BIOPROSPECTING OF THRAUSTOCHYTRIDS FROM DIFFERENT MARINE HABITATS

A Dissertation for

Course code and Course Title: MMI-651 Discipline Specific Dissertation

Credits: 16

Submitted in partial fulfilment of Master's Degree

M.Sc. in Marine Microbiology

by

SANISHA VASANT SATARKAR

Seat number: 22P0390012

ABC ID: 964-787-102-913

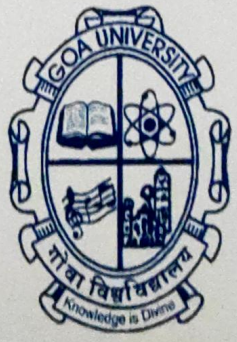
PR number: 201910425

Under the Supervision of

DR. VARADA SAMIR DAMARE

School of Earth, Ocean and Atmospheric Sciences

Master's in Marine Microbiology



GOA UNIVERSITY

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Examined by:

Seal of the school

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "BIOPROSPECTING OF THRAUSTOCHYTRIDS FROM DIFFERENT MARINE HABITATS" is based on the results of investigations carried out by me in the M.Sc. Marine Microbiology at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the Supervision of Dr. Varada S. Damare 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 be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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Satarkar

Sanisha Vasant Satarkar

Seat no:22P0390012

Date 02/05/2024

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report **"BIOPROSPECTING OF THRAUSTOCHYTRIDS FROM DIFFERENT MARINE HABITATS"** is a bonafide work carried out by **Miss. Sanisha Vasant Satarkar** under my supervision in partial fulfilment of the requirements for the award of the degree of **Master of Science** in the Discipline Marine Microbiology at the School of Earth, Ocean and Atmospheric Sciences, Goa University.

Dr. Varada S. Damare

Date: 02/05/2024 31/5/24

Sr. Prof. Sanjeev C. Ghadi
School of Earth, Ocean and Atmospheric Sciences
Date:
Place: Goa University

SCHOOL OF EARTH, OCEAN AND ATMOSPHERIC SCIENCES GOA UNIVERSITY TALEIGAO PLATEAU GOA

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Satarkar

Sanisha Vasant Satarkar

CONTENTS

PREFACE

The research carried out for the dissertation titled **"Bioprospecting of thraustochytrids from different marine habitats"**, we are basically trying to understand thraustochytrids that help to possess antimicrobial activity and degradation of hydrocarbons present in the marine environments. Thraustochytrids were first discovered in 1934, and since the 1960's they have been increasingly studied for their beneficial and deleterious effects.

Multidrug resistance is a common phenomenon observed in the recent year which leads to several deaths. Therefore, search for a novel antimicrobial compound is needed. Also hydrocarbon contamination in marine environment occurs due to natural processes like seepage from the oil wells in the seafloor, by chronic pollution in harbors and oil tanker routes or as a result of acute periodic inputs such as oil tanker accidents. Contamination by hydrocarbons is detrimental to the health of marine organisms and can be devastating to the ecosystem. Hence a green technology using microorganisms that can bioremediate such pollutants will be beneficial in the long run.

Bioprospecting is a continuous pursuit of discovering new natural sources. Thraustochytrids have caught their attention because they have been found to produce substances that exhibit antimicrobial properties. The study aims to investigate the antimicrobial activity of thraustochytrids in marine habitats, with the potential to combat drug-resistant pathogens and enhance human health.

In addition to their antimicrobial potential, thraustochytrids also play a crucial role in the degradation of hydrocarbons in marine habitats. Thraustochytrids have the ability to break down

complex hydrocarbon molecules into simpler forms. This is important in the context of oil spills or other instances of hydrocarbon pollution in the ocean.

By studying how thraustochytrids degrade hydrocarbons, we hope to gain understanding and improving natural degradation processes in marine environments. This knowledge could be used to develop more effective strategies for cleaning up oil spills and minimizing the environmental impact of hydrocarbon pollution.

So, the bioprospecting of thraustochytrids is an exciting field of research that explores their antimicrobial activity and their role in hydrocarbon degradation in marine habitats. It's all about exploiting the potential of these microorganisms to benefit human health and protect our oceans.

FIGURES

ABBREVIATIONS USED

Entity	Abbreviation
Bushnell Haas broth	BHB
Centi metre	Cm
Degree Celsius	°C
Folin ciocalteu	FC
Grams	G
Micro litre	µl
Milli metre	Mm
Minimal salt medium	MSM
Minute	Min
Modified Vishniac	MV
Optical density	OD
Revolutions per minute	Rpm
Room temperature	RT
Second	S
Sodium chloride	NaCl
Sodium hydroxide	NaOH

ABSTRACT

Thraustochytrids are marine organisms with potential antimicrobial activity for use in pharmaceutical, aquaculture, and human health sectors due to their contribution to the marine environment. The present study on the antimicrobial activity of thraustochytrids was conducted due to the limited availability of reports on this topic. Thraustochytrids isolates exhibited the highest antibacterial activity with the zone of inhibition of 1.0-2.5 cm against *Pseudomonas* sp. and the lowest 1.2-2.0 cm against *Salmonella* sp. Although thraustochytrids protists are known to be of widespread occurrence in the sea, their hydrocarbon-degrading abilities have never been investigated so the present study were carried out. The Thraustochytrids isolates like 8-B Red, OMD-4, MC-4, and OMS-4 showed a more than 60% decrease in sodium benzoate levels in flasks containing 0.1% sodium benzoate, while at concentration 0.5% they showed a decrease of less than 30%. The biomass in all the flasks generally showed the same trend for all the isolates. Further, biosurfactant properties of thraustochytrids isolates were also tested. Tested isolate gave positive results indicating that they all are producing biosurfactants

KEYWORDS

Antimicrobial, marine habitats, hydrocarbons, biosurfactants

CHAPTER 1

INTRODUCTION

INTRODUCTION

Thraustochytrids are unicellular oleaginous, eukaryotic-stramenopilan protists under the Kingdom Stramenopila, consisting of oomycetes and diatoms. (Bongiorni, 2012; Dellero et al., 2018). Thraustochytrids were initially classified based on the presence of biflagellated zoospores (Huang et al., 2003) and later based on morphological characteristics, molecular sequence studies, polyunsaturated fatty acids, and pigment profiles. Thraustochytriaceae comprises 11 genera and 35 species, with over 15 species belonging to Thraustochytrium sp. (Kalidasan et al., 2021b). They are abundantly present in coastal water and estuarine habitats especially algae, plants, and sediments, including decaying mangrove leaves (Marchan et al., 2018; Wang et al., 2019). Thraustochytrids are key decomposers, facilitating nutrient recycling and providing food to detritus-feeding organisms in mangrove habitats through decaying litter. (Raghukumar, 2002; Kathiresan et al., 2011; Taoka et al., 2017). Thraustochytrids are microorganisms that enhance soil fertility and enrich nutrients in mangrove and estuarine environments (Kathiresan et al., 2014; Kalidasan et al., 2019). Thraustochytrids are capable of synthesizing nanoparticles (Kalidasan et al., 2021a) that exhibit antimicrobial and antioxidant properties (Kalidasan et al., 2015a; Kalidasan et al., 2015b; Kalidasan et al., 2021b; Kalidasan et al., 2021b; Kalidasan et al., 2022). Thraustochytrids have been studied in various coastal, estuarine, mangrove, and marine environments across China (Mohan et al., 2022), Vietnam (Hien et al., 2022), Thailand (Aini et al., 2022), Japan (Taoka et al., 2017), Sweden (Patel et al., 2021), Italy (Russo et al., 2021), Korea (Saini et al., 2023), and Taiwan (Chauhan et al., 2023). The study of thraustochytrids in Indian mangrove areas is limited to Goa (Raghukumar, 2017), Kerala

(Jaseera et al., 2018), Mumbai (Pawar et al., 2021), Andaman Islands (Kalidasan et al., 2021b), and Tamil Nadu (Kalidasan et al., 2021a).

Thraustochytrids metabolites have shown promising antimicrobial properties, playing a pivotal role in antibiotic drug discovery, helping the pharmaceutical industry overcome single-resistant determinants. Multiple resistant mechanisms limit antimicrobial use, increasing demand for effective, non-toxic therapeutics due to increased bacterial infections incidence. (Kalidasan et al., 2014). Antimicrobials are crucial in preventing, treating, and controlling diseases caused by pathogens like *E. coli, S. aureus, Vibrio, Salmonella, Pseudomonas* and *Bacillus* (Hoflack et al., 2001; Krausse and Schubert, 2010).

One of the studies has shown oil spills have become a significant environmental concern in the last century, spreading hydrocarbons horizontally on ground/water surfaces, groundwater, soil pore airspace, and soil particle surfaces (Plohl et al., 2002). Thraustochytrids may play an ecological role in degrading petroleum hydrocarbons, which can cause significant soil and groundwater contamination (Raghukumar, 1995). Hydrocarbons in seawater can be sourced naturally, through chronic pollution in harbours and oil tanker routes, or through acute periodic inputs like oil tanker accidents (Raikar, 2001). Abiotic factors significantly influence the environmental fate of petroleum hydrocarbons, affecting microbial growth and enzymatic activities that determine their utilization rates. Abiotic factors, such as carbon and nitrogen sources, play a crucial role in the functioning of organisms (Leahy and Collwell, 1990). Petroleum hydrocarbons can persist indefinitely in one environment, while under certain conditions, they can be completely biodegraded within hours or days (Atlas and Bartha, 1992). The marine

environment can sustainably degrade oil through the presence of mixed populations of hydrocarbon-degrading microorganisms (Leahy and Collwell, 1990).

Crude oil, a blend of hydrophobic components like n-alkanes, aromatics, resins, and asphaltenes, contains some fractions that can be toxic to living organisms. Certain crude oil fractions are known to be harmful to living organisms (Costello, 1979). The leakage of crude oil into soil, causing damage to microorganisms and plants, can lead to an increase in toxic levels beyond 3% (Onuoha et al., 2003). Bioremediation is a set of technologies that uses biological activity to remove or reduce the harmful effects of contaminants in hydrocarbon management (Silva et al., 2015). Unique microorganisms like bacteria, microalgae, and fungi can help clean contaminated sites by either being isolated from different environments, introduced into the site, or enriching existing organisms (Adebusoye et al., 2007). Bioremediation is a preferred method for treating hydrocarbons due to its non-toxic and safer products, such as carbon dioxide and water, produced by microbes using crude oil as a carbon substrate (Toledo et al., 2006). Microbial remediation is an eco-friendly, practical, and economical method for mineralizing hydrocarbons into carbon dioxide and water, making it a promising and practical solution for contaminated sites (Wang et al., 2015).

