राष्ट्रीय ध्रुवीय एवं समुद्री अनुसंधान केन्द्र
पृथ्वी प्रणाली विज्ञान संगठन
पृथ्वी विज्ञान मंत्रालय (भारत सरकार)
हेडलैण्ड सडा, वास्को-डा-गामा, गोवा-४०३ ८०४, भारत

NATIONAL CENTRE FOR POLAR AND OCEAN RESEARCH
Earth System Science Organisation
Ministry of Earth Sciences, (Goverment of India)
Headland Sada, Vasco-da-Gama, Goa - 403 804, INDIA

CERTIFICATE

This is to certify that Mr. **Krupa Varaprasad O** student of Goa University, Taleigao Plateau, Goa, has successfully completed a winter internship from **National Centre for Polar and Ocean Research** in the field of Arctic Ecology and Biogeochemistry from 01/12/2022 to 30/12/2022 under the guidance of **Dr. K.P. Krishnan** and under the supervision of **Dr. Anand Jain** and **Ms. Yuga Ghotge.**

During the period of his internship program with us, he had been exposed to various techniques and skills in the laboratory.

We wish him every success in his life and career.

05/04/2023
Supervisor

Archana Singh / अर्चना सिंह
Scientist / वैज्ञानिक
ESSO-National Centre for Antarctic and Ocean Re...
इ.एस.एस.ओ.-राष्ट्रीय अंटार्कटिक एवं ...ुद्री अनुसंधान ...
Ministry of Earth Sciences (Govt. of India)
पृथ्वी विज्ञ... मंत्रालय (भारत सरकार)
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हेडलैंड सडा, वास्को ड गामा, गोवा- 403 804

Archanajit
05.04.2023
Authorized Signature
Scientist-in-charge
Arctic Ecology & Biogeochemistry
Arctic Operations

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Internship Report

Internship Program at:

National Centre for Polar and Ocean Research (NCPOR)

Date: 1st December 2022 to 30th December 2022

Submitted by:
Name of Student: O. Krupa Varaprasad
Roll No: 21P050016
Class and Subject: MSc Marine Biotechnology
Number of days completed: 30 days

Acknowledgment

I would like to express my sincere gratitude to Dean of the School of Biological Sciences and Biotechnology Dr. Savita Kerkar, and the Programme Director of Marine Biotechnology Dr. Sanjeev Ghadi for allowing me to do the internship.

I would like to extend my appreciation and gratitude to Department of Biotechnology (DBT) for providing me with an opportunity to work as an intern.

I would like to extend my gratitude towards the Management of NCPOR, Goa for providing me an opportunity to experience my winter internship in their respected institute. I would like to extend my gratitude to Dr. K.P. Krishnan, Scientist F, NCPOR, for allowing us to work in his prestigious lab. My sincere thanks to Dr. Anand Jain and Ms. Yuga Ghotge for providing me this opportunity to associate myself with them for my training and also like to express my sincere gratitude to them for providing me the most valuable guidance and affable treatment given to me at every stage to boost my morale and helping me in learning the way of working at a reputed institution, which helped me to add a feather in my cap.

I am especially thankful to Prof. Samantha Fernandes and Dr. Meghanath Prabhu, for providing me an opportunity and for encouraging me to do my internship at National centre for polar and ocean research. Without whose guidance and support this would have not been possible. It was because of them due to which I could intern in such a prestigious institution and nurture our interest in research and academia.

This internship experience has been invaluable in my professional development and I am grateful for the guidance and support provided by department.

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1. Introduction:

National Centre for Polar and Ocean Research, Goa, is India's premier R&D institution responsible for the country's research activities in the Polar and Southern Ocean realms. It conducts geoscientific surveys of the country's EEZ and its extended continental shelf beyond 200M, deep-sea drilling in the Arabian Sea basin through the IODP, exploration for ocean non-living resources such as the gas hydrates and multi-metal sulphides in mid-ocean ridges. There are two Indian Antarctic Research Bases, Maitri and Bharati, and the Indian Artic base Himadri.

The Indian scientists first kept their steps on the Antarctica continent in 1981. There are two groups which play a role in the Indian Antarctic Program, these are Antarctic Scientific Division and the Antarctic Logistics Division. Antarctic Regions hold an important place while answering the key questions about the global climate change such as contribution towards global sea – level, the background aerosol properties, variability in the sea ice cover and phenomenon like Antarctic haze and Ozone concentrations. Attempts have been made to address some of these issues has helped mitigating on some important problems concerning human life and well-being.

