DIVERSITY AND DISTRIBUTION OF CULTURABLE AND NON-CULTURABLE FORMS OF MARINE ACTINOBACTERIA AND ITS ROLE IN SOUTHERN OCEAN BIOGEOCHEMISTRY

PROJECT COMPLETION REPORT

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PROJECT COMPLETION REPORT

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- **EXECUTIVE SUMMARY :**

Southern Ocean (SO) comprises the southernmost waters of the World Ocean, encircling Antarctica, and is easily subjected to rapid climate change. The SO is intensively investigated for its significance in global biogeochemical cycling. Microbes, being the main drivers of biogeochemical cycles, play an essential role in ecosystem functioning. This has led to the focus on microbial community structure analysis of SO microbes. Actinobacteria, being the largest contributor of natural product discovery, still remains untapped in the ecosystems of SO. The focus of our study was to explore the diversity of Actinobacteria in different depths of water and sediment samples from SO. Culture dependent and independent studies were performed to determine the diversity of actinobacterial genera. Rare actinobacteria such as *Chryseoglobus indicus, Micrococcus luteus, Rhodococcus erythropolis, Brachybacterium* sp., *Microbacterium oxydans, Streptomyces harbinensis* and *Brevibacterium casei* were obtained as a result of 16S rRNA sequencing of strains cultivated in the laboratory. Metagenomic analysis revealed presence of numerous unknown genes. *Agromyces, Brachybacterium, Corynebacterium, Dietzia, Glutamicibacter, Gordonia, Microbacterium, Mycobacterium, Nocardioides, Psuedonocardia, Rhodococcus* and *Sva0996 marine groups* were the most prominent genus of Actinobacteria obtained from metagenomic analysis. These results promote the idea of tapping the actinobacteria for novel metabolites of medical, food and industrial applications. This brings us closer to finding novel drugs against unfamiliar infections of the modern world. Further study involving bioprospecting of such rare actinobacterial genera will be a boon to the natural product drug discovery.

INTRODUCTION

Encircling the Antarctic continent at about 35°S lies the Southern Ocean (SO), a unique oceanographic environment having strong winds that flow over its surface due to lack of continental barriers to block the wind flow (Chapman et al., 2020). Further, these waters are transported across longitudes by a strong circumpolar current called the Antarctic Circumpolar Currents (ACC), resulting in redistribution of nutrients, salts, heat and organic matter. The oceanographic settings of the SO coupled with high productivity makes it a sink of atmospheric carbon dioxide of global significance (Sarkar et al., 2019). Owing to its significance in global biogeochemical cycling and recent warming in response to climate change (Henley et al., 2020), the SO is currently subjected to intensive investigations. The microbiology of SO, especially the analysis of microbial community structure, has gained great attention in recent times as microbes are the main drivers of biogeochemical cycles playing an essential role in ecosystem functioning (Singori et al., 2014). Yet, the abundance and diversity of microorganisms in the SO is not fully understood (Pinkerton et al., 2021).

Actinobacteria are one among the largest bacterial phylum with high ecological and industrial significance. They are well recognized for its contribution in natural products discovery notably antibiotics (Liu et al., 2023). The distribution of actinobacteria in different marine regions of marine environment is also well documented (van Bergeijk et al., 2023; Brahimi et al., 2020). Marine derived actinobacteria are recognized as an important source for diverse bioactive compounds (Solanki et al., 2008). Nonetheless, only a limited number of studies are carried out to understand the diversity of actinobacteria in the SO. A few culturable forms of actinobacteria were isolated from the High Nutrient Low Chlorophyll (HNLC) waters of the Indian sector of the SO (ISO) (van Bergeijk et al., 2023). Gupta et al. (2015) explored a small portion of microbiota from the SO waters through culture-dependent method and even isolated few bacteria that have not been cultured and characterized before. Also, Sivasankar et al. (2018) used GIS to map the diversity of Actinobacteria in the Polar Frontal waters of the ISO and described the dominance of *Streptomyces*.

An amplicon microbiome study by Sadaiappan et al. (2020) revealed that the overall community structure of unculturable actinobacteria is remarkably similar in Polar and Sub-polar waters and their study also showed the domination of *Streptomyces* in the SO. Nevertheless, the study by Sadaiappan et al. (2020) was carried out using surface water samples. Thus, the actinobacterial community structure in the water samples at different depths of the SO is not known. In this context, this study aims to analyze the actinobacterial diversity using water samples collected at different depths of the SO. In addition, this study also analyzes the actinobacterial diversity in the sediment samples of the SO which is still undetermined. This research work is primarily designed to create a baseline report on unculturable Actinobacteria from the ISO and the outcome of the study will help to isolate culturable forms of actinobacteria and also to understand the ecology of Actinobacteria in the ISO.

REVIEW OF LITERATURE & RATIONALE FOR STUDY

National Status

Identification of microorganisms plays a key role in the determination of the composition of microbial diversity for bioprospecting of biotechnologically important biomolecules. Bhave et al., (2012) had published the first report of isolation of *Streptomyces radiopugnans* from Antarctica. To date, there is only one publication regarding *S. radiopugnans* R97T isolated from radiation-polluted soil. Like the type strain, Antarctica isolate was also thermotolerant and radio tolerant, but in addition, it required salts for growth and did not degrade phenol. We envisaged that metabolic pattern of the same species varies based on acclimatization in its native ecological habitat. Additionally, Antarctica isolate had produced novel antibacterial compounds (patent-US2012/ 0156295). The study highlighted that least explored extreme regions like Antarctica are rich resources of novel microbial strains producing novel bioactive compounds.

Tale et al., (2015) isolated actinomycetes from Antarctic water samples. All the isolated actinomycetes were evaluated for their ability to synthesize various enzymes. Results indicated that four (RGI-1 to RGI-4) out of five isolates were positive for amylase production. Two (RGI-1 and RGI-2) isolates screened positive for protease activity. Two (RGI-1 and RGI-5) were positive for cellulase activity. Significantly one of the isolates (RGI-1) could produce four different types of enzymes.

Sivasankar et al., (2018) reported the Geographic Information System (GIS) to map and catalogue the data on the actinobacterial diversity of the Southern Ocean waters was completed through sampling and analysis. Water samples collected at two sampling stations viz. Polar Front 1 (Station 1) and Polar Front 2 (Station 2) during 7th Indian Scientific Expedition to the Indian Ocean Sector of the Southern Ocean (SOE-2012-13) were used for analysis. At the outset, two different genera of Actinobacteria were recorded at both sampling stations. Streptomyces dominated with a high score (> 60%), followed by *Nocardiopsis* (< 30%) at both the sampling stations-Polar Front 1 and Polar Front 2-along with other invasive genera such as *Agrococcus*, *Arthrobacter*, *Cryobacterium*, *Curtobacterium*, *Microbacterium*, *Marisediminicola*, *Rhodococcus* and *Kocuria*. This data will help to discriminate the diversity and distribution pattern of the Actinobacteria in the Polar Frontal Region of the Southern Ocean waters. It is a novel approach useful for geospatial cataloguing of microbial diversity from extreme niches and in various environmental gradations. Furthermore, this research work will act as the milestone for bioprospecting of microbial communities and their products having potential applications in healthcare, agriculture and beneficial to mankind. Hence, this research work would have significance in creating a database on microbial communities of the Antarctic ecosystem.

Digitalization is the process that solve discrepancies in microbial identification and cataloguing their diversity in distinct ecological habitats. In view of this connection, the psychrophilic and psychrotolerant actinobacteria were isolated from the water samples of the Polar Frontal region of the Southern Ocean. 16S rRNA gene sequencing for identification of psychrophiles was carried out and sequences were deposited in NCBI GenBank. 16S rRNA gene sequences were used to create QR codes, CGR, FCGR and GC plot. This generated digital data help to relate the diversity amongst the isolated actinobacterial strains. The digital data showed considerable divergence among the actinobacterial strains. This generated

bioinformatics data is helpful in the delimitation of the psychrophilic and psychrotolerant actinobacteria. Thus, the present study is a robust and accurate method for the identification of Polar microorganisms in a fixed boundary. Hence, this work will help to assign a unique digital identity to microorganisms in near future (Rekadwad et al., 2018).

