Studies on Plastic-Associated Marine Fungi from Mangrove Environment

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

ANUSHKA ANAND NAIK

Seat Number : 22P039003

ABC ID: 783741446803

PRN : 201905757

Under the Supervision of

DR. PRIYA M. D'COSTA

School of Earth, Ocean and Atmospheric Sciences

Marine Microbiology

GOA UNIVERSITY

MAY 2024

Examined by:

Seal of the School

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **"Studies On Plastic-Associated Marine Fungi From Mangrove Environment"** is based on the results of investigations carried out by me in the Marine Microbiology Discipline at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the Supervision of Dr Priya M. D'Costa. 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 / College will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

I hereby authorize the University/college authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Anushka Anand Naik

22P0390003

Date: 02/04/2024

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report " **Studies On Plastic-Associated Marine Fungi From Mangrove Environment** " is a bonafide work carried out by **Ms Anushka A. Naik** 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.

Date: 02/04/2024

Dr. Priya M. D'Costa
Marine Microbiology
School of Earth, Ocean and Atmospheric Sciences

31/7/24

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

SEOAS, GOA UNIVERSITY TALEIGAO PLATEAU GOA

School Stamp

CONTENTS

Chapter	Particulars	Page numbers
	Preface	i
	Acknowledgments	ii
	Tables and Figures	iii
	Abbreviations used	iv
	Abstract	v
1.	Introduction	1-5
	1.1 Background	
	1.2 Aim and Objectives	
	1.3 Hypotheses/ Research question	
	1.4 Scope	
2.	Literature Review	6-16
3.	Methodology	17-22
4.	Analysis and Conclusions	23-44
	References	45-51
	Appendix I	52
	Appendix II	59

PREFACE

Plastic pollution is an emerging concern for several decades. There are many organisms recent noticed on plastic called Plastisphere, which are found attached to plastics and not just bacteria but eukaryotic organisms like fungi are found attached to plastic marine debris. The research carried out for dissertation titled "**Studies On Plastic-Associated Marine Fungi From Mangrove Environment**" focuses on two mangroves of Goa, along west coast of India and gives us an insights of fungal diversity attached to the plastic marine debris in mangrove environment and their ability to produce enzymes namely Lipases and Laccases. This study would help in devising strategies for fungal-mediated remediation of plastic waste.

ACKNOWLEDGEMENTS

Firstly, I would like to thank God for his omnipotent presence and grace during the course of the completion of this project. I would like to thank Sr. Prof. Sanjeev C. Ghadi, Dean and Dr. Varada S. Damare, Programme Director, Marine Microbiology, School of Earth, Ocean and Atmospheric Sciences, for giving me the opportunity to carry out this project as well as providing me with all the necessary facilities I required. I am also profoundly thankful to my project guide, Dr. Priya M. D'Costa, Assistant Professor, School of Earth, Ocean and Atmospheric Sciences, for her guidance and overwhelming encouragement which has helped me throughout this project and without her help this project would not have been a success. I would like to thank Dr. Nikita Lotlikar and Dr. Varada s. Damare, Assistant Professor, School of Earth, Ocean and Atmospheric Sciences for their valuable inputs and suggestions regarding culturing and identification of fungi that helped me in the course of this project. I am also grateful to Ms. Vaishali Merchant, laboratory assisstant, Mr. Rohan, laboratory attendants as well as Ms. Imrana Shaikh, PhD research scholar and the entire staff of the department of Marine Microbiology, for all their help and assistance throughout the year. I also extend my gratitude to Dr. Samir Damare, Principle Scientist, National Institute of Oceanography (NIO), Goa, for his assistance in providing me necessary reagents that were required for my practical work. I thank Mr. Madhusudan Lanjewar, Technical Officer, Instrumentation Center, Goa University, for carrying out the SEM analysis of the required cultures.

I deeply thank my family for their moral and economic support throughout this journey. Last but not the least, I would like to thank all my friends and classmates for their encouragement and support in making all this possible.

