Study of Polysaccharide Degrading Bacteria from Schirmacher Oasis, Antarctica

A Dissertation for

Course code and Course Title: MBT-651 Dissertation

Submitted in partial fulfilment of Master of Science Degree
in Marine Biotechnology

by

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Date: 8 April, 2024

Examined by:

Seal of the School

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Study of Polysaccharide Degrading Bacteria from Schirmacher Oasis, Antarctica**" is based on the results of investigations carried out by me in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Prof. Sanjeev C Ghadi and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation. I hereby authorize the University authorities to upload this dissertation to the dissertation repository or anywhere else as the UGC regulations demand and make it available to anyone as needed.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Study of Polysaccharide Degrading Bacteria from Schirmacher Oasis, Antarctica**" is a bonafide work carried out by **Mr Ghanshyam Sharma** under my supervision in partial fulfilment of the requirements for the award of the degree of Masters of Science in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University.

Prof. Sanjeev C Ghadi

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Prof. Bernard F. Rodrigues

Sr. Professor and Dean of

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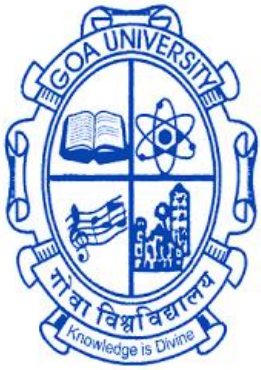

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PREFACE

The investigation of extremophiles, particularly bacteria thriving in frigid landscapes of Antarctica, represents a frontier of scientific possibilities. The focus of this dissertation lies in the identification and characterization of polysaccharide-degrading bacteria indigenous to Antarctica. Polysaccharides serve as vital substrates for energy and carbon recycling in polar ecosystems. Understanding the enzymatic capabilities and metabolic pathways of bacteria specialized in polysaccharide degradation in this extreme environment is crucial for elucidating their ecological significance and exploring potential biotechnological applications. This dissertation endeavours to employ meticulous scientific methodologies to isolate, characterize, and identify polysaccharide-degrading bacteria native to Antarctica. By integrating classical microbiological techniques, molecular biology tools, and bioinformatics analysis, this study aims to unravel the taxonomic diversity of bacteria in extreme cold conditions of Antarctica. This research seeks to contribute substantially to our understanding of microbial ecology in polar regions and unlock the biotechnological potential inherent in these unique bacterial communities.

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Firstly, I would like to express my gratitude to God for all the blessings he has bestowed upon me throughout my dissertation journey.

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ABBREVIATIONS

◦ C	Degree Celsius
µg/L	Microgram per litre
cm	Centimetre
g	Gram
mL	Milli litter
M	Molar
%	Percentage
LAF	Laminar airflow
R2A	Reasonar's 2A agar
CMC	Carboxy Methyl Cellulose
ABM	Antarctic Bacterial Media
PCR	Polymerase Chain Reaction
DNA	Deoxy-ribonucleic acid
EEO	Electro-endosmosis
M9 media	Minimal media
kb	Kilo Base pair
ISEA	Indian Scientific Expedition to Antarctica

ABSTRACT

Antarctic microbial communities provide a unique source of cold-adapted enzymes with potential applications in biotechnology. In cold environmental conditions, bacteria that degrade polysaccharides are essential for the breakdown of organic materials. This work aims to isolate and describe bacteria that break down polysaccharides from the Antarctic environment. Polysaccharide degradation activity was determined in bacterial isolates and molecular identification studies were performed. The diversity of bacteria that break down polysaccharides in the Antarctic environment and their capacity to produce enzymes in frigid temperatures were investigated in this study. The results advance our knowledge of cold-adapted enzymatic activity and its possible applications.

CHAPTER - I

1. INTRODUCTION

1. INTRODUCTION

1.1 BACKGROUND

Antarctica also known as the southernmost continent is completely covered with ice throughout the year. It is the fifth-largest in size and is said to be the coldest, driest and windiest place on Earth. (Cowan et al., 2014 and Luis et al., 2013).

Most Antarctic regions are still poorly studied, except those in the arid deserts of the McMurdo Dry Valleys and the Transantarctic region of Victoria Land (Thompson et al., 2020). Schirmacher Oasis stands out as a notable plateau region in East Antarctica. This area is characterized by the presence of over a hundred freshwater lakes. These lakes serve as valuable indicators, preserving evidence of climatic variations and the deglaciation history since the Last Glacial Maximum, which occurred approximately 19 to 24 thousand years ago. There are three types of lakes present in Schirmacher Oasis- (i) Epishelf lakes(E), (ii) Landlocked lakes(L), and (iii) Proglacial lakes(P). Epishelf lakes are associated with ice shelves and are connected to the ocean waters. Landlocked lakes are inland bodies of water without any direct connection to the ocean. Proglacial lakes are formed near the terminus of glaciers and are influenced by glacial dynamics. Each type of lake in Antarctica has unique characteristics based on its location, formation processes, and hydrological connections (Ravindra et al., 2021).

Extremophiles are organisms known to survive in extremely harsh conditions such as low or high temperatures, high pressure, high acidity and high salinity (Rampelotto, 2013).

Psychrophiles also called, cold-loving organisms thrive well at lower temperatures. They can be either facultative or obligatory. Facultative psychrophiles are capable of thriving in cold environments, but their growth is not strictly limited to low temperatures only. Their optimum temperatures for survival range from 20-30°C showing maximum growth around

30-35°C (Puhakka et al., 2002). Obligate psychrophiles are microorganisms that exclusively flourish in cold environments. Their optimal growth is at temperatures ranging from 15-18°C (Kushner, 1976).

Colwellia psychrerythraea is identified as an obligate psychrophile, characterized as a Gram-negative bacteria. Its ideal growth temperature ranges from -1°C to 10°C and is motile even at temperatures as low as -10°C (Mudge et al. 2021). The initial cultivated psychrotolerant representative, identified as strain TUM19329^{T}, was isolated from an Antarctic Lake (Shimada et al., 2021).

Microbial degradation of complex organic matter by psychrophiles plays an important role in the global carbon cycle. Polysaccharides can have a long life as they are not easily degradable due to their complex structure (Sichert et al., 2021).

Phylum *Firmicutes, Actinobacteria, Proteobacteria* and *Bacteroidetes* are responsible for the degradation of various polysaccharides. *Planococcus, Marinobacter, Bacillus, Salinibacterium*, and *Alcaligenes* sp. are also known to degrade a wide range of polysaccharides such as cellulose, pectin, alginate, chitin, xylene etc. (Vishnupriya et al.,2023).

Bacteria with the ability to degrade agar were isolated from the surfaces of macroalgae in King George Island, Antarctica. A total of 30 pigmented, agarolytic bacteria were identified from the Antarctic macroalgae. The agarolytic isolates were classified within several genera, including *Algibacter, Arthrobacter, Brachybacterium, Cellulophaga, Citricoccus, Labedella, Microbacterium, Micrococcus, Salinibacterium, Sanguibacter,* and *Zobellia* (Alvarado et al., 2017).

Psychrophilic bacteria provide cold-adapted enzymes that offer significant economic advantages due to their higher productivity, opening up numerous biotechnological applications across various industries such as food and feed, cheese ripening, detergents and cleaning, bio-bleaching in the paper and pulp sector, as well as pharmaceutical, medical, and domestic applications (Cavicchioli et al., 2011).

Enzymes produced by polysaccharide-degrading psychrophilic microbes have garnered attention for various applications across industries, pharmaceuticals, medicine, and the food and feed sector (Yadav, A. N., et al., 2019).

1.2 AIM AND OBJECTIVES: -

Aim:-To Isolate Polysaccharide Degrading Bacteria from Schirmacher Oasis, Antarctica.

Objectives: -

1. Screening of polysaccharide-degrading bacteria from Antarctica.
2. Determining the psychrophilic nature of the bacterial isolates.
3. Identification of selected polysaccharide-degrading bacteria.

1.3 RESEARCH HYPOTHESIS: -

This hypothesis proposes that extreme cold conditions of Antarctica have been selected for the identification of novel bacterial strains for the study of specific adaptations of these bacteria to degrade complex polysaccharides. The study primarily focuses on CAZymes activity at low temperatures. This research would involve isolating and identifying bacteria from Antarctic Lake samples, followed by testing their ability to degrade various polysaccharides. By comparing these results to bacteria from temperate zones, researchers can determine if there's a significant difference in polysaccharide degradation potential and the applications of the same.

1.4 SCOPE: -

The scope of this study is to screen for novel psychrophilic polysaccharide-degrading bacteria from Antarctica. This study focuses on the identification of polysaccharide-degrading bacteria from Antarctica and their biochemical characterization. Molecular identification studies of selected strains showing utilization of polysaccharides with promising future potential biotechnological applications in various industries such as cosmetic, pharmaceutical, paper pulp, and food etc.

CHAPTER - II

2. LITERATURE REVIEW

2. LITERATURE REVIEW: -

Freshwater lakes in Antarctica, comprise less than 5% of the area occupied by the Antarctic landmass. They form closed ecosystems with simple trophic structures. Many of these lakes originated from epipelagic ponds that avoid freezing at the bottom during harsh Antarctic winters. In Antarctica's Don Juan, an oligotrophic lake contains the presence of microorganisms like *Bacillus, Micrococcus,* and *Corynebacterium*. Researchers have also reported the presence of yeasts such as *Candida* and *Cryptococcus* (Matondkar et al., 1983).

"Schirmacher Oasis" is an area on East Antarctica's Princess Astrid Coast. It is located between latitudes 70° 44' 33"S and 70° 46' 30"S and longitudes 11° 22' 40"E and 11° 54' 00" E (Dharwadkar et al., 2018). The polar ice sheet borders this region on the south, while an ice shelf borders it on the north. It encompasses erosional landforms such as valleys, hills, and roche moutonnee, showcasing typical glacial features like polishing and striations. Depositional landforms such as terraces, moraines, and patterned ground are also seen in the area.

Proteases, amylases, lipases, alginate lyases etc. are examples of enzymes that are used in many biotechnological processes (Zhang et al., 2010). Enzymes participating in the breakdown of polysaccharides, including alginates, agar-agar, and agarose, have been identified in diverse mesophilic bacterial genera. *Alteromonas, Bacillus, Flavobacterium* and *Pseudomonas* sp. belong to this category. It has been reported that *Flavobacterium* can hydrolyze alginate and agar. Notably, it was recently discovered that the Antarctic *Flavobacterium* sp. known as INACH002 is an agarase-producing strain. This strain is closely related to *Flavobacterium faecale* and *Flavobacterium algicola* (Lavín et al., 2016).