India started its research in the Arctic in 2008. The aim of India's Arctic programme is to contribute to the development, consolidation and dissemination of the current understanding of climate change, its impacts and adaptations in the Norwegian Arctic, Svalbard. The research includes atmospheric, biological, marine and earth sciences and glaciological studies.

2. Objectives:

2.1. Objective of the internship:

To gain knowledge and work experience in the research field, which will help me later in my career.

2.2. Objective of the experiments conducted during the internship:

To know about the microbial population in the fjords and co relate the growth of bacteria to the macroalgal density in the Arctic environment.

3. Brief overview of the work done:

I had worked in the Polar Biology lab in NCPOR for my internship during the entire time period that I was there. The scientists in the Polar Biology lab conducted experiments on the samples collected from the arctic regions to obtain information on the environmental factors that are known to affect the vegetation in the arctic region. They are trying to learn how the carbon cycle of the Arctic region is different from the other parts of other parts of the world and how it plays a role in the environmental conditions and the growth of bacterial communities in that region.

3.1. Cell counting and cell volume determination

The experiments were conducted in various steps. During the first two weeks, cell counting and cell volume determination of the given samples from the Arctic was done using fluorescent microscopy. The samples were collected from 10 different stations in the arctic region. The samples were filtered and some were kept unfiltered to do the cell counting, this helped to differentiate the cell growth, cell size and cell volume of the same sample when it is filtered and when it is left unfiltered.

The biomass of the marine bacterioplankton was estimated using cell count and cell volume estimation. 12 different samples were used. DAPI stain was used to visual the samples. DAPI is extensively used in fluorescence microscopy ass it binds strongly to adenine-thymine rich regions in the DNA. It can be used to stain both viable and non-viable cells as it can pass through intact cell membranes. The fluorescence microscope excites a specimen with a specific wavelength of light and forms an image with the fluorescence light emitted by the object. The microorganisms are stained with fluorochrome-labelled probes or fluorochromes that bind specific cell membranes.

The most commonly used fluorescence microscopy is epifluorescence microscopy, also called incident light or reflected light fluorescence microscopy. Epifluorescence microscopes employ an objective lens that also acts as a condenser so that the specimen is illuminated from above rather below. A mercury vapor arc lamp or other source produces an intense beam of light that passes through an excitation filter. The exciter filter transmits only the desired wavelength of light. The excitation light is directed down the microscope by the dichromatic mirror. This mirror reflects light of shorter wavelength but allows light of longer wavelengths to pass through. The excitation light continues down, passing through the

objective lens to the specimen, which are stained with fluorochromes. The fluorochrome absorbs light energy from the excitation light and fluoresces brightly. The emitted fluorescent light travels up through the objective lens into the microscope. And passes through the barrier filter to the eye piece.

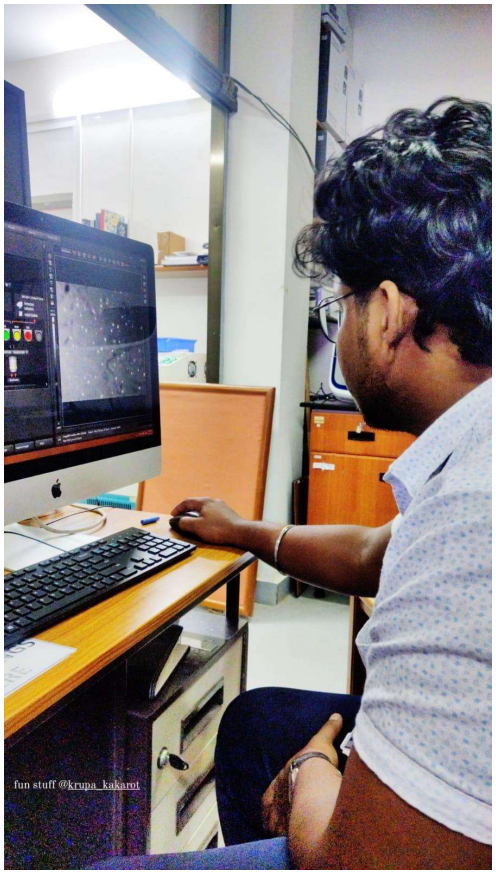
Images of the cells observed under fluorescence microscope were taken in LAS X software. Leica Application Suite X (LAS X) is a software platform for Leica microscopes. It integrates confocal, wide field, stereo, super resolution, and light sheet instruments.

Cell volume was calculated to study the selective grazing by bacteriovores and to find out how the bacterioplankton contribute to the optical properties of the seawater. A magnifier having 0.1 mm interval was used to measure the size of the bacterioplankton. The various shapes of the cells were observed and the readings were noted down.

