Microbes associated with algal mats from Schirmacher Oasis, Antarctica

A Dissertation for

Course code and Course title: GBT 651 & Dissertation

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Submitted in partial fulfilment of Master's Degree

in Biotechnology

by

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I hereby declare that the data presented in the dissertation report entitled, **"Microbes associated with algal mats from Schirmacher Oasis, Antarctica"** is based on the results of investigations carried out by me in the Department 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.

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This is to certify that the dissertation report **"Microbes associated with algal mats from Schirmacher Oasis, Antarctica"** is a bonafide work carried out by Ms. Veda V.S Kakodkar under my supervision in partial fulfilment of the requirements for the award of the degree Master's of Science in the Discipline of Biotechnology at the School of Biological Science and Biotechnology, Goa University.

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PREFACE

The Antarctic continent, with its extreme cold and high UV radiation presents a unique and challenging environment for life. Despite harsh conditions, certain microbial communities thrive in association with algal mats in these extreme environments. These mats, comprise of a complex assemblage of algae, bacteria, and other microorganisms. They play a crucial role in the Antarctic ecosystem contributing to primary production in the nutrient cycle.

This study focuses on microbial diversity within algal mats from Schirmacher Oasis, Antarctica, aiming to isolate and characterize novel microbial cultures. By these molecular based identification studies, we hope to gain insights into unique adaptations of microorganisms in this extreme environment. Understanding the microbial diversity and potential biotechnological applications of these isolates could offer valuable contributions to various fields such as bioremediation, pharmaceuticals, cosmetic industry, astrobiology and food industry.

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LIST OF ABBREVIATIONS

SR NO	ABBREVATION	FULL FORM
1	℃	Degree Celsius
2	G	Gram
3	g/L	Gram per litre
4	mL	Milli litre
5	%	Percentage
6	SEM	Scanning Electron Microscopy
7	ABM	Antarctic Bacterial Medium
8	R2A	Reasoners 2A Agar
9	CMC	Carboxy Methyl Cellulose
10	PCR	Polymerase Chain Reaction
11	bp	Base Pair

ABSTRACT

Antarctica's harsh environmental conditions support a vast range of microbial life, which often thrive in specific niches such as algal mats. This study focuses on isolating and characterizing microbial cultures from Antarctic algal mats. This helps in understanding variety, adaptability, and possible biotechnological applications of these psychrophilic microbes. Morphological, biochemical, and genetic characterisation of the isolated microbial cultures were carried out their ability to degrade multiple polysaccharides was checked.

CHAPTER 1

Introduction

1.1 Background

Antarctica is the coldest area on Earth, with average annual temperature levels of -20℃ or less. The majority of the Antarctic region is permanently covered in ice because of its region's harsh weather conditions, which include year-round below-freezing temperatures (Toro et al., 2007). Approximately 60-70% of freshwater on the Earth's surface is observed in Antarctica (Wilkins et al., 2013). Antarctica's microbial diversity is highly affected seasonal fluctuations of temperature, light, winds, nutrients, and salts (Cavicchioli R et al.,2015). The only continent where microorganisms solely are responsible for driving carbon's biogeochemical transformations is Antarctica. Different microbial populations are associated with a wide diversity of physical and chemical characteristics found in various freshwater lakes in Antarctica (Siddiqui et al.,2013). About 0.4% of the continent's surface is made up of exposed soil patches, which are mostly found along the coast. A variety of "extreme" abiotic conditions can affect soil, such as extremely low temperatures, high soil salinity, poor water availability, nutrient levels, high UV radiation levels, and strong, chilly winds coming down from glaciers or mountain summits (Zablocki et al., 2014). In Schirmacher Oasis there are over 180 lakes in the 20 km long and 3 km wide area. The presence of three different types of lakes is noted: land-locked lakes (L) which are primarily freshwater fed by snow and glacial melts, epi-shelf lakes (E) are mostly made up of ocean tidal water mixed with fresh water from glacier melts and snowmelts, and pro-glacial lake (P) are formed by melting glaciers (Mojib et al., 2009).

Microorganisms surviving in extreme environmental conditions are known as extremophiles. These microbes are vital to Antarctic ecosystem's nutrient recycling process and often display remarkable adaptation and survival capabilities (Huang et al., 2010).

Psychrophilic microbes have effectively colonized every persistently frigid habitat known, including deep seas, mountains, and the polar regions. Some of these species are also referred to as psychrotolerant or psychrotrophic, depending on the temperature at which they thrive best. While psychrotolerant bacteria can survive at low temperatures like 0°C, their development rate gradually slows down as the temperatures drop. Psychrophiles prefer growing temperatures of ≤15°C, however they can grow as high as 20°C. Nonetheless, a large number of psychrotolerant microbes may grow actively at temperatures as high as 40 degrees (Mojib et al., 2009). Psychrophiles are also capable of breaking down a range of proteins, lipids, and carbohydrates (Straka & Stokes, 1960).

Microbial mats are densely packed with vertically layered communities in which microorganisms position themselves in response to light and chemical gradients at the micrometer scale. Such communities are usually dominated by cyanobacteria or algae (Wilkins et al., 2013). Periphyton is the name for algae populations that adhere to the surfaces of mud and undersea rock. These communities are typically dominated by cyanobacteria in Antarctica, which create vast mats made up of billions of tiny microscopic filaments held together by mucilage released by the bacteria's cells (Broady, 2015). Mat surfaces are mostly orange-brown as the presence of carotenoids protect and shield them from harmful UV of the benthic region. Cyanobacteria are a diverse and ecologically significant group of photosynthetic prokaryotes. The are mostly obligatory photoautotrophs found predominant in freshwater habitats. Despite being widespread throughout Antarctica, microbial mats can contain a wide variety of organisms. The following groupings are frequently found in Antarctic mats: Cyanobacteria, especially those belonging to the *Nostocales* and *Oscillatoriales* families, which mostly include

Leptolyngbya and Phormidium frigdium (Taton et al., 2003, Fernandez-Carazo et al., 2011). Although *Phormidium* is frequently the main component, the mats also contain algae, protozoa and invertebrate creatures (Broady, 2015). Bacterial cultures include *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes* which are commonly found in association with algal mats and play important roles in nitrogen cycling and organic matter breakdown (Collins et al., 2003).

The dynamics of Antarctica's ecology depends heavily on the cycling of nutrients by algae. Antarctica's marine and terrestrial habitats both rely heavily on algae, especially phytoplankton and microalgae, for primary production and nutrient cycling. These algae use nutrients which are frequently scarce in cold region like iron, phosphorus, and nitrogen to support their development and reproduction (Kohler, T.J & Lundquist, J.D 2018). Phytoplankton, algae, and other primary producers rely on nutrients like nitrogen and phosphorus for growth. These organisms, in turn, support higher trophic levels, including krill, fish, seals, and whales ((Middelburg et al., 1995).

The biggest pools of resources that can be metabolized that microbial populations come into contact with in their surroundings are complex polysaccharides. Remineralization of particulate organic matter is one of the main biogeochemical processes that is driven by the breakdown of these polysaccharides by bacteria. There are two main methods by which bacteria break down polysaccharides. The first tactic is to use periplasmic enzymes for degradation, which will reduce the amount of hydrolysis products lost to the extracellular environment and second includes degradation by cell surface attachment (D’Souza et al., 2023). Due to their antitumor, antioxidant, anticoagulant, and anti-inflammatory properties, marine polysaccharides and the hydrolysed products have become important in

the development of pharmaceuticals, cosmetics, functional foods, and agriculture. These applications have significant positive effects on the economy and society (Shang et al., 2018). Chitin is an environmentally friendly biopolymer that is found in large quantities in nature. The cell walls of macroalgae include a wide variety of sulphated polysaccharides (mostly carrageenan), which are an important source of food for species in the surrounding environments (Aquino et al., 2005).