International Status

A study was carried out to identify the actinomycetes isolated from the soils of the Antarctic Island Livingston and to investigate their potential to synthesize antibacterial agents against phytopathogens. Twenty-three actinomycete strains were the object of this study. Using PCR (polymerase chain reaction) amplification, all strains were affiliated to genus Streptomyces. The sequencing of the 16S rRNA for three of the strains showed greatest similarity to *Streptomyces tendae* for one of them, and revealed that the other strains had closest relations to streptomycetes isolated from anthropogenically unaltered regions including Antarctica. The isolates were studied for production of antibacterial substances both by molecular and culture methods. PCR targeting specific biosynthetic genes involved in the production of some groups of antibiotics was performed. The screening showed that all strains possessed the gene for Type-II polyketide synthase, 11 strains for non-ribosomal peptide synthetase; 6 strains for polyene antibiotics; and 4 strains for glycopeptide antibiotics. The production of antibacterial substances by the strains was tested *in vitro* against phytopathogenic bacteria. The strains differed in the number of inhibited test bacteria and in their spectrum of action. Four strains showed a wide range of activity against Gram-positive and Gram-negative phytopathogens. The results obtained revealed that the Antarctic soils are potential source for isolation of streptomycetes producing antibiotics from different groups (Malinova et al., 2014).

Tonkova et al., (2014) reports a partial characterization and biosynthetic abilities of 26 thermophilic actinobacteria isolated from soil and penguin excrement samples from Livingston Island, Antarctica. About 15% of the tested Antarctic actinobacteria were able to tolerate up to 4% NaCl in the growth medium. We found that all strains are sensitive to eight, and 77% to ten of the 12 tested antibiotics suggesting relatively low anthropogenic impact in this Antarctic region. The Antarctic actinobacteria were tested for hydrolytic enzymes activity, antibiotic and hemolytic activity. It was found that all strains were able to hydrolyze starch, 81% - tributyrin, and 65% -casein. All Antarctic actinobacteria demonstrated hemolytic activity, and about 27% - antimicrobial activity against some common bacterial pathogens. The results obtained revealed promising strains that are producers of industrially important thermostable enzymes and antibiotic compounds.

Three *Streptomyces* strains isolated from Antarctic soils were screened for *in vitro* and *in vivo* antibacterial activity towards causative agents of bacterial spot of pepper – *Xanthomonas vesicatoria, Xanthomonas euvesicatoria* and *Xanthomonas gardneri*. Cell-free filtrates of cultural media and mycelium extracts obtained in 120 h of the cultivation of the streptomycetes were used. The *in vitro* antimicrobial activity was determined by the agar diffusion method. All streptomycetes showed antibacterial effect which varied for different test-bacteria and the producer strains. The extracts from the mycelia of the strains were more active. The potential of the streptomycetes for *in vivo* control of phytopathogenes was investigated by treatment of pepper seeds infected with *X. vesicatoria, X. euvesicatoria* and *X. gardneri* using streptomycetes cell-free filtrates and mycelium extracts. The cell-free extracts did not show activity, whereas the extracts manifested high decontamination activity

(between 94 and 100%) decreasing seed germination slightly (between 8 and 30%) (Malinova et al., 2015).

A study reported the microbial diversity and edaphic drivers of community structure, across Browning Peninsula which is an ice-free polar desert situated in the Windmill Islands, Eastern Antarctica site, using traditional cultivation methods, a novel approach termed soil substrate membrane system (SSMS), and culture-independent 454-tag pyrosequencing. The measured soil environmental and microphysical factors of chlorine, phosphate was found to be significant drivers of the bacterial community, while none of the soil parameters analyzed were significantly correlated to the fungal community. Overall, Browning Peninsula soil harbored a distinctive microbial community in comparison to other Antarctic soils comprised of a unique bacterial diversity and extremely limited fungal diversity. Tag pyrosequencing data revealed the bacterial community to be dominated by Actinobacteria (36%), followed by Chloroflexi (18%), Cyanobacteria (14%), and Proteobacteria (10%). For fungi, Ascomycota (97%) dominated the soil microbiome, followed by Basidiomycota. As expected, the diversity recovered from culture-based techniques was lower than that detected using tag sequencing. However, in the SSMS enrichments, that mimic the natural conditions for cultivating oligophilic "k-selected" bacteria, a larger proportion of rare bacterial taxa (15%), such as *Blastococcus, Devosia, Herbaspirillum, Propionibacterium* and *Methylocella* and fungal (11%) taxa, such as Nigrospora, Exophiala, Hortaea, and Penidiella were recovered at the genus level. At phylum level, a comparison of OTU's showed that the SSMS shared 21% of Acidobacteria, 11% of Actinobacteria and 10% of Proteobacteria OTU's with soil. For fungi, the shared OTUs was 4% (Basidiomycota) and <0.5% (Ascomycota). This was the first known attempt to culture microfungi using the SSMS which resulted in an increase in diversity from 14 to 57 microfungi OTUs compared to standard cultivation. Furthermore, the SSMS offers the opportunity to retrieve a greater diversity of bacterial and fungal taxa for future exploitation (Ferrari et al., 2017).

RATIONALE OF THE STUDY

The Southern Ocean is currently subjected to intensive investigations, mainly related to its importance for global biogeochemical cycling and its alarming rate of warming in response to climate change. Microbes play an essential role in the functioning of this ecosystem and are the main drivers of the biogeochemical cycling of the elements. Yet the diversity and abundance of microorganisms in this system remain poorly studied. Actinobacteria are the ecologically and industrially important group of bacteria which are widely distributed in terrestrial and marine ecosystems. Sivashankar et al., (2018) reported the psychrophilic and psychrotolerant actinobacterial strains from polar front waters collected from Indian part of the Southern Ocean. Except this, there is no encouraging report on actinobacteria from the Indian sector of Southern Ocean. In this context, the proposed work will open up a new avenue in actinobacterial research in the Indian sector of Southern Ocean.

AIMS & OBJECTIVES

Aim:

To study the diversity and distribution of marine actinobacteria in the Indian sector of Southern Ocean and their role in biogeochemical cycling

Objectives:

1. Collection of water samples from the Indian sector of Southern Ocean and their physico-chemical characterization
2. Determination of unculturable actinobacterial diversity through metagenomic approach
3. Determination of culturable actinobacteria diversity
4. Characterization and taxonomy of actinobacterial strains isolated from Indian sector of Southern Ocean and their *ex-situ* conservation

**No deviations were made in the committed objectives except the number of stations covered.*

- **METHODOLOGY**

Objective 1: Collection of water samples from the Indian sector of Southern Ocean and their physico-chemical characterization

Sample collection:

Overall, 20 stations were proposed to study the cultural and uncultured diversity of actinobacteria from the Indian sector of Southern Ocean *[Ref No: NCPOR/2019/PACER-POP/BS-08 dated 05-07-2019]*. During the 11th Indian Expedition to Southern Ocean, vertical water samples were collected from 11 stations along the cruise track in the Indian Sector of Southern Ocean to study the culturable and unculturable actinobacterial diversity. This included 3 stations between 40°S and Prydz Bay, 4 from coastal region and 4 samples from different fronts (PF2, PF1, Sub Antarctic Front (SAF) and Sub Tropical Front (STF)] **(Figure 1)**. Both the samples collected for culturable actinobacterial study and unculturable diversity study were received and safely preserved at 4°C and -20°C respectively, at NCPOR, Goa.

From each location, 3 samples were be collected from three different depths (surface water, sub surface chlorophyll maxima and base of the photic depth) **(Figure 2).** Physico-chemical properties of all the samples will also be studied at NCPOR. The collected samples will be filtered in duplicates through 0.2 µm filters and the samples shall be used to study non-culturable actinobacteria. Aliquots of seawater samples shall also be used for isolation of culturable forms of actinobacteria. The taxonomic identification of culturable forms shall be carried out following standard protocols to understand its diversity.

Objective 2: Determination of uncultured actinobacterial diversity through metagenomic approach

Isolation of e-DNA

DNA from the particulate matter trapped on the membrane filters was extracted by adopting CLU (combination of lysis and ultrasonication) method described by Djurhuus et al. (2017). The concentration and purity of the DNA was evaluated with a Nanodrop 2000 spectrophotometer.

PCR amplification

In the first step, the total DNA isolated was used as template and amplified using actinobacteria specific primers 243F (5'-GGATGAGCCCGCGGCCTA-3') and A3R (5'-CCAGCCCCACCTTCGAC-3') to amplify actinobacterial 16S rRNA genes (1.2 kb) (Tian et al., 2007). The PCR products was further amplified with the primers S-d-Bact-0341-b-S-17 (5'-

CCTACGGGNGGCWGCAG-3') and S-d-Bact-0785-aA-21 (5'-GACTACHVGGGTATCTAATCC-3') targeting the V3-V4 hyper variable regions of bacterial 16S rRNA genes (average length of ~450 bp) (Caparaso et al., 2012). DNA amplification was done using NEB Phusion high fidelity DNA polymerase. Following amplification, 2 µL of PCR product was used to verify amplification success with 2% agarose gel electrophoresis and eluted using PureLink gel extraction kit.