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental contaminants primarily produced by natural combustion processes and human activities. The accumulation of pollutants (PAHs) in marine environments is largely due to human activities such as atmospheric deposition, industrial emissions, oil spills, ship traffic, urban runoff, and illegal industrial effluent discharge. High concentrations of PAHs have been found in coastal sediments near urban and industrial cities (Shiaris & Jambard-Sweet, 1986). Mangrove ecosystems, the crucial inter-tidal estuarine wetlands along tropical and sub-tropical

coastlines, are significantly impacted by human activities and are susceptible to PAH contamination. Over the past two decades, there has been a growing concern over the contamination of marine environments with persistent, toxic, mutagenic, and carcinogenic PAHs . PAH is can be eliminated into the environment through processes like volatilization, photo-oxidation, chemical oxidation, bioaccumulation, and adsorption on sediment particles, minimizing their adverse effects. The primary method for effectively eliminating PAHs from the environment is through microbial transformation and degradation (Wilson and Jones, 1993). PAH-contaminated sediments may contain microorganisms capable of degrading hydrocarbons, which could be utilized for PAH elimination (Catallo and Portier, 1992). Certain bacteria in hydrocarbon-contaminated environments showed biodegradation potentials as active or even higher than those from non-contaminated sediments, possibly due to acclimatization and adaptation (Wild and Jones, 1986; Chaineau et al., 1999; Ramsay et al., 2000). The indigenous community in mangrove sediments has a high number of aromatic-degraders, with potential for oil degradation. Factors like salinity, initial PAH concentration, and simple carbon source may influence biodegradation potential. Mixed culture conditions, including co-metabolism or antagonism, may influence the biodegradation potential of toxic organic compounds, highlighting the importance of carbon sources like glucose in enhancing biodegradation (Kobayashi and Ritmann, 1982).

Biosurfactants are amphiphilic compounds that can accumulate between fluid phases and are produced on microbial cell surfaces or secreted extracellularly. Biosurfactants have hydrophilic moiety, which can be carbohydrate, amino acid, phosphate group, or similar compounds, and hydrophobic moiety, primarily fatty acid carbon chain. This property reduces interfacial and surface tensions, making them potential candidates for improving oil

recovery. Microorganisms produce various types of biosurfactants, including fatty acid and polymeric biosurfactants. The surface tension of water was reduced from 72 mN/m to 27 mN/m. Surfactin has been found to enhance the treatment of residual hydrocarbon from ship bilge waste. Factors like carbon and nitrogen sources, phosphorus, iron, manganese, magnesium, pH, temperature, agitation, and operation mode significantly impact the production and quality of biosurfactants (Nayarisseri et al., 2018). Biosurfactants offer superior biodegradability, lower toxicity, environmental compatibility, foaming ability, selectivity and specific activity, while synthetic surfactants have a more economic but environmentally impactful nature. Biosurfactants face significant disadvantages due to high production costs and difficulties in purification due to the expensive biotechnological processes involved.

Marine thraustochytrids, despite their widespread presence, are poorly understood for their biomass and biological activities in the marine environment. Hence, the current study was made on antibacterial activity, potentiality to degrade hydrocarbons, to investigate the bio degradation potential of the enriched mixed thraustochytrids cultures obtained from different marine environment to test any relationship of PAH contamination in marine environment, naphthalene biodegradation and identify and characterize new strains producing biosurfactants from marine sources and production of biomass.

1.1 Background

Thraustochytrids are a group of marine protists that have gained attention for their unique characteristics. They are single-celled organisms that thrive in various marine environments, such as sediments and seawater.

When it comes to antimicrobial activity, thraustochytrids have shown promising results in combating harmful microbes. They produce bioactive compounds, including antimicrobial agents, that can inhibit the growth of bacteria, fungi, and even some viruses. These bioactive compounds have the potential to be used in medicine, agriculture, and food preservation to fight against microbial infections.

In terms of hydrocarbon degradation, thraustochytrids possess the remarkable ability to break down and metabolize hydrocarbon compounds. They can target a wide range of hydrocarbons, including crude oil and diesel. This makes them valuable in environmental remediation efforts, particularly in areas affected by oil spills or contamination. Thraustochytrids contribute to the natural degradation of hydrocarbons, helping to restore ecosystems and minimize the impact of pollution.

Thraustochytrids are actively studied to better understand their mechanisms of antimicrobial activity and hydrocarbon degradation. By uncovering the underlying processes and compounds involved, they hope to harness the full potential of these organisms for various applications. (Shiaris & Jambard-Sweet, 1986). Overall, thraustochytrids have shown great promise in both antimicrobial activity and the degradation of hydrocarbons. Their unique characteristics and abilities make them a fascinating area of research, with the potential to make significant contributions in medicine, environmental cleanup, and beyond.

1.2 AIM AND OBJECTIVE

AIM

The main aim of the dissertation to study bioprospecting of thraustochytrids from different marine habitats.

OBJECTIVES

- To check if Thraustochytrids from different sources can display any antimicrobial property.
- To check if Thraustochytrids from different sources can grow in the presence of aromatic hydrocarbon compounds.

1.3 HYPOTHESES

(1) Thraustochytrids should display antimicrobial properties. Thraustochytrids are part of the microbial loop and they are decomposers in the marine environment. They probably compete with other decomposers like bacteria for nutrients, and in order to achieve this, they probably possess antimicrobial activity.

(2) Thraustochytrids may have the potential to degrade aromatic hydrocarbons. Thraustochytrids have hydrophobic cell walls to which hydrocarbons can bind. They also have tendency to form biofilm, so they might attach to the tar balls and eventually degrade hydrocarbons present therein.

1.4 SCOPE

Thraustochytrids have a wide scope when it comes to antimicrobial activity and the degradation of hydrocarbons. These organisms have shown impressive abilities in both.

In terms of antimicrobial activity, thraustochytrids have demonstrated effectiveness against various types of bacteria, fungi, and even some viruses. They have the potential to be used in medicine, agriculture, and food preservation to combat harmful microbes. Thraustochytrids offer a natural and promising alternative to traditional antimicrobial agents.

When it comes to the degradation of hydrocarbons, thraustochytrids have shown their ability to break down and metabolize different types of hydrocarbon compounds, including crude oil and diesel. This makes them valuable in environmental remediation efforts, particularly in areas affected by oil spills or contamination. Thraustochytrids contribute to the restoration of ecosystems and the reduction of pollution caused by hydrocarbons.

Overall, thraustochytrids have a significant role to play in both antimicrobial activity and the degradation of hydrocarbons. Their potential applications in medicine, agriculture, and environmental cleanup make them a great interest for further research and exploration. It's truly interesting to see how these tiny organisms can have such a positive impact on our world.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

Thraustochytrids, are marine microorganisms in the stramenopiles phyla, found in oceans and sediments, particularly in nutrient-rich mangrove forests, playing crucial ecological roles for carbon recycling (Sparrow, 1936). They are also marine decomposers, single-celled eukaryotes that secrete enzymes like amylases, proteases, and phosphatases, widely distributed in marine ecosystems (Taoka et al., 2009; Taoka et al., 2017; Lin et al., 2020). Mangroves are crucial ecologically due to their abundant presence in regions rich in detritus and decaying plant material, which aids in nutrient cycling through decomposing decaying matter. They significantly contribute to the synthesis of omega-3 PUFAs, DHA and EPA, essential for crustacean growth and reproduction (Raghukumar,2008). Thraustochytrids, a member of the Labyrinthulea class, were mistakenly classified as fungi due to their similar appearance and lifestyle. Thraustochytrids have unique characteristics such as extracellular non-cellulosic scales cell wall, heterokont flagella zoospores, and a bothrosome-produced ectoplasmic net for extracellular digestion (Hamamoto and Honda., 2019). Thraustochytrids exhibit variable morphology throughout their life cycle, with a main vegetative asexual cycle varying by genus. Sexual reproduction in this group is poorly understood. Thraustochytrids are highly beneficial for human health due to their high concentrations of DHA, palmitic acid, carotenoids, and sterols. Scientists are exploring ways to increase DHA, fatty acids, and squalene concentrations in thraustochytrids through genetic modification or medium composition/conditioning (Jain et al., 2007). Advancements in gene transfers to plant species have made oil isolation easier and cost-effective. Thraustochytrids are being cultured for their use in fish feed and dietary supplements for humans and animals (Hu et al., 2007Kiy,1998). Scientists are exploring innovative methods to convert waste water into

valuable products like squalene, which can be utilized for biofuel production (Mackiewicz et al., 2010).

Thraustochytrids, specifically the genera *Schizochytrium*, *Thraustochytrium*, and *Ulkenia*, are increasingly being of interest in bio-technology (Raghukumar 2008; Gupta et al. 2012; Lee Chang et al. 2014). The three genera belong to the Thraustochytriaceae family, which is characterized by ovoid thalli and an anchoring and feeding network of ectoplasmic threads (Perkins 1972; Beakes et al. 2009; Adl et al. 2012; Anderson & Cavalier-Smith, 2012).

Thraustochytriaceae, along with other families, form the order Thraustochytrida, which, along with Labyrinthulida and Amphitremida, belongs to the Labyrinthulomycota class (Beakes et al. 2009; Adl et al. 2012; Anderson & Cavalier-Smith, 2012; Gomaa et al. 2013; Ruggiero et al. 2015; Pan et al. 2016). Labyrinthulomycota are marine, saprotrophic, unicellular organisms lacking a plasmid, characterized by a bothrosome, tubulocristate mitochondria, and Golgi-derived scales (Perkins 1972; Porter 1972; Honda et al. 1999; Leander & Porter 2001; Raghukumar 2002; Adl et al. 2012). *Bigyra*, a basal clade of non-plastidial, unicellular organisms within the kingdom of Stramenopila/Heterokonta, includes Bicosoecids, Placidida, Opalinata, and Blastocystis (Tsui et al. 2009; Adl et al. 2012; Ruggiero et al. 2015). Stramenopila are eukaryotic protists known for their asymmetrically bifurcated zoospores. Stramenopiles consist of the osmotrophic sister-clades Oomycota and Hypochytriomycota, both fungus-like and non-plastidial, and the photosynthetic Ochrophyta. The Ochrophyta are a monophyletic clade comprising golden-brown pigmented algal clades like Bacillariophyceae, Phaeophyceae, Chrysophyceae, Eustigmatophyceae, and Xanthophyceae. (Brown & Sorhannus 2010; Ruggiero et al. 2015).

Thraustochytrids are present in mangrove leaf litter, along with other saprophytic microbes (Kalidasan et.al., 2019). In order to compete with other microbes for survival and multiplication, the thraustochytrids have to produce antimicrobial substances (Kalidasan et al., 2015a). However, only a two reports are available on the antimicrobial activity of thraustochytrids. So, this present study examined the antimicrobial activity of different isolates of thraustochytrids.

Bahnweg G, et al. (1979) has shown that thraustochytrids have the capability to utilize a wide range of organic nitrogen and carbon compoundsfor their nutrition. However, the capability of these protists to degrade hydrocarbons has not been examined so far. So, this present study investigated how different isolates of thraustochytrids break down hydrocarbons.

History of Thraustochytrids:-

Thraustochytrids, often misnomers, are marine fungi, protists, heterokonts, chromists, or heterotrophic microalgae. Despite efforts to establish their taxonomic position, confusion persists regarding doubtful strains and group members. In 1934, Thraustochytrium proliferum was first described as a thraustochytrid from the marine alga Bryopsis plumose in coastal waters near Woods Hole, Massachusetts (Sparrow, 1936). Initially, Thraustochytriaceae was mistakenly classified as Phycomycetes due to their ability to release biflagellate zoospores and form rhizoid-like structures, known as the ectoplasmic net (Ellenbogen et al., 1969; Sparrow, 1960). The thraustochytrids were later assigned to the Oomycetes (Sparrow, 1973, Cavalier-Smith et al., 1994). The study confirmed thraustochytrids as not true fungi or Oomycetes, while other authors identified common features between thraustochytrids, labyrinthulids, and aplanochytrids, suggesting

interrelationships(Alderman et al., 1974; Moss, 1986; Perkins, 1973a). The International Code of Nomenclature for algae, fungi, and plants, Labyrinthulomycetes, has been expanded upon by increasing studies supporting these interrelationships (Von Arx, 1974) or

Labyrinthulea (International Code of Zoological Nomenclature, ICZN) (Cavalier-Smith, 1986; Olive, 1975). The three groups of microorganisms (thraustochytrids, labyrinthulids, and aplanochytrids) were systematically categorized and characterized (Cavalier-Smith, 1989; Porter, 1990).