Using a combination of culturable methods, Terminal Restriction Fragment Length Polymorphism (T-RFLP), and 16S rRNA gene clone libraries, the bacterial diversity within three

different depths (18–22 cm, 60–64 cm, and 100–104 cm) of a 136-cm sediment core extracted from an Antarctic freshwater lake was evaluated. Using culture-independent molecular techniques based on small subunit rRNA, investigations into microbial ecology within freshwater sediments revealed five major phylogenetic groups in the 18 cm library: namely *Gammaproteobacteria* (62.1%), *Betaproteobacteria* (6.8%), *Bacteroidetes* (28.8%), *Firmicutes* (0.8%), and *Actinobacteria* (1.5%), as determined by BLAST analysis (Shivaji et al., 2011).

The Bacteroidetes phylum is also known for its proficiency in degrading diverse complex carbohydrates, contributing to its dominance in various environments. Polysaccharide degradation in many environmental species within this phylum is closely associated with the type IX secretion system (T9SS), exclusive to Bacteroidetes. This system is utilized for secreting specific enzymes and is intricately connected to gliding motility (McKee et al., 2021).

In the vicinity of Syowa Station on East Ongul Island in the Antarctic, ice-free regions host numerous small freshwater lakes that are oligotrophic. Strain 107-E2^T was isolated from this area, and exhibits growth between 5-25°C. The pH range for its growth was found to be between 6.0 - 9.0. Furthermore, a freshwater lake in Skarvsnes, Antarctica yielded the isolation of a novel bacteria, *Lysobacter oligotrophicus* (Fukuda et al., 2013). This bacteria is also known to thrive well in oligotrophic environments. Over a 12-month study period, the phytoplankton productivity and biomass in Crooked Lake and Lake Druzhby, two large freshwater lakes in eastern Antarctica, were assessed. Despite Crooked Lake being sampled at a single site and Lake Druzhby featuring a more detailed investigation due to its complex structure, both lakes exhibited ultra-oligotrophic conditions, with concentrations of chlorophyll consistently below 1 µg/L (Henshaw et al., 2002).

In Kongsfjorden (Arctic), bacterial isolates demonstrated a notable capacity for producing cold-active extracellular enzymes, encompassing amylase, pectinase, alginate lyase, xylanase, and carboxymethyl (CM)-cellulase. This study revealed that 52% of the bacterial isolates exhibited positive extracellular enzyme activities at 4°C, emphasizing their cold-adapted enzymatic capabilities. Additionally, 41% of the isolates maintained such activities at 20°C, further highlighting their versatility in enzyme production across different temperatures (Jain et al., 2017).

Tsomgo Lake in Sikkim, located in the Eastern Himalayas, exhibit Proteobacteria as the predominant taxa. Its CAZymes (Carbohydrate Active enzymes) are involved in carbohydrate degradation, and they encompass Glycoside Hydrolases (GHs), Polysaccharide Lyases (PLs), Carbohydrate Esterases (CEs), Glycosyl Transferases (GTs), protein with noncatalytic Carbohydrate-Binding Modules (CBMs), and enzymes with Auxiliary Activities (Rai et al., 2021). Microbes like *C. psychrerythraea, Psychrobacter cryohalolentis K5, Psychrobacter arcticus*, and *Desulfotalea psychrophila* produce psychrophilic CAZymes with diverse applications. These enzymes not only play a role in carbohydrate metabolism but also exhibit applications as antioxidants, antimicrobials, and photo-protectants (Pantanella et al., 2006).

Fourteen cultivable psychrophilic bacteria from Antarctica samples, including certain *Pseudomonas* species like the N25 strain, demonstrate potential for biohydrogen production (Alvarado-Cuevas et al., 2015).

Cellulolytic bacteria isolated from Stain House Lake in Antarctica specifically isolates CMAA 1184 and CMAA 1185, demonstrated significant cellulase activity. These bacteria belong to *Bacillus* sp. 16S ribosomal RNA gene subclade and produce cold-active cellulase. The high cellulase levels, along with psychrotolerant and pH-adaptive features, warrant further

investigation into their role in cellulose breakdown and potential as organic matter degraders in extreme freshwater environments (Melo et al., 2014).

All isolates from lakes of Stornes Peninsula were identified as psychrophilic, revealing three novel species within the *Pseudomonas* genus: *Pseudomonas antarctica* sp. nov., *Pseudomonas meridiana* sp. nov., and *Pseudomonas proteolytica* sp. nov. These classifications were established through phenotypic and chemotaxonomic characteristics. These species help in synthesizing cryoprotectors by psychrophilic and psychrotolerant microorganisms and are known to have many applications in agriculture, cosmetics, and medicine (Chauhan et al., 2015).

CHAPTER –III

3. MATERIALS AND METHODOLOGY

3. MATERIALS AND METHODS: -

3.1 Revival of samples: -

Samples were collected from freshwater lakes in Schirmacher Oasis, Antarctica during the 42nd ISEA (Nov 22-Feb 23). Various samples collected from different Antarctic lakes were revived on respective R2A agar and M9 plates supplemented with 1% polysaccharide by streaking. The samples were incubated at 4°C for two months. Revived samples were re-streaked on ABM plates for faster growth (Harmesh et al., 2012).

3.2 Growth on different polysaccharide media: -

For screening of different multiple polysaccharide degrading bacteria, isolated colonies were streaked on multiple plates for detection of their ability to degrade multiple polysaccharides. Modified R2A and M9 media supplemented with 1% polysaccharide as the sole carbon source were used to check the polysaccharide degrading ability of the various bacterial isolates. (Monge et al., 2020). Gel rite was used as a gelling agent and as a substitute for agar to detect polysaccharide degrading activity of all polysaccharides except agarose (Lin et al., 1984).

All streaked plates of different media were incubated at 4°C. After three months of incubation, all plates were checked by flooding the plates using different dyes such as Lugol's iodine for agar-degrading bacteria (Kwon et al., 2020) , Phenol red or 10% cetyl pyridinium chloride for carrageenan degrading bacteria (Chauhan et al., 2016), 0.1% Congo red for xylene and carboxymethyl-cellulose degrading bacteria (Sazci et al., 1986), and 5N HCl for pectin-degrading bacteria (Jain et al., 2017).

3.3 Determining the psychrophilic nature of bacterial isolates: -

For the determination of obligatory psychrophiles, all cultures were streaked in triplicates of two sets of each. One set was incubated at 4°C and another one was incubated at 22°C. After one month of incubation period bacterial growth was observed.

3.4 Identification of the isolated bacterial cultures: -

3.4.1 Gram staining:-

Gram staining was performed to initially characterize and identify bacterial species by analyzing the composition of their cell walls.

On a clean slide, a bacterial smear of overnight grown culture was prepared. This was followed by air-drying and heat fixation of the culture. The Gram staining protocol was followed and the slide was examined under a 100X objective lens with oil immersion (Gephardt et al.,1981).

3.4.2 DNA isolation of the selected strains: -

Bacterial Genomic DNA was isolated using MB505 HiPurA® Bacterial Genomic DNA Purification Kit. The isolated DNA was then run on 0.7% Low EEO Agarose gel. The bands obtained were checked using a UV transilluminator, and the isolated DNA concentration was determined using a Qubit® 2.0 Fluorometer.

3.4.3 PCR (Polymerase Chain Reaction): -

A PCR reaction to amplify the 16S rRNA gene of the selected bacterial isolates was performed. “27F and 1492R” primers were used to amplify genomic DNA.

Table 1: - Primer sequence of forward and reverse primer

Sr. No.	Primer name	Primers Sequence	References
1.	27F	5' AGAGTTTGATCCTGGCTCAG 3'	(Dos Santos et al., 2019).
2.	1492R	5' TACGGTTACCTTGTTACGACTT 3'	

The following parameters were used for the PCR reaction.

Table 2: - PCR parameters for 30-cycle reaction

Sr. No.	Parameters	Temperature	Time	No. of Cycles
1.	Initial Denaturation	95°C	2 minutes	1
2.	Denaturation	95°C	30 second	30
3.	Annealing	51.9°C	30 second	
4.	Extension	72°C	1 minute 33 second	
5.	Final extension	72°C	8 minutes	1

1. Concentration of L49A template DNA: -

Table 3.1 = PCR reaction mixture components for L49A DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	38

2.	10X Taq buffer	5
3.	dNTPs (10mM)	1
4.	Template DNA (~50ng)	3
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

2. Concentration of P7B template DNA: -

Table 3.2 = PCR components for P7B DNA

Sr. No.	PCR Components	Volume(µL)
1.	Sterile Nuclease Free water	36
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template(~50ng)	5
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

3. Concentration of L6C template DNA: -

Table 3.3 = PCR components for L6C DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	40.3
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template(~50ng)	0.7
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

4. Concentration of L5D template DNA: -

Table 3.4 = PCR components for L5D DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	40.5
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	0.5
5.	Forward Primer	1

6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

5. Concentration of L6E template DNA: -

Table 3.5 = PCR components for L6E DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	32
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	9
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

6. Concentration of L49F template DNA: -

Table 3.6= PCR components for L49F DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	31

2.	10X Taq buffer	5
3.	dNTPs 10Mm	1
4.	Template (~50ng)	10
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

7. Concentration of L49G template DNA: -

Table 3.7 = PCR components for L49G DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	40
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	1
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

8. Concentration of L5H template DNA: -

Table 3.8 = PCR components for L5H DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	39.5
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	1.5
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

9. Concentration of L27I template DNA: -

Table 3.9 = PCR components for L27I DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	40
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template(~50ng)	1
5.	Forward Primer	1

6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

10. Concentration of L32J template DNA: -

Table 3.10 = PCR components for L32J DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	39.5
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template(~50ng)	1.5
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

11. Concentration of L27K template DNA: -

Table 3.11 = PCR components for L27K DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	39

2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	2
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

12. Concentration of L32L template DNA: -

Table 3.12 = PCR components for L32L DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	39
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	2
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

13. Concentration of P7M template DNA: -

Table 3.13 = PCR components for P7M DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	40
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	1
5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

14. Concentration of L49N template DNA: -

Table 3.14 = PCR components for L49N DNA

Sr. No.	PCR Components	Volume (µL)
1.	Sterile Nuclease Free water	39
2.	10X Taq buffer	5
3.	dNTPs 10mM	1
4.	Template (~50ng)	2

5.	Forward Primer	1
6.	Reverse Primer	1
7.	Taq DNA Polymerase	1
8.	Total Volume	50

3.4.4 <u>Biochemical Identification</u>: -

The carbohydrate utilization test was conducted using the HiCarboTM Kit from HiMedia Pvt. Ltd., comprising parts A, B, and C. Following the manufacturer's standard methodology, 50µL of 5-day grown bacterial culture was inoculated into the wells of the kit containing different carbohydrates. Colour changes were observed and noted after an incubation period of 5 days. The guidance provided by the KB009 kit was utilized to interpret the results.