DNA sequencing

The sequencing libraries was generated using NEB Next®Ultra™ DNA Library Prep Kit for Illumina. The library quality was assessed on the Qubit @ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina MiSeq platform. The DNA isolation, PCR amplification and DNA sequencing was carried out by the AgriGenome Labs Pvt. Ltd., Cochin, India.

Bioinformatic analysis

The raw 16S rRNA amplicon sequences were included in the bioinformatic analysis as described earlier (Ramakodi et al., 2021). In brief, the data was processed on the DADA2 tool (Callahan et al., 2016) with the following criteria: the first 25 bases were removed from forward and reverse reads; the forward and reverse reads were truncated to the length 245bp; the reads were preprocessed with the maximum expected error (maxEE) of 2 for forward and reverse reads; the cleaned reads were denoised using the error rates learnt by the DADA2 package; the denoised reads were merged and chimeric sequences were removed. Finally, the amplicon sequence variants (ASVs) were inferred. The taxonomy classification was carried out using the RDP classifier (Wang et al., 2007) as implemented in the DADA2 package. The database SILVA version 138 was used for taxonomy assignment as SILVA was found to provide good toxonomic resolution in environmental microbiome studies (Ramakodi 2022). The alpha and beta diversity were analyzed using Phyloseq version 1.30.0 (McMurdie and Holmes, 2013). All the bioinformatic analysis was carried out on R version 3.6.3 with the following dependencies/ packages: tidyverse, ggplot2, dplyr, ape, DESeq2, microbiome, reshape2, vegan, pals and RcolorBrewer.

Objective 3: Determination of diversity of culturable forms of actinobacteria

Isolation of culturable actinobacteria:

Culturable actinobacterial forms present in the vertical water samples collected from the Indian sector of Southern Ocean was isolated onboard **(Figure 3)** as well as in the laboratory after the cruise. The cells retained in the filter paper was dislodged in a 50 ml of sterile water and a 100 µl of the sample was spread plated on different selective media viz., Actinomycetes Isolation Agar (AIA), starch casein agar (SCA), Nutrient Agar. Plating was done in triplicate and one un-inoculated plate will be kept as control. Water samples was also directly plated on the above-mentioned isolation media. All the agar plates were incubated for 60 days at 6°C for the isolation of psychrophilic actinobacteria and at 20°C for the isolation of psychrotolerant actinobacteria **(Figure 4)**. After 60 days incubation, the population of the Actinobacteria appearing on agar was counted and expressed in terms of Colony Forming Unit per milliliter (CFU/mL) after calculation. Only the isolates displaying negative in KOH string test (preliminary confirmatory test for selective Gram-Positive bacteria inc. Actinobacteria) was isolated and stored for further studies.

Objective 4: Characterization and taxonomic identification of culturable actinobacteria and their ex-situ conservation:

Phenotypic and chemotaxonomic analysis: Phenotypic characteristics such as micromorphology, cultural, carbon utilization and physiological characteristics of potential actinomycete strain was studied by the method described by Shirling and Gottileb (1966). Based on the results of phenotypic and chemotaxonomic characteristics, distinct actinobacterial strains were identified at genus level with the help of Nonomura keys (1974).

Molecular characterization, phylogenetic analysis of actinobacteria: Actinobacterial DNA was isolated by adopting the method described by Cook and Meyers (2003) with some modifications, whenever necessary. The purified DNA obtained was suspended in TE buffer and stored at -20^{0}C. The PCR amplification of 16S ribosomal RNA gene was optimized using Thermal cycler (Gene AMP 2720 – Applied Biosystem). Universal and genus specific primers was used for amplification of 16 rRNA gene. Sanger Dideoxy chain Terminator sequencing was done in an automated DNA sequencer.

16S rRNA sequence analysis and multiple sequence alignment *:* The closely related homologs were identified through phylogenetic analysis by comparing the almost complete 16s rRNA gene sequence of actinobacteria (1400 nt) with the non-redundant database of nucleotide sequences deposited at NCBI web server (www.ncbi.nlm.nih.gov), through Basic Local Alignment Tool (BLAST) program (http://www.ncbi.nim.nih.gov/blast/). Dataset of potential orthologs was prepared by considering those database sequences which had >98% sequence identity with the query sequence. Phylogenetic analysis and nucleotide conservation of the data set sequences were studied through multiple sequence alignment program *viz*., Mega 5.0 and clustering was calculated with the neighbour-joining method. The dendrogram/phylogram was prepared using method of phylogenetic tree construction. Distances between the studied sequences help in understanding the evolutionary distance among the species.

Criteria for species identification*:* Identification of species was performed through sequence similarity basis following the criteria used by Bosshard et al. (2003) which states the following selection parameters. 1) When the percentage similarity of the query sequence and the reference sequence is 99% or above, the unknown isolate is assigned to reference species; 2) when the percentage similarity is between 99-95%, the unknown isolate is assigned to the corresponding genus, and 3) when percentage similarity is less than 95%, the unknown isolate is assigned to a family. ***Nucleotide accession number:*** The 16s rRNA sequence of the potential actinobacterial strains were submitted to Genbank to get the accession number.

Ex situ **conservation of actinobacteria by different preservation methods**

Taxonomically different actinobacterial cultures were selected for *ex situ* conservation. Actinobacterial cultures were preserved in triplicate by slant culture method using respective agar, glycerol stock using 20% glycerol broth and also by lyophilization.

- **SUMMARY OF THE RESULTS**

Objective 1: Collection of water samples from the Indian sector of Southern Ocean and their physico-chemical characterization

During 11th ISESO (Jan-Mar 2020), water samples from 11 vertical stations **Table 1**) at 3 different depths (surface, DCM and 200m) along the cruise track and processed on-board to study the culturable and unculturable actinobacterial diversity. All the samples were transported and stored at 4°C (for culturable study) and –20°C (for unculturable study) at NCPOR in May 2020.

In addition, during SOE11, a total of 264 psychrotolerant bacterial colonies were recovered from vertical water samples (surface, DCM, 200m) collected from 6 sampling stations which were also preserved at 4°C at NCPOR Goa. Due to lack of adequate incubation time, we were unable to recover and subculture the Psychrophilic bacterial colonies on-board. Hence, we transported the isolation agar plates (Fig. 2) containing the psychrophilic colonies to NCPOR along with the isolated psychrotolerant bacterial cultures.

Table 1. List of stations covered during first expedition (ISOSE - 11)

S. No	Station No	Lat.	Long
1.	SOE11-1	45˚00.15	72˚00.26
2.	SOE11-2	56˚59.49	78˚00.19
3.	SOE11-3	65˚00.01	80˚00.72
4.	SOE11-7	67˚32.38	76˚50.94
5.	SOE11-9	65˚00.00	76˚59.90
6.	SOE11-15	66˚50.31	71˚00.06
7.	SOE11-25	64˚59.86	65˚00.18
8.	SOE11-36	57˚00.11	57˚29.91
9.	SOE11-37	50˚24.99	57˚29.54
10.	SOE11-38	46˚59.98	57˚29.93
11.	SOE11-39	43˚00.37	57˚30.32

Objective 2: Determination of unculturable actinobacterial diversity through metagenomic approach

A data of 1.13 GB was received from the 30 water samples sequenced using Illumina MiSeq platform. The Illumina raw reads summary is given in **table 2 & fig. 3**. The number of reads obtained in the range between 98,963 to 1,85,984. The GC % was ranged between 51.5 to 56.9 and the obtained mean rad length is 250 base pairs. The detailed analysis of uncultured actinobacterial diversity in Southern Ocean waters is given in **Figure 5 and 6.**

Table 2. Summary of Illumina raw reads for metagenomics analysis to study the uncultured actinobacterial diversity in SO vertical water samples

Sample	Read orientation	Mean read quality (Phred score)	Number of reads	% GC	% Q < 10	% Q 10–20	% Q 20–30	% Q > 30	Number of bases (MB)	Mean read length (bp)

