter</i> sp.	<i>Cellulomonas</i> sp.	35.4
<i>Rhodococcus fascians</i>	<i>Flavobacterium</i> sp.	73.6
<i>Actinomycetales</i> bacterium	<i>Arthrobacter</i> sp.	84.7
<i>Actinomycetales</i> bacterium	<i>Arthrobacter</i> sp.	71.3
<i>Sphingomonas faeni</i>	<i>Janthinobacterium</i> sp.	68.8
<i>Flavobacterium</i> sp.	<i>Muricauda</i> sp.	87.4

Ice-oil-81 showed similarity with *Psychrobacter* sp., whereas uncultured bacterium showed similarity with *Arthrobacter* sp. It was also observed that the bacteria present in the Arctic Ocean and Antarctic Iceland showed more than 90% similarity, e.g. *Serratia* sp. from the Arctic showed 99.4% similarity with *Hafnia* sp. from the Arctic Ocean. In the Antarctic region *Oxalobacteraceae* and *Burkholderiales* were 98.7% similar in their DNA sequences.

Phyla *Actinobacter*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* are common in cold environment<sup>21,22</sup>. With the help of RDP, phylogram interpretation was done

and it was found that *Actinobacteria* and  $\gamma$ -*Proteobacteria* occupied 55.2% and 50.9% respectively. Apart from  $\gamma$ -*Proteobacteria*,  $\alpha$ -*Proteobacteria*, *Acintobacteria*, *Flavobacteria* and  $\beta$ -*Proteobacteria* were also present, whereas *Deinococci* and *Bacteroidetes* were less abundant and present only in the Antarctic region (Figure 3). Abundance of *Sphingobacteria* was more or less same in both the Antarctic and Arctic regions. The detailed region-wise descriptions are given in Table 2.

Diversity index value depends upon the type and evenness of bacterial species in a community. The Shannon–Weiner index ( $H'$ ) of the Arctic Ocean, Southern Ocean

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**Table 2.** Abundance (no.) of bacterial class in Antarctic Iceland, Arctic Ocean and Southern Ocean

Class of bacteria	Antarctic Iceland	Arctic Ocean	Southern Ocean
<i>Actinobacteria</i>	124	142	0
<i>Bacilli</i>	27	8	0
<i>Deinococci</i>	1	0	0
<i>Betaproteobacteria</i>	28	48	0
<i>Alphaproteobacteria</i>	87	9	3
<i>Gammaproteobacteria</i>	158	131	25
<i>Bacteroidetes</i>	1	0	0
<i>Sphingobacteria</i>	10	8	0
<i>Flavobacteria</i>	56	48	1

and Antarctic Iceland was 1.4821, 0.4787 and 1.6926 respectively. In the Arctic and Antarctic regions the abundance of bacterial species is unequal, viz. in the Antarctic region  $\gamma$ -*Proteobacteria* are abundant, whereas in the Arctic Ocean, *Actinobacteria* are abundant followed by  $\gamma$ -*Proteobacteria*.

When bacteria were separated during continental drift, the isolated species may have evolved through environmental selection. Biodiversity study of bacterial community in the Arctic Ocean, Southern Ocean and Antarctic Iceland is important to understand the distribution pattern, evolution and existence of threatened species. Bacterial biogeography is in its infancy and it is difficult to obtain information regarding their diversity. In the present study, we focused on the distribution pattern and diversity of bacterial species in the two poles and Southern Ocean, based on their 16S rDNA sequence information readily available in the public domain and also based on our laboratory sequence data. The results of the study suggest that bacteria of class *Actinobacteria* are dominant in the Arctic Ocean. In the Antarctic region  $\gamma$ -*Proteobacteria* are abundant followed by *Actinobacteria*. This may be due to variation in environmental conditions (may include temperature, pressure and different selection mechanism)<sup>24</sup>. It was also observed that some of the bacteria were present at both the poles. Hence our study supports the fact that most of the microbes are geographically distributed, while some follow the law of endemism.

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