CHAPTER –IV

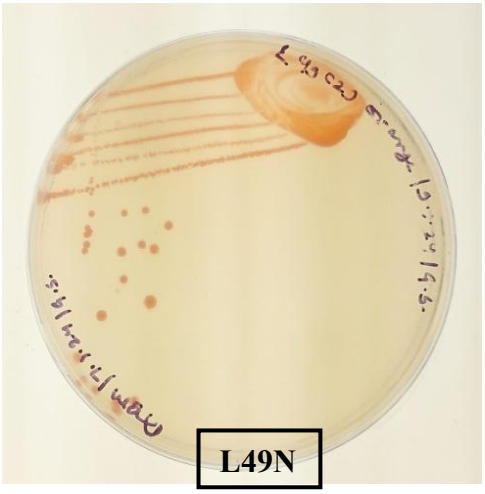
4. ANALYSIS AND CONCLUSION

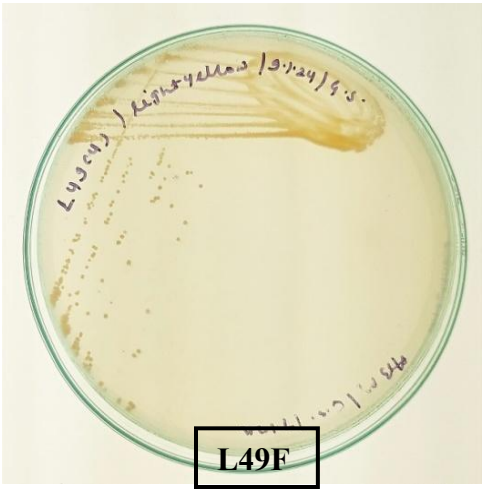
4. ANALYSIS AND CONCLUSION:-

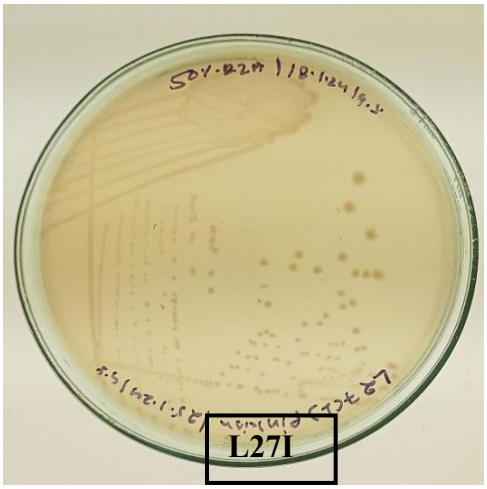
4.1 Revival of samples: 14 colonies were isolated in ABM and R2A media plates at 4°C colony morphology was observed and colony characteristics were mentioned in Table 4.

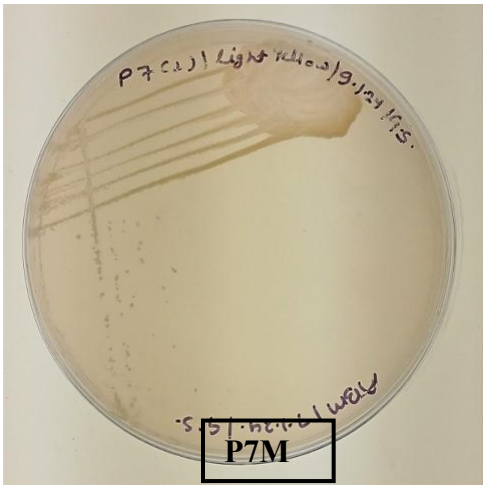
Table 4: - Colony morphology characteristics of isolates

Sr. No.	Lake	Margin	Colour	Elevation	Texture	Shape
1.	L49N	Entire	Orange	Umbonate	Shiny	Round
2.	L5H	Entire	Dark Yellow	Raised	Shiny	Round
3.	P7B	Entire	Lime Yellow	Raised	Shiny	Round
4.	L49A	Entire	Whitish	Convex	Shiny	Round
5.	L32J	Lobate	Creamish	Flat	Slimy	Filamentous
6.	L27O	Entire	Pinkish	Convex	Dry	Round
7.	L6C	Entire	Creamish	Convex	Mucoid	Round
8.	L32L	Entire	Creamish	Convex	Shiny	Pinpoint Round
9.	L49F	Entire	Light Yellow	Pulvinate	Smoothy	Round
10.	P7M	Entire	Light Yellow	Flat	Shiny	Punctiform
11.	L27I	Entire	Pinkish	Umbonate	Translucent	Round
12.	L49G	Entire	Yellowish	Pulvinate	Shiny	Round
13.	L27K	Entire	Dark Yellow	Raised	Shiny	Round
14.	L6E	Entire	Yellow	Pulvinate	Shiny	Round
15.	L5D	Entire	Creamish	Flat	Mucoid	Round









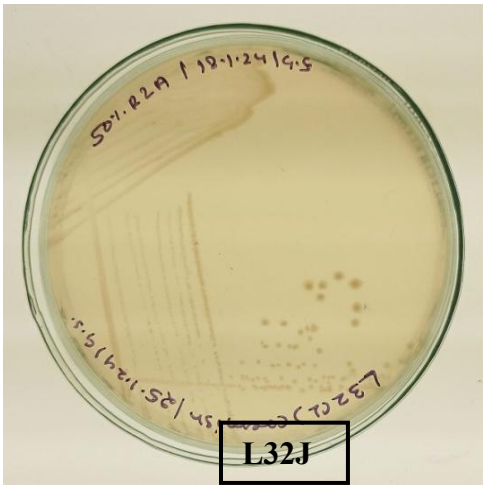


Figure 1.1:- Diagram showing isolated colonies of various Polysaccharide-degrading bacteria from Antarctica

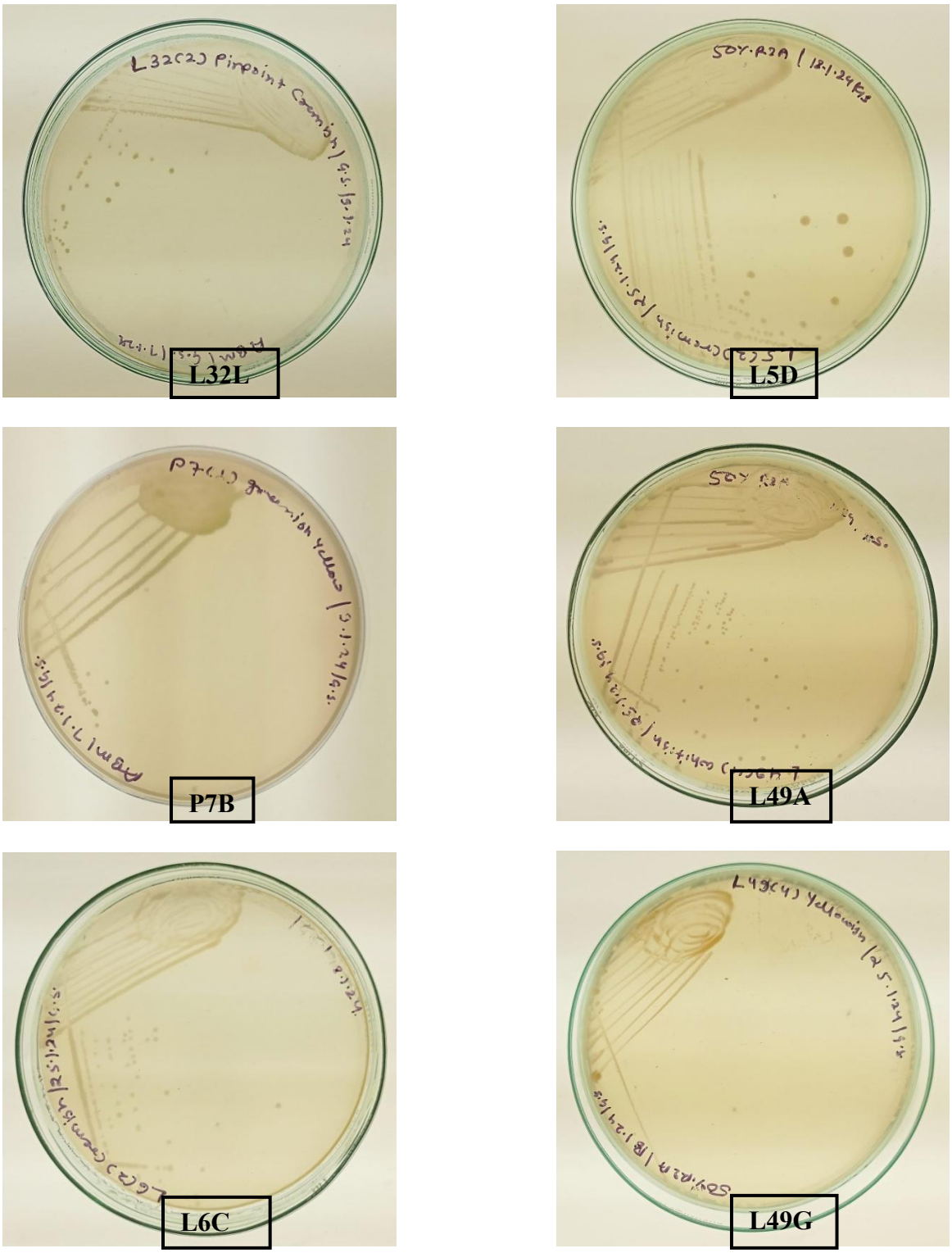
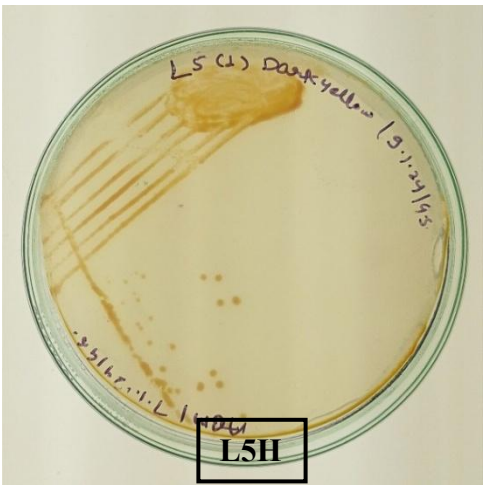
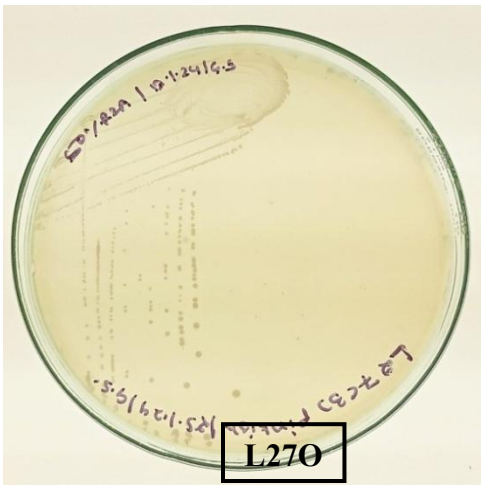


