DECEMBER-2022

INTERNSHIP REPORT

Submitted By:-

Mayuri B. Singh. (MBT Part II)

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पृथ्वी प्रणाली विज्ञान संगठन
पृथ्वी विज्ञान मंत्रालय (भारत सरकार)
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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 Ms. **Mayuri B. Singh** 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 period of her internship program with us, she had been exposed to various techniques and skills in the laboratory.

We wish her every success in her life and career.

05/04/2023
Supervisor

Archana Singh
05.04.2023
Authorized Signature
Scientist-In-Charge
Arctic Ecology & Biogeochemistry
Arctic Operations

Archana Singh / अर्चना सिंह
Scientist / वैज्ञानिक
ESSO-National Centre for Antarctic and Ocean Research
ई.एस.एस.ओ.-राष्ट्रीय अंटार्कटिक एवं समुद्री अनुसंधान केन्द्र
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Finally, I would like to express my gratitude and appreciation to all who helped me directly and indirectly to complete this project.

INTRODUCTION

The National Centre for Polar and Ocean Research (NCPOR) is a research and development institute of India Contributing to polar and southern oceanic regions. It mainly involves studies of biological and physical changes in the polar environment. The Himansh station in Spiti, Himachal Pradesh, and the Himadri and IndARC Arctic research stations in Svalbard, Norway, all are run by NCPOR.

Biological studies of any region in the world are mainly related to the microbial flora of that area. Here also we are estimating micro-organisms over other species in an environment. For this objective various techniques like isolation and incubation of microbes on minimal media, fluorescence and electron microscopy, DNA extraction and amplification, sequencing, and searching for similarities among them and existing species.

Micro-organisms are the species found in a diverse environment like polar regions (Arctic or Antarctic circle), hot springs (volcanoes, hydrothermal vents), saline regions (sea, salt pans), or alkaline regions (salt pans). Organisms show different defense mechanisms to adapt to this adverse condition. Organisms living in colder regions are generally called psychrophiles. The optimum temperature for psychrophiles is 15°C.

To maintain structural integrity micro-organisms growing in colder regions modify their cellular and metabolic structure. When the laboratory organism *Escherichia coli* is compared with a psychrophilic organism it is found that microbes of colder regions lack lipid bilayer in their cell membrane. Also, it is seen that cell membranes contain a large number of branched fatty acids arranged in a disorderly. This results in high membrane fluidity. In the case of enzymes, microbes show more adaption in their tertiary or quaternary structures. Enzymes are made up of proteins that show loose, more flexible, and conformationally different to withstand cold environment.

Nowadays, microorganisms are useful to mankind in their industrial applications. Thus, an experiment for psychrophilic analysis is also designed to find its use in food, medicine, cosmetics, or any other preparation. In this project,

we mainly focused on the genetic studies of bacteria. For this Polymerase Chain Reaction (PCR amplification) technique is used. But before this, we need to isolate the bacteria with help of a suitable medium that fulfill all optimum conditions of microbes. After this with the use of bacterial culture DNA extraction is done which extends with PCR amplification for sequence similarities studies.

Polymerase Chain Reaction is a method in which the sample is first heated to induce the DNA to denature or separate into two pieces of single-stranded DNA, to amplify a segment of DNA using PCR. Finally, using the original strands as templates, an enzyme widely recognized as "Taq polymerase" constructs two new strands of DNA. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA segment.