SOE11-2-200M	R1	35.93	116,330	52.55	0	5.4	2.49	92.1	29.08	250
	R2	34.82	116,330	52.83	0.04	8.72	3.73	87.52	29.08	250
SOE11-2-DCM	R1	36.34	98,923	51.5	0	4.31	2.09	93.6	24.73	250
	R2	35.29	98,923	51.39	0.04	7.34	3.27	89.35	24.73	250
SOE11-2-SURFACE	R1	36.28	129,254	53.65	0	4.36	2.17	93.47	32.31	250
	R2	35.16	129,254	53.61	0.03	7.51	3.51	88.95	32.31	250
SOE-11-3-200M	R1	36.15	136,876	52.86	0	4.75	2.23	93.01	34.22	250
	R2	35.17	136,876	52.9	0.03	7.53	3.37	89.07	34.22	250
SOE-11-3-DCM	R1	35.92	107,582	56.13	0	4.86	2.61	92.53	26.9	250
	R2	34.62	107,582	56.47	0.03	8.47	4.35	87.15	26.9	250
SOE-11-3-SURFACE	R1	36.03	112,894	55.18	0	4.9	2.47	92.63	28.22	250
	R2	34.6	112,894	55.72	0.03	8.89	4.31	86.77	28.22	250
SOE-11-7-200M	R1	36.1	176,952	55.87	0	4.55	2.38	93.07	44.24	250
	R2	33.7	176,952	56.27	0.03	11.29	5.64	83.05	44.24	250
SOE-11-7-DCM	R1	36.46	133,812	52.54	0	3.83	1.93	94.24	33.45	250
	R2	35.43	133,812	52.53	0.04	6.74	3.11	90.11	33.45	250
SOE-11-7-SURFACE	R1	36.25	149,411	55.5	0	4.26	2.19	93.55	37.35	250
	R2	35.12	149,411	55.68	0.04	7.41	3.62	88.93	37.35	250
SOE-11-9-200M	R1	36.26	132,788	54.33	0	4.31	2.21	93.47	33.2	250
	R2	33.94	132,788	54.54	0.03	11.08	5.04	83.85	33.2	250
SOE-11-9-DCM	R1	36.42	143,252	54.26	0	3.99	1.93	94.09	35.81	250
	R2	34.31	143,252	54.43	0.03	10.19	4.42	85.35	35.81	250
SOE-11-9-SURFACE	R1	36.37	168,229	55.04	0	4.02	1.97	94.01	42.06	250
	R2	33.81	168,229	55.32	0.04	11.34	5.16	83.47	42.06	250
SOE-11-15-200M	R1	35.82	126,596	55.65	0	5.72	2.65	91.62	31.65	250
	R2	32.6	126,596	56.9	0.03	14.87	6.92	78.18	31.65	250
SOE-11-15-DCM	R1	36.24	140,131	55.18	0	4.45	2.1	93.44	35.03	250
	R2	34	140,131	55.37	0.03	10.9	4.85	84.22	35.03	250

SOE-11-15-SURFACE	R1	36.27	146,537	55.38	0	4.13	2.1	93.78	36.63	250
	R2	33.8	146,537	55.55	0.03	11.29	5.21	83.47	36.63	250
SOE-11-25-200M	R1	36.13	154,168	55.23	0	4.73	2.24	93.03	38.54	250
	R2	34.84	154,168	55.42	0.03	8.43	3.84	87.7	38.54	250
SOE-11-25-DCM	R1	36.37	145,979	53.98	0	4.09	2	93.91	36.49	250
	R2	35.38	145,979	54.19	0.04	6.85	3.23	89.89	36.49	250
SOE-11-25-SURFACE	R1	36.31	127,458	54.13	0	4.27	2.12	93.62	31.86	250
	R2	33.9	127,458	54.35	0.03	11.25	5.07	83.64	31.86	250
SOE-11-36-200M	R1	36.39	117,084	54.55	0	3.86	1.98	94.16	29.27	250
	R2	35.41	117,084	54.33	0.03	6.73	3.19	90.05	29.27	250
SOE-11-36-DCM	R1	36.39	108,697	54.29	0	4.03	1.95	94.01	27.17	250
	R2	35.31	108,697	54.36	0.04	7.1	3.25	89.62	27.17	250
SOE-11-36-SURFACE	R1	36.08	142,200	56.28	0	4.7	2.3	93	35.55	250
	R2	34.88	142,200	56.46	0.03	8.16	3.78	88.03	35.55	250
SOE-11-37-200M	R1	35.91	121,387	55.9	0	5.23	2.67	92.11	30.35	250
	R2	34.51	121,387	56.29	0.04	9.24	4.43	86.29	30.35	250
SOE-11-37-DCM	R1	36.49	185,984	55.13	0	3.61	1.79	94.6	46.5	250
	R2	35.58	185,984	55.06	0.03	6.26	2.92	90.78	46.5	250
SOE-11-37-SURFACE	R1	36.21	154,448	54.73	0	4.61	2.08	93.31	38.61	250
	R2	35.11	154,448	54.77	0.03	7.76	3.45	88.76	38.61	250
SOE-11-38-200M	R1	35.95	145,238	55.04	0	5.3	2.51	92.19	36.31	250
	R2	34.99	145,238	55.41	0.03	7.99	3.69	88.29	36.31	250
SOE-11-38-DCM	R1	36.11	133,627	55.04	0	4.77	2.23	93	33.41	250
	R2	35.25	133,627	55.14	0.03	7.24	3.24	89.49	33.41	250
SOE-11-38-SURFACE	R1	35.68	171,666	54.62	0	6.3	2.79	90.9	42.92	250
	R2	34.58	171,666	55.19	0.03	9.28	4.22	86.47	42.92	250
SOE-11-39-200M	R1	36.12	138,292	55.6	0	4.6	2.36	93.05	34.57	250
	R2	35.12	138,292	55.83	0.03	7.2	3.74	89.03	34.57	250
SOE-11-39-DCM	R1	35.49	133,182	57.06	0	6.42	3.04	90.55	33.3	250

	R2	34.1	133,182	57.93	0.02	10.36	4.97	84.65	33.3	250
SOE-11-39-SURFACE	R1	35.71	165,999	56.25	0	5.81	2.82	91.37	41.5	250
	R2	34.48	165,999	56.82	0.03	9.4	4.37	86.2	41.5	250

Objective 3: Determination of culturable actinobacterial diversity

All the psychrophilic and psychrotolerant bacterial cultures, isolated on-board and in laboratory, were sub cultured on respective culture media viz., Actinomycetes Isolation Agar (AIA), Starch Casein Agar (SCA) and Nutrient Agar (NA). All the culture plates were incubated at both 4°C & 28°C for 10-20 days. Summary of the bacterial cultures isolated is given in table 3.

Table 3 Southern Ocean bacterial isolates from vertical water samples

Stations	Growth	Total	KOH string test –ve	Gram Positive
SOE11-1,2,3,7,9,15,25,36,37, 38, & 39	Psychrotolerant	142	75	18
	Psychrophilic	113	69	28

Objective 4: Characterization and taxonomy of actinobacterial strains isolated from Indian sector of Southern Ocean and their *ex-situ* conservation

- The KOH string test negative, Gram positive as well as Gram's variable cultures were selected for further characterization and preservation.
- Totally 41 bacterial strains were sequenced using 16SrRNA gene sequencing. Among them 51.2% of the strains is gram positive. Also 19.5% of the strains are coming under actinobacterial phyla which are *Chryseoglobus indicus, Micrococcus luteus, Rhodococcus erythropolis, Brachybacterium* sp., *Microbacterium oxydans, Streptomyces harbinensis* and *Brevibacterium casei*. **(Figure 7 and 8)**
- Taxonomic position of the Southern Ocean bacterial isolates is given in table 4.
- Fig. 4 depicts the phylogenetic analysis of 16S rRNA sequences of SO bacterial isolates.
- Cultural morphology including pigmentation of all the selected cultures was recorded (fig. 5 & table 5).

Table 4 Molecular taxonomy of Southern Ocean bacterial isolates based of 16SrRNA gene sequencing

S.No	Strain no.	*Genus*	Gram's reaction	Phylum
1	SOE11-25-PP15	*Vibrio*	Gram Negative	Proteobacteria
2	SOE11-21-PP2	*Bacillus*	Gram Positive	Bacillota
3	SOE11-25-PP16	*Fictibacillus sp.*	Gram Positive	Bacillota
4	SOE11-25-PP13	*Bacillus marisflavi*	Gram Positive	Bacillota
5	SOE11-09-PP13	*Staphylococcus*	Gram Positive	Bacillota
6	SOE11-GC1-PP10	*Bacterium strain NBTE-R22*	Unknown	Unknown
7	SOE11-01-PP2	*Staphylococcus hominis*	Gram Positive	Bacillota