Figure 1.2:- Diagram showing isolated colonies of various Polysaccharide-degrading bacteria from Antarctica





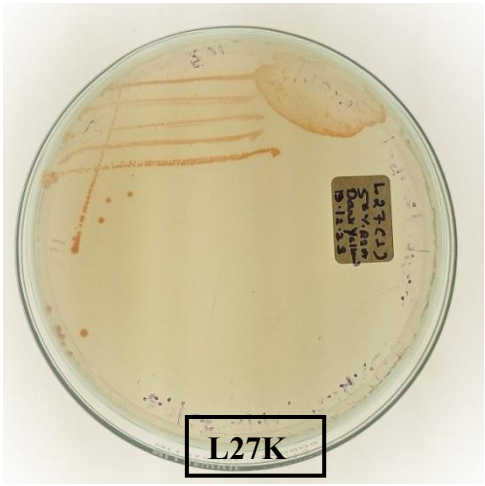


Figure 1.3: - Diagram showing isolated colonies of various Polysaccharide-degrading bacteria from Antarctica

4.2 <u>Gram staining</u>:-

Gram staining technique was performed for all the isolates, revealing their Gram character. Bacteria that appear purple after undergoing the Gram staining process are classified as Gram-

positive, whereas those that appear pink are categorized as Gram-negative (Bartholomew et al., 1952). The Gram characteristics are mentioned in table 5.

Table 5: - Gram characteristics of isolates.

Sr. No.	Colony	Gram Characteristics
1.	L49F	Negative, Rod Shape
2.	L5H	Negative, Rod Shape
3.	L27K	Negative, Rod Shape
4.	L6E	Negative, Rod Shape
5.	L49G	Positive, Rod Shape
6.	L49N	Negative, Rod Shape
7.	L5D	Negative, Rod Shape
8.	L32L	Positive, Rod Shape
9.	L49A	Negative, Rod Shape
10.	L27I	Negative, Rod Shape
11.	L27O	Positive
12.	P7M	Positive, Rod Shape
13.	P7B	Negative, Cocci
14.	L6C	Negative, Rod Shape
15.	L32J	Negative, Rod Shape

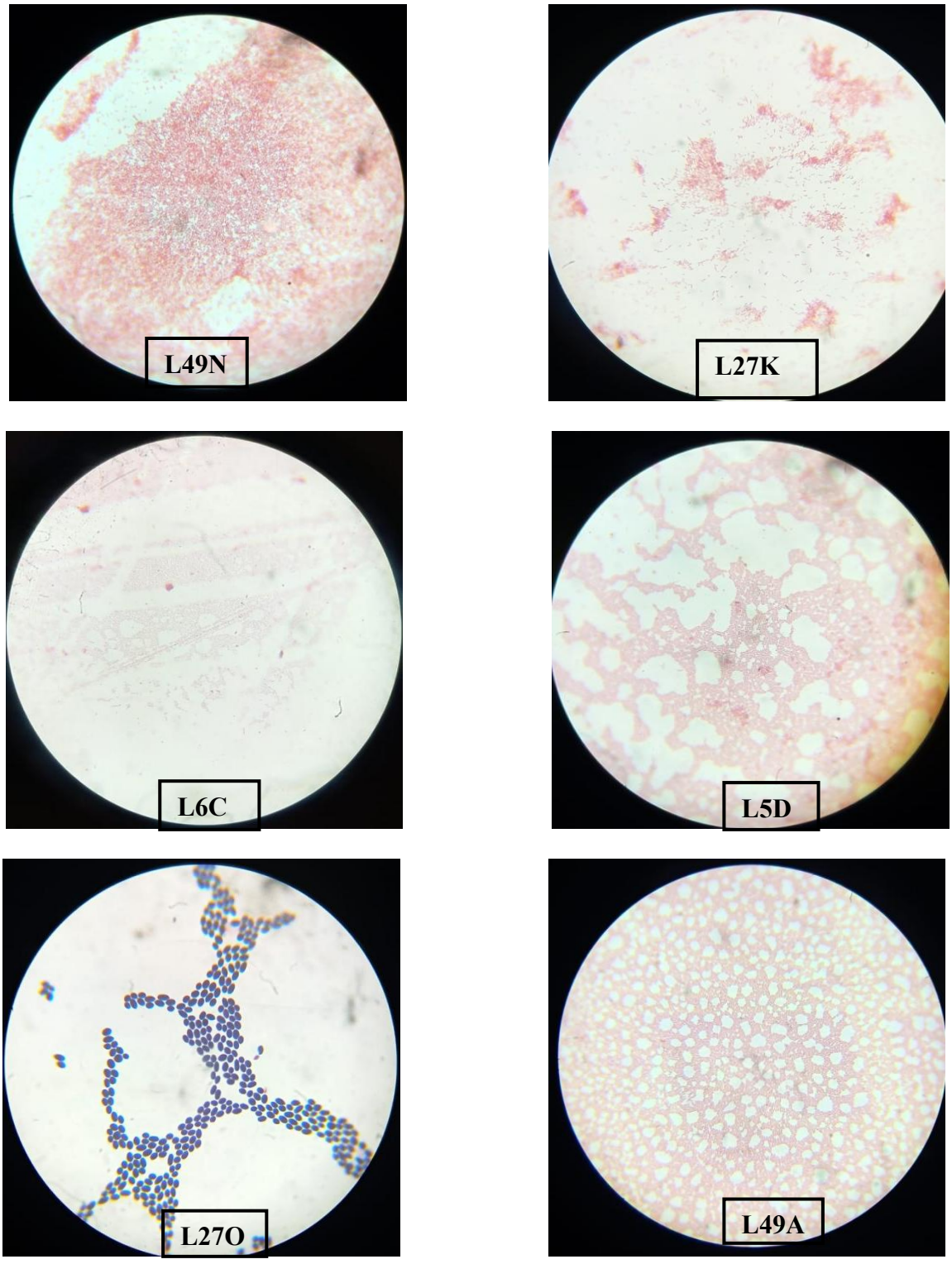


Figure 2.1: - Microscopic images depicting Gram character of isolates

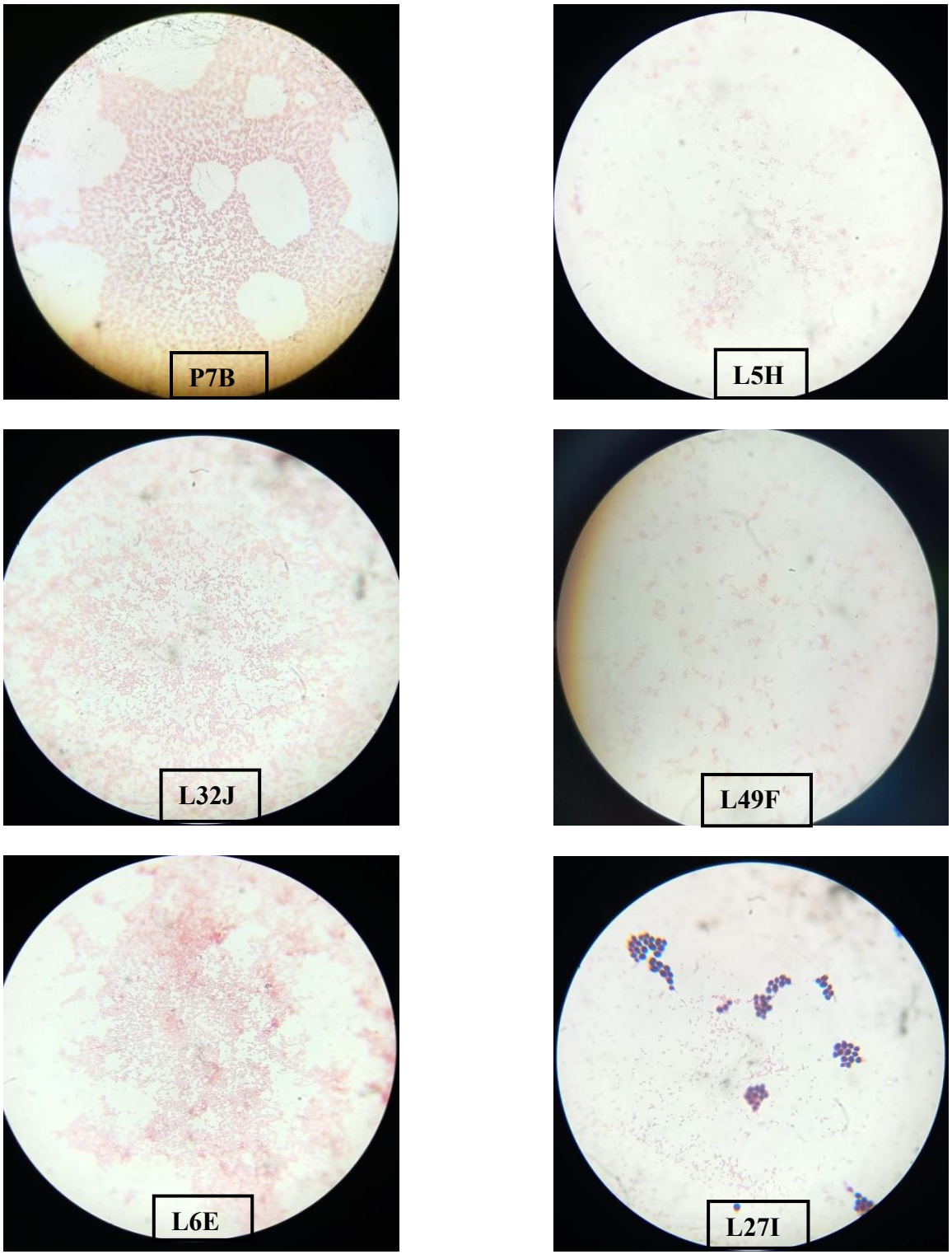
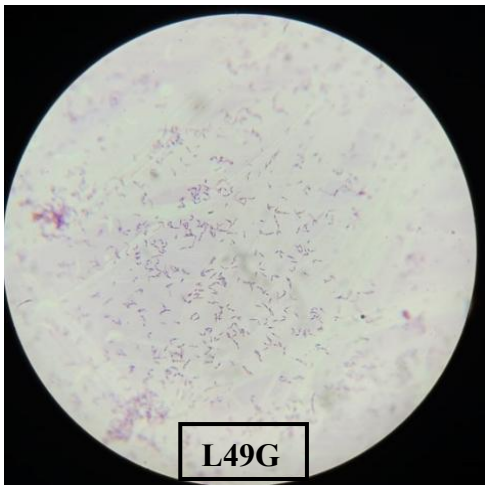
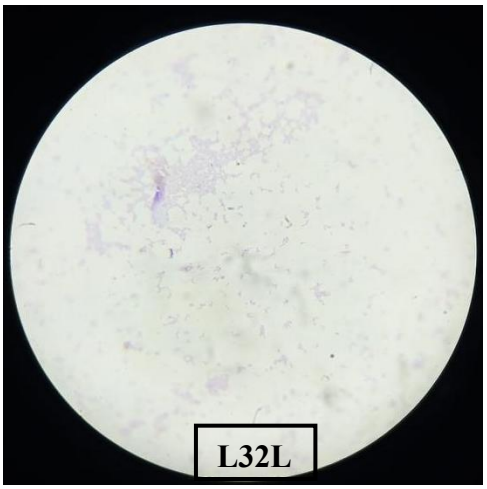