TABLES

Table No.	Description	Page No.
4.1	Selected location sites and no. of fungal isolates recovered from there	23
4.2	Parameters measured at study sites	23
4.3	Details of plastisphere fungal isolates from Ribandar mangroves	25
4.4	Details of plastisphere fungal isolates from Chorao mangroves	26
4.5	Details of fungal isolates from plastic marine debris from Ribandar mangroves	33
4.6	Details of fungal isolates from plastic marine debris from Chorao mangroves	34
4.7	Enzymes detected in plastic-associated fungal isolates from Ribandar	35
4.8	Enzymes detected in plastic-associated fungal isolates from Ribandar	36

FIGURES

Figure No.	Description	Page No.
3.1	Ribandar Mangroves	17
3.2	Chorao Mangroves	17
3.3	Sample collection from Ribandar mangroves	18
3.4	Sample collection from Chorao mangroves	19
4.1	Fungal isolation growing attached to plastic samples	24
4.2	Number of fungal isolates on different media	24
4.3	Morphological diversity of plastic-associated fungal colonies from Ribandar mangroves	27
4.4	Morphological diversity of plastic-associated fungal colonies from Chorao mangroves	28
4.5	Microscopic images of fungal isolates from Ribandar mangroves	29-30
4.6	Microscopic images of fungal isolates from Chorao mangroves	31-32
4.7	Fungal isolates showing positive results for Lipase activity	37-39
4.8	Scanning Electron Micrograph of fungal isolates on plastic	40-41

ABBREVIATIONS USED

Entity	Abbreviation
Room Temperature	RT
Sabourauds Dextrose Agar	SDA
Potato Dextrose Agar	PDA
Czapek Dox Agar	CDA
Malt Extract Agar	MEA
Degree celsius	°C

ABSTRACT

Plastic pollution, often referred to as 'white pollution', is recognized as a global concern in present times. Plastics are notorious for their recalcitrance to microbial breakdown; this is attributed to them being high molecular weight, hydrophobic, long-chain molecules with only a single functional group. Yet, intriguingly, plastics offer an excellent attachment substrate to a wide range of organisms, including bacteria, fungi, algae, etc., collectively called the 'plastisphere'. This study explores the diversity of plastisphere fungi from Ribandar and Chorao mangroves in Goa, along the west coast of India. A range of media (Czapek Dox Agar, 2% Malt Extract Agar, Potato Dextrose Agar, Sabouraud's Dextrose Agar) were used to detect fungi associated with plastic debris. Total 56 fungal isolates were recorded. Interestingly, the most common fungus attached to the Ribandar plastic debris samples was the filamentous fungus –*Aspergillus sp.* And *Circinella sp*. were also observed. Chorao samples showed the presence of *Aspergillus sp., Fusarium sp., Penicillium sp., Cladosporium sp. and Circinella sp.* This study further examines the fungal isolates which are having the ability to produce enzymes which are involved in the degradation of plastic polymers such as Lipases and Laccases. SEM analysis further confirmed the presence of branched fungal hyphae on the surface of plastics. Further studies on these fungal enzymes involved in degradation of plastic polymers would point the way to commercialization of the technology involved, and lead to potentially effective remediation strategies for plastic waste.

KEYWORDS

Plastic marine debris, Plastisphere, Fungal diversity, Mangroves, Enzymes, *Aspergillus*

CHAPTER 1: INTRODUCTION

1.1 Background

Plastic products are widely used, and the composition of the plastic polymers employed in them varies according to the intended uses of the objects that they are used to produce. Polyethylene terephthalate (PET), low-density polyethylene (LDPE), and high-density polyethylene (HDPE) are examples of common plastics (Plastics Europe, 2021). Roughly 367 million tonnes of plastic goods were manufactured as of 2020. Since the 1990s, there has been a significant growth in the volume of plastic produced (Plastics Europe, 2021). However, according to Geyer et al. (2017), only around 9% of total plastic trash is recycled, with the remaining 60% ending up as litter in terrestrial habitats or buried in landfills.