1.2 Aim

To isolate microbial cultures from algal mats present at Schirmacher Oasis, Antarctica

1.3 Objectives

- Isolation of culturable microbes from the algal mat
- Determining the polysaccharide degrading capability of microbial cultures
- Identification of polysaccharide degrading bacteria

1.4 Scope

Algal mats, in particular, provide a habitat for a multitude of microorganisms. This study aims to isolate and characterize microbial cultures from Antarctic algal mats to explore their diversity, adaptations, and potential biotechnological applications. Enzymes and temperature-sensitive compounds secreted by psychrophiles have an important use in pharmaceutical industry These psychrophilic bacteria play a role in maintaining soil health by decomposing plant materials and returning nutrients to the ecosystem. Enzymes from Antarctic bacteria are used as biocatalysts in various industrial processes that require low-temperature conditions, such as food processing, detergents, and textiles. Polysaccharide-degrading bacteria can be used for bioremediation in cold environments contaminated with oil spills or other pollutants. They break down organic pollutants into simpler, less harmful compounds.

1.5 Hypothesis

Algal mats from Antarctica harbour a diverse array of microbial species, adapted to the extreme environmental conditions of the continent. It is expected that a diverse range of microbial cultures will be isolated from Schirmacher Oasis, representing various taxonomic groups. Given the frigid Antarctic environment, we expect the isolates to demonstrate adaptations to cold temperatures and possess certain novel properties with respect to, cold-active enzymes and cryoprotectants. It is hypothesized that microbial cultures from Schirmacher Oasis will produce cold-adapted enzymes, such as alginate, pectin, CMC, citrate, amylases, proteases, and lipases, which remain active at low temperatures. These enzymes have potential applications in biotechnology and pharmaceutical industries that require low-temperature processes.

CHAPTER 2

Literature Review

The diversity of algae found in Antarctic lakes has been divided into five groups: diatoms, dinoflagellates, cryptophytes, chlorophytes, and prokaryotic cyanobacteria (Izaguirre et al., 2021). Algal mats are mostly dominated by filamentous and coccoid Cyanobacteria, which ingest other species (like diatoms) in their mucilage, which help in binding sand grains and silt together (Bonaventura et al., 2006). A large number of the variations in the makeup of algae species and mat shape can be attributed to variations in light, temperature, conductivity, oxygen lakes, and their in-situ habitats. These algae can use solar energy to create organic molecules from carbon dioxide and water. Various algal groups such as Chrysophytes, Chlorophytes, Prasinophytes, and Cryptophytes, are significant primary producers highly prevalent in Antarctica (Izaguirre et al., 2021). Studies conducted by Wharton et al(1983) state that algal mats are abundant in most benthic environments in Antarctica, mostly composed of blue-green algae (cyanobacteria) such as *Phormidium frigidum* and *Lyngbya martensiana*.

Water and soil samples from Schirmacher Oasis, Antarctica, have a diverse range of psychrotrophic bacteria and yeasts belonging to genera *Pseudomonas, Sphingobacterium, Micrococcus, Planococcus*, and *Arthrobacter*. Yeast from this area mostly comprise of *Bullera*, *Candida*, and *Rhodotorula* genera. According to biochemical investigations, these psychrotrophs have cold-active, heat-labile, and freeze-thaw-resistant enzymes and are capable of translation and transcription even at 0°C. (Shivaji & Chatiopadhyay, 1994).

Antarctica's bacteria have revealed a wide range of variations in shape and size. Additionally, a diverse population of bacteria comprising rods, cocci, clusters of cocci, chains of rods, and a single long, filamentous bacterium can be found in the soils of Schirmacher Oasis (Shivaji et al., 1989).

Psychrophilic bacteria have extracellular enzyme activities actively playing a major role in the synthesis of biomass, the cycle of nutrients, and the breakdown of litter (Hatha et al. 2013). The high percentages of isolates that can produce multiple polysaccharide-degrading enzymes suggest that microbiota has evolved exceptionally to thrive in cold conditions(Shivaji et al., 1989).

Arctic bacterial isolates from Kongsfjorden have demonstrated extraordinary ability to manufacture cold-active extracellular enzymes such as xylanase, pectinase, amylase, and carboxymethyl (CM)-cellulase and every isolate that tested positive for extracellular enzyme activity belonged to the *Gammaproteobacteria* microbial class. The four genera with the most isolates were *Shewanella, Pseudoalteromonas, Pseudobacter*, and *Pseudodomonas.* Polysaccharides-degrading bacteria play a pivotal role in breaking down complex organic compounds, such as algal polysaccharides, into simpler forms. This process releases essential nutrients like carbon, nitrogen, and phosphorus back into the environment (Cavicchioli et al., 2019). It also contributes to carbon cycling in Antarctic ecosystems which means carbon is utilized by bacteria for sustaining microbial communities and ecosystem productivity. Polysaccharide degrading bacteria also assist in the decomposition of these mats. This decomposition process releases nutrients that support the growth of other organisms, contributing to ecosystem health and productivity (Bowman, 2015).

CHAPTER3

Methodology

3.1 Pre-treatment of Algal Mats

Algal mats used in this study wwere collected during 42nd ISEA from Schirmacher Oasis during November 2022 – February 2023. Algal mats samples were mixed with sediments and soil .

A small piece of the mat was taken and cleaned. They were teased and washed with 0.8% of saline to remove excess sediments and were observed under the microscope at 40X.

3.2 Scanning electron microscopic analysis

Algal mats were analysed by SEM using Carl-Zeiss scanning electron microscope (SEM) at Goa University (USIC) for observing morphological characteristics. They were teased and fixed on a slide with 2.5% glutaraldehyde and kept overnight. The next day the samples were treated with 10%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% ethanol for 10 minutes respectively. After 100% ethanol dehydration, the samples were air dried and slides were sputter coated with gold and then processed.

3.3 Isolation of microbial cultures from algal mats

3.3.1 Direct plating

Approximately 0.1 g of algal mat was weighed and teased in saline under aseptic conditions. 0.1mL of this sample was spread plated on different R2A and M9 media plates supplemented with 1% of different polysaccharides such as pectin, carrageenan, citrate, Xylan, CMC ang alginate as a sole carbon source and gel rite were used as substitute for other polysaccharide except agar kept for incubation at 4℃ for 2-3 months.

3.3.2 Enrichment of culture

Different tubes of R2A and M9 media containing different polysaccharides(0.2%) were prepared and approximately 0.1g of the mat was added in each tube and mixed vigorously. The tubes were incubated at 4℃ for 2-3 months under shaking conditions until growth was observed.

3.3.3 Isolation of pure colonies

Each isolated colony was picked and streaked on different polysaccharide plates of R2A and M9 media supplemented with 1% polysaccharide as sole carbon source for 3 months at 4 ℃ until growth was observed. Cultures which showed maximum degradation were used for further studies. Isolated colonies were also streaked on ABM plates for faster growth.

3.4 Gram staining

Microscopic observation of isolated bacteria was carried out using gram staining technique developed by Hans Christian Gramin in 1985. A thin smear was made of different cultures on different slide and heat fixed. observed under oil immersion

3.5 Screening for Polysaccharide degradation activity

For activity of polysaccharide degrading enzymes, such as pectinase, alginate, CMC, carrageenan, culture was streaked on respective polysaccharide plates and were incubated at 22^0C for 2 weeks. The zone of clearance around bacterial colonies was observed by flooding the plates with appropriate reagent. Agar containing plate was flooded with Lugol's iodine, alginate was flooded with 10% Cetyl pyridinium chloride, carrageenan was flooded with phenol red and pectin and CMC was flooded with 0.2% Congo red (Jain & Krishnan, 2017).

3.6 Genomic DNA extraction

Five cultures were selected based on their ability to degrade multiple polysaccharides. Of all those five cultures DNA was isolated using HiPurA® Bacterial Genomic DNA purification kit and was run on a 0.7% low EEO agarose gel and observed using a UV transilluminator. The concentration of the extracted DNA was determined using the Qubit 2.0 Fluorometer.

3.7 PCR

A PCR reaction was setup to amplify the 16s rRNA gene of the selected bacterial isolates. This was carried out using 16s primers and resultant PCR products were subjected to gel extraction.