The steps that we had followed for this experiment are as follows:

1. Sample filtration
2. Staining the sample with DAPI stain
3. Incubating the sample at 4°C for 20 minutes.
4. PBS wash to the filter paper
5. Fixation of the sample using 2% formaldehyde
6. Filtration using a black stained 0.2 µm pore size nucleopore filter
7. Place on a slide
8. Cover the sample with paraffin oil
9. View under 1000X (oil immersion) on an epifluorescence microscope.
10. For measurements, the cells were photographed.
11. Bacterioplanktons size were categorized into spheres (volume = $4/3\pi r^3$) and rods (volume = πr^2[L-2/3r]).

The number of cells found in the unfiltered sample was more than the filtered sample, indicating that the biomass in the unfiltered sample was greater than the filtered sample. It implies that bacterioplankton might not be the only thing present in the sample but there might also be some other organic molecules which were leading to the increase in biomass.



3.2. Media preparation

During the third week, the samples were cultured. The samples were serially diluted and 10^2, 10^3, 10^4 concentrations were used to plate. Modified basal media was prepared to culture the plates. To mimic the conditions of sea water, basic salt composition was added. To this, trace metals and vitamins were added. To check the optimum media wherein the growth of the microorganisms would be maximum, four different kinds of media were prepared, i.e., modified basal media along with sodium alginate and agar; modified basal media, laminarin and agar; modified basal media plus mannitol and agar; modified basal media and agar. Dipotassium phosphate was added to the media after autoclaving. The pH was adjusted to 7 by using 1N NaOH. The samples were spread plated and kept in a low temperature incubator for 15 days.

3.3. DNA extraction, PCR amplification and gel elution.

During the fourth week, DNA extraction, PCR amplification and gel elution of the samples was conducted. DNA extraction is a method which is used to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components. DNA extraction techniques include organic extraction (phenol-chloroform method), non-organic method (salting out and proteinase K treatment) and adsorption method (silica gel membranes).

The PCR technique is based on the enzymatic replication of DNA. A short segment of DNA is amplified using primer mediated enzymes. PCR is based on the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. Because DNA polymerase can add a nucleotide only onto a pre-existing 3'OH group, it needs a primer to which it can add the first nucleotide. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies.

PCR requires a series of repeated reactions, called cycles. Each cycle precisely executes three steps in a machine called a thermocycler. In the first step, the DNA containing the sequence to be amplified is denatured by raising the temperature to about 95°C. Next the temperature is lowered to about 50°C so that the primers can anneal to complementary DNA on both sides of the target sequence. Finally, the temperature is raised, usually to 68 to 72°C, so that the DNA polymerase can extend the primers and synthesize copies of the target DNA sequence using dNTPs. Only polymerases that function at the high temperatures can be used. The most commonly used thermostable enzyme is Taq polymerase.

Gel elution is a technique used to isolate a desired fragment of intact DNA from an agarose gel following agarose gel electrophoresis. Elution is a process of extracting one material from another by washing with a solvent. It helps in the extraction of sample material into the solution so that it can be tested easily. DNA is separated by the process of agarose gel electrophoresis. The DNA extracted from elution is amplified by PCR.

The DNA obtained was first measured using spectrophotometer, following which it was amplified using PrimeSTAR PCR kit by Takara Bio. PrimeSTAR is an optimized mixture composed of PrimeSTAR HS DNA Polymerase developed by TaKaRa Bio Inc. PrimeSTAR HS DNA Polymerase has a matchless proof-reading activity due to very strong 3' to 5' exonuclease activity, and besides it's amplification efficiency is higher than that of Taq DNA Polymerase.

Gel Elution was performed using the Wizard SV Gel and PCR Clean Up System made by Promega. The Wizard SV Gel and PCR Clean-Up System is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. After electrophoresis to separate the DNA fragments, the bands of interest is excised and dissolved in the presence of guanidine isothiocyanate. DNA can be isolated using microcentrifugation to force the dissolved gel slice or PCR product through the membrane while simultaneously binding the DNA on the surface of the silica.