Antimicrobial activity of bacteria:

Bacillus genus members produce diverse antimicrobial compounds, making them widespread across various ecological niches (Cazorla et al. 2007; Deleu et al., 2008). Bacillus subtilis isolates produce a diverse array of peptides. (Ongena and Jacques, 2008) and non-peptide (Hamdache et al. 2011; Wise et al. 2012). The use of anti-microbial compounds has led to the registration of certain B. subtilis strains as biopesticides, such as Serenade® and Kodiak, for controlling plant pathogens. (Chan et al., 2009; Chooi et al., 2010;Etchegaray et al., 2008). Cyclic lipopeptides (CLP), a class of antimicrobial compounds, are particularly significant in B. subtilis due to their fatty acyl chain and cyclic peptide ring structure (Raaijmakers et al., 2010). *B. subtilis* produces CLPs, which are categorized into three families: (Cao et al., 2012; Fickers et al., 2009) surfactins, fengycins, and iturins, each with distinct antimicrobial properties (Ongena and Jacques, 2008). CLPs have demonstrated potential in controlling plant pathogenic microorganisms through indirect mechanisms, such as inducing host plant resistance (Jourdan et al., 2009). or direct via effects on plant pathogen biological membranes (Patel H et al., 2011; Patel A. et al., 2021). This study explores the specific mechanisms of action of CLP and the potential uses and

ecological relevance of *B. subtilis* as CLP-producing biocontrol agents. (Buchoux et al., 2008).

Plant ecosystems are intricate, containing various microbial communities, and natural ecosystems typically reach a microbial community climax where all populations have reached a steady state. (Bélanger and Avis, 2002). The natural balance in artificial systems can be significantly altered by changes in the microbial community. For example, an artificial system such as agriculture can select for non-desirable microorganisms (e.g.plant pathogens) while repressing the growth of naturally- occurring beneficial microorganisms (Avis et al., 2008). Changes in the natural microbial balance can significantly impact plant health and productivity, as noted by (McCromick, 2013). Adding beneficial microorganisms to disturbed ecosystems can mitigate plant pathogen issues, but only highly competitive antagonists can adapt to these environments. CLPs offer numerous advantages in plant-soil environments, including protection from competing microorganis. (Cho et al., 2003; Henry et al., 2011; Francius et al., 2008). Humans use CLP-producing B. subtilis as biocontrol against plant pathogens in various plant production settings.

The study of (J. Falardeau et al. 2013) of CLP mechanisms, their different activities, and potential synergistic effects is aiding in the effective use of bacterial antagonists for controlling plant pathogens. Recent advancements in CLP knowledge highlight the need for further research to effectively utilize CLP-producing B. subtilis strains for plant protection. (Bais et al., 2004; García et al., 2013) Further research is needed to understand the precise action modes of each CLP and how these mechanisms influence the spectrum of activity of B. subtili. (Elkahoui et al., 2012; Eeman et al., 2009; Heerklotz et al., 2007). Antimicrobials potential to reduce genetic resistance in sensitive microorganisms has been hypothesized,

but no definitive mechanistic work has been conducted to fully understand this benefit. (Avis & Bélanger, 2001; Ahimou et al., 2000). The potential benefits of producing multiple CLP families from an ecological and plant protection perspective are yet to be fully understood, necessitating further research on additive, synergistic, and mutual inhibition effects.(Additional research should explore the full potential benefits of CLP-producing B. subtilis as plant pathogen control agents.

Antimicrobial activity of thraustochytrids:

In marine and estuary settings, such as the water column, sediments, algae, mangroves, and invertebrates, thaustochytrids are widely distributed (Raghukumar S. et al. 2002). In mangrove environments, these heterotrophic, unicellular protists are primarily responsible for breaking down leaf litter (Kalidasan K. et al. 2019). Accordingly, the thraustochytrids are essential to the mangrove ecosystem's food web enhancement, nutrient enrichment, and litter decomposition (Raghukumar S. et al. 2002). The production of omega-3 polyunsaturated fatty acids (PUFA) with antimicrobial, antioxidant, and nanoparticle qualities from microbial sources is promising for the aquaculture, nutraceutical, and human health industries.

In the study by Kalidasan et al,. (2023), the antibacterial efficacy of *A. mangrovei* and *Thraustochytrium* sp., was tested against human clinical bacterial pathogens such as *Klebsiella pneumonia*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Vibrio cholera*, obtained from Pathology Department, Rajah Muthaiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India. The antibacterial activities were evaluated by agar disc diffusion method. *Thraustochytrium* sp.,

exhibited the highest antibacterial activity with the zone of inhibition of 78.77% against *Staphylococcus aureus* and the lowest (20.95%) against *Klebsiella pneumonia*e.

Thraustochytrids are marine fungus that are zoosporic, unicellular, and a part of the chromistan protist family. They are commonly found in the water. They have been isolated from a wide range of environments, including seawater and sediments, live algae, animal guts, and living animals. Occasionally, they reach biomass levels comparable to that of bacteria. Despite this, not much is known about their ecological importance in the water. Seawater contains hydrocarbons due to a variety of sources, including natural processes like seepage from oil wells in the seafloor, chronic pollution in harbors and oil tanker routes, and acute, sporadic inputs like oil ship accidents (Rosenberg E, et al. 1992). Numerous microorganisms are known to play a significant role in hydrocarbon degradation in the sea, including cyanobacteria, fungus, and bacteria (Leahy and Collwell, 1990).

In the study by Raikar et al. (2001) all the thraustochytrid cultures used were isolated from six sites along the coast of Goa (15°25'N; 73°95'E), which were exposed to chronic oil pollution due to the presence of boat jetties, dockyard or harbour Dona Paula jetty; Betim jetty; Cortalim boatyard; Malim jetty; Marmagoa harbour and Siridao beach. According to Rosenberg E, et al. (1992) microorganisms capable of predominantly utilizing soluble hydrocarbons may not adhere to oil and have little need for hydrophobicity of cells. On the contrary, organisms that adhere to high molecular weight hydrocarbons by virtue of their hydrophobic cell walls are potential degraders of hydrocarbons that have a poor solubility in water. The MATH assay has commonly been used to test cell wall hydrophobicity (Rosenberg M, et al. 1991) Judging by this assay, all 3 thrausochytrids tested appeared to be potential hydrocarbon degraders, with fractions of adherence ranging from 0.3 to 0.4.

CHAPTER 3

METHODOLOGY

METHODOLOGY

Culture and Growth Conditions: -

Eighteen isolates of thraustochytrids (OMD-1, OMD-2, OMD-3, OMD-4, OMS-2, OMS-4, OGS-2, MC-1, MC-4, RD-1, 8B Red, 5 Long Padina, DB Sarg, ZB-6, AKN3, A8 Ulva, 9B, A3 Brown) were provided for this study. The thraustochytrid isolates were preserved in Modified Vishniac broth.

Antibacterial activity: Antibacterial potential of thraustochytrids was assessed on *E. coli, S. aureus, Salmonella* sp., *Vibrio* sp., *Pseudomonas* sp., and *Bacillus* sp. isolated from seawater of (Cacra beach). Nutrient Agar plates were prepared using distilled water. All 6 bacteria were inoculated into sterile Nutrient Broth flasks prepared using distilled water. The bacterial cultures were incubated at 37º C for 24 hours. From the broth 100 µl was spread plated on the labelled Nutrient Agar plates. Using the rear end of sterile 1ml tips, wells were created in the plates and they were filled with 50 µl of the sample to be tested for antibacterial activity.

Antibacterial activity in culture supernatant: The sample used to test this was 7-day old thraustochytrid culture broth. The plates were kept in refrigerator for 15 minutes and further incubated at 37º C for 24 hours. The diameter of the zone of inhibition was measured after the incubation period (Chandrasekaran et al. 2008).

Antibacterial activity in cell pellet: Cell pellet of each thraustochytrid isolate was harvested after 7 days of incubation by centrifuging at 4500 rpm for 20 mins. The cell pellet was suspended in sterile seawater and sonicated. Sonication was carried out using ultrasonic processor with titanium alloy probe of 6 mm diameter (Labman, Model PRO-650, S. No. L35431), 30 % power rate and 2 rounds of 30 s with 6 equal steps of 3 s pulse 'on' and 2 s pulse 'off'. After sonication, the suspension was added into the well and the rest of the procedure was followed as mentioned above.

Hydrocarbon degrading ability of thraustochytrids

Degradation of sodium benzoate by Volumetric Titration method:-

The comparative ability of 18 isolates (OMD-1, OMD-2, OMD-3, OMD-4, OMS-2, OMS-4, OGS-2, MC-1, MC-4, RD-1, 8B Red, 5 Long Padina, DB Sarg, ZB-6, AKN3, A8 Ulva, 9B, A3 Brown) to degrade sodium benzoate was tested. The cultures were inoculated in MV broth using 1% inoculum and incubated at room temperature (RT) on a rotary shaker at 105 rpm for 7 days. These were used as inoculum for experimental flasks. For the degradation experiments, 100 ml conical flasks containing 20 ml of MV broth and two different concentrations of sodium benzoate, i.e., 0.1% and 0.5% separately were prepared. These were again inoculated with 1% inoculum. The culture flasks were incubated at RT at 105 rpm for 7 days (till visible growth was observed). Uninoculated media served as a control. All the experiments were carried out in triplicates.

After incubation, flasks were centrifuged for 4500 rpm/20 min. Pellet was used for determination of biomass produced by each isolate in the presence of sodium benzoate.

Supernatent was used to determine the decrease in sodium benzoate due to degradation by the isolates. For this, 10 g of samples (supernatant) from the control and experimental flasks containing sodium benzoate were taken in separate beakers and 1 ml of 10 % NaOH and 12 g NaCl were added to each of these. Sufficient distilled water was added to bring the volume up to 50 ml and were allowed to stand for 30 minutes with frequent shaking. One drop of phenolphthalein was added (the colour changed to pink), following which few drops of HCl were added until the mixture in the beaker turned colourless . Three ml HCl was added in excess. Contents in the flasks were added to separating funnels containing 25 ml chloroform. This was followed by frequent shaking for 20 minutes. The funnels were then allowed to stand for 24 hours. Approximately 12.5 ml of the chloroform layer (i.e. lower layer) was transferred into conical flasks and the chloroform was allowed to evaporate for 24 hours. After evaporation, 50 ml of 50 % ethanol solution was added in the conical flasks. The resulting solutions from each conical flasks were titrated with 0.05 M NaOH and 1 drop of phenolphthalein was added as an indicator (Battung et al. 2011). After titration was completed the amount of sodium benzoate in the samples was calculated using the following formula.