Table 3.7.1 – 16S primers

16 s	Forward Primer	27F :- 5'-AGAGTTTGATCCTGGCTCAG-3'	
	Reverse primer	1492R:- 5'-TACGGTTACCTTGTTACGACTT-3'	(Chen et al., 2015)

Table 3.7.2 Parameters for amplification of DNA Obtained

Steps	Temperature	Time	Cycles
Initial Denaturation	95^0c	2min	1

Denaturation	95^0C	30sec	30
Annealing	51.9^0C	30 sec	
Extension	72^0C	1 min 30 sec	
Final Extension	72^0C	8 mins	1

Reaction mixture of components for Amplification of DNA

Table 3.7.3 Reaction mixture of components of isolate PYa

Components of PCR	Volume (µL)
Nuclease free water	40.7
10X Taq B	5
dNTPS	1
Template DNA(~50ng)	0.3
Forward primers	1
Reverse primers	1
Taq polymerase	1

Table 3.7.4 Reaction mixture of components of bacterial isolate PYb

Components of PCR	Volume (µL)

Nuclease free water	40.6
10X Taq B	5
dNTPS	1
Template DNA (~50ng)	0.4
Forward primers	1
Reverse primers	1
Taq polymerase	1

Table 3.7.5 Reaction mixture of components of bacterial isolate L55

Components of PCR	Volume (µL)
Nuclease free water	40.6
10X Taq B	5
dNTPS	1
Template DNA (~50ng)	0.4
Forward primers	1
Reverse primers	1
Taq polymerase	1

Table 3.7.6 Reaction mixture of components of bacterial isolate L27(1)

Components of PCR	Volume (µL)
Nuclease free water	40.3
10X Taq B	5
dNTPS	1
Template DNA (~50ng)	0.7
Forward primers	1
Reverse primers	1
Taq polymerase	1

Table 3.7.7 Reaction mixture of components of bacterial isolate L32

Components of PCR	Volume (µL)
Nuclease free water	40
10X Taq B	5
Dntps	1
Template DNA (~50ng)	1
Forward primers	1
Reverse primers	1

Taq polymerase	1

3.8 Biochemical Analysis

Hi Carbohydrate TM Kit KB009 from Hi Media were utilized to know whether certain isolates utilize carbohydrate or not. These kits employ a standard identification approach. The culture was added to the kits, and they were then incubated at 12°C.

3.9 Sequencing

Trimmed and aligned 16S rRNA contig sequences were used as queries in BLAST (Basic Local Alignment Search Tool Nucleotide) to find the closest identical match in NCBI's GenBank database (Altschul et al., 1990).

CHAPTER 4

Analysis And Conclusion

4.1 Microscopic Examination

According to (De Los Ríos et al., 2015) microscopic examination reveals the presence of different filaments indicating mixed algal mat.

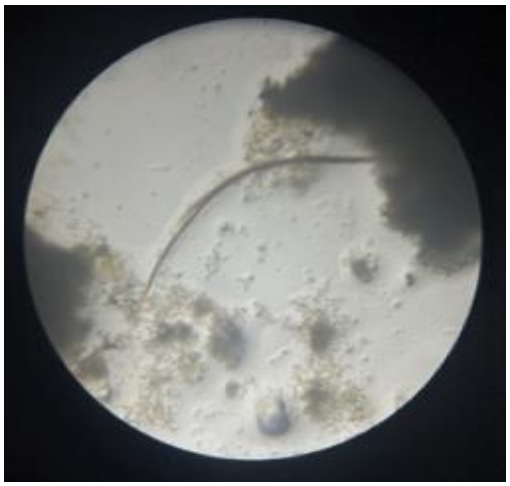

Fig 4.1.1 Microscopic possible mix mat image of mat L32 images

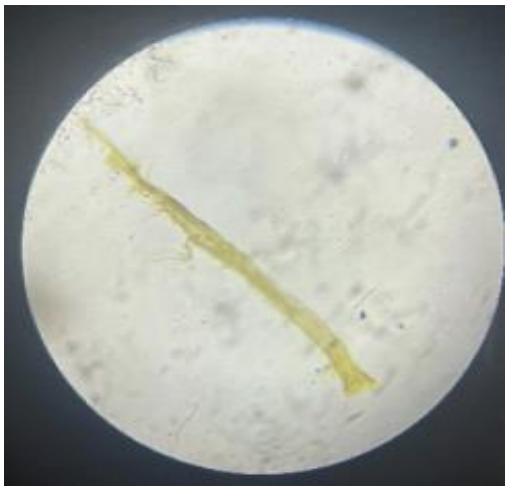

Fig 4.1.2 Microscopic images of unidentified algal species from L41C

Fig4.1.3 Microscopic image of unidentified algal species from L41C mat

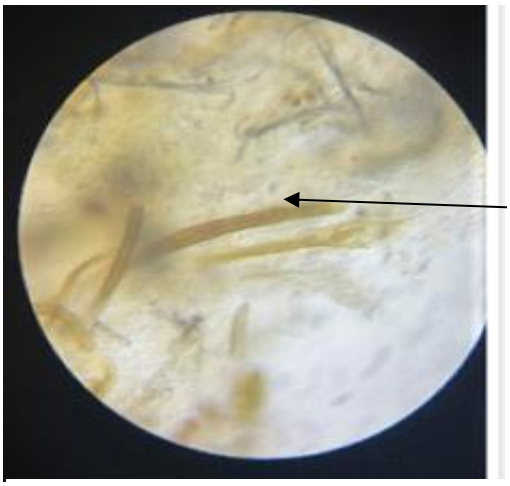

Fig 4.1.4 Microscopic image of predominantly algal species

Fig 4.1.1 to 4.1.4 depicts microscopic images of different algal mat observed under 40X power

4.2 Analysis of SEM Images

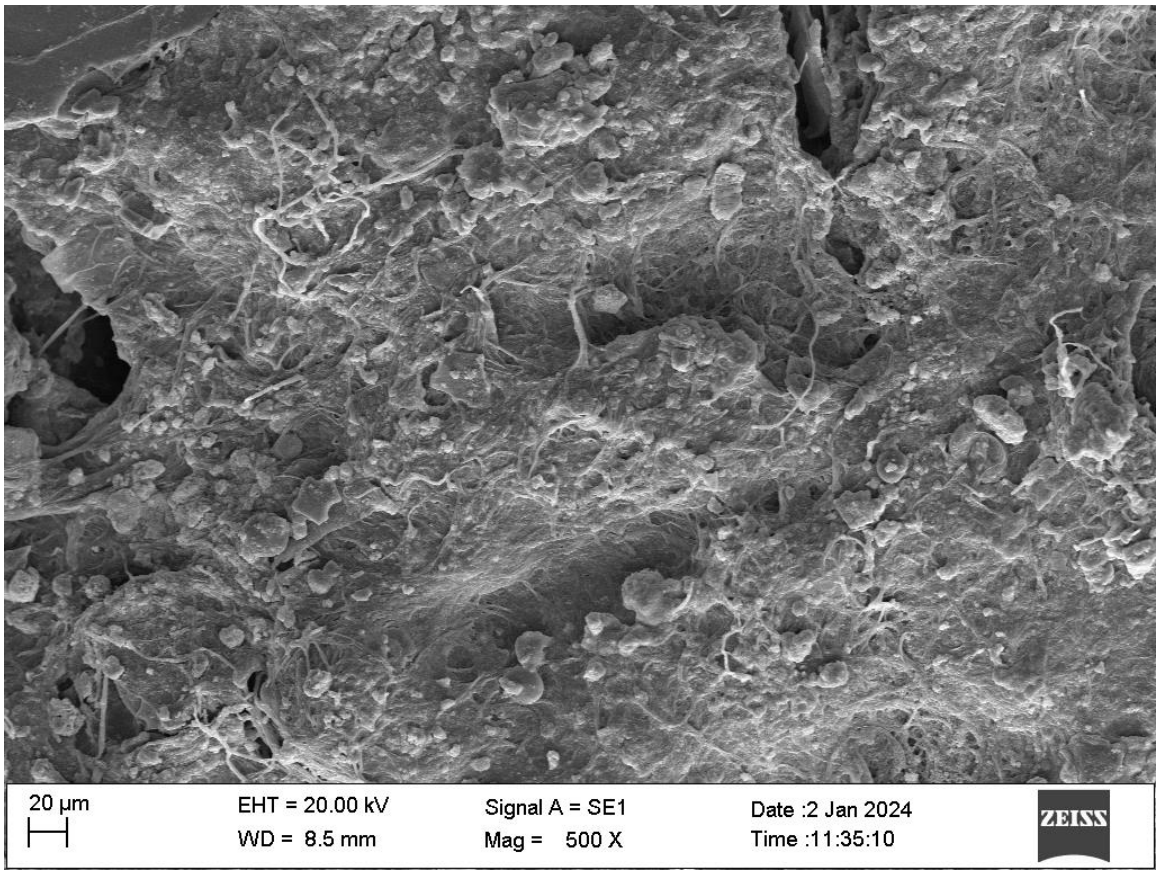

Fig 4.2.1 SEM image of microbial cultures associated with algal mat and its possible degradation