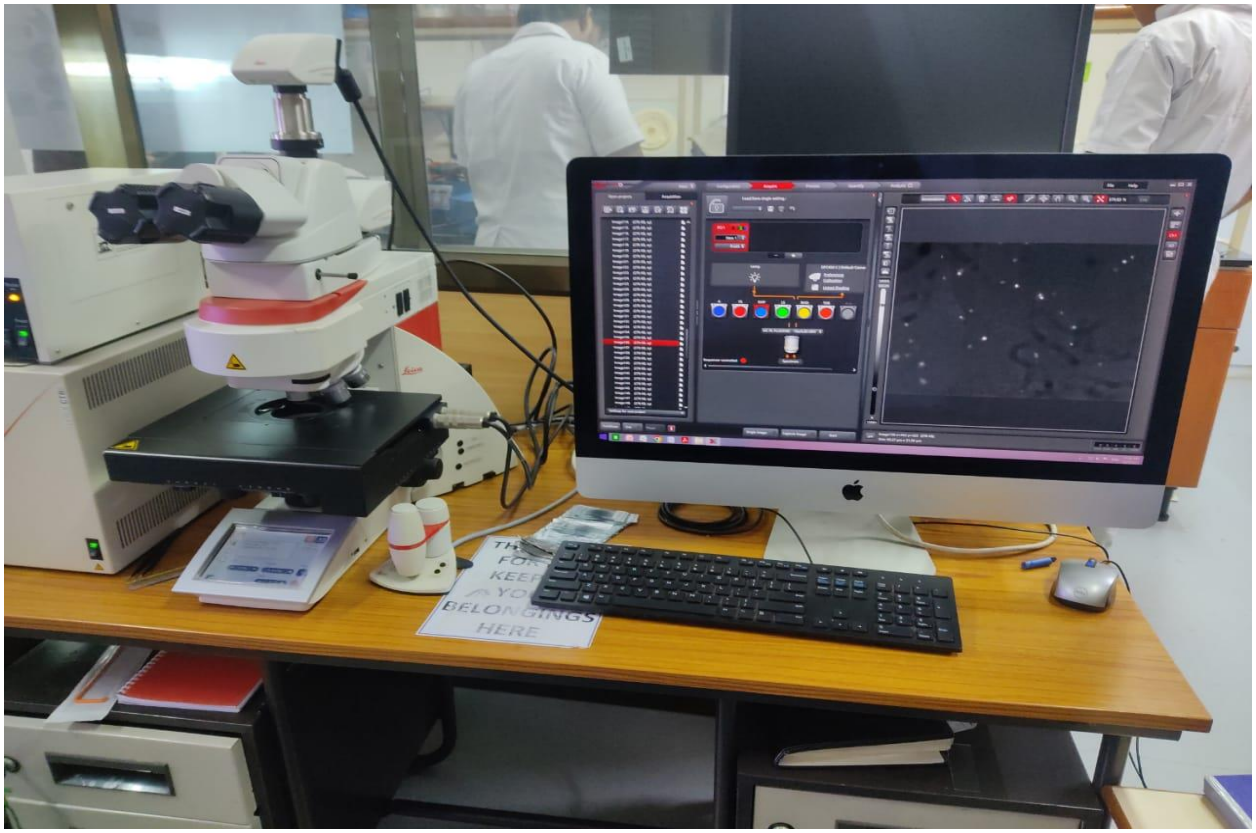
PCR's entire cycling methodology is automated and can be finalized in a short amount of time. It is governed by an instrument called a **thermalcycler**, which is programmed to change the reaction's temperature every few minutes to facilitate DNA synthesis and denaturation.

INSTRUMENTS

1. FLOURESCENCE MICROSCOPY :-

Using the appropriate fluorophores, we can easily quantify microbial populations and tell the difference between living and dead cells using fluorescence microscopy techniques. These characteristics are crucial from a technology perspective because they enable the evaluation of a starter culture's metabolic state prior to use or the tracking of cell viability.