The DNA was extracted from the sample using fastDNA SPIN kit which is commercially available. The steps for this process are as follows:

1. The filter paper which contained the sample was cut using sterile scissors and forceps.
2. The cut sample was added to the lysing matrix E tube.
3. 978 µl sodium phosphate buffer was added to the tube.
4. 122 µl MT buffer was added to the tube.
5. The sample was vortexed for 5 minutes.
6. The sample was then centrifuged at 14000 rpm for 5-10 minutes.
7. Discard the pellet.
8. The supernatant was transferred to a sterile 2 ml centrifuge tube.
9. 250 µl protein precipitation solution was added to the supernatant.
10. The tube was mixed by inverting for 10 times.
11. Sample was centrifuged at 14000 rom for 5 minutes.
12. Discard pellet.
13. Transfer supernatant to 15 ml centrifuge tube.
14. Add the binding matrix in the tube.
15. To allow the binding of DNA, it was placed on the rotator for 2 minutes.
16. The tube was kept still at room temperature for 3 minutes to allow the settling of the silica matrix.
17. 500 µl of supernatant was discarded.
18. Binding matrix was resuspended in the remaining amount of supernatant.
19. 600 µl of the mixture was transferred to the SPIN filter.
20. Centrifuged at 14000 rpm for 1 minute.
21. Cycles of centrifugation and discarding took place.
22. The SPIN filter was air dried for 5 minutes.
23. The binding matrix was suspended in 25 µl of DNase/pyrogen free watere.

24. Centrifuge at 14000 rpm for 1 minute. The DNA is eluted.
25. Discard the spin filter.
26. Store at -20°C.

For DNA analysis by Agarose Gel Electrophoresis, the following protocol was followed:

1. 5 µl of the DNA eluted from the well of a 1% agarose gel in 0.5X TBE electrophoresis buffer was loaded.
2. Etbr loading dye, a 10X solution of bromophenol blue and sucrose solution, was added. PCR Amplification of the given sample was conducted.

For gel elution, the following protocol was used:

1. The bands from the gel were cut and added to 2 ml centrifuge tubes.
2. 10 µl MBS was added and the tubes were vortexed
3. The tubes were incubated at 56°C until the gel had dissolved.
4. Transferred it into a sterile 2 ml collection tube.
5. 700 µl MSW was added.

6. Centrifuged at 15000 rpm for 1 minute.
7. Discard the filtrate.
8. Transfer to a new collection tube.
9. 500 µl MWS was added.
10. Centrifuged for 15000 rpm for 1 minute.
11. Centrifuge again at 15000rpm for 5 min.

3.4. Qubit Fluorometer

The eluted DNA was measured using Qubit Fluorometer. The Invitrogen Qubit 4 Fluorometer accurately and quickly measures the concentration of DNA, RNA, or protein in a single sample. It can also be used to assess RNA integrity and quality. The Qubit fluorometer uses fluorescent dyes to determine the concentration of either nucleic acids or proteins in a sample. The most common method of measuring the concentration of nucleic acids and protein is the UV-absorbance method. This method uses a spectrophotometer to measure the natural absorbance of light at 260 nm (for DNA and RNA) or 280 nm (for proteins).

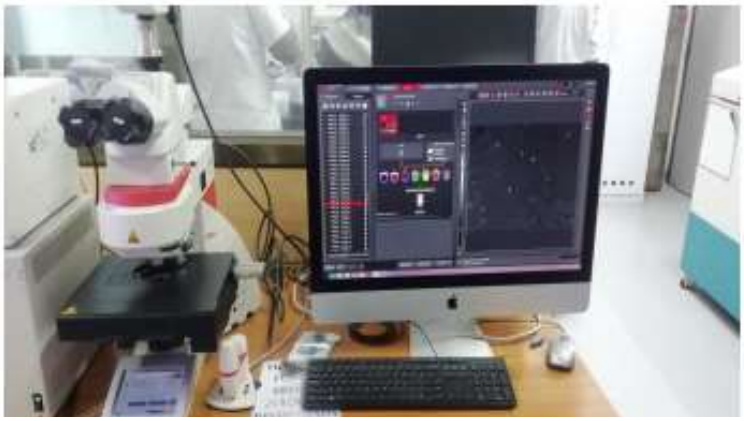
3.5. Ion Chef System

Ion Chef System was used for library preparation. The Ion Chef System is the next generation of workflow simplification products for the Ion GeneStudio S5 Systems. The Ion Chef System provides automated preparation of the library, template preparation, and chip loading for users. The Ion Chef System provides a convenient walk-away workflow resulting in equalized, pooled libraries ready for templating. The automated workflow supports Ion AmpliSeq one- and two-pool designs. Automating sample preparation workflows on the Ion Chef System results in significantly higher productivity.