1ml of 0.05 M NaOH → 0.0072 g sodium benzoate

X ml NaoH → ? gm of sodium benzoate

Degradation of naphthalene:-

Three thraustochytrid isolates (OMD-4, OGS-2 and ZB-6) were tested for degradation of naphthalene. Experiments were carried out in 10ml of MSM (Minimal Salt Medium)

containing 0.1% naphthalene. Several controls were maintained; the first one was MV with 0.1 % naphthalene, the second one was MV without naphthalene and the third one was MSM with 0.4 % glucose and no naphthalene. All the culture flasks were incubated at RT on a rotary shaker at 105 rpm for 7 days (till visible growth was observed). All the experiments were carried out in duplicates.

FC assay (Bhatawadekar et al. 2023): After incubation, the culture broth was aliquoted in different tubes and centrifuged for 10 minutes to collect cell pellet. One aliquot of the cell pellet was washed twice with respective sterile media and resuspended in the same sterile medium and incubated at RT at 105 rpm. After 24 hours of incubation, 100 µl of cell suspension from each flask was transferred into 24-well microtitre plate. To this, 100 µl of Folin Ciocalteau (FC) reagent was added, followed by the addition of 80 µl of 10% sodium bicarbonate. This reaction mixture was incubated in the dark for 30 minutes and observed for the colour change. Phenol solution (0.05%) was used as a positive control.

Microbial adhesion to hydrocarbon (MATH) assay (Nayarisseri et al. 2018):

The second aliquot of the cell pellet obtained from the above experiment were washed twice with sterile phosphate buffer and suspended in a phosphate buffer and diluted using the same phosphate buffer to an optical density (OD) of ~ 0.5 at 610 nm. To the cell suspension (2 ml) in test tubes 100 µl chloroform was added and vortexed for 3 min. After vortexing, chloroform and aqueous phases were allowed to separate for 1 hour. OD of the aqueous phase was then measured at 610 nm in a spectrophotometer (Shimadzu UV-1780, S. No. A119161). From the OD values, the percentage of cells attached to chloroform was calculated using the following formula:

$$\%\ of\ bacterial\ cell\ adherence = (1 - \frac{OD\ of\ suspension\ shaken\ withhydrocarbon}{OD\ original\ suspension})x\ 100$$

Emulsification Index (Nayarisseri et al. 2018): The culture supernatant obtained from the above experiment was used to find emulsification index. For this, 1ml of culture suspension was taken in a sterile test tube and overlayed with equal amount of crude oil. Then, the mixture was vortexed at high speed for 1 min and allowed to stand for 24 hours. Distinct layers were seen and height of the layers was measured to calculate the emulsification index.

$$Emulsification\ index = \frac{Height\ of\ the\ emulsion\ layer}{Total\ height}\ x\ 100$$

Oil Spreading Assay (Nayarisseri et al. 2018): On a clean glass slide 20 µl of distilled water was added. To the surface of the distilled water, 20 µl of sterile crude oil was added. Ten µl of culture supernatant obtained after centrifugation was placed on the centre of the oil layer. Oil-free clearing zone indicated biosurfactant production and the diameter of this clearing zone was then measured. SDS was used as positive control.

Drop Collapse Assay (Nayarisseri et al. 2018): The wells of a cavity slide were coated with sterile crude oil and were kept aside for 24 hours. After 24 hours, a drop of the culture supernatant was placed on the oil-coated well. Flattening of the drop or its collapse indicated biosurfactant production.

Biomass determination: The third aliquot of cell pellet was used for biomass determination in the presence of naphthalene. The wet thraustochytrids cells (pellet) were placed in the oven for 24 hours to dry before weighing in an analytical balance (Nayarisseri et al. 2018). Biomass was expressed as grams per ml of the medium.

Degradation of crude oil:-

Quantitative estimation of crude oil degradation using gravimetry method: The comparative abilities of 3 isolates (OMD-4, OGS-2 and ZB-6) to degrade crude oil were tested. The inoculum was prepared in 10ml of MV broth. Experiments were carried out in Bushnell Haas Broth (BHB) as well as MV containing 0.1 % crude oil. Plain MV broth was used as culture control. All the culture flasks were incubated at RT on a rotary shaker at 105 rpm for 7 days (till visible growth was observed). Uninoculated media served as negative control. All the experiments were carried out in triplicates.

Following incubation, the residual crude oil was sequentially extracted from the culture medium with dichloromethane (1:1). Sample with dichloromethane was placed in a separating funnel and shaken continuously for 30 minutes. After this the contents were allowed to settle for 24 hours; two layers were formed: aqueous layer and organic dichloromethane layer containing the residual crude oil. The organic layer was decanted in pre-weighed petriplates and air dried. After dichloromethane evaporation, the residual oil was quantified gravimetrically (Barnes et al. 2018). The percentage degradation of the crude oil was then calculated as below equation.

$$\%\ degradation\ of\ crude\ oil = \frac{weight\ of\ crude\ oil\ (initial) - weight\ of\ crude\ oil\ (after\ treatment)}{weight\ of\ crude\ oil\ (initial)}\ x\ 100$$

On incubation of cultures with crude oil, biomass estimation was done after 7 days for which cell pellet was collected, washed with sterile seawater and centrifuged again to determine the wet weight and dry weight. Biomass was expressed as g/ml.

CHAPTER 4

ANALYSIS AND CONCLUSIONS

RESULTS

Antibacterial activity: -

Out of the 18 thraustochytrid isolates, 16 showed possessed antibacterial activity. All the 16 isolates showed the highest antibacterial activity against *Pseudomonas* while lowest against *Salmonella* based on zone of inhibition. OMD-2 and A3Brown did not show any antibacterial activity against all the bacteria tested. The pellet of OMS-2, 8B Red, RD-1 and DB Sarg also did not show any antibacterial activity. Interestingly 61% of the isolates showed antibacterial activity in both culture pellet as well as supernatant **(Figs. 4.1 and 4.2)**. The zone of inhibition for *E. coli* ranged from 1.1-2.4 cm **(Fig. 4.3)**, for *S. aureus* from 1.2-2.1 cm **(Fig. 4.4)**, for *Bacillus* sp. from 0.9-2.3 cm **(Fig. 4.5)**, for *Vibrio* sp. from 1-2.1 cm **(Fig. 4.6)**, for *Pseudomonas* sp. from 1-2.5 cm **(Fig. 4.7)** and for *Salmonella* sp. from 1.2-2 cm (Fig. 4.8). The chloramphenicol zone ranged from 2.2-4.5 cm **(Fig. 4.3-4.8)**.

Hydrocarbon degrading ability of thraustochytrids:-

Degradation of sodium benzoate by Volumetric Titration method:-

8-B Red, OMD-4, MC-4, and OMS-4 exhibited more than 60 % decrease in sodium benzoate in experimental flasks containing 0.1 % of sodium benzoate. However, at a concentration of 0.5%, they showed a decrease of less than 30 % **(Fig. 4.9)**. On the other hand, OMD-1, MC-1, A3Brown, and A8Ulva demonstrated a decrease of more than 30% in 0.5% sodium benzoate. Additionally, OMD-4, ZB-6 and OGS-2 also showed a decrease in sodium benzoate levels. The biomass in all the flasks generally showed the same trend for all the isolates **(Fig. 4.10)**.

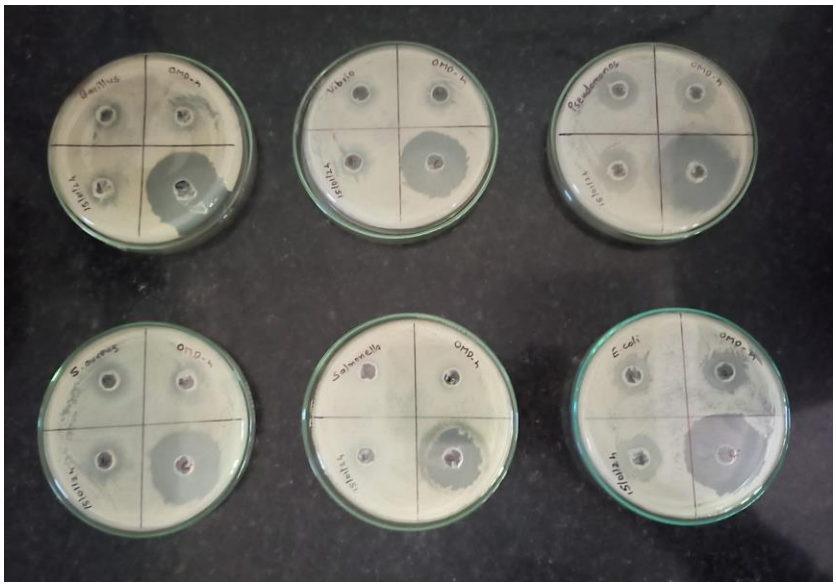

Fig. 4.1. Antibacterial test of OMD-4 isolate (culture supernatant)

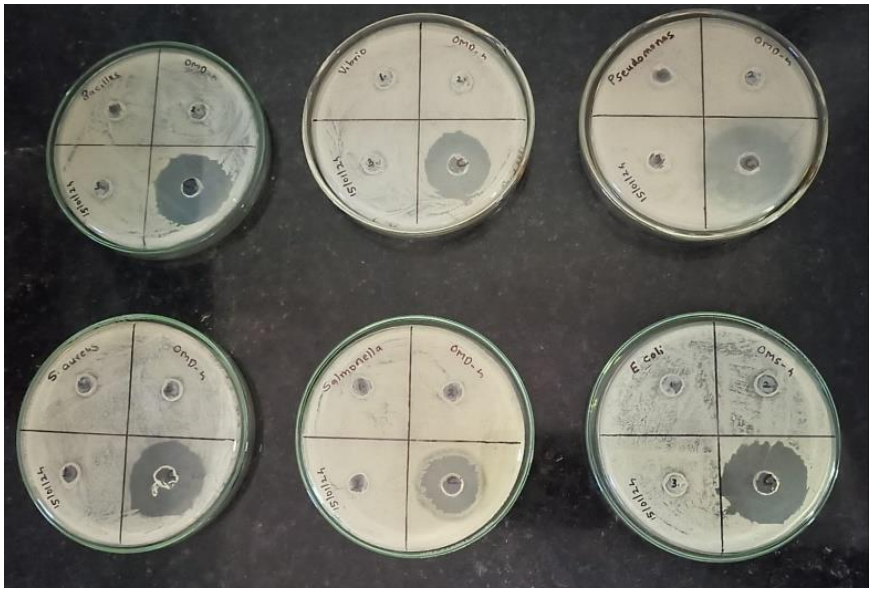

Fig. 4.2. Antibacterial test of OMD-4 isolate (pellet)