8	SOE11-03-PP34a	*Salinicola*	Gram Negative	Proteobacteria
9	SOE11-25-PP28	*Metabacillus indicus*	Gram Positive	Bacillota
10	SOE11-21-PP5	*Marine arctic deep-sea bacterium FF7a*	Unknown	Unknown
11	SOE11-21-PP9	*Chryseoglobus indicus*	Gram Positive	Actinobacteria
12	SOE11-21-PP14	*Aureimonasaltamirensis*	Gram Negative	Proteobacteria
13	SOE11-21-PP15	*Micrococcus luteus*	Gram Positive	Actinobacteria
14	SOE11-21-PP11	*Erythrobacter sp.*	Gram Negative	Proteobacteria
15	SOE11-27-PP3	*Rhodococcus erythropolis*	Gram positive	Actinobacteria
16	SOE11-21-PP16	*Erythrobacter sp.*	Gram-negative	Proteobacteria
17	SOE11-21-PP10	*Erythrobacter sp.*	Gram-negative	Proteobacteria
18	SOE11-02-PP9	*Clostridium amygdalinum*	Gram positive	Bacillota
19	SOE11-03-PP3	*Staphylococcus epidermidis*	Gram positive	Bacillota
20	SOE11-03-PP13	*Staphylococcus hominis*	Gram positive	Bacillota
21	SOE11-27-9	*Micrococcus luteus*	Gram Positive	Actinobacteria
22	SOE11-1-4	*Brachybacterium sp.*	Gram Positive	Actinobacteria
23	SOE11-1-7	*Pseudoalteromonas*	Gram Negative	Proteobacteria
24	SOE11-2-6	*Salinicolasalarius*	Gram Negative	Proteobacteria
25	SOE11-3-3	*Salinicola sp.*	Gram Negative	Proteobacteria
26	SOE11-5-37	*Microbacterium oxydans*	Gram Positive	Actinobacteria
27	SOE11-7-42	*Pseudomonas lactis*	Gram Negative	Proteobacteria
28	SOE11-9-26	*Martelella sp.*	Gram Negative	Proteobacteria
29	SOE11-15-57	*Streptomyces harbinensis*	Gram Positive	Actinobacteria
30	SOE11-1-27	*Klebsiella pneumoniae*	Gram Negative	Proteobacteria
31	SOE11-9-4	*Halomonastitanicae*	Gram Negative	Proteobacteria
32	SS3-9	*Stahylococcus hominis*	Gram positive	Bacillota
33	SS7-4	*Bacillus paralicheniformis*	Gram positive	Bacillota
34	SS3-15	*Salinicola salaries*	Gram-negative	Proteobacteria
35	SS7-13	*Bacillus aryabhattai*	Gram positive	Bacillota
36	SS3-11	*Bacillus subtilis*	Gram positive	Bacillota
37	SS7-1	*Altererythrobacter sp.*	Gram Negative	Proteobacteria
38	SO6	*Brevibacteriumcasei*	Gram positive	Actinobacteria
39	Seaice-9	*Klebsiella pneumoniae*	Gram Negative	Proteobacteria
40	Seaice-43	*Klebsiella sp.*	Gram Negative	Proteobacteria
41	Sea Ice- 5	*Klebsiella pneumoniae*	Gram Negative	Proteobacteria

Table 5 *Ex-situ* conservation of Southern Ocean bacterial strains

S.No	Isolate name	Sample depth (m)	Growth	Temp	Colony consistancy	AMC	Gram Staining
1	SOE11-01-PP1	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval
2	SOE11-01-PP2	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval

3	SOE11-01-PP3	Surface	Good	4-7°C	Leathery	Orange	Gram positive, spherical
4	SOE11-01-PP7	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval
5	SOE11-02-PP9	DCM	Good	4-7°C	Leathery	Pink	Gram positive, Oval
6	SOE11-02-PP13	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
7	SOE11-02-PP15	500	Good	4-7°C	Leathery	Yellow	Gram positive, spherical
8	SOE11-02-PP16	500	Good	4-7°C	Leathery	Pink	Gram positive, Oval
9	SOE11-02-PP18	500	Good	4-7°C	Leathery	Pink	Gram positive, Oval
10	SOE11-03-PP1	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval
11	SOE11-03-PP3	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval
12	SOE11-03-PP5	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval
13	SOE11-03-PP7	Surface	Good	4-7°C	Leathery	Creamy	Gram positive, Oval
14	SOE11-03-PP9	Surface	Good	4-7°C	Leathery	Pink	Violet, Elongated
15	SOE11-03-PP13	Surface	Good	4-7°C	Leathery	Pink	Gram positive
16	SOE11-03-PP15	Surface	Good	4-7°C	Leathery	Creamy	Gram positive, Oval
17	SOE11-03-PP16	Surface	Good	4-7°C	Leathery	Orange	Gram positive, Oval
18	SOE11-03-PP32	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Oval
19	SOE11-03-PP34	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Elongated
20	SOE11-09-PP9	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
21	SOE11-09-PP11	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
22	SOE11-09-PP13	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
23	SOE11-09-PP15	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
24	SOE11-09-PP46	Surface	Good	4-7°C	Leathery	Pink	Gram positive, Elongated
25	SOE11-09-PP65	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
26	SOE11-09-PP66	DCM	Good	4-7°C	Leathery	Yellow	Gram positive, Oval
27	SOE11-09-PP67	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous

28	SOE11-09-PP68	DCM	Good	4-7°C	Powdery	White	Gram positive, filamentous
29	SOE11-01-4	Surface	Good	4/28°C	Leathery	Yellowish white	Gram positive, spherical
30	SOE11-01-7	Surface	Good	4/28°C	Leathery	Peach	Gram positive, Oval
31	SOE11-01-21	Surface	Good	4/28°C	Oily	Oil yellow	Gram positive, spherical
32	SOE11-01-23	Surface	Good	4/28°C	Watery	Dirty white	Gram positive, spherical
33	SOE11-01-27	500m	Good	4/28°C	Leathery	Dirty white	Gram positive, filamentous
34	SOE11-02-2	Surface	Good	4/28°C	Spoty leathery	Pink spots	Gram positive, spherical
35	SOE11-02-4	Surface	Good	4/28°C	Watery	Dirty white	Gram positive, filamentous
36	SOE11-02-6	Surface	Good	4/28°C	Leathery	Golden yellow	Gram positive, filamentous
37	SOE11-02-61	1000m	Good	4/28°C	Rough Leathery	Oil yellow	Gram positive, spherical
38	SOE11-03-3	Surface	Good	4/28°C	Spoty leathery	Pink spots	Gram positive, spherical
39	SOE11-03-6	Surface	Good	4/28°C	Soft velvety	Pink	Gram positive, Oval
40	SOE11-03-44	500m	Good	4/28°C	Oily	Dirty white	Gram positive, spherical
41	SOE11-03-61	500m	Good	4/28°C	Creamy	Pale white	Gram positive, spherical
42	SOE11-07-38	Surface	Good	4/28°C	Soft velvety	Rose	Gram positive, Oval
43	SOE11-07-42	200m	Good	4/28°C	Rough Oily	Oil yellow	Gram positive, Oval
44	SOE11-09-4	Surface	Good	4/28°C	Leathery	White	Gram positive, spherical
45	SOE11-09-5	1500m	Good	4/28°C	Spongy rough	White	Gram positive, spherical
46	SOE11-09-26	Surface	Good	4/28°C	Spoty rough	Dirty yellow	Gram positive, spherical

ACHIEVEMENTS AND HOW THE DELIVERABLES HAVE CONTRIBUTED IN THE OVERALL PROGRAMS OF THE MINISTRY

- Except few *Streptomyces* colonies, there were no sporulating actinobacterial colonies observed on all the three media used for isolation from water samples during 45 days of incubation. Usually, according to literature, water logging condition is un / less favorable for actinobacterial sporulation.
- Several pigmented colonies were observed on the isolation agar plates inoculated with surface water samples (in comparison with DCM and 200 m). This might be a protection mechanism against radiation since colonies from deep sea are not much exposed to sun light.
- Most of the pigmented colonies observed on the isolation plates resemble colony morphology of rare / non sporulating actinobacterial genera previously reported in the published literature. Previous studies have also reported pigmented actinobacteria isolated from Polar Regions.
- Despite KOH string test and Gram staining, the 16S rRNA sequencing showed few gram-negative strains.
- Most of the gram-positive strains were also observed to be of the phylum Proteobacteria and Bacillota.
- *Micrococcus, Microbacterium, Brachybacterium, Rhodococcus, Streptomyces*, and *Chryseoglobus* were the actinobacterial genus isolated.

- **SALIENT OUTCOME**

Water samples were collected from selected locations in the Indian Sector of Southern Ocean (ISO) during 11th Indian Scientific Expedition to Southern Ocean (ISESO). Duplicates of samples were filtered for both culture dependent and culture independent study. Microorganisms were isolated from water samples of few stations on-board while few were isolated in the laboratory. Over 200 psychrophilic and psychrotolerant bacteria was isolated from on-board cultivation, whereas less than 50 isolates were recovered from laboratory cultivation. Moreover, loss of viability was observed in the isolated cultures over a period of time, despite proper maintenance. This also applied for culture independent study using metagenomics. Unexplored environment like the Southern Ocean holds the potential for novel microbes with unique properties. Delay in cultivation and delay in processing might take away our chances of identifying newer organism and/or newer compounds. Studies must focus on stable growth and storage conditions of the microorganisms, especially the psychrophiles.