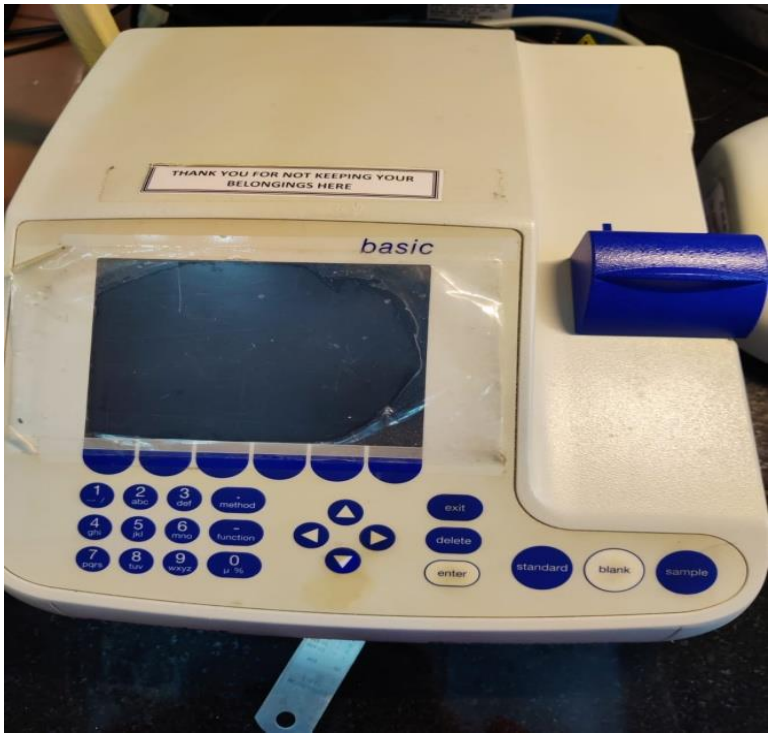
For nuclear morphology detection is done by staining the microbes with 4,6-diamidino-2-phenylindole (DAPI), dye mainly stains minor-groove of double-stranded DNA at adenine and thymine residues. It is anticipated that 4,6-Diamidino-2-phenylindole won't be seen when standard fluorescein or green fluorescent protein filter cubes are used. In this study, we demonstrate that 4,6-Diamidino-2-phenylindole may become sensitive to the blue/cyan excitation used in fluorescein/green fluorescent protein filter cubes after being observed using UV or violet excitation.

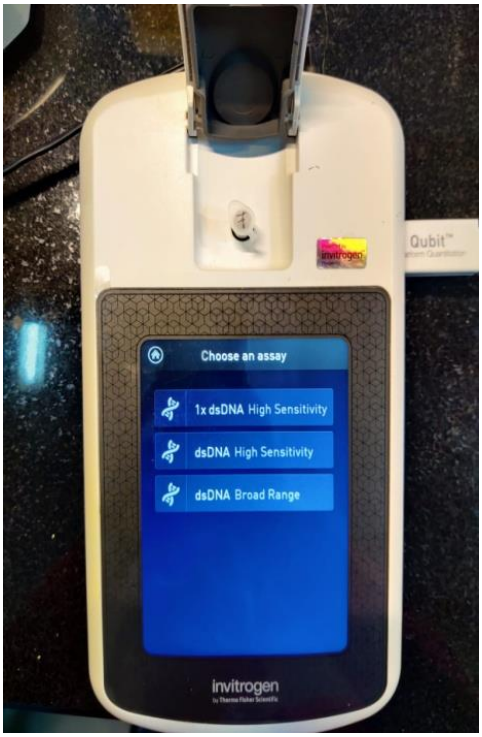


2. NANODROP SPECTROPHOTOMETER :-

By utilising fibre optic technology and the natural surface tension properties of the sample, the NanoDrop microvolume sample retention system (Thermo Scientific NanoDrop Products) is able to capture and hold very small amounts of sample without the use of conventional containment tools like cuvettes or capillaries. It uses method of A260 for estimating concentration of nucleic acid. Ratio of A260/A280 gives the accuracy of instrument and protocol followed.

Fluorescent measurements can be made with as little as 2 µL of material by employing the same microvolume sample retention technology, reducing the volume requirements for fluorescent assays significantly. Using a microvolume fluorospectrometer, it is now possible to conduct such microreactions with a volume of no more than 10 µL.





3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:-

The distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (the column's packing material) is the foundation of HPLC's separation principle. The rate at which the molecules move through the stationary phase varies according to the analyte's chemical structure. The time a sample's molecules spend "on-column" is determined by the particular intermolecular interactions they have with the packing material. As a result, the sample's constituents are eluted at different rates.

As a result, the sample ingredients are separated. After leaving the column, the analytes are recognized by a detection unit, such as a UV detector. The signs are changed over and recorded by an information the executives framework and afterward displayed in a chromatogram. The mobile phase can be subjected to additional detector units, a fraction collection unit, or waste after passing through the detector unit.

4. ANION EXCHANGE CHROMATOGRAPHY :-

Anion exchange chromatography is a type of ion exchange chromatography (IEX),that uses molecule's net surface charges to separate them. More specifically, anion exchange chromatography makes use of an ion exchange resin that has a positive charge and is drawn to molecules with net negative surface charges. A wide range of molecules, from amino acids and nucleotides to large proteins, can be separated using anion exchange chromatography, which is used for both preparative and analytical purposes. The preparative anion exchange chromatography of proteins is our primary focus here.