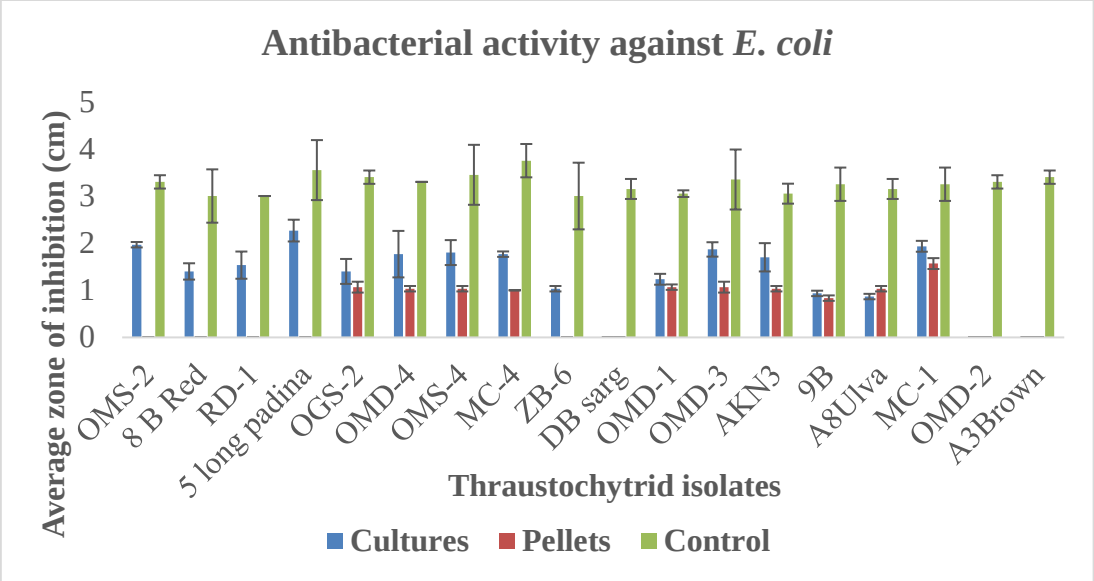

Fig. 4.3. Antibacterial activity of 18 thraustochytrid isolates against *E. coli*. The blue bar represents the activity shown by culture supernatant, the red bar represents the activity shown by cell pellet, and the green bar represents the activity shown by chloramphenicol.

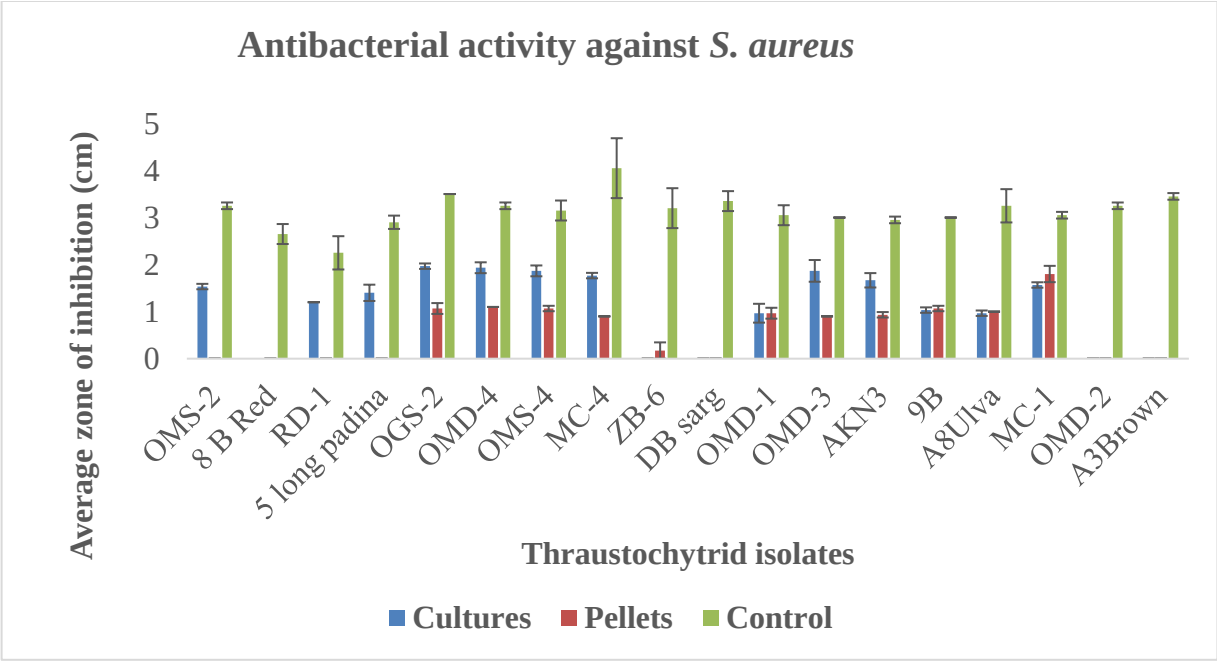

Fig. 4.4. Antibacterial activity of 18 thraustochytrid isolates against *S. aureus*.

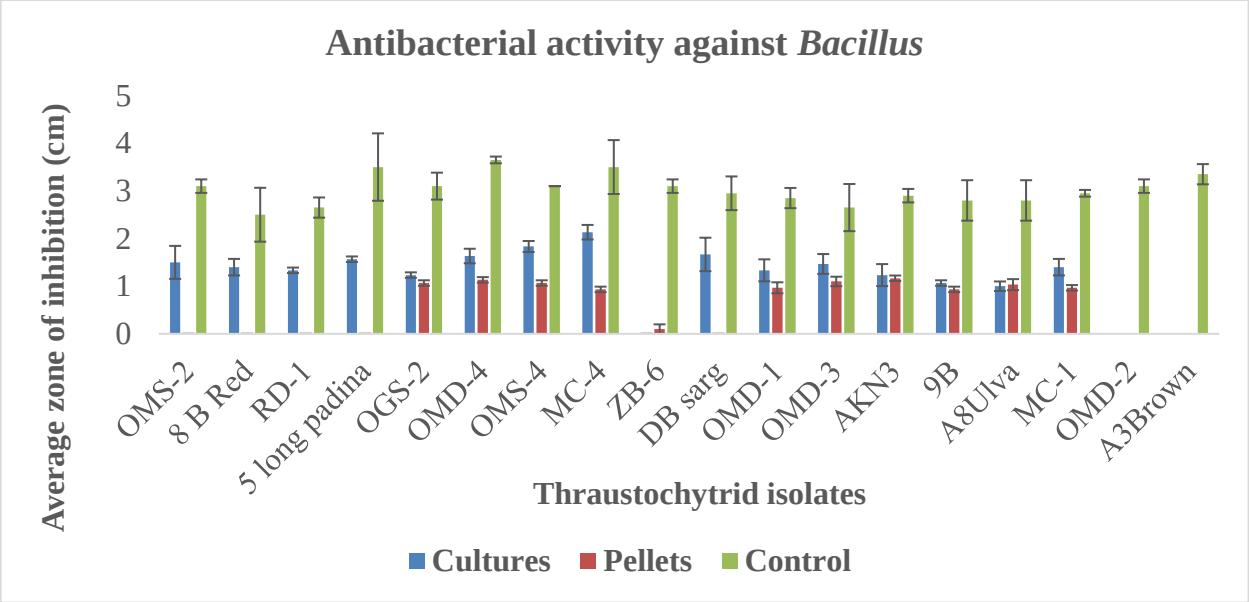

Fig. 4.5. Antibacterial activity of 18 thraustochytrid isolates against *Bacillus* sp. The blue bar represents the activity shown by culture supernatant, the red bar represents the activity shown by cell pellet, and the green bar represents the activity shown by chloramphenicol.

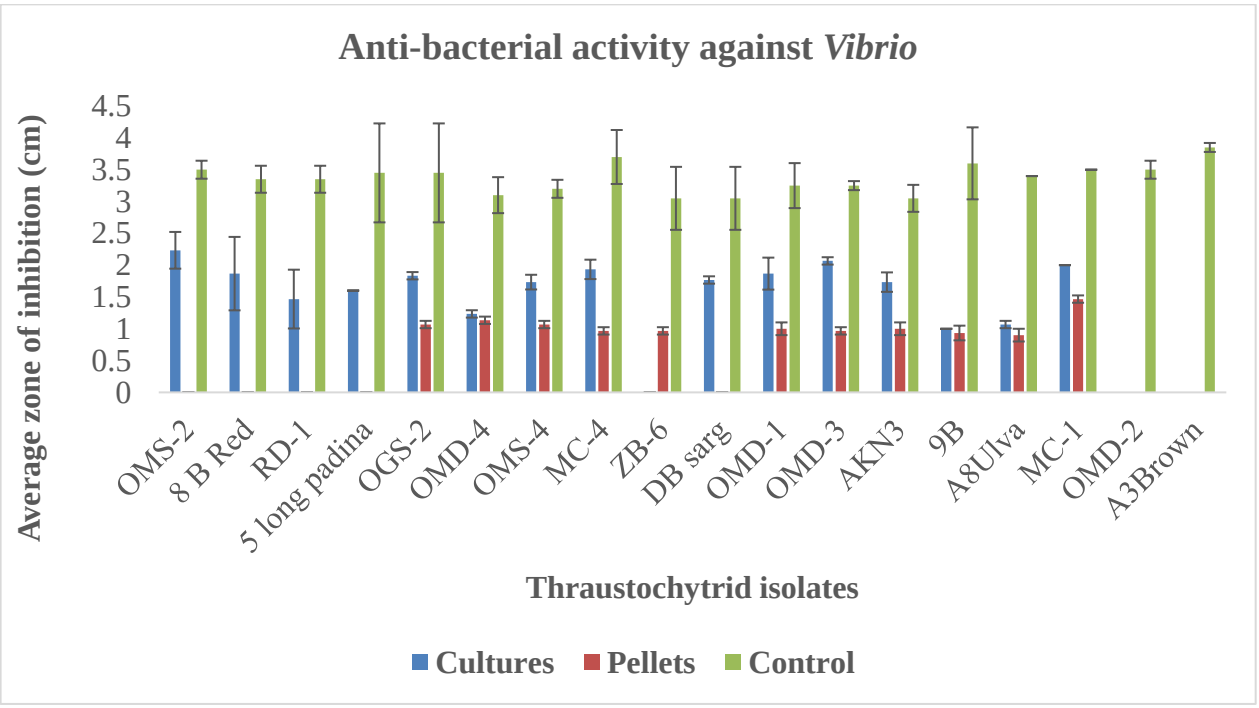

Fig. 4.6. Antibacterial activity of 18 thraustochytrid isolates against *Vibrio* sp.