- **SCOPE FOR FUTURE**

Availability of data on actinobacterial diversity of Southern Ocean environments is less compared to other Polar environments. The few data reported, also highlights the uncultured genus of Actinobacteria. The data generated in this study depicts the presence of numerous rare actinobacterial genera. The possibility of obtaining highly potential secondary metabolites with novel properties from such rare Actinobacteria is enticing in the field of Drug Discovery. Bioactivity of the obtained actinobacterial strains is yet to be studied. Future research will focus on bioprospecting potentials of the rare actinobacterial genera from unexplored regions of Southern Ocean.

- **PUBLICATION OF RESULTS/PRESENTATION OF PAPERS**
  - **Articles published:**
    1. Abirami B., K. Manigundan, M. Radhakrishnan, V. Gopikrishnan, Jerrine Joseph, J & Bhaskar, P. V. (2021). Anti freeze proteins (Afp): Properties, sources and applications–A review. *International Journal of Biological Macromolecules*, *189*, 292-305. [IF. 8.03]
    2. Manigundan K.,, Radhakrishnan M, & Abirami B (2022). Exploring Newer Biosynthetic Gene Clusters in Marine Microbial Prospecting. *Marine Biotechnology*, 1-20. [IF. 3.8]
    3. Namitha, R., B. Abirami, B.S. Anoop, D.V. Diya Dominic, Ahna Ameer, K. Manigundan, M. **Radhakrishnan*,** P. Santhiya, Parli V Bhaskar, K. Govindaraju*, I.S. Bright Singh and G. Dharani. Oct 2023. Synthesis and characterization of gold nanoparticles using *Brevibacterium casei* (SOSIST-06) isolated from Southern Ocean water samples and their *in vitro* and *in silico* anti-WSSV activity. *Aquaculture* (IF. 4.5)
  - **Book chapter :**
    1. Abirami, B., Manigundan, K., Radhakrishnan, M., Gopikrishnan, V., Bhaskar, P. V., Shanmugasundaram, T., & Dastager, S. G. (2021). Diversity and bioprospecting potentials of antarctic (polar) microbes. In *Microbiome-Host Interactions* (pp. 349-365). CRC Press.
    2. Baskaran, A., Radhakrishnan M., & Bhaskar, P. V. Microbial symbionts in polar animals. In *Microbial Symbionts.*
  - **Manual chapters :**
    1. Kaari, M., Baskaran, A., Venugopal, G., **Manikkam, R.,** & Bhaskar, P. V. (2022). Isolation of Psychrophilic and Psychrotolerant Actinobacteria. In *Methods in Actinobacteriology* (pp. 141-144). Humana, New York, NY.
    2. Baskaran, A., **Manikkam, R.,** Kaari, M., Venugopal, G., Thirugnanasambandham, S., & Bhaskar, P. V. (2022). Screening and Production of Antifreeze Proteins from Actinobacteria. In *Methods in Actinobacteriology* (pp. 467-470). Humana, New York, NY.
  - **<u>Ms Communicated</u>**:
    1. **Radhakrishnan Manikkam***, Manigundan Kaari, Abirami Baskaran, Meganathan P. Ramakodi, Gopikrishnan Venugopal[1] and Parli Venkateswaran Bhaskar. Dec 2023. Diversity of actinobacteria in the sediment and water column in the Indian sector of Southern Ocean (ISO) as revealed by 16S rRNA-based metagenomic analysis (*Submitted to Brazilian J Microbiology*)

- **CAPACITY BUILDING - NO. OF B.TECH/M.TECH/PH.D.S ETC. OUT OF THE PROJECT**

Under graduate students trained : 7
Post graduate students trained : 3
Ph.D student trained : 2

- **NO. OF WORKSHOPS CONDUCTED IF ANY, RELATED TO THE PROJECT ALONG WITH DATE & NAME OF THE WORKSHOP**
    1. National Conference on Microbes in Polar Environment during 25-28, Aug 2021
    2. Faculty Development Programme on Opportunities and obstacles in Actinobacterial Research during 13-18, Sep 2021
    3. Faculty Development Program (FDP) & Student Training Program (STP) on New Avenues and Challenges in Actinobacterial Research during 29 Sep – 6 October 2021

- **ABSTRACT IN 300 WORDS FOR POSSIBLE PUBLICATION ON MOES NEWSLETTER/WEBSITE.**

Southern Ocean (SO) comprises the southernmost waters of the World Ocean, encircling Antarctica, and is easily subjected to rapid climate change. The SO is intensively investigated for its significance in global biogeochemical cycling. Microbes, being the main drivers of biogeochemical cycles, play an essential role in ecosystem functioning. This has led to the focus on microbial community structure analysis of SO microbes. Actinobacteria, being the largest contributor of natural product discovery, still remains untapped in the ecosystems of SO. The focus of our study was to explore the diversity of Actinobacteria in different depths of water and sediment samples from SO. Culture dependent and independent studies were performed to determine the diversity of actinobacterial genera. Rare actinobacteria such as *Chryseoglobus indicus, Micrococcus luteus, Rhodococcus erythropolis, Brachybacterium* sp., *Microbacterium oxydans, Streptomyces harbinensis* and *Brevibacterium casei* were obtained as a result of 16S rRNA sequencing of strains cultivated in the laboratory. Metagenomic analysis revealed presence of numerous unknown genes. *Agromyces, Brachybacterium, Corynebacterium, Dietzia, Glutamicibacter, Gordonia, Microbacterium, Mycobacterium, Nocardioides, Psuedonocardia, Rhodococcus* and *Sva0996 marine groups* were the most prominent genus of Actinobacteria obtained from metagenomic analysis. These results promote the idea of tapping the actinobacteria for novel metabolites of medical, food and industrial applications. This brings us closer to finding novel drugs against unfamiliar infections of the modern world. Further study involving bioprospecting of such rare actinobacterial genera will be a boon to the natural product drug discovery.



Dr. M. Radhakrishnan
(Principal Investigator)