Figure 2.2: - Microscopic images depicting Gram character of isolates





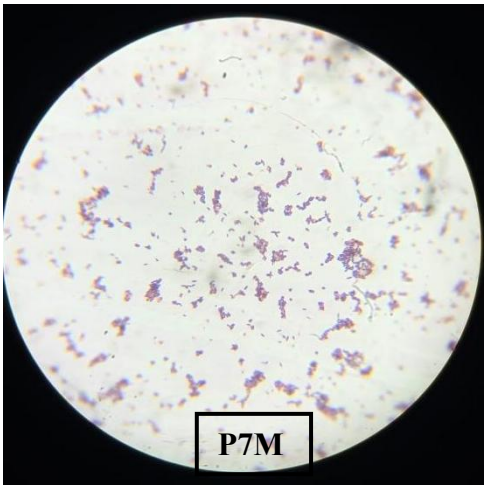


Figure 2.3: - Microscopic images depicting Gram character of isolates

4.3 Polysaccharide-degrading capability of bacteria isolated from Antarctica:-

After a three-month incubation period at 4°C, bacterial colony growth was observed on different media as mentioned in Table 6 and screening for polysaccharide degradation was carried out by flooding the plates with respective dyes as mentioned above.

Table 6: - Growth of isolates on different polysaccharide

Sr. No	Colony	M9 Agar	M9 Pectin	M9 Xylene	M9 CMC	M9 Carrageenan	M9 Alginate
1.	L49F	+	+	+	+	+	-
2.	L5H	+	+	+	+	+	-
3.	L27K	+	+	+	-	+	+
4.	L6E	+	+	+	+	+	+
5.	L49G	+	+	-	-	-	+
6.	L49N	+	+	+	-	+	+
7.	L5D	+	+	+	-	-	+
8.	L32L	+	+	+	-	-	-
9.	L49A	+	+	+	+	+	+
10.	L27I	+	+	+	-	+	+
11.	L27O	+	+	+	-	+	+
12.	P7M	+	-	+	-	+	+
13.	P7B	+	+	+	-	+	+
14.	L6C	+	+	+	+	+	-
15.	L32J	+	+	+	-	-	+

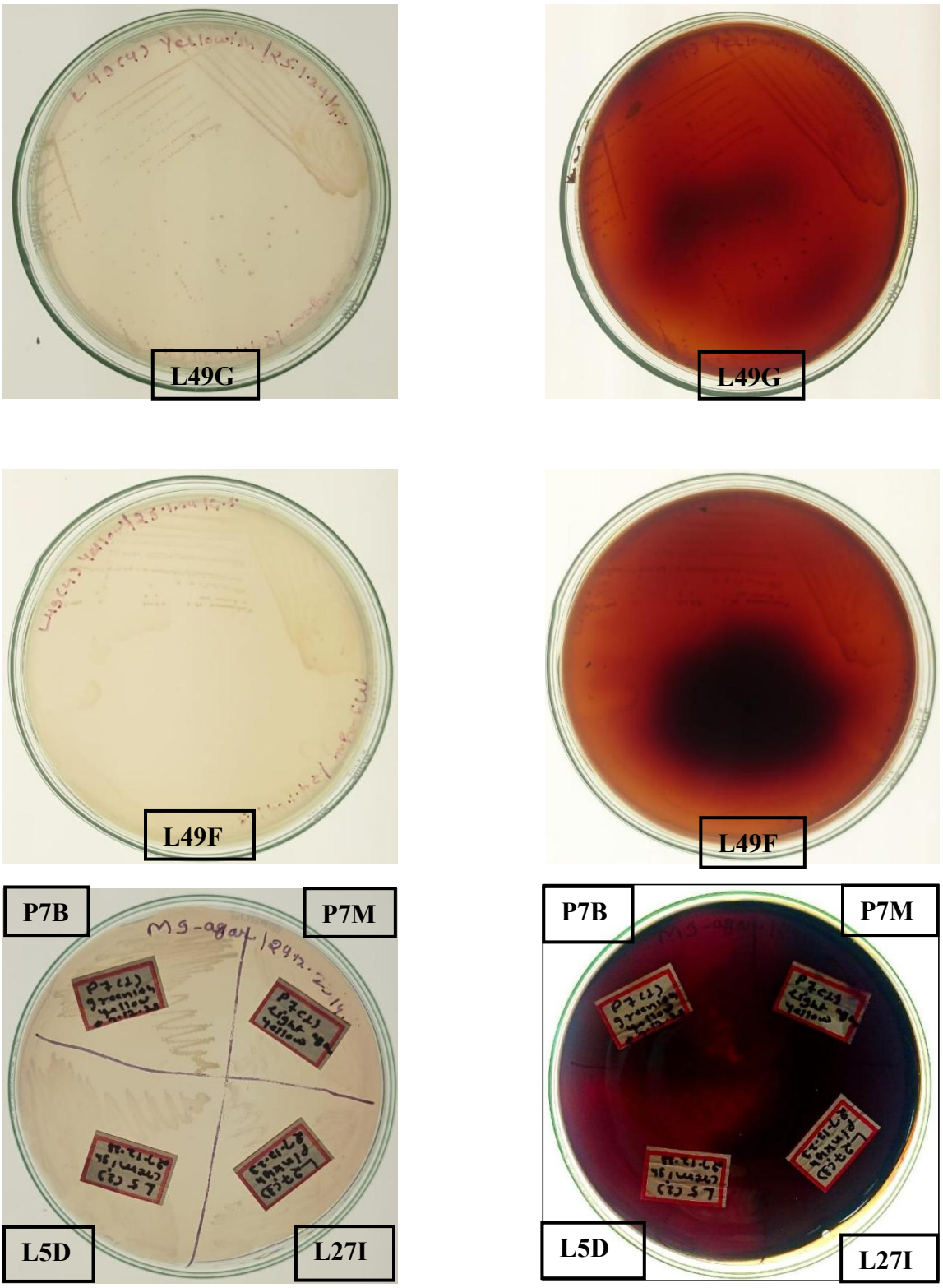


Figure 3.1: - Colony on M9-agar media plate before and after flooding Lugol's iodine.