Every protein posses isoelectric pH(pI) on which it has no net charge. The buffer which gives pH above pI changes the protein into negatively charged molecules. When the protein of interest has a net negative charge at the working pH, a positively charged anion exchange resin is chosen because proteins with different pI values will have different affinities for the positively charged surface groups on the particles of the anion exchange media; As a result, the resin will attract a variety of proteins at varying strengths, making their separation easier.

5. SANGER SEQUENCER :-

The growth of NGS technologies has accelerated research in genomics. With low-input DNA, NGS can simultaneously sequence more than 100 genes and entire genomes. Due to its numerous advantages, Sanger sequencing is still widely used in the sequencing field. Low cost for sequencing individual genes and accuracy of 99.99%, making it ideal for site-directed mutagenesis or cloned insert verification sequencing.

In Sanger sequencing, a DNA groundwork corresponding to the layout DNA (the DNA to be sequenced) is utilized to be a beginning stage for DNA blend. Four deoxynucleotide triphosphates (dNTPs) are present: A, G, C, and T), the polymerase adds the complementary dNTP to the template DNA strand to extend the primer. Four dideoxynucleotide triphosphates (ddNTPs) are used to determine which nucleotide is included in the chain of nucleotides. The synthesis reaction is stopped by using a distinct fluorescent dye labeled with ddATP, ddGTP, ddCTP, and ddTTP). DdNTPs can't link to the next nucleotide because it has an oxygen atom removed from the ribonucleotide, unlike dNTPs.

METHODS

Micro-organisms sampled from five different stations of the Arctic living in different environments like microalgae dominant sites or outside to it. All samples are isolated using Modified basal media by four quadrant method and incubated at 15°C for 3-4 days. After incubation colonies are observed for appearances like colour, margin, elevation, and others. Isolated colonies from the culture plate are used for further estimation.

Bacteria isolated from samples are first visualized by using a fluorescence microscope. Organisms are stained with 4,6-diamidino-2-phenylindole (DAPI), for visualization. We applied two different methods for staining. First, sonicate the sample for two minutes, this result in the disaggregation of organisms that gives complete visualization of each organism. Secondly, samples are filtered through a 0.75 microns filter and the filtered sample is used for staining. After this two different approaches remaining method for staining and visualization is the same. The sample is filtered through 0.22 microns of black filter paper and then the paper is placed on a slide for visualization. This method is called slide preparation.

After slide preparation, all slides are visualized under a 100X lens by using a fluorescence microscope. These slides are used for counting organisms present in a particular slide site. Also, the same slides are used for counting volumes of bacteria present in them. If slides are prepared correctly it is used for understanding the shapes of organisms. By using biostatics method we can calculate the organisms present at sampling site by using slides made for fluorescence microscopy.

After fluorescence microscopy, molecular biology techniques are used for further studies. In molecular biology first, we have to isolate the genetic material of an organism and sequence it for comparison with other organisms. Bioinformatic tools are used to find out adaptations that occurred in organisms due to changes in atmosphere or nutrition.

In the case of bacteria, genetic material is Deoxyribonucleic acid (DNA) isolated by using DNA isolation kits. Here we used the FastDNA spin kit for the isolation of DNA from sample bacteria. A kit is used isolation process is easy and the complete protocol is fixed. According to the given protocol, DNA isolation is

done. The concentration of DNA is found using Nanodrop along with Qubit. DNA purity is also identified using the same. After DNA isolation we move towards the gel separation method using an agarose gel. This will give us the pure and the strands of the same length which are easy to amplify using the PCR amplification technique.