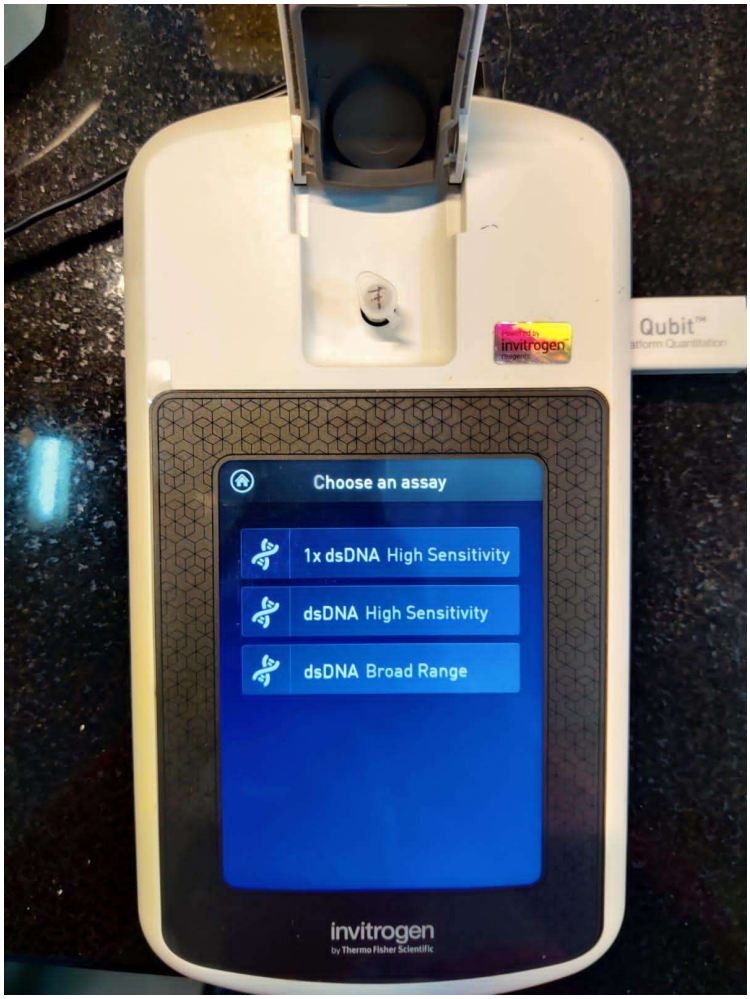
cryobiology lab

HPLC (high performance liquid chromatography)

Ion-exchange chromatography



Fluorescent Microscopy

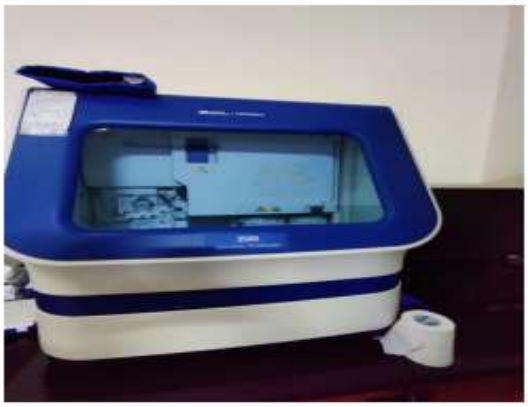


QUBIT FLUOROMETER

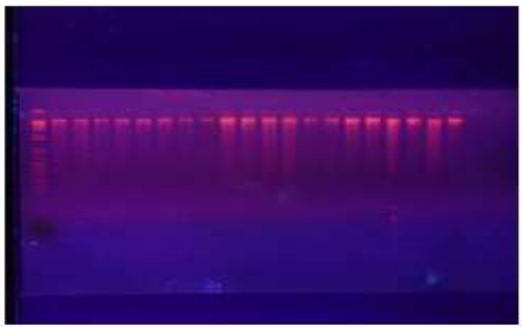
GENETIC ANALYZER

PCR RESULTS

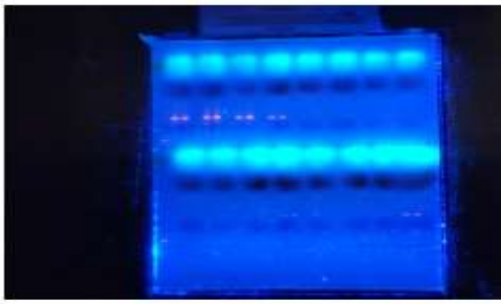
Gene sequencer / sanger sequencer



Gene



DNA isolation from the given sample using fast DNA SPIN kit



4. Conclusion:

The work experience I encountered during the internship allowed me to enhance my scientific skills. The hands-on experience to perform the experiments helped to improve my techniques while working. I have learnt a great and gathered a lot of experience which will be helpful to me in the future. The scientists initiated my curiosity and interest regarding the research conducted. This internship has been an excellent and rewarding experience. Thus, I have completed my internship with great satisfaction.