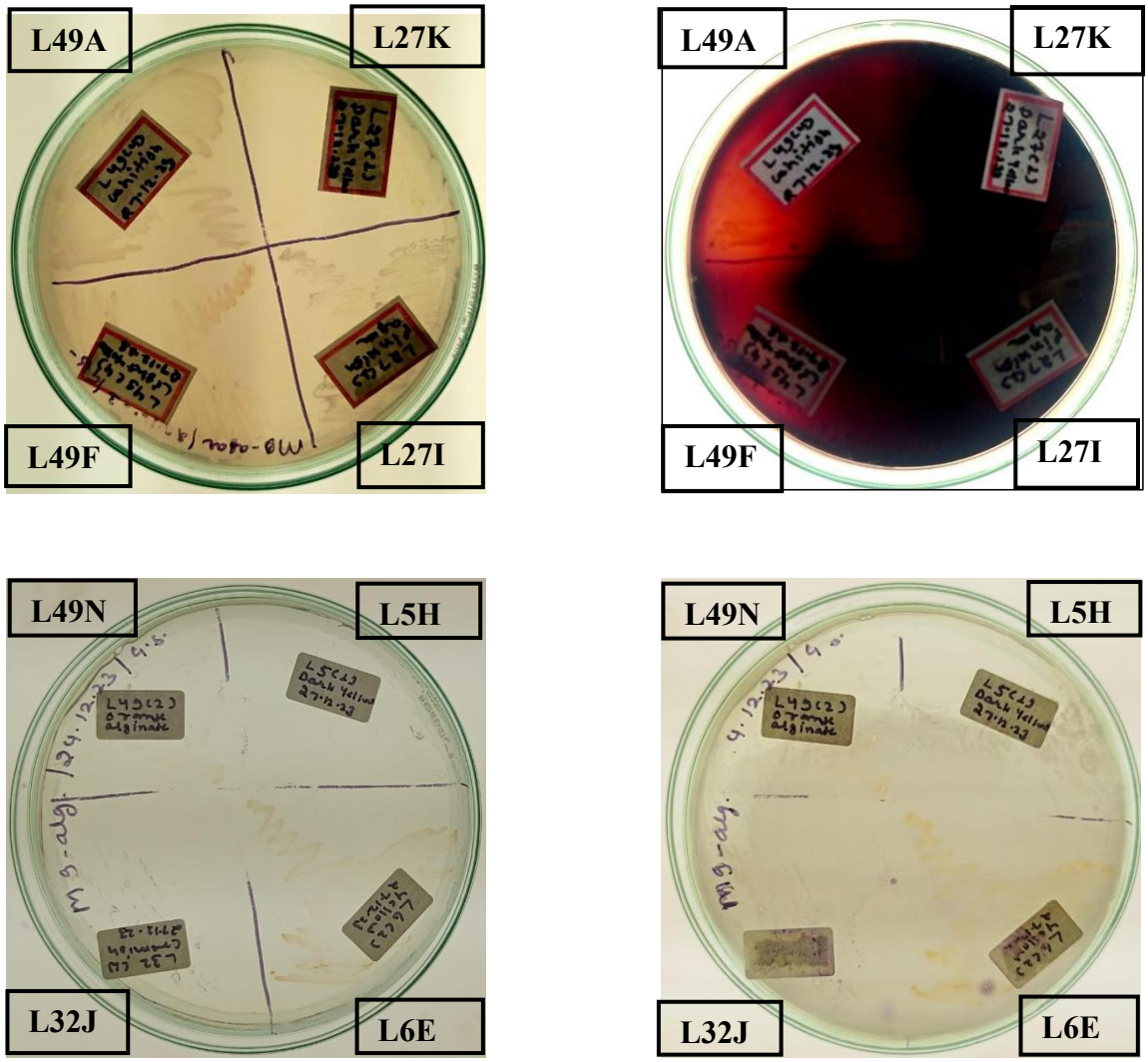


Figure 3.2: - Colony on M9-agar media plate before and after flooding Lugol's iodine and M9-alginate media plate before and after flooding 10% CPC.

4.4 <u>Determining the psychrophilic nature of bacterial isolates</u>: -

After an incubation period of one month, several cultures demonstrated faster growth at 22°C, while one culture (L6C strain) exhibited faster growth at 4°C. The culture exhibiting faster growth at 4°C can be classified as an obligatory psychrophile, whereas the cultures showing faster growth at 22°C can be categorized as psychrotolerant.

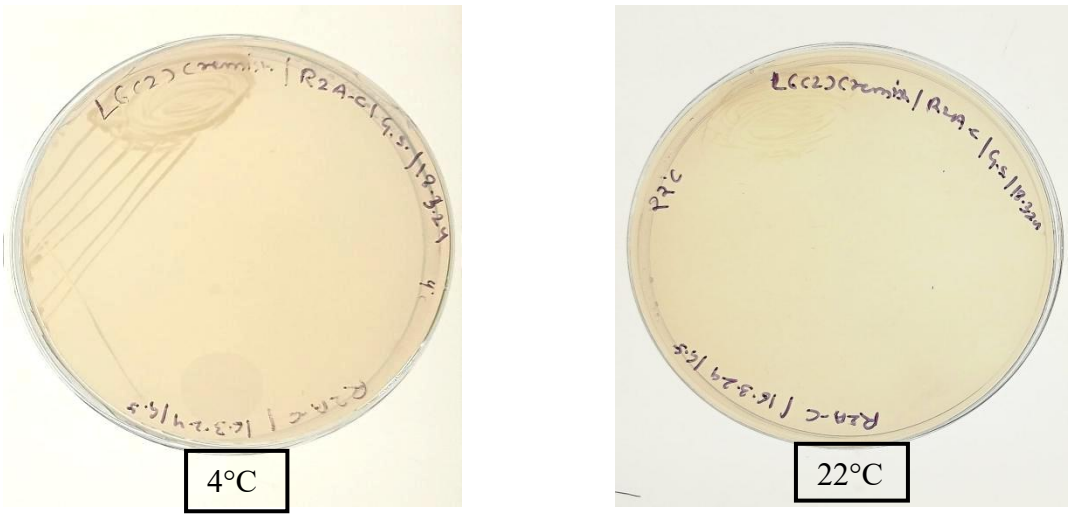
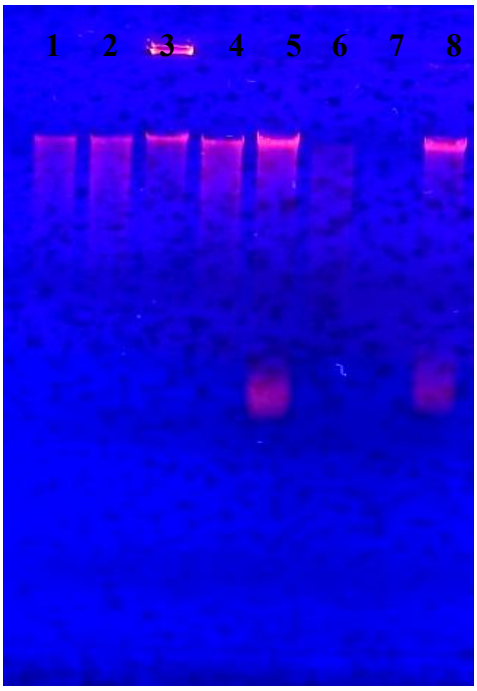


Figure 4: - L6C colony growth at 4°C and 22°C

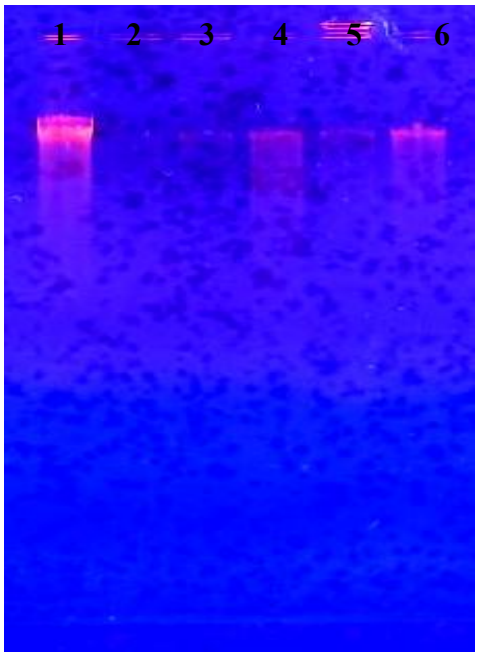
4.5 <u>DNA isolation and PCR amplification</u>: -

Genomic DNA of the bacterial isolates was successfully carried out and the bands obtained were observed using a UV transilluminator.



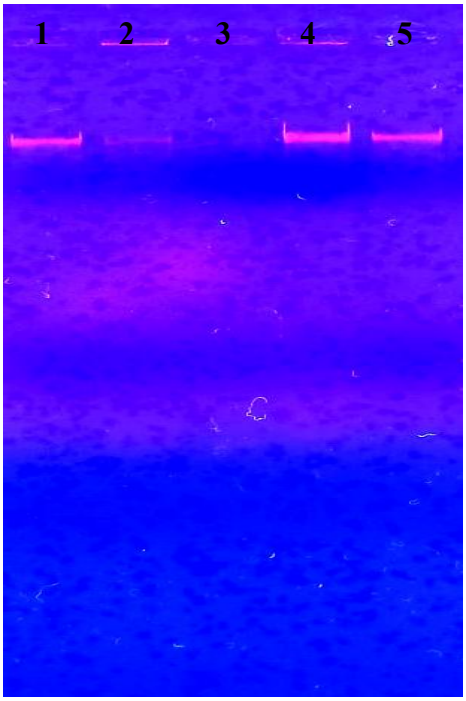
Lane1:-L49N
Lane2:-L27K
Lane3:-L5D
Lane4:-L5H
Lane5:-L32J
Lane6:-L49G
Lane7:-L27O
Lane8:-L27I

Figure 5.1: - Genomic DNA Bands on 0.7% agarose gel



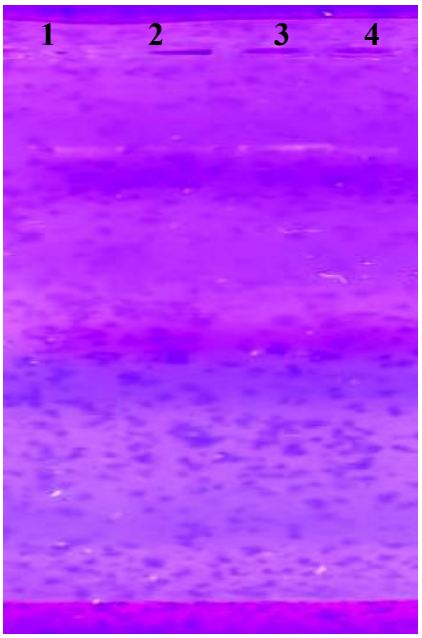
Lane 1:- P7M
Lane 2:- P7B
Lane 3:- L5D
Lane 4:- L32L
Lane 5:- L49A
Lane 6:- L49G

Figure 5.2: - Genomic DNA Bands on 0.7% agarose gel



Lane 1:- L49A
Lane 2:- P7B
Lane 3:- L6E
Lane 4:- L6C
Lane 5:- L5D

Figure 5.3: - Genomic DNA Bands on 0.7% agarose gel



Lane 1:- L49A

Lane 2:- L6E

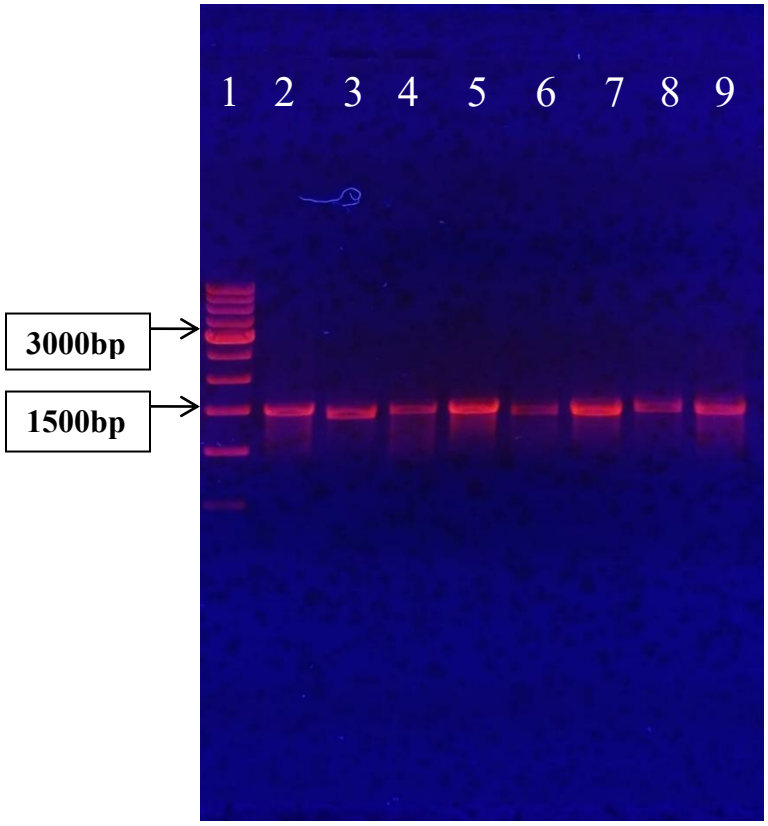
Lane 3:- L49F

Lane 4:- P7B

Figure 5.4: - Genomic DNA Bands on 0.7% agarose gel

DNA yield was found to be low for L49A, L6E, L49F and P7B. The probable reasons could be, incomplete lysis of cells and high GC content etc (Eriksson et al., 2017).