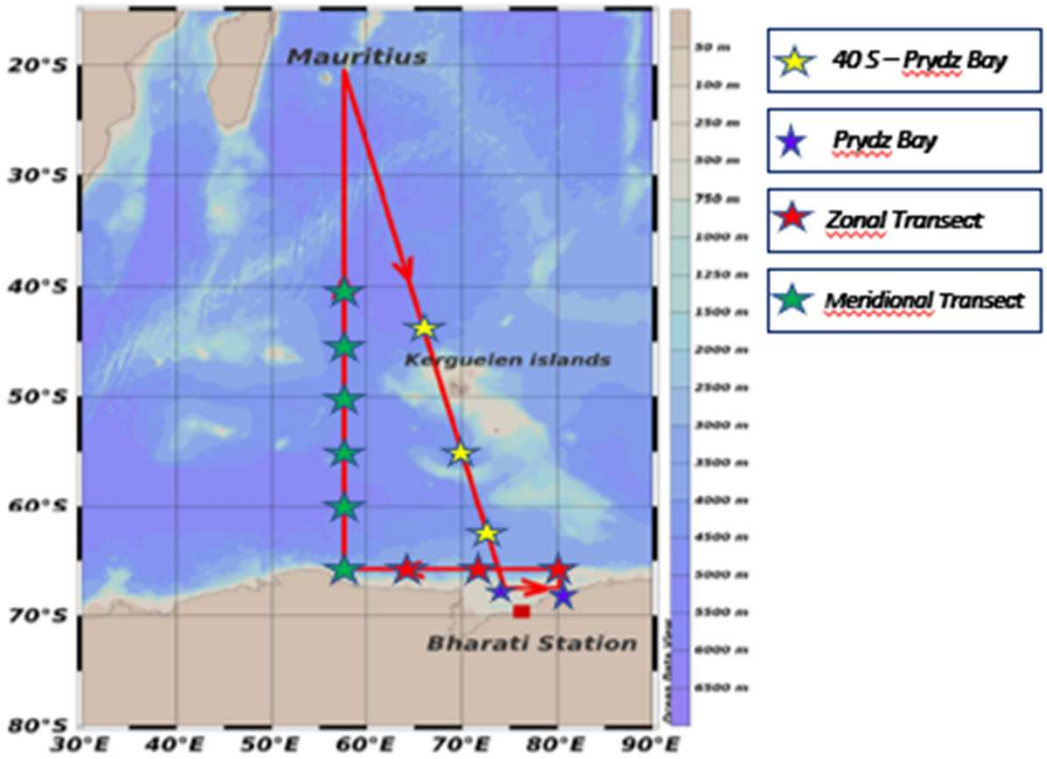


Figure 1. Cruise track for Southern Ocean Expedition (SOE) 2019-20

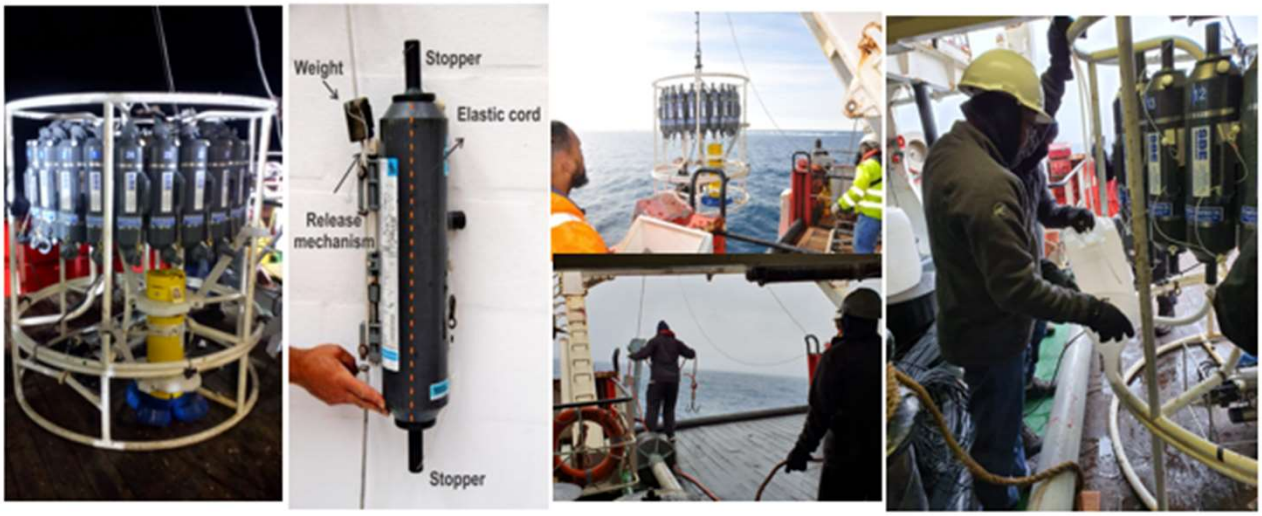


Figure 2. Collection of water samples using CTD-Niskin sampler



Figure 3. Lab space on SA Agulhas for the isolation of actinobacteria and sample processing