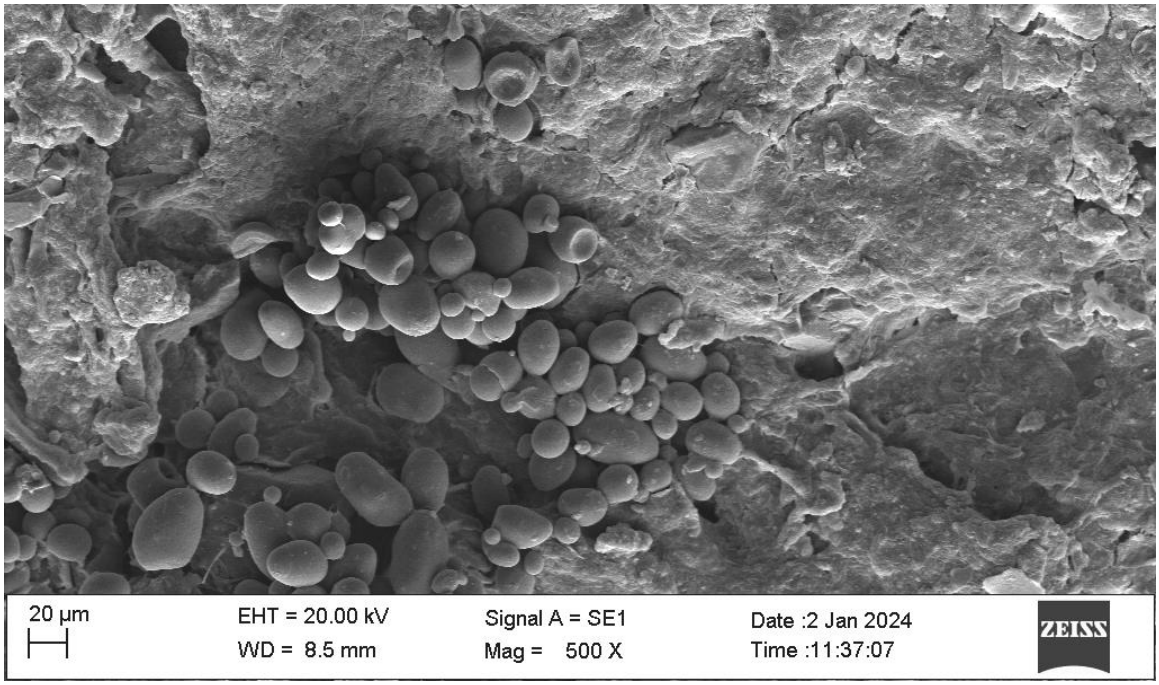

Fig 4.2.2 Sem image of possible yeast associated microbes from algal mat(P12)

4.3 Analysis of isolation of microbial cultures from algal mats

4.3.1 Analyses of direct plating

After spread plating of the sample, the plates were incubated at 4°C for 2 months and growth was observed on the plates. Majority of colonies were translucent colonies with unique colour such as orange, pink, yellow, lime yellow and dark yellow.