Following the isolation of genomic DNA, amplification of DNA bands was carried out using the forward primer "27F" and the reverse primer "1492R". The resulting amplified fragment had an approximate length of 1.5kb and a 500 bp DNA ladder was used for comparing desired amplified DNA band length.



Lane 1:- 500 bp DNA Ladder

Lane 2:- L32J

Lane 3:- L27K

Lane 4:- L27I

Lane 5:- L32L

Lane 6:- L5H

Lane 7:- L49N

Lane 8:- P7M

Lane 9:- L49G

Figure 6.1: - 1.5kb Amplified fragment of Genomic DNA on 0.7% agarose gel

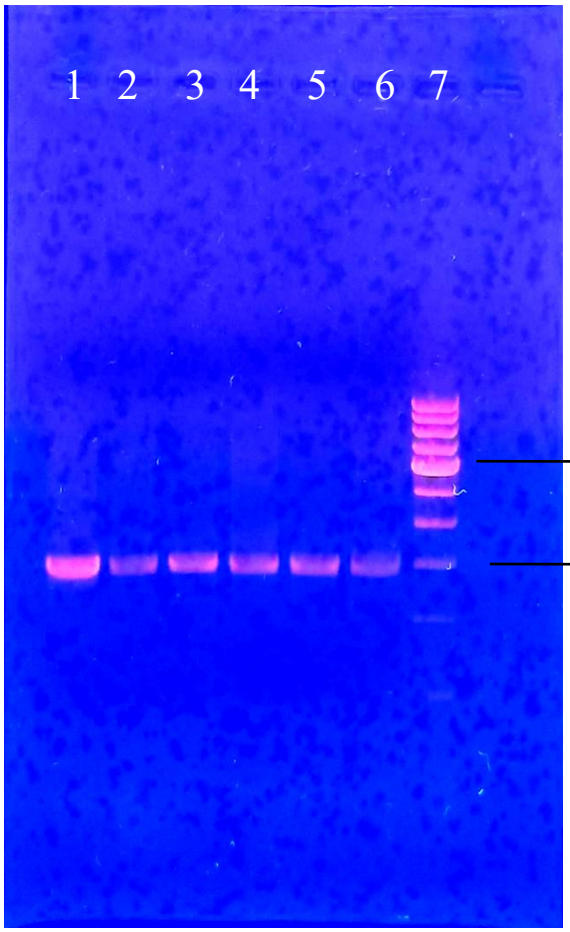


Figure 6.2: - 1.5 kb Amplified fragment of Genomic DNA on 0.7% agarose gel

4.6 Molecular identification:-

NCBI Nucleotide Blast analysis of the 16S rRNA gene sequencing following bacterial strains showing homology with the following species**.**

Table 7: - Blast analysis of 16S rRNA gene sequencing

Sr. No.	**Bacterial strains**	**Suggested Homology**
1.	L49A	***Arthobacter* sp.**
2.	P7B	***Brachybacterium* sp.**
3.	L6C	***Crayobacterium* sp.**
4.	L5D	***Pseudomonas* sp.**
5.	L49F	***Mycobacterium* sp.**
6.	L5H	***Sphingomonas* sp.**
7.	L27I	***Pseudomonas* sp.**
8.	L32J	***Pseudomonas* sp.**
9.	L27K	***Sphingomonas* sp.**
10.	L32L	***Nocardia* sp.**
11.	P7M	***Bravibacterium* sp.**
12.	L49N	***Pseudomonas* sp.**

4.7 <u>Carbohydrate utilization test</u>: -

A carbohydrate utilization test was carried out by using the Hicarbo™ Kit and the carbohydrate utilization by bacteria was confirmed by observing the wells for the colour change. The bacterial strain given a positive test for carbohydrate utilization indicated as '+' and negative test indicated as '-' and the intermediate result indicated as 'v'.

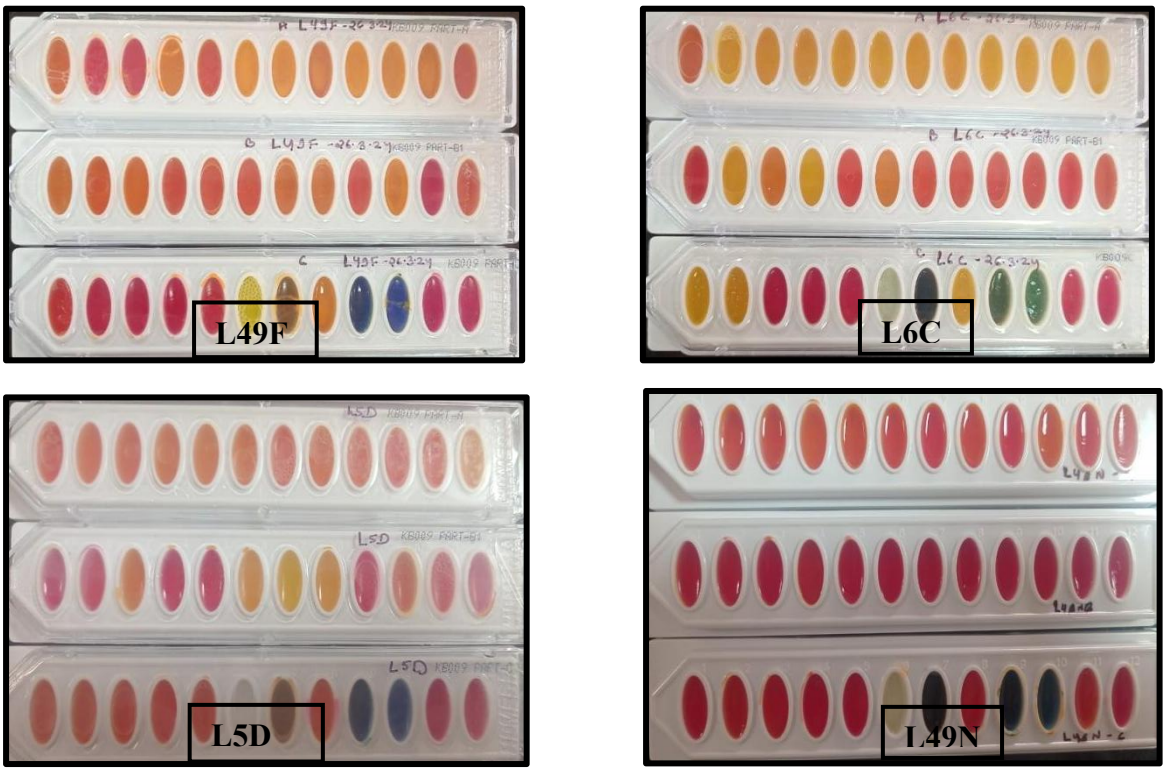


Figure 7: - HiCarbo™ Kit after inoculation of bacterial culture showing colour change.

Table 8: - Utilization of various carbohydrates for growth by bacterial isolates

Sr. No.	Test	L49F	L6C	L5D	L49N
1	Lactose utilization	V	-	V	V
2	Xylose utilization	-	+	V	V
3	Maltose utilization	-	+	V	V
4	Fructose utilization	+	+	V	V
5	Dextrose utilization	-	+	V	V
6	Galactose utilization	+	+	V	V
7	Raffinose utilization	+	+	-	V
8	Trehalose utilization	V	+	V	V
9	Malibiose utilization	+	+	V	V
10	Sucrose utilization	+	+	V	V
11	L- Arabinose utilization	+	+	V	V
12	Mannose utilization	-	+	-	V
13	Inulin utilization	V	-	-	-
14	Sodium gluconate utilization	V	+	-	-
15	Glycerol utilization	V	V	V	-
16	Salicin utilization	V	+	-	-
17	Dulcitol utilization	+	-	-	-
18	Inositol utilization	V	V	+	-
19	Sorbitol utilization	V	-	+	-
20	Mannitol utilization	V	-	+	-
21	Adonitol utilization	-	-	-	-
22	Arabitol utilization	+	-	V	-
23	Erythritol utilization	-	-	-	-
24	Alpha – Methyl – D glucoside utilization	+	-	-	-
25	Rhamnose utilization	V	+	V	-
26	Cellobiose utilization	-	+	V	V
27	Melezitose utilization	-	-	V	V

28	Alpha – Methyl – D Mannoside utilization	-	-	V	-
29	Xylitol utilization	-	-	V	-
30	ONPG activity	+	-	-	-
31	Esculin hydrolysis	+	+	-	+
32	D – Arabinose utilization	+	+	V	-
33	Citrate utilization	+	-	+	+
34	Malonate utilization	+	-	+	+
35	Sorbose utilization	-	-	-	V
36	Control	-	-	-	-

4.8 <u>CONCLUSION</u>: -

In conclusion, this research has offered significant insights into the capability of 14 bacterial strains isolated from Antarctica to degrade polysaccharides. Utilizing dye-based plate assays, we examined the Agarolytic and Alginolytic activity of bacterial isolates.

FUTURE PROSPECTS

Future Prospects: -

1. Whole Genome Sequencing of Psychrophilic strains.

2. Molecular identification of Yeast strains.

3. Bio-prospecting of potential polysaccharide degrading strains.

REFERENCES: -

1. Alvarado, R., & Leiva, S. (2017). Agar-degrading bacteria isolated from Antarctic macroalgae. *Folia Microbiologica*, *62*, 409-416.

2. Alvarado-Cuevas, Z. D., López-Hidalgo, A. M., Ordoñez, L. G., Oceguera-Contreras, E., Ornelas-Salas, J. T., & De León-Rodríguez, A. (2015). Biohydrogen production using psychrophilic bacteria isolated from Antarctica. *International Journal of Hydrogen Energy*, *40*(24), 7586-7592.