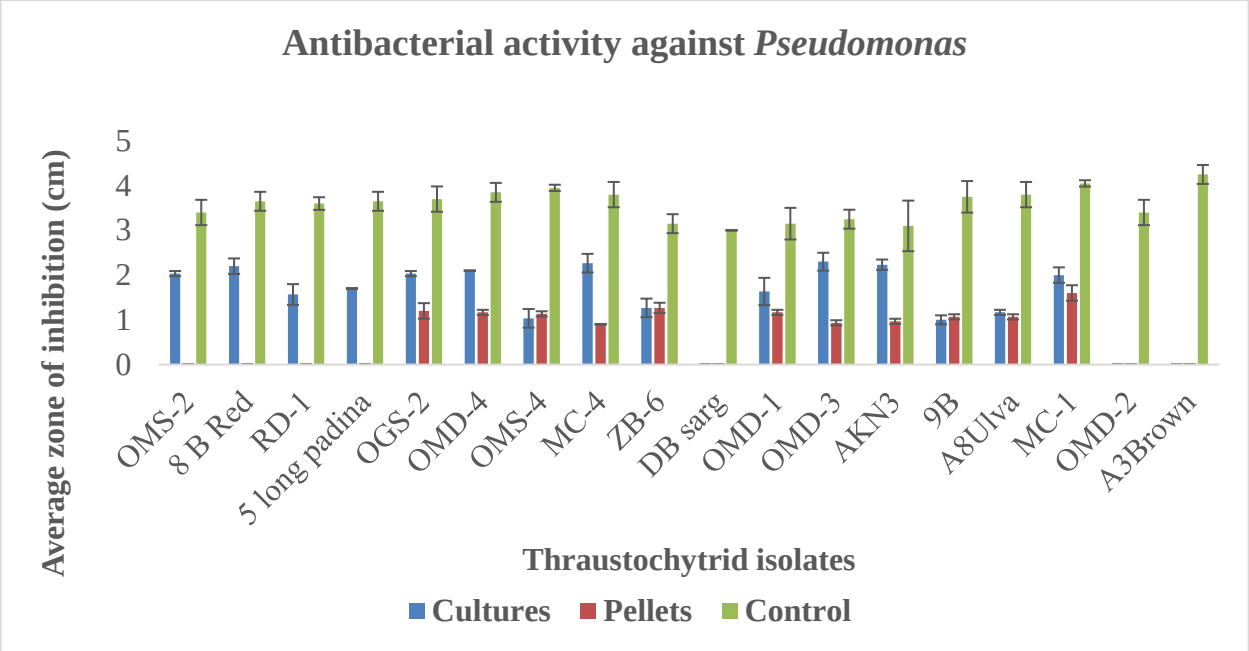

Fig. 4.7. Antibacterial activity of 18 thraustochytrid isolate against *Pseudomonas* sp. The blue bar represents the activity shown by culture supernatant, the red bar represents the activity shown by cell pellet, and the green bar represents the activity shown by chloramphenicol.

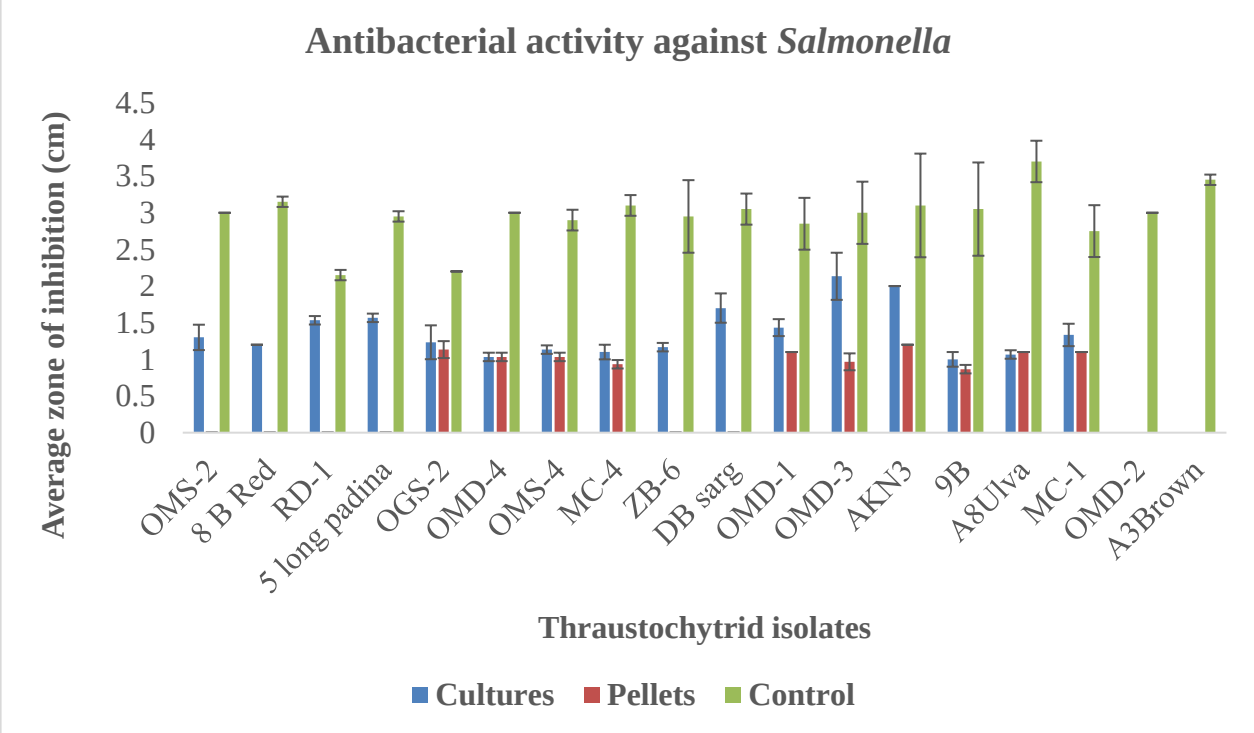

Fig. 4.8. Antibacterial activity of 18 thraustochytrid isolates against *Salmonella* sp.

The control flasks without any sodium benzoate showed the best growth and, hence, maximum biomass (0.0132 g/ml), followed by the flasks containing 0.1 % sodium benzoate. Biomass in the presence of 0.5 % sodium benzoate was the least.

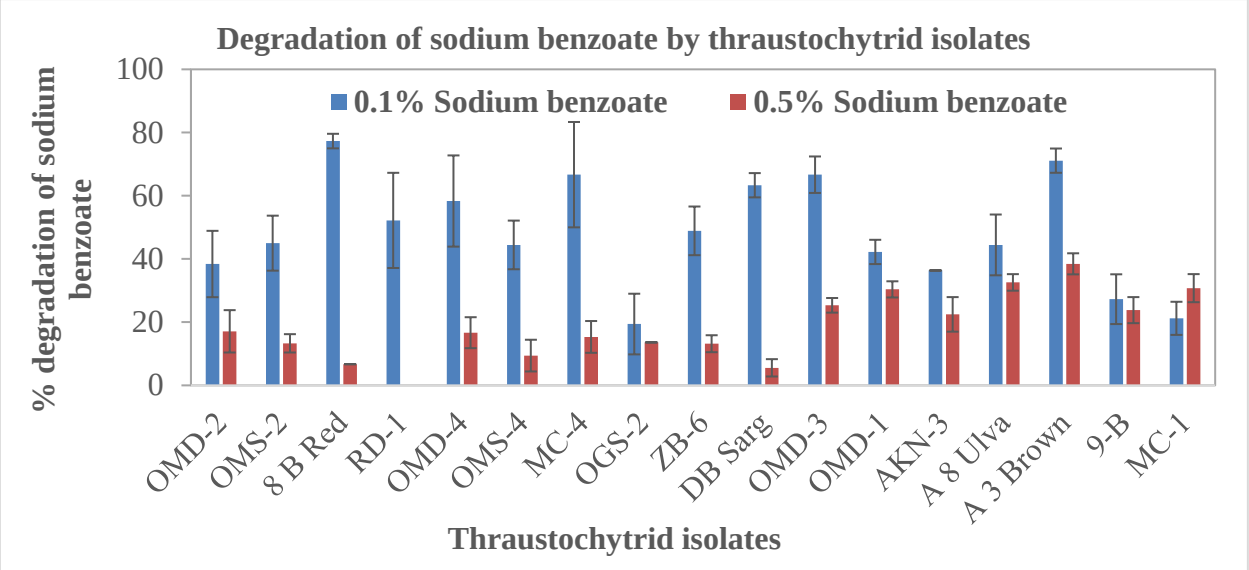

Fig. 4.9. Degradation of sodium benzoate by thraustochytrid isolate

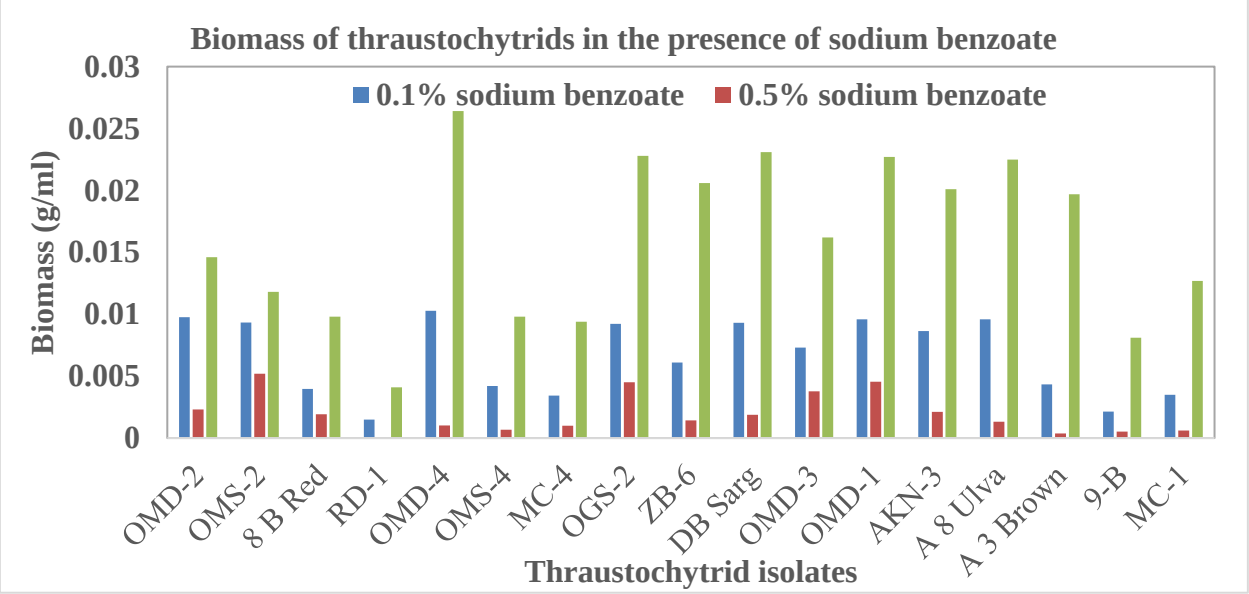

Fig. 4.10. Biomass of thraustochytrids in the presence of 0.1% and 0.5% sodium benzoate.

Degradation of naphthalene:-

FC assay: OMD-4, OGS-2 and ZB-6 isolates showed blue colouration by Folin Ciocalteu assay in the presence of naphthalene, which indicates that they have degraded naphthalene after 7 days of incubation at room temperature **(Fig. 4.11)**. Flasks without naphthalene did not produce blue colour indicating no biosurfactant production in the absence of hydrocarbon.

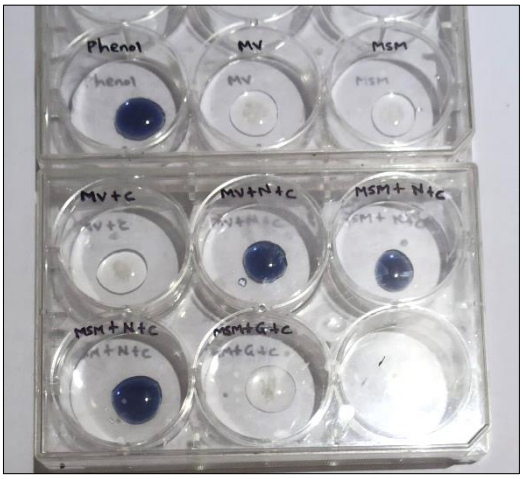

Fig. 4.11. Folin Ciocalteu assay of OMD-4 isolate.