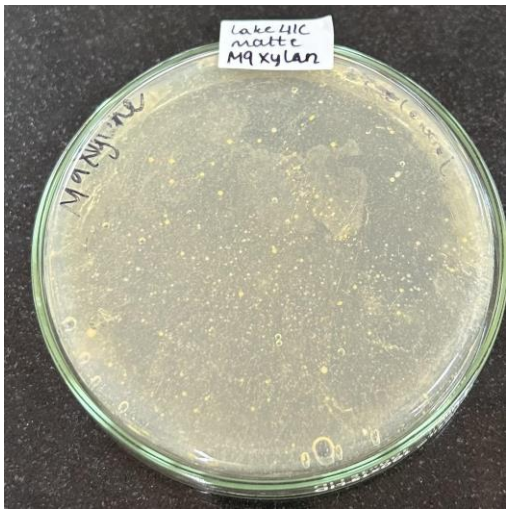

Fig 4.3.1.1 Isolated colonies after spread plating on R2A Agar plate

Fig 4.3.1.1 Isolated colonies after spread plating on M9 Xylan

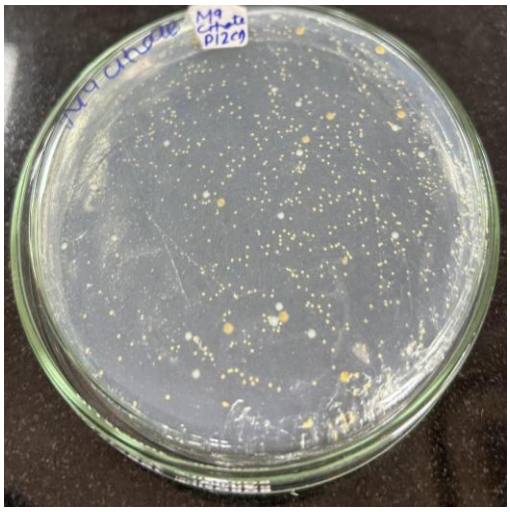

Fig 4.3.1.3 Isolated colonies after spread plating on M 9 citrate

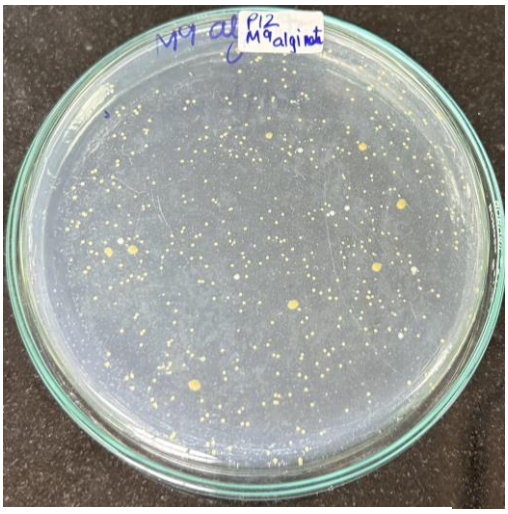

Fig 4.3.1.3 Isolated colonies after spread plating M9 Alginate

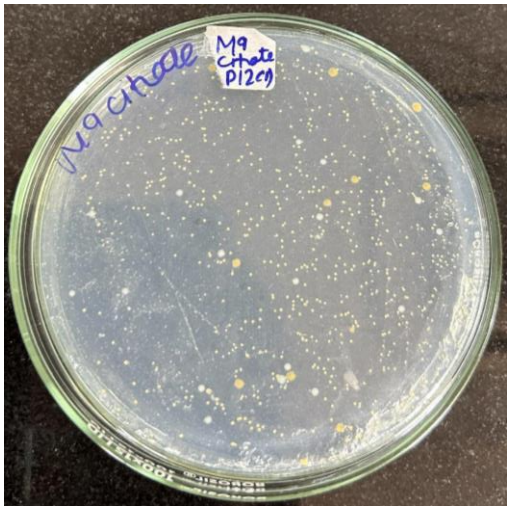

Fig 4.3.1.5 Isolated colonies after spread plating on M 9 citrate

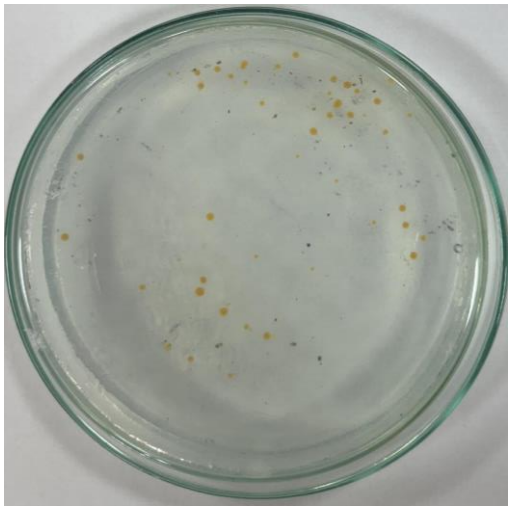

Fig 4.3.1.6 Isolated colonies after spread plating on R2A Alginate

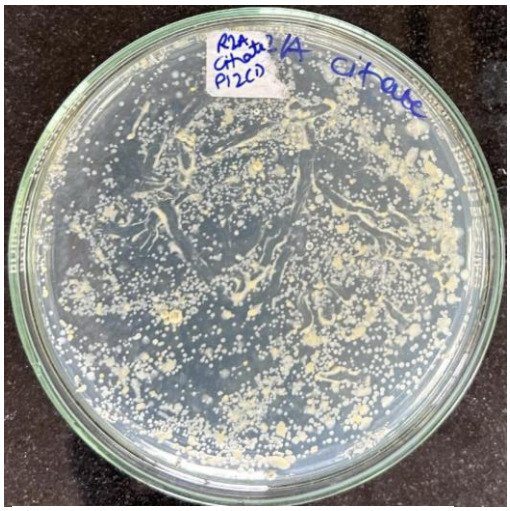

Fig 4.3.1.7 Isolated colonies after spread plating on R2A citrate

Fig 4.3.1.8 Isolated colonies after spread plating on M9 Alginate

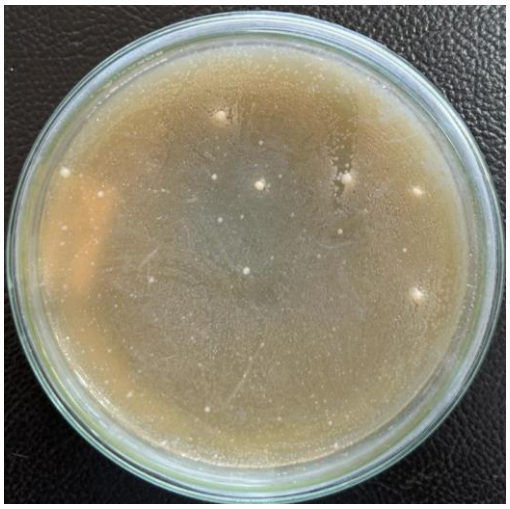

Fig 4.3.1.9 Isolated colonies after spread plating on M9 Pectin

Fig 4.3.1.1 to Fig 4.3.1.9 Indicates plates containing colonies grown on different polysaccharides

4.3.2 Enrichment Method

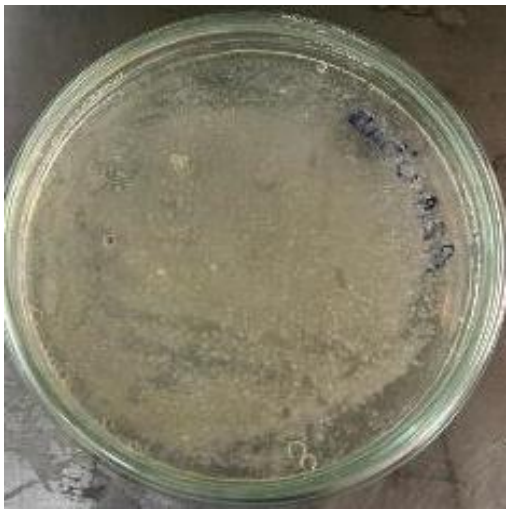

Fig 4.4.2.1 Spread plated plate of R2A Citrate L27(1)

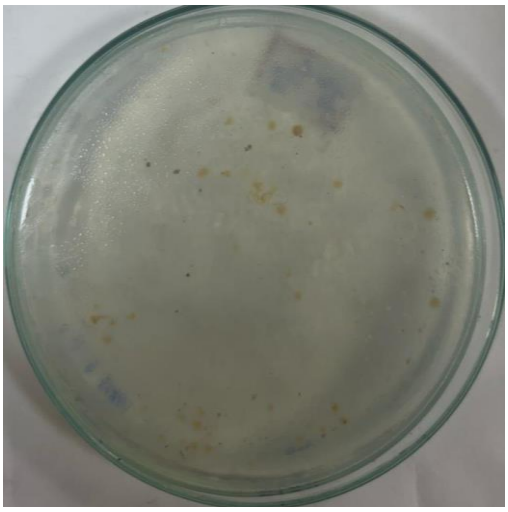

Fig 4.4.2.2 Spread plated plate of R2A Alginate L32

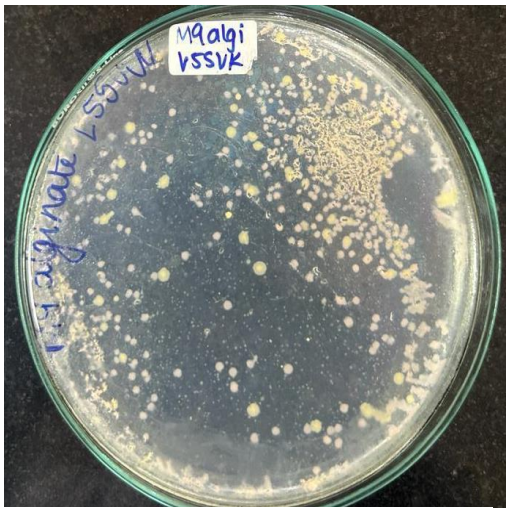

Fig 4.4.2.3 Spread plated plate of M9 Alginate L55

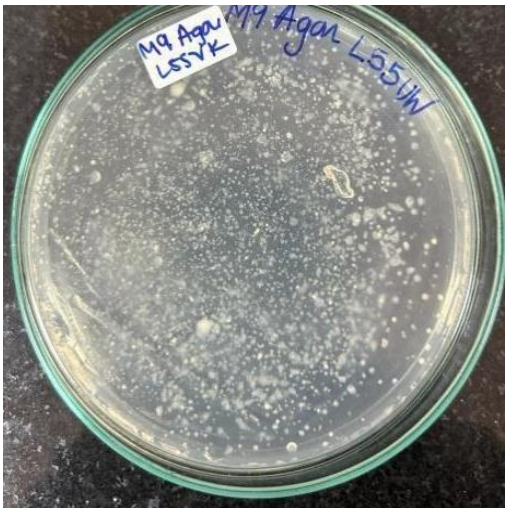

Fig 4.4.2.4 Spread plated plate of M9 Agar L55

Fig 4.4.2.5 Spread plated plate of M9 CMC L41C2

Fig 4.3..2.1 to Fig 4.3.2.5 indicates spread plating of broth medium on different polysaccharides