3. Bartholomew, J. W., & Mittwer, T. (1952). The gram stain. *Bacteriological Reviews*, *16*(1), 1-29.

4. Brooks, S. T., Jabour, J., Van Den Hoff, J., & Bergstrom, D. M. (2019). Our footprint in Antarctica competes with nature for rare ice-free land. Nature Sustainability, 2(3), 185-190.

5. Bugno, Adriana, Adriana Aparecida Buzzo Almodóvar, and Tatiana Caldas Pereira. 2010. "Enumeration of Heterotrophic Bacteria in Water for Dialysis: Comparison of the Efficiency of Reasoner'2 Agar and Plate Count Agar." *Brazilian Journal of Microbiology* 41(1):15–18.

6. Canene-Adams, Kirstie. 2013. "General PCR." Pp. 291–98 in *Methods in Enzymology*. Vol. 529. Elsevier.

7. Chauhan, A., Bharti, P. K., Goyal, P., Varma, A., & Jindal, T. (2015). Psychrophilic pseudomonas in the Antarctic freshwater lake at Stornes peninsula, larsemann hills over east Antarctica. Springerplus, 4, 1-6.

8. Cavicchioli, R., Charlton, T., Ertan, H., Omar, S. M., Siddiqui, K. S., & Williams, T.(2011).Biotechnological uses of enzymes from psychrophiles. *Microbial biotechnology*, *4*(4), 449-460

9. Chauhan, P. S., & Saxena, A. (2016). Bacterial carrageenases: an overview of production and biotechnological applications. 3 Biotech, 6(2), 146..

10. Chown, S. L., & Brooks, C. M. (2019). The state and future of Antarctic environments in a global context. Annual Review of Environment and Resources, 44, 1-30.

11. Cowan, D. A., Makhalanyane, T. P., Dennis, P. G., & Hopkins, D. W. (2014). Microbial ecology and biogeochemistry of continental Antarctic soils. Frontiers in microbiology, 5, 78994.

12. Dharwadkar, A., Shukla, S. P., Verma, A., & Gajbhiye, D. (2018). Geomorphic evolution of Schirmacher Oasis, central dronning Maud land, East Antarctica. Polar Science, 18, 57-62.

13. Dos Santos, H. R. M., Argolo, C. S., Argôlo-Filho, R. C., & Loguercio, L. L. (2019). A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. BMC microbiology, 19, 1-14.

14. Eriksson, P., Mourkas, E., González-Acuna, D., Olsen, B., & Ellström, P. (2017). Evaluation and optimization of microbial DNA extraction from faecal samples of wild Antarctic bird species. *Infection ecology & epidemiology*, *7*(1), 1386536

15. Feller, G., & Gerday, C. (2003). Psychrophilic enzymes: hot topics in cold adaptation. Nature Reviews Microbiology, 1(3), 200-208.

16. Forsberg, Z., Sørlie, M., Petrović, D., Courtade, G., Aachmann, F. L., Vaaje-Kolstad, G., ... & Eijsink, V. G. (2019). Polysaccharide degradation by lytic polysaccharide monooxygenases. Current Opinion in Structural Biology, 59, 54-64.

17. Fukuda, W., Kimura, T., Araki, S., Miyoshi, Y., Atomi, H., & Imanaka, T. (2013). Lysobacter oligotrophicus sp. nov., isolated from an Antarctic freshwater lake in Antarctica. International journal of systematic and evolutionary microbiology, 63(Pt_9), 3313-3318.

18. Gephart, P., R.G. E. Murray, R. N.Costilow, E.W., Nester, W.A. Wood, N.R. Krieg, and G. B Phillips. 1981. Manual of Methods for Downloaded from www.asmscience.org by IP: 71.127.236.37 On: Mon, 12 Aug 2019 17:45:19 American Society for Microbiology © 2016 8 General Bacteriology, ASM Press, Washington D.C. Gram. C.1884. Ueber die isolirte Färbung der Schizomyceten in Schnitt-und Trockenpräparaten. Fortschritte der Medcin, (2):185-189

19. He, Z., Ding, B., Ali, Q., Liu, H., Zhao, Y., Wang, X., ... & Juan, Y. (2023). Screening and isolation of cold-adapted cellulose-degrading bacterium: A candidate for straw degradation and De novo genome sequencing analysis. Frontiers in Microbiology, 13, 1098723.

20. Henshaw, T., & Laybourn-Parry, J. (2002). The annual patterns of photosynthesis in two large, freshwater, ultra-oligotrophic Antarctic lakes. Polar Biology, 25, 744-752.

21. Jain, A., & Krishnan, K. P. (2017). A glimpse of the diversity of complex polysaccharide-degrading culturable bacteria from Kongsfjorden, Arctic Ocean. Annals of Microbiology, 67(2), 203-214.

22. Kushner, D. J. (1976). Microbial life at low temperatures. In Chemical Evolution of the Giant Planets (pp. 85-93). Academic Press.

23. Kwon, M., Jang, W. Y., Kim, G. M., & Kim, Y. H. (2020). Characterization and application of a recombinant exolytic GH50A β-agarase from Cellvibrio sp. KY-GH-1 for enzymatic production of neoagarobiose from agarose. ACS omega, 5(45), 29453-29464..

24. Atala, C., De la Iglesia, R., & Trefault, N. (2016). Isolation and characterization of an Antarctic Flavobacterium strain with agarase and alginate lyase activities.

25. Lin, C. C., & Casida Jr, L. E. (1984). GELRITE is a gelling agent in media for the growth of thermophilic microorganisms. Applied and environmental microbiology, 47(2), 427-429.

26. Luis, A. J. (2013). Past, present and future climate of Antarctica.

27. Matondkar, S. P., & Gomes, H. R. (1983). Biological studies on the ice shelf and in freshwater lakes at Princess Astrid coast, Dronning Maud Land, Antarctica.

28. McKee, L. S., La Rosa, S. L., Westereng, B., Eijsink, V. G., Pope, P. B., & Larsbrink, J. (2021). Polysaccharide degradation by the Bacteroidetes: mechanisms and nomenclature. Environmental Microbiology Reports, 13(5), 559-581.

29. Melo, I. S., Zucchi, T. D., Silva, R. E., Vilela, E. S., Sáber, M. L., Rosa, L. H., & Pellizari, V. H. (2014). Isolation and characterization of cellulolytic bacteria from the Stain house Lake, Antarctica. Folia microbiologica, 59, 303-306.

30. Morita, Richard Y. 1975. "Psychrophilic Bacteria." *Bacteriological Reviews* 39(2):144–67.

31. Mudge, M. C., Nunn, B. L., Firth, E., Ewert, M., Hales, K., Fondrie, W. E., ... & Junge, K. A. (2021). Subzero, saline incubations of Colwellia psychrerythraea reveal strategies and biomarkers for sustained life in extreme icy environments. Environmental microbiology, 23(7), 3840-3866.

32. Pesciaroli, C., Cupini, F., Selbmann, L., Barghini, P., & Fenice, M. (2012). Temperature preferences of bacteria isolated from seawater collected in Kandalaksha Bay, White Sea, Russia. Polar Biology, 35, 435-445.

33. Rai, A., & Bhattacharjee, A. (2021). Molecular profiling of microbial community structure and their CAZymes via metagenomics, from Tsomgo Lake in the Eastern Himalayas. Archives of Microbiology, 203, 3135-3146.

34. Rampelotto, P. H. (2013). Extremophiles and extreme environments. Life, 3(3), 482-485.

35. Vishnupriya, S., Jabir, T., Adarsh, B. M., Kattatheyil, H., Kabeer, S., Krishnan, K. P., ... & AA, M. H. (2023). Diversity of complex polysaccharide degrading bacteria from the sediments of interlinked high Arctic fjords, Svalbard. Regional Studies in Marine Science, 63, 102989.

36. Sazci, A., Erenler, K., & Radford, A. (1986). Detection of cellulolytic fungi by using Congo red as an indicator: a comparative study with the dinitrosalicyclic acid reagent method. Journal of Applied Microbiology, 61(6), 559-562.

37. Sahay, H., Babu, B. K., Singh, S., Kaushik, R., Saxena, A. K., & Arora, D. K. (2013). Cold-active hydrolases produce bacteria from two different sub-glacial Himalayan lakes. Journal of Basic Microbiology, 53(8), 703-714.

38. Shivaji, S., Kumari, K., Kishore, K. H., Pindi, P. K., Rao, P. S., Srinivas, T. N. R., ... & Ravindra, R. (2011). Vertical distribution of bacteria in lake sediment from Antarctica by

culture-independent and culture-dependent approaches. Research in microbiology, 162(2), 191-203.

39. Sichert, A., & Cordero, O. X. (2021). Polysaccharide-bacteria interactions from the lens of evolutionary ecology. Frontiers in Microbiology, 12, 705082.

40. Zhang, C., & Kim, S. K. (2010). Research and application of marine microbial enzymes: status and prospects. Marine drugs, 8(6), 1920-1934.

APPENDIX: -

1. **R2A AGAR COMPOSITION: -** Dissolve 18.1 g of R2A agar in 1 L of distilled water and the pH of the media is 7.2.

Serial No.	Ingredients	Quantity (gL^{-1})
1.	Yeast extract	0.5
2.	Protease peptone	0.5
3.	Casein hydrolysate	0.5
4.	Glucose	0.5
5.	Soluble starch	0.5
6.	Sodium pyruvate	0.3
7.	Dipotassium hydrogen phosphate	0.3
8.	Magnesium-sulphate anhydrous	0.024
9.	Agar-agar	15.0

2. **ABM media composition:- The pH of the media is 6.9.**

Sr. No.	Component	Quantity (w/v)
1.	Yeast extract	0.1%
2.	Peptone	0.5%
3.	Agar-agar	2%

3. M9 media composition(5X):- Dissolve 56.4 g of media in 1 L of distilled water.

Sr. No.	Ingredients	Quantity(gL^{-1})
1.	Disodium hydrogen phosphate	33.90
2.	Potassium dihydrogen phosphate	15
3.	Sodium chloride	2.5
4.	Ammonium chloride	5

4. Instruments: -

- pH meter (pH 700, Eutech Instruments, Thermo Fisher Scientific, India)
- Autoclave
- Laminar Air Flow
- Hot air oven (i-therm AI-7981)
- Refrigerator
- Incubator shaker (HALLY INSTRUMENTS™)
- Weighing balance (Shimadzu ATX224)
- Thermal cycler (SureCycler 800)