Figure 4. On board isolation of actinobacteria

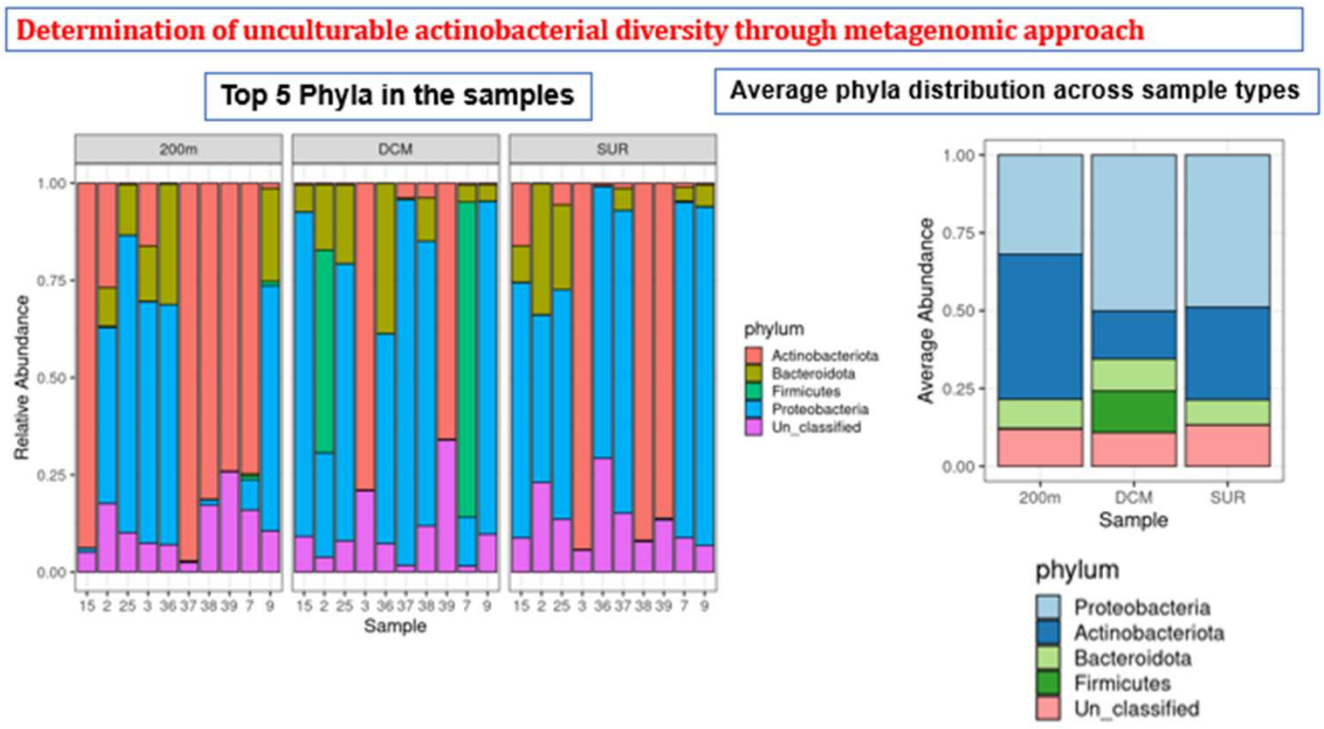


Figure 5. Top five bacterial phyla in the Southern Ocean waters

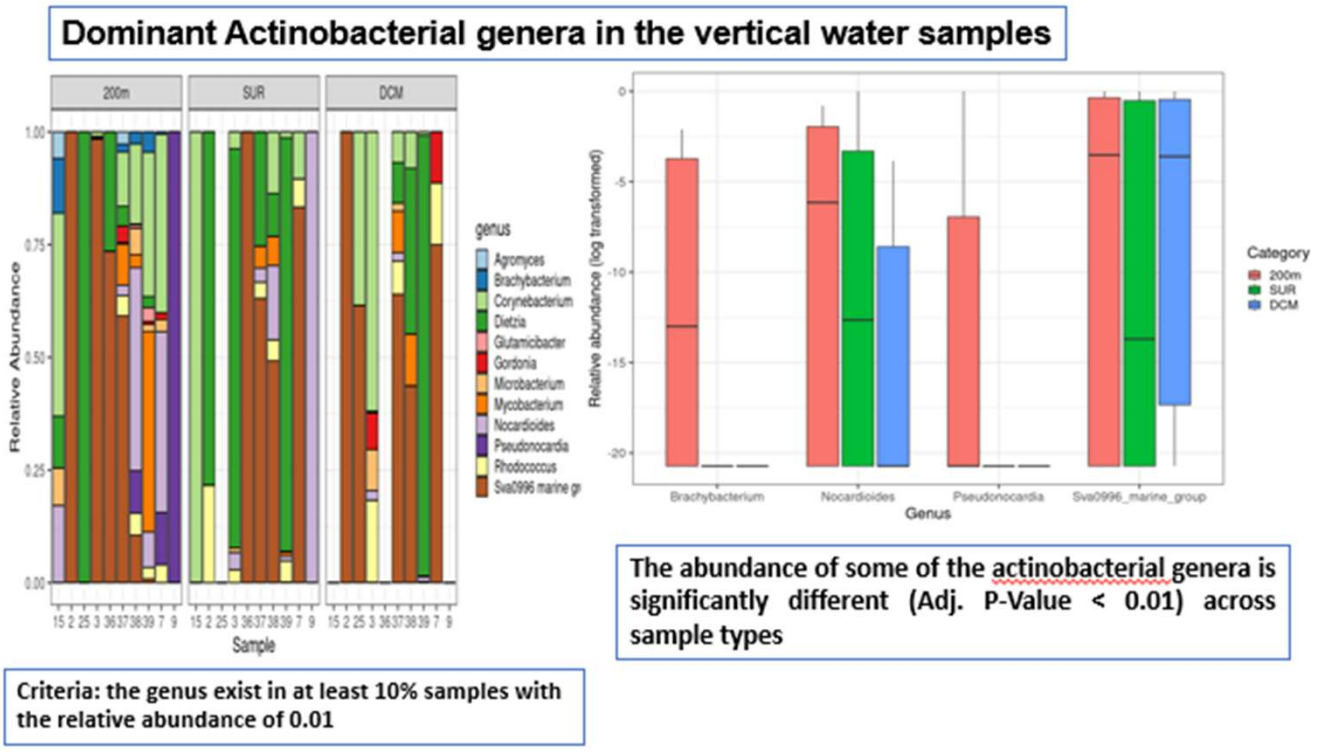


Figure 6 Dominant uncultured actinobacterial genera in the Southern Ocean waters

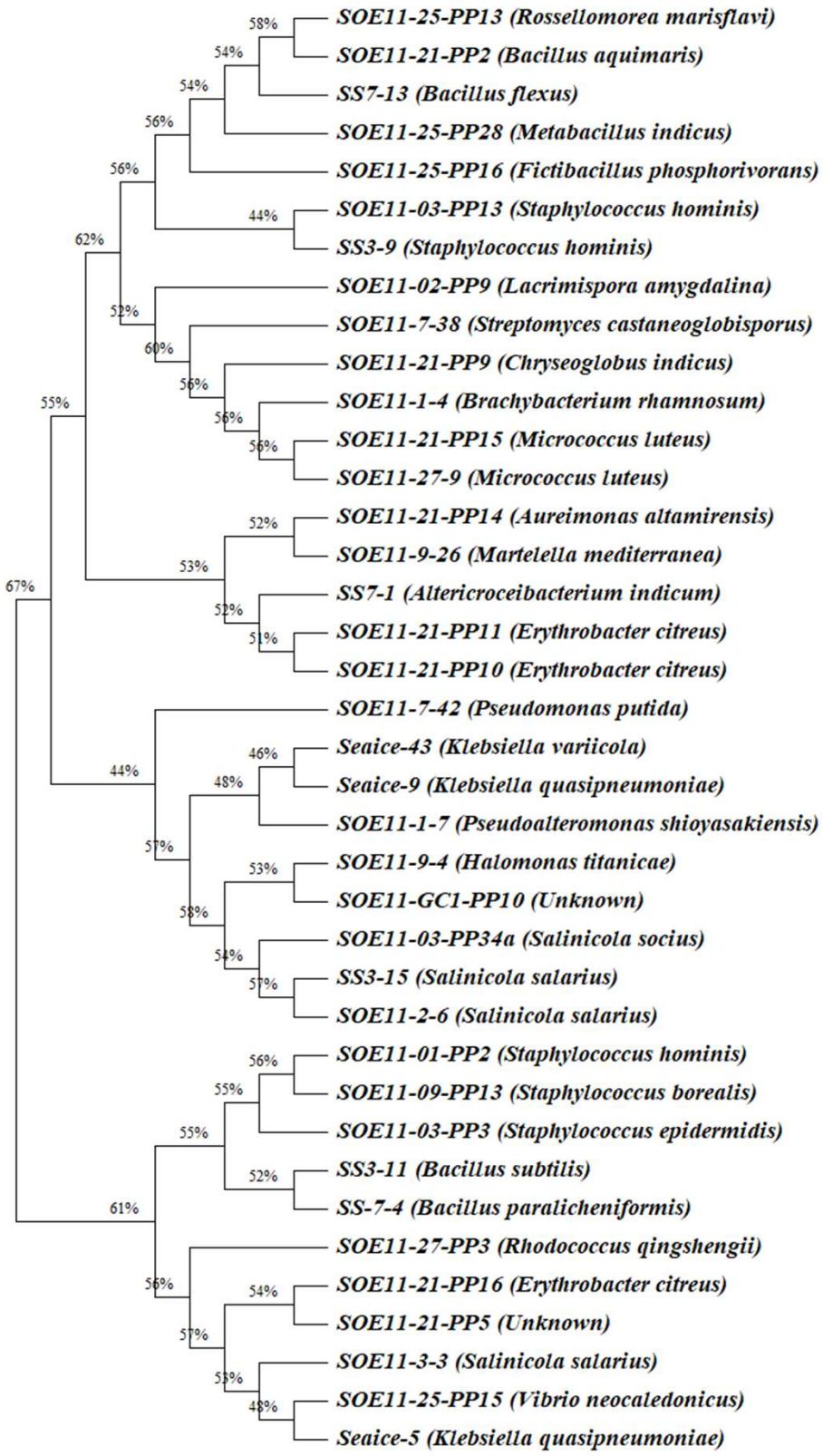


Figure 7. Phylogenitic analysis of Southern Ocean bacterial 16S rRNA gene sequences based on Neibour-Joining method constructed in MEGA11

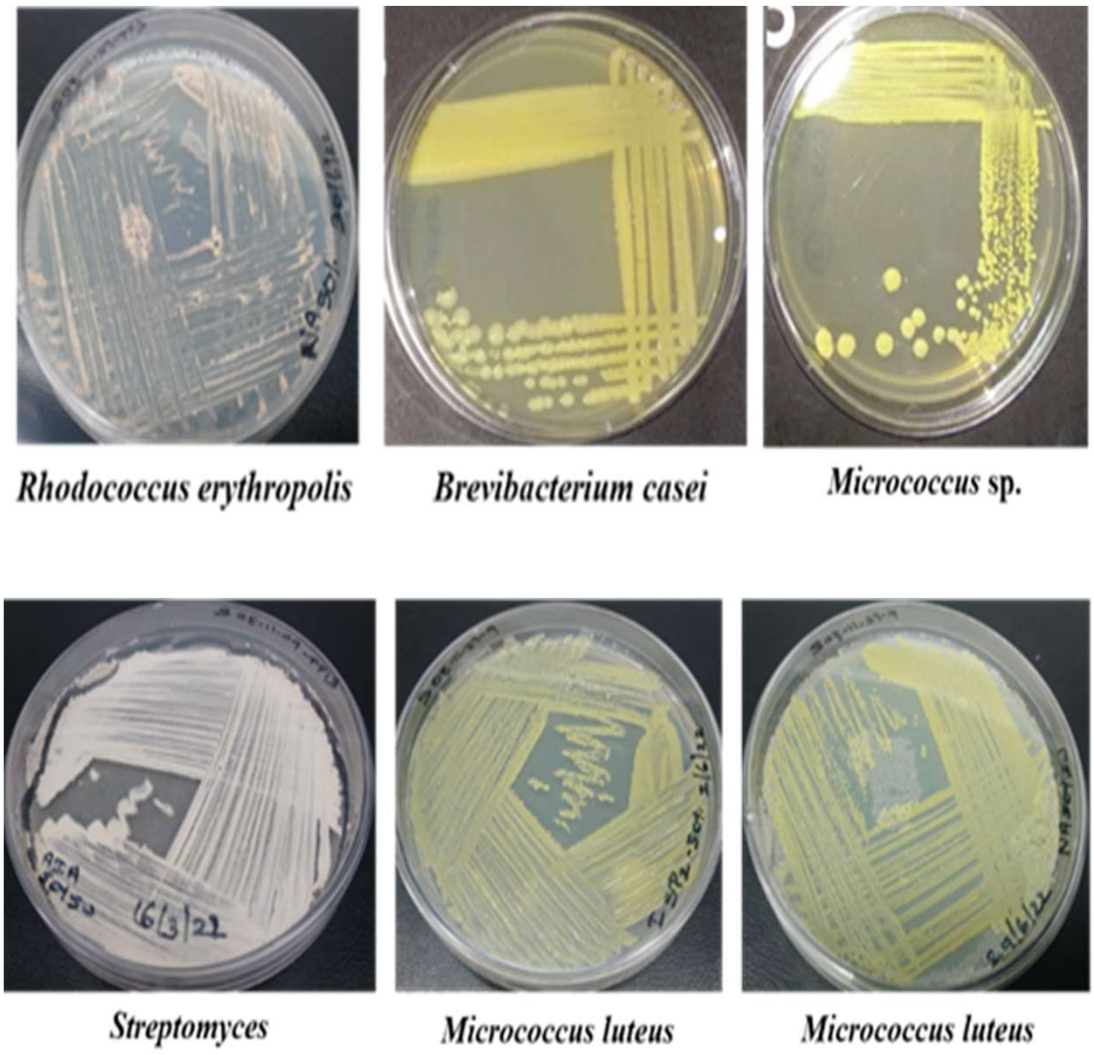


Figure 8. Cultural morphology of different actinobacterial genera isolated from Southern Ocean waters