4.3.3 Purification

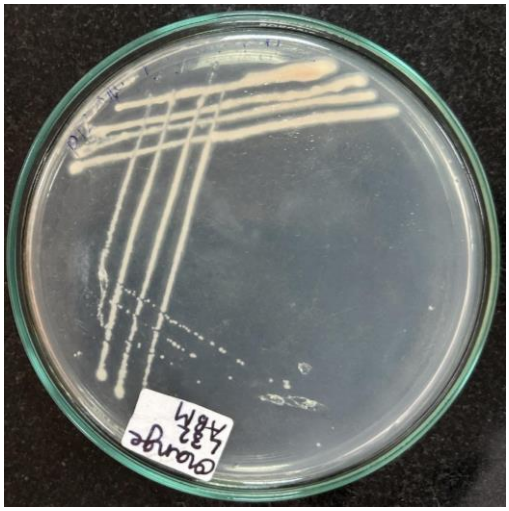

Fig 4.3.3.1 Purified plate of L32A

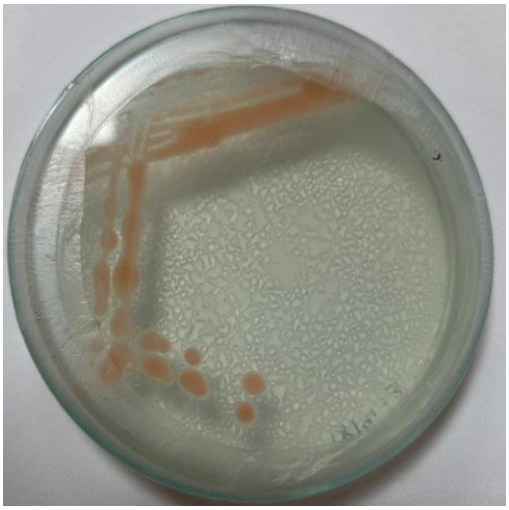

Fig 4.3.3.2 Purified culture of P12B

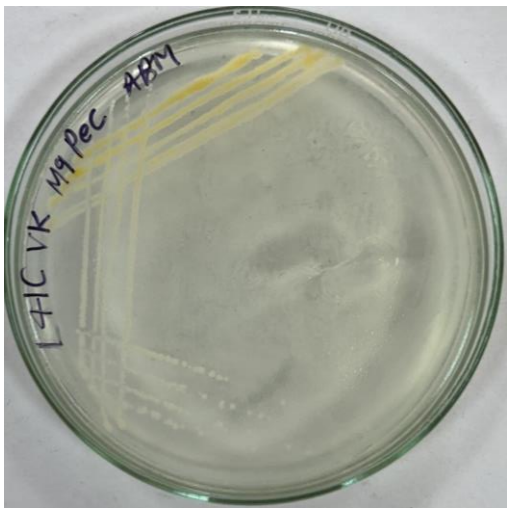

Fig 4.3.3.3 Purified L27(1)B Culture

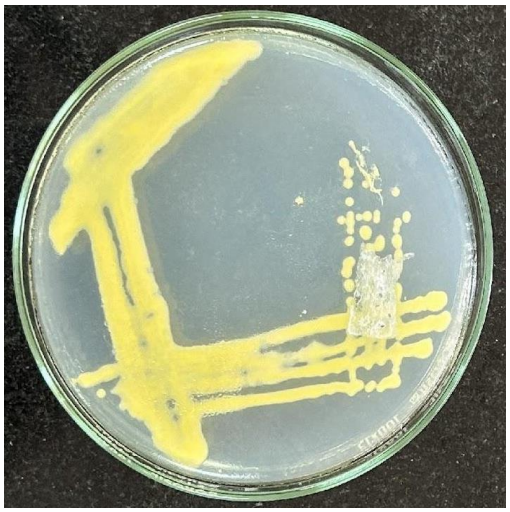

Fig 4.3.3.4 Purified L41C A

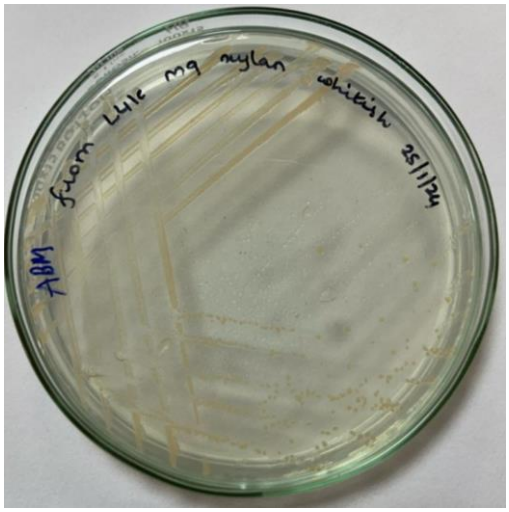

Fig 4.3.3.5 Purified L27(1) A culture

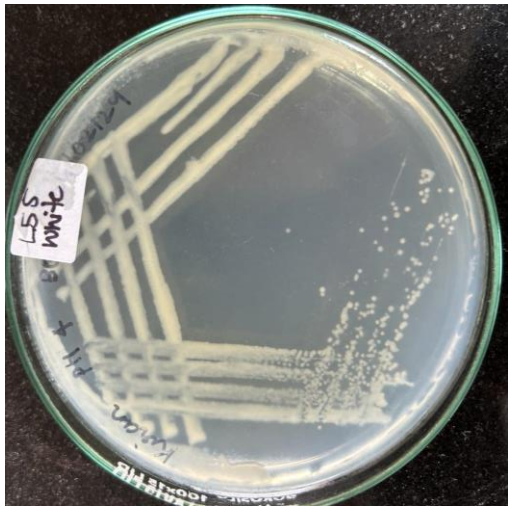

Fig 4.3.3.6 Purified L55 culture

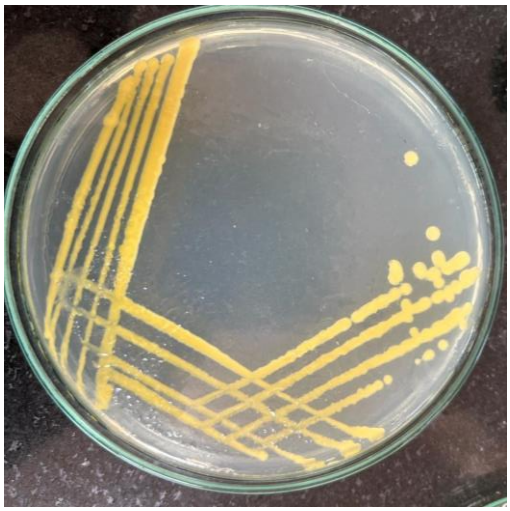

Fig 4.3.3.7 Purified plate of P12 C

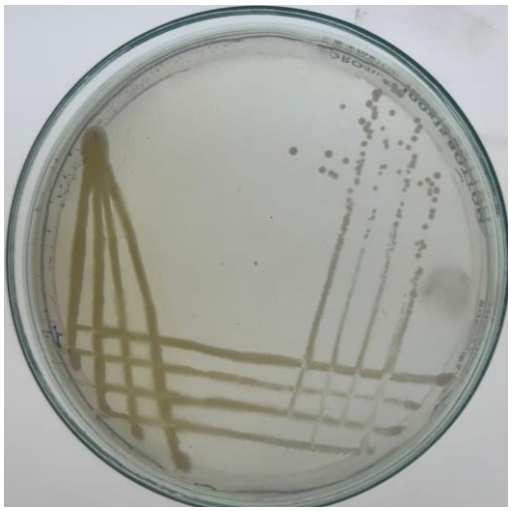

Fig 4.3.3.8 Purified plate of L41C C

Fig 4.3.3.9 Purified L32C plate

Fig 4.3.3.10 Purified P12 C Culture plate

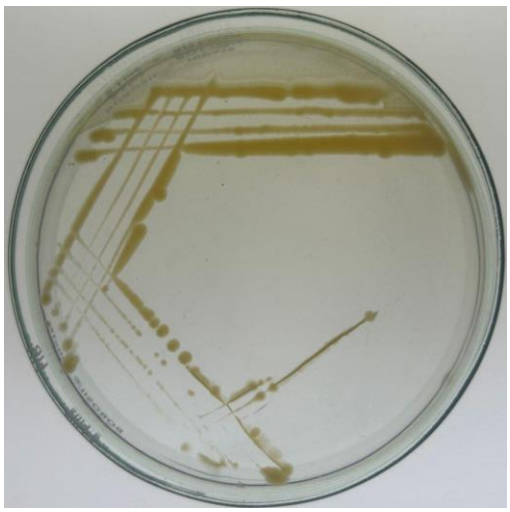

Fig 4.3.3.11 Purified L41CA Culture

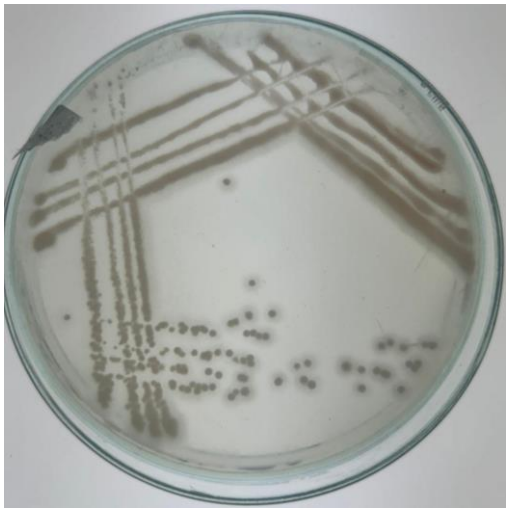

Fig 4.3.3.12 Purified P12A Culture

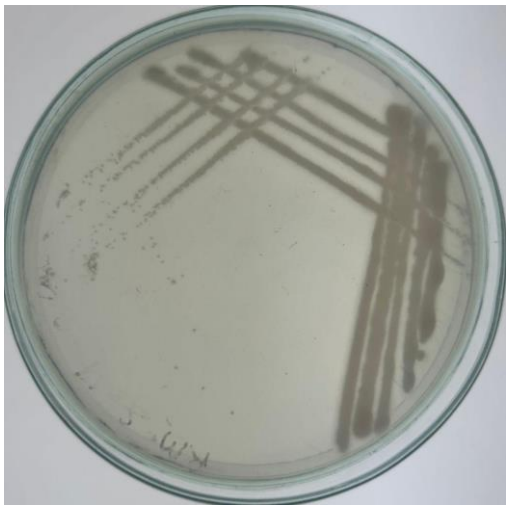

Fig 4.3.3.13 Purified P12 B Culture

Fig 4.3.3.1 – 4.3.3.13 - Purification of cultures done on ABM plates

4.3.4 Morphological characteristics

The colony characteristics of the selected isolates are summarized in table 4.3.5.1

Table 4.3.4.1 : Colony Characteristics of the purified colonies obtained from different algal mats

Colony	Margin	Colour	Elevation	Texture	Shape	Opacity
L32 A	Entire	Orange	Raised	Smooth	Circle	Translucent
L32 B	Entire	Pink	Raised	Buttery	Circle	Translucent
L32 C	Entire	white	Raised	Creamy	Circle	Translucent
P12 A	Entire	Pink	Raised	Buttery	Circle	Translucent
P12 B	Entire	Orange	Raised	Smooth	Circle	Translucent
P12 C	Entire	Dark yellow	Raised	Smooth	Circle	Translucent
L41C A	Entire	Dark yellow	Raised	Smooth	Circle	Translucent
L41C B	Entire	Lime yellow	Raised	Smooth	Circle	Translucent