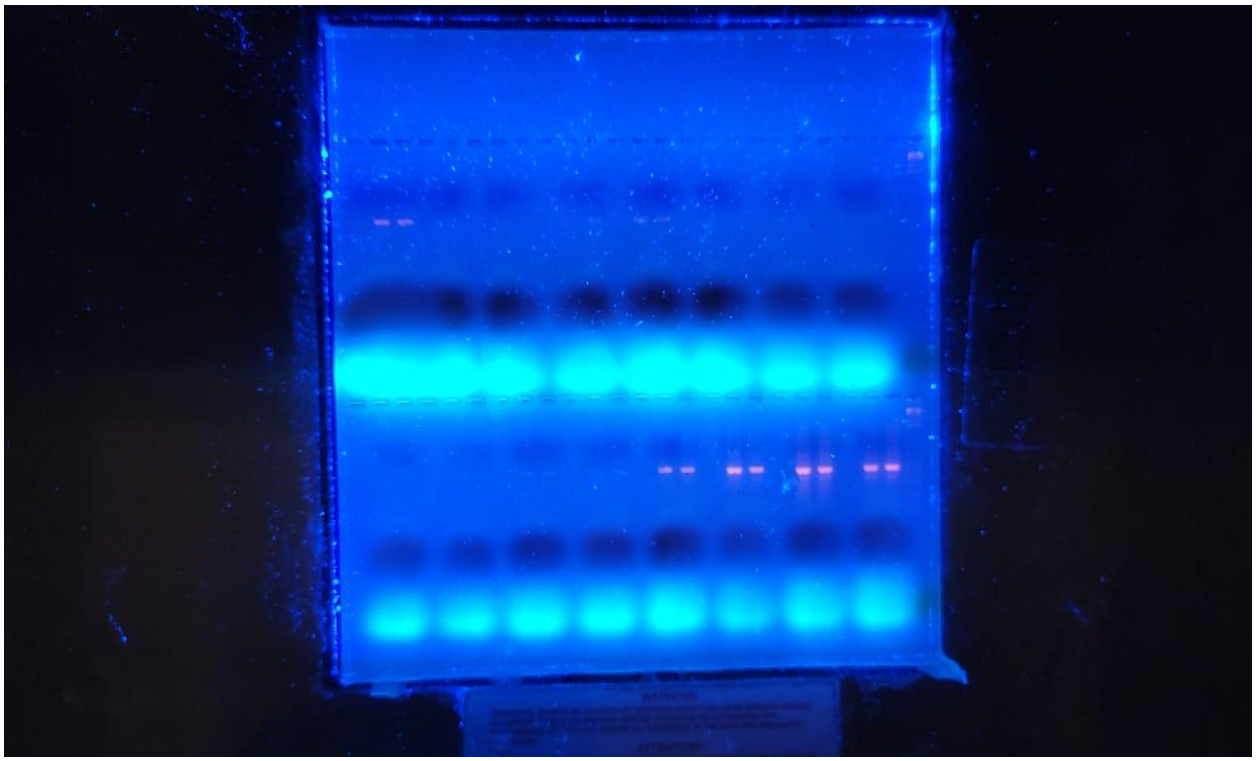
PCR amplification is done using the PrimeStar Amplification kit, which provides the prepared solution mixture of dNTPs, Taq polymerase, primers, and buffer containing the required ions. In it sample DNA i.e. template is added and kept in the PCR amplifier. Here we used the nested PCR amplification method. In it, we used the protocol for 35 cycles of amplification in which denaturation time is 2 min for 95°C, annealing at 56°C for 15 sec, and extention of the template at 72°C for 5 minutes.

After amplification, PCR products are separated and visualized using gel electrophoresis with 2% agarose gel. And then these same gel-separated products are extracted from the gel using the Promega gel extraction kit. Extracted PCR product concentration is determined using Qubit. After this sequencing is done using a Sanger sequencer for further studies.

RESULT and CONCLUSION

By using a Modified Basal Media plate, isolated colonies are obtained. These colonies are observed for their morphology and abundance of bacteria using fluorescence microscopy at the specific site. Microscopy indicates more coccus and some rod-shaped bacteria. By calculating the radius of cocci and the length and breadth of rod volume of bacteria is identified. Also, the same slide gave the approx number of organisms present at that site.

After this DNA of the bacteria is isolated successfully and PCR amplified for 16S rRNA. This will allow us to compare the isolated bacteria with already sequenced organisms. PCR products are run along with a molecular marker. This gives the molecular weight of each band separated on a gel. Thus it is easy to identify the 16S rRNA band of DNA and extracted it.



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