Microbial adhesion to hydrocarbon (MATH): Highest cell adherence was observed with ZB-6 (88.47 %) followed by OMD-4 (87.31 %) and OGS-2 (87.29 %). Positive cell hydrophobicity indicated biosurfactants production.

Emulsification Index: Low emulsification index was observed for all the three isolates (OMD-4, OGS-2 and ZB-6) in MSM with naphthalene, indicating the production of biosurfactant that breaks down the emulsion. (**Figs. 4.12** and **4.13**).

Oil spreading assay: All the three isolates gave positive results i.e. oil spreading with a clearance zone of 1.5-1.8mm for the culture supernatant grown in the presence of naphthalene **(Fig. 4.14)**.

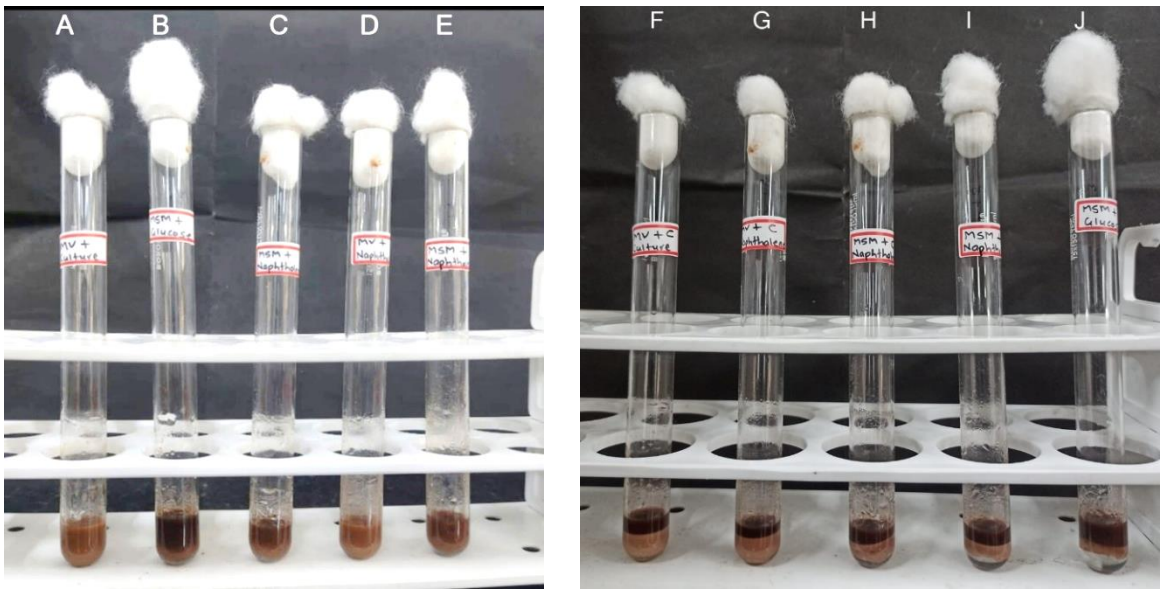

Fig. 4.12. Tubes containing culture supernatant and crude oil for estimation of emulsification index.

A: MV + culture after vortex.

B: MV + culture + naphthalene after vortex

C: MSM + culture + naphthalene after vortex

D: MSM + culture + naphthalene after vortex

E: MSM + culture + naphthalene after vortex

F: MV + culture after 24 h

G: MV + culture + naphthalene after 24 h

H: MSM + culture + naphthalene after 24 h

I: MSM + culture + naphthalene after 24 h

J: MSM + culture + glucose after 24 h

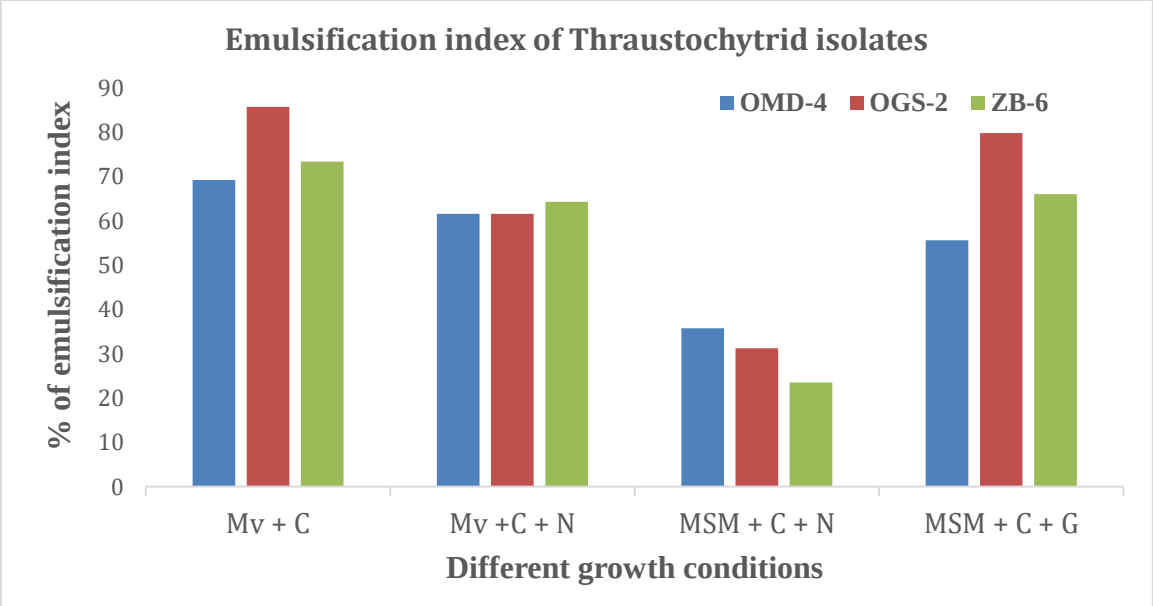

Fig. 4.13. Emulsification index of thraustochytrids isolates

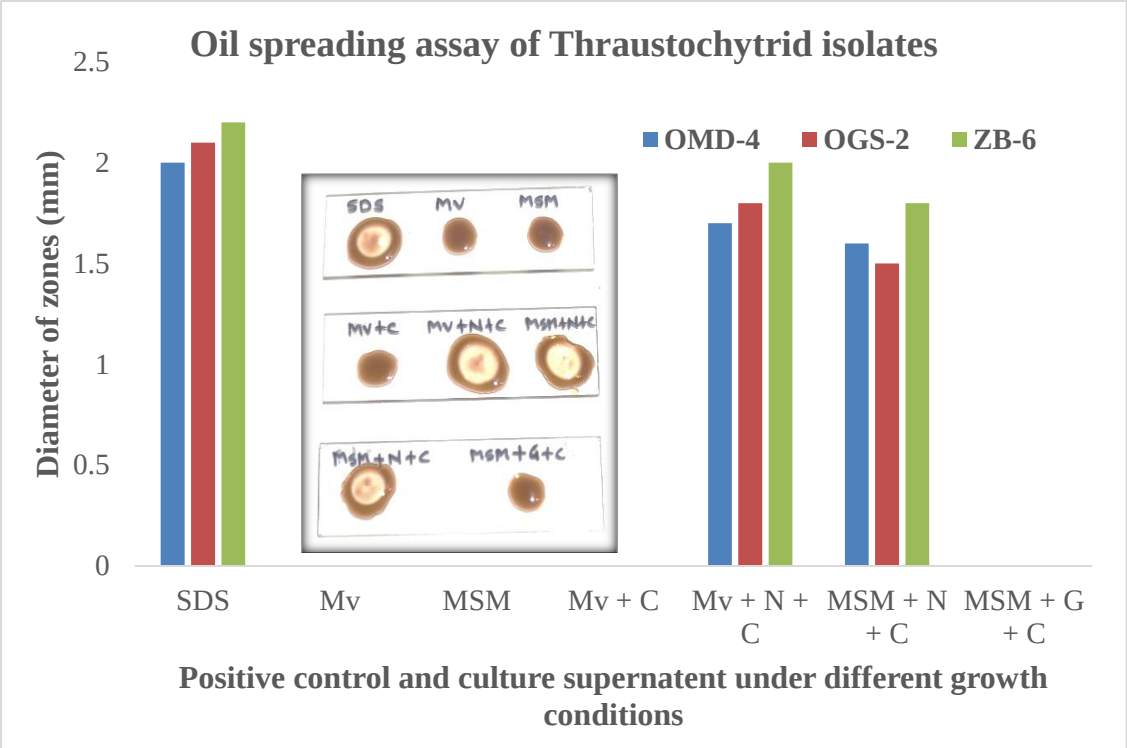

Fig.4.14. Oil spreading assay of thraustochytrids isolates. The picture inserted within the figure represents the same for the isolate OMD-4. (N=naphthalene, C=culture, G=glucose)

Drop collapse assay: All three isolates showed flattening of the drop when grown in the presence of naphthalene, a respective is shown in **Fig 4.15**.

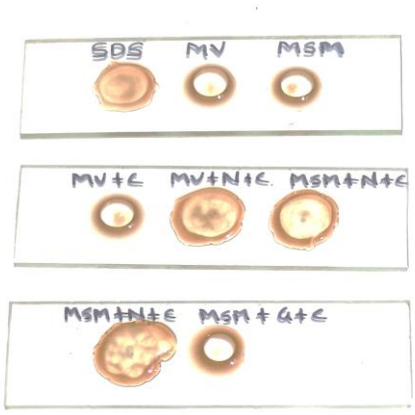

Fig. 4.15. Drop collapse assay of Thraustochytrid isolates

Biomass determination: All the isolates grew well in the presence of naphthalene but showed better growth in its absence (**Fig. 4.16**).

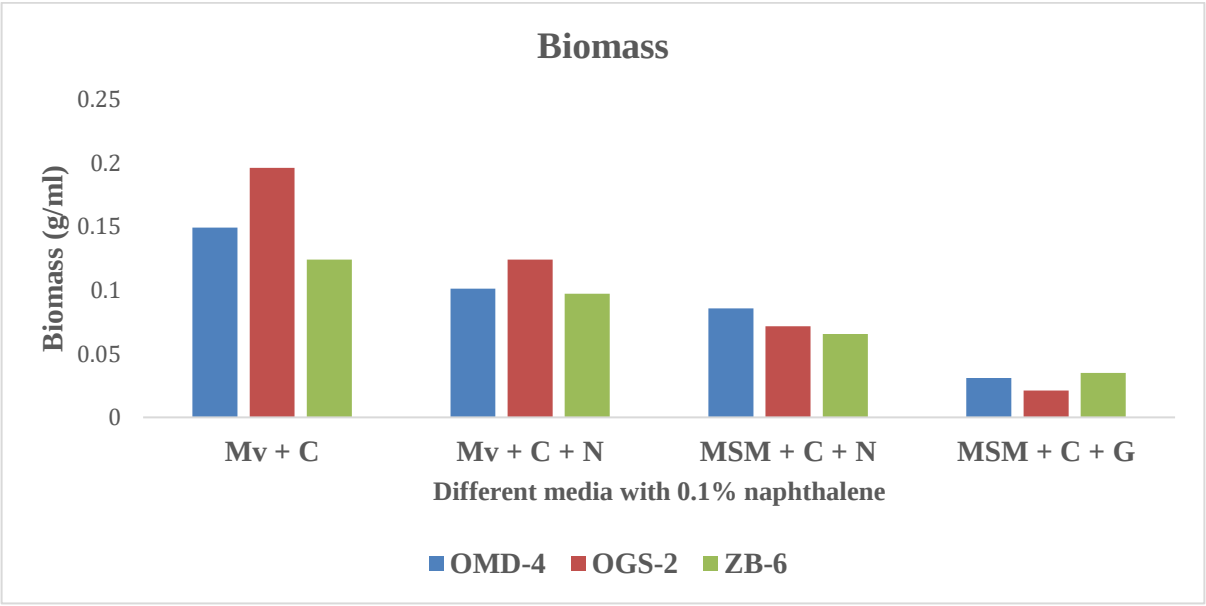

Fig. 4.16. Biomass of Thraustochytrid isolates

Degradation of crude oil: ZB-6 showed maximum ability to utilize crude oil, giving the highest percentage degradation of 75.95% followed by OMD-4 (71.31%) and OGS-2 (62.79%) (**Fig. 4.17**). This was correlating with the biomass (**Fig. 4.18**).