L41C C	Wavy	White	Raised	Smooth	Circle	Translucent
L55	Entire	white	Raised	Smooth	Circle	Translucent
L27(1)A	Wavy	White	Elevated	Smooth	Circle	Translucent

L27(1)B	Entire	Yellow	Raised	Smooth	Circle	Translucent

4.4 Gram staining

The gram features of the bacterial isolate were revealed through the application of the Gram staining technique. It was observed majority of the isolates had Gram negative character and were having short rod shape, where as two isolates were Gram positive in character and oval in shape

Isolate	Gram staining
L32 A	Gram negative
L32 B	Gram positive
L32 C	Gram negative
P12 A	Gram positive
P12 B	Gram negative
P12 C	Gram negative
L41C A	Gram negative
L41C B	Gram negative
L41C C	Gram negative
L55	Gram negative
L27(1) A	Gram negative

L27(1) B	Gram negative

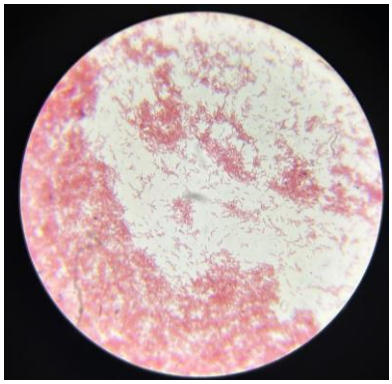

Fig 4.4.1 Gram staining of P12 C

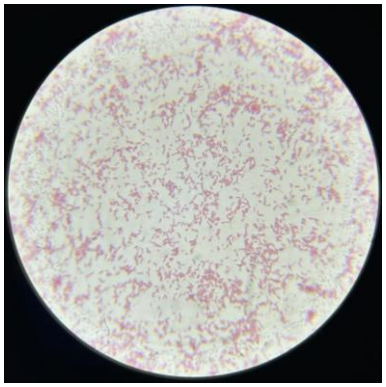

Fig 4.4.2 Gram staining of L32 C

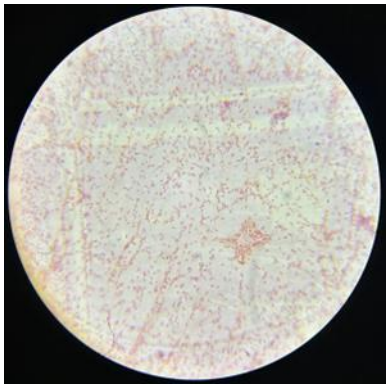

Fig 4.4.3 Gram Staining of L41C

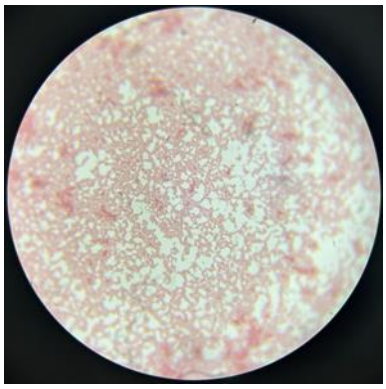

Fig 4.4.4 Gram staining of L27(1)

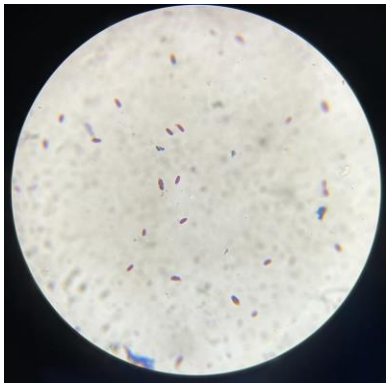

Fig 4.4.5 Gram staining of P12 a

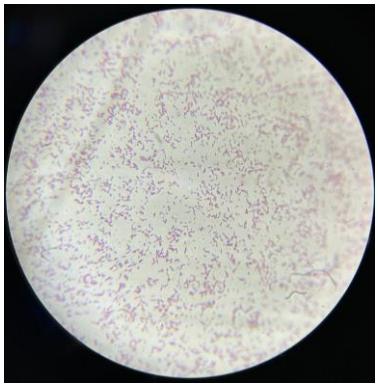

Fig 4.4.6 Gram staining of L55

Fig(4.4.1),(4.4.2),(4.4.3),(4.4.4),(4.4.6) indicates Gram positive rods and Fig (4.4.5)-Gram negative

4.5 Polysaccharide degradation

Colony	M9 Agar	M9 CMC	M9 Pectin	M9 citrate	M9 Alginate	M9 Carra
L27(1) A	+	+	+	+	+	-
L27(1) B	+	+	+	+	+	+
L55	+	+	+	+	+	+
P12 C	+	+	+	-	+	+
P12 B	+	+	+	+	+	-
P12 A	+	-	-	+	-	-
L32 C	+	+	+	+	+	-
L32 B	+	+	+	+	+	-
L32 A	+	-	-	+	-	+
L41C A	+	+	+	+	+	-
L41C B	+	+	+	+	+	-
L41C C	-	+	-	+	+	+

4.6 DNA Isolation

Genomic DNA was isolated using MB505 HiPurA® Bacterial Genomic DNA Purification Kit

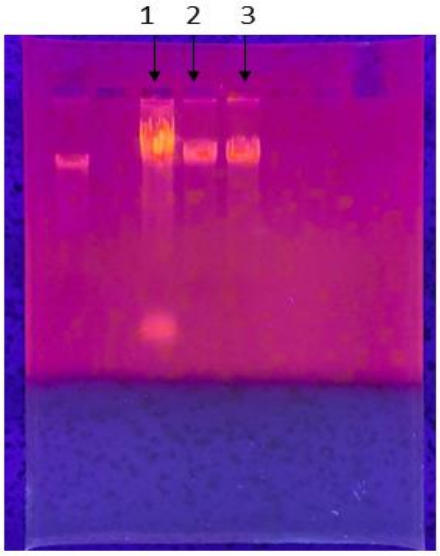

Fig 4.6.1

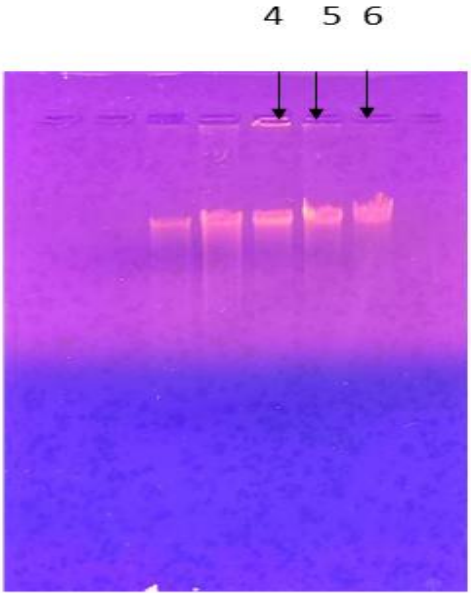

Fig 4.6.2

Fig 4.6.1 Gel image observed under UV transilluminator

Well no 1- DNA band of isolate from PY A

Well no 2- DNA band of isolate from PY B

Well no 3-DNA band of isolate from L55

Fig 4.6.2 Gel image observed under UV transilluminator

Well no 4 - DNA band of isolate from L41C

Well no 5 – DNA band of isolate from L32

Well no 6 – DNA band of isolate from L27(1)

4.7 PCR

DNA was amplified Using PCR and bands were obtain at 1.5 kb

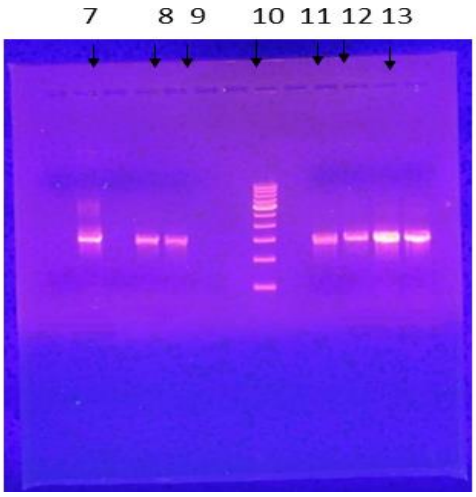

Fig 4.7.1

Fig 4.7.1 Gel image of PCR products
Well no 7 - P12
Well no 8 - P12
Well no 9 – L55
Well no 10 – L41C
Well no 11 – L27(1)
Well no 12 – L32

4.8 Biochemical test

Carbohydrate utilized by bacteria was confirmed by observing the wells foe colour change

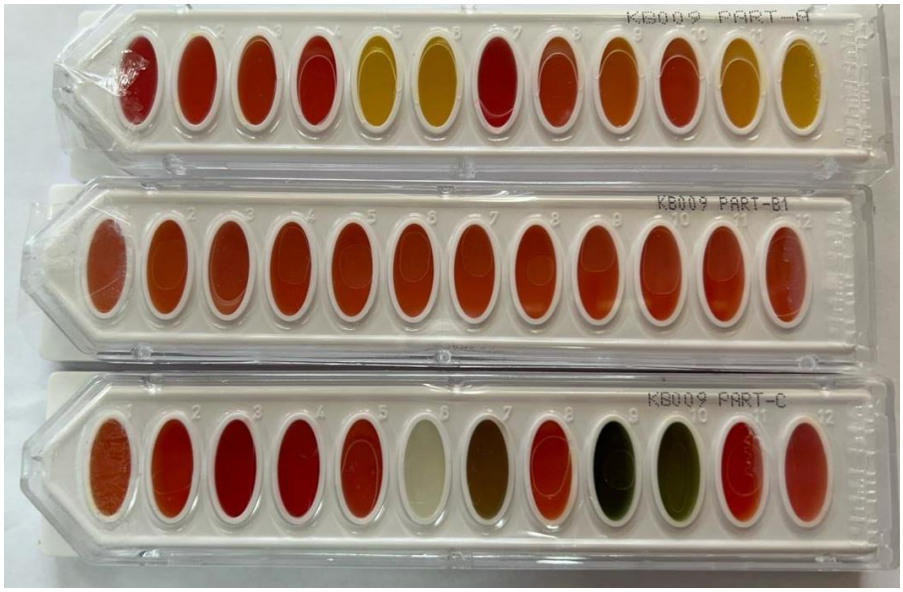

Fig 4.8.1 Results of Sugar utilized byL55

S. No	Test	Observation
1	Lactose utilization	-
2	Xylose utilization	-
3	Maltose utilization	-
4	Fructose utilization	-
5	Dextrose utilization	+
6	Galactose utilization	+

7	Raffinose utilization	-
8	Trehalose utilization	-
9	Malibiose utilization	-
10	Sucrose utilization	-
11	L- Arabinose utilization	V
12	Mannose utilization	V
13	Inulin utilization	V
14	Sodium gluconate utilization	V
15	Glycerol utilization	V
16	Salicin utilization	V
17	Dulcitol utilization	V
18	Inositol utilization	V
19	Sorbitol utilization	V
20	Mannitol utilization	V
21	Adonitol utilization	V
22	Arabitol utilization	V
23	Erythritol utilization	V
24	Alpha- Methyl- D- glucoside utilization	V

25	Rhamnose utilization	V
26	Cellobiose utilization	-
27	Melezitose utilization	-
28	Alpha- Methyl- D- Mannoside utilization	-
29	Xylitol utilization	-
30	ONPG activity	-
31	Esculin hydrolysis	-
32	D- Arabinose utilization	-
33	Citrate utilization	-
34	Malonate utilization	-
35	Sorbose utilization	-
36	Control	-

4.8.2 Results of sugars utilized by P12C

S. No	Test	Observation
1	Lactose utilization	V
2	Xylose utilization	V
3	Maltose utilization	+
4	Fructose utilization	+
5	Dextrose utilization	+
6	Galactose utilization	+
7	Raffinose utilization	+

8	Trehalose utilization	+
9	Malibiose utilization	+
10	Sucrose utilization	+
11	L- Arabinose utilization	+
12	Mannose utilization	+
13	Inulin utilization	V
14	Sodium gluconate utilization	V
15	Glycerol utilization	V
16	Salicin utilization	-
17	Dulcitol utilization	-
18	Inositol utilization	-
19	Sorbitol utilization	-
20	Mannitol utilization	-
21	Adonitol utilization	-
22	Arabitol utilization	-
23	Erythritol utilization	-
24	Alpha- Methyl- D- glucoside utilization	-
25	Rhamnose utilization	V

26	Cellobiose utilization	+
27	Melezitose utilization	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	-
36	Control	-

4.9 Sequencing

NCBI Nucleotide Blast analysis of the genomic sequence of bacteria strain L55 and L41C suggested homology with *Pseudomonas sp*. The top hits include *Pseudomonas sp*. with 81.4-84% similarity

Table No:4.9.1- Blast analysis of 16S rRna gene sequencing

Sr no	Bacterial strain	Suggested Homology
1	L55	*Pseudomonas sp*
2	L41C A	*Pseudomonas sp*

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APPENDIX

R2A Media Composition g/L

Caesin Hydrolysate	0.5
Protease peptone	0.5
Yeast extract	0.5
Sodium pyruvate	0.3
Dipottasium hydrogen phosphate	0.3
Magnessium sulphate anhydrous	0.3
Agar	15

M9 Media composition g/L

Disodium hydrogen phosphate	33.90
Pottasium Dihydrogenate	15.00
Sodium chloride	2.50
Ammonium Chloride	5