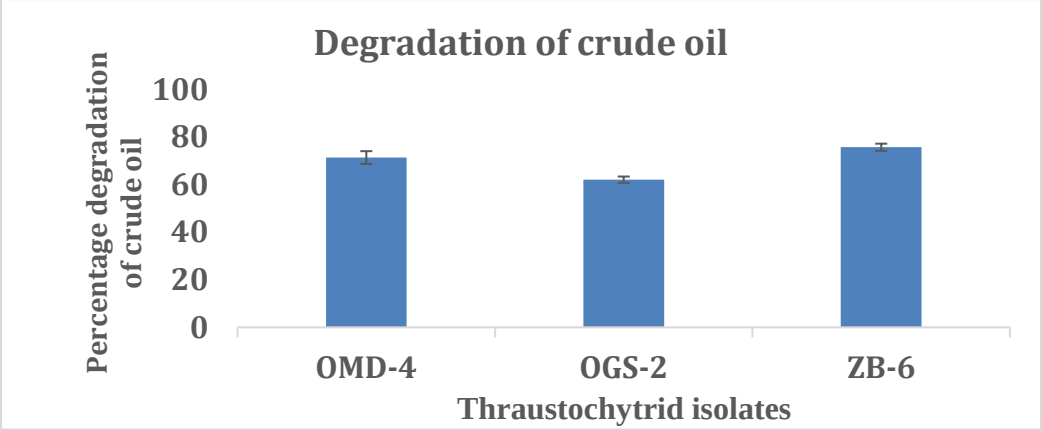

Fig. 4.17. Degradation of crude oil by thraustochytrid isolates.

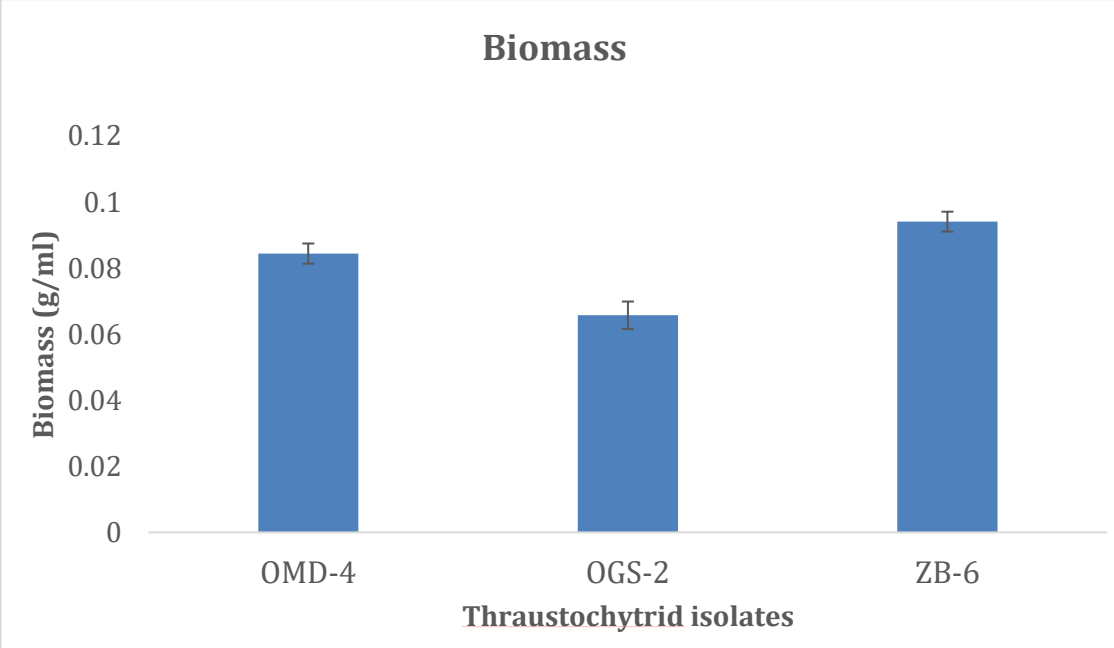

Fig. 4.18. Biomass of Thraustochytrid isolates in the presence of crude oil.

DISCUSSION

Thraustochytrids are unicellular oleaginous protists, classified based on morphological characteristics. They along with other saprophytic microbes, are found in mangrove leaf litter (Kalidasan et al., 2019). These organisms have several biotechnological applications as revealed by several studies (Raghukumar, 2008; Marchan et al., 2018). Bioprospecting of these organisms for several applications has helped reveal their significance in industry. Bioprospecting is the systematic exploration of natural biochemical and genetic information to create commercially valuable products for various industries (Raaijmakers et al. 2010).

Thraustochytrids produce metabolites that exhibit promising antimicrobial properties, aiding in antibiotic drug discovery. However, only a few reports are available on the antimicrobial activity of thraustochytrids (Vu et al., 2022; Kalidasan et al., 2021; Kalidasan et al., 2015). Thraustochytrids are part of microbial loop and they are the decomposer in the marine environment. They probably compete with other decomposers like bacteria for nutrients and in order to achieve this they probably possess antimicrobial activity. This is supported by results in the present study. Out of the 18 thraustochytrid isolates, 16 showed antibacterial activity against various bacteria tested. Another aspect in bioprospecting is the potential of thraustochytrids to be used for bioremediation purposes. One such bioremediation application can be remediation of hydrocarbons from polluted environment. (Bahnweg et al. 1979) showed that thraustochytrids have the capability to utilize a wide range of organic nitrogen and carbon compounds for their nutrition. However, the capability of these protists to degrade hydrocarbons has been examined only once (Raikar et al., 2001). So, this present study investigated how different isolates of thraustochytrids break down hydrocarbons.

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental contaminants, primarily produced by natural combustion processes and human activities. They accumulate in marine environments, particularly coastal sediments near urban and industrial cities (Nayarisseri et al. 2018). The present study found OMD-4, OGS-2, and ZB-6 isolates degraded naphthalene after 7 days of incubation at room temperature. This was confirmed by the blue colour in the FC assay which was not seen in the absence of naphthalene.

Biosurfactants, amphiphilic compounds with hydrophilic and hydrophobic moiety, can reduce interfacial and surface tensions, making them potential for improving oil recovery in microbial cell surfaces (Ongena and Jacques 2008). Microbial cell adherence assay is used to test cell wall hydrophobicity. The positive strains indicate the affinity of cells towards hydrophobic substrate. The study found that ZB-6 had the highest cell adherence of 88.47%, followed by OMD-4 at 87.31% and OGS-2 at 87.29%, indicating the production of biosurfactant.

Lower the emulsification index is good because it indicates that isolates are producing biosurfactants, and biosurfactants reduce the emulsion formation or break down the emulsion. The emulsification index was low in MSM with naphthalene because the isolates is using naphthalene as C substrate for growth in MSM, but the emulsification index was greater in MV with naphthalene because in MV, the isolates might be utilizing organic substrates present in MV instead of naphthalene for growth. The study found that all three isolates (OMD-4, OGS-2, and ZB-6) had a low emulsification index in MSM with naphthalene, indicating the production of a biosurfactant.

The oil displacement area is directly proportional to the surface-active compound in the solution. However, in this study only the qualitative study to check the presence of surfactant

was carried out. The present study found that all three isolates showed positive results in oil spreading with a clearance zone of 1.5-1.8 mm for culture supernatant grown in naphthalene presence. OMD-4, OGS-2 and ZB-6 gave positive results with MATH assay and drop collapse assay. Adherence of thraustochytrid cells to hydrocarbon can be attributed to their cell wall hydrophobicity (Raiker et al. 2001). The present study found that three isolates exhibited drop flattening when grown in the presence of naphthalene. This further confirms that all three isolates are producing biosurfactants. Thus, they grow in the presence of hydrocarbon by utilizing it and producing biomass, though it was lesser than that seen in its absence. These organisms can therefore be used for bioremediation. Bioremediation produces non-toxic, safer products like carbon dioxide and water, making it an eco-friendly, practical, and economical solution for contaminated sites. The present study found that isolate ZB-6 demonstrated the highest degradation percentage of 75.95% for crude oil utilization, followed by OMD-4 (71.31%) and OGS-2 (62.79%) as seen by gravimetry analysis. This correlated with their biomass during the experiment.

CONCLUSION

Thraustochytrids not only exhibit antimicrobial activity but also possess the remarkable ability to degrade hydrocarbons. This means that they have the potential to be used in various applications, such as combating microbial infections and aiding in the cleanup of oil spills or other hydrocarbon-contaminated environments. With their dual capabilities, thraustochytrids offer a promising avenue for both medical and environmental advancements.

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APPENDIX

1. Nutrient agar

Ingredients	Grams/Liter
Peptone	5.000 g
Sodium chloride	5.000 g
HM peptone B#	1.500 g
Yeast extract	1.500 g
Agar	15.000 g
Final pH (at 25°)	7.4±0.2

2. Nutrient broth

Ingredients	Grams/Liter
Peptone	10.000 g
Beef extract	10.000 g
Sodium chloride	5.000 g
pH after sterilization	7.3±0.1

3. Minimal salt media

Ingredients	Grams/Liter
Dipotassium hydrogen phosphate	0.173 g
Potassium dihydrogen phosphate	0.068 g
Magnesium sulfate heptahydrate	0.01 g
Sodium chloride	0.4 g
Ferrous sulfate heptahydrate	0.003 g

Ammonium nitrate	0.1 g
Calcium chloride	0.002 g

4. Bushnell Haas broth

Ingredients	Grams/Liter
Magnesium sulphate	0.200 g
Calcium chloride	0.020 g
Monopotassium phosphate	1.000 g
Dipotassium phosphate	1.000 g
Ammonium nitrate	1.000 g
Ferric chloride	0.050 g
pH after sterilization	7.3±0.1

5. Modified Vishniac

Ingredients	Grams/Liter
Liver infusion powder	0.001 g
Yeast extract	0.01 g
Peptone	0.15 g
Dextrose	0.4 g

6. Phosphate buffer

Ingredients	Grams/Liter
Dipotassium hydrogen phosphate	87.09 g
Potassium dihydrogen phosphate	68.045 g