Premium benefits. The majority of marine plastic debris is made up of improperly disposed-of land-based plastic waste that has found its way into the oceans by rivers, tides, wind, and wastewater outflows. Land-based sources accounted for 4–12 million tonnes of marine plastic debris in 2010 (Jambeck et al. 2015). Furthermore, rivers were the source of 1.15–2.41 million tonnes of marine plastic debris (Lebreton et al. 2017).

Marine ecosystems have suffered both directly and indirectly by the buildup of plastic garbage. Marine species that swallow plastic fragments suffer internal organ damage (Ahrendt et al. 2020; Wright et al. 2013). Additionally, plastic wastes that have broken down into tiny fragments and fibres are known as microplastics, and they are produced by weathering, wave action, and other processes (Thompson et al. 2004). This poses additional risks. Microplastics float on ocean surfaces and contain Persistent Organic

Pollutants (POPs), which are endocrine disruptors that hinder the survival rate of marine organisms (Rios et al. 2007). Microplastics also transport microbial pathogens and alien species, hazardous to marine ecosystems in other regions (Arias-Andres et al. 2018; Beloe et al. 2022; Bowley et al. 2021). Thus, plastic in marine ecosystems has become a serious environmental issue. However, there are no policies or solutions in place that effectively mitigate the plastic waste problem. Recent studies have explored chemical degradation, recycling, and biodegradation as potential marine plastic waste remediation measures.

Much research attention has been directed toward plastic waste degradation by microorganisms. Plastic waste has persisted in natural environments for decades. Plastic debris that is inhabited and partially decomposed by the microbial community is now referred to as the "plastisphere" (Zettler et al. 2013) and numerous different microorganisms are found in it (Hirota et al. 2021; Amaral-Zettler et al. 2020). Some of them were reported to degrade plastic by various kinds of tests (Badahit et al. 2018; Sangeetha Devi et al. 2019; Hou et al. 2022; Kumari et al. 2019; Muhonja et al. 2018; Yamada-Onodera et al. 2001): and enzymatic activities involved in plastic degradation have been investigated extensively (Temporiti et al. 2022). Fungi comprise only about 3% of all eukaryotic organisms in the plastisphere, although they play a vital role as decomposers in the environment (Rogers et al., 2020). Numerous plastic-degrading fungi have been detected and identified in the landfill (terrestrial) plastisphere including Aspergillus spp. (Cosgrove et al. 2007; Muhonja et al. 2018; Zahra et al. 2010), Fusarium spp. (Kanelli et al. 2015; Zahra et al. 2010), and *Penicillium simplissimum* (Yamada-

Onodera et al. 2001). Previous studies on plastic-degrading fungi in marine environments concentrated primarily on several specific taxa such as *Aspergillus sp*. (Sarkhel et al. 2020) and *Zalerion maritimum* (Paço et al. 2017).

The polymers are used by microbes as their main source of carbon as they attach to the polymer's surface and keep multiplying. Next is the depolymerization process known as "biofragmentation," extracellular enzymes and bacterially generated free radicals catalyze the breakdown of biodegraded polymers into smaller pieces. Next step is assimilation in which the biofragmented smaller molecular weight compounds are then transported into the microbial cytoplasm. The last step is mineralization, which involves the successful delivery of these plastic derivatives into cells and a sequence of enzymatic reactions that cause them to completely decompose into oxidized metabolites including CO2, N2, CH4, and H2O. Numerous enzyme activities, including peroxidases, lipases, esterase, cutinase, and laccase, are necessary for the complete mineralization process (Varshney et al.,2023). Hydrolases, mainly esterase, cutinase, lipase, and protease can hydrolyse the plastic polymer into simpler carbon molecules that can be absorbed into the cell of fungi for metabolism (Ghosh et al., 2019). Whereas the secreted enzymes break down the carbon chains and weaken plastic polymers by creating cracks, pits, and weak points in the structure, the mycelial hyphae permeate the broken sites and colonize the detached parts thus allowing it to augment the degradation process by exerting mechanical pressure onto these broken compartments as it releases more degrading enzymes (Hyde et al., 2019).

In the past, (Lee et al. 2019; Park et al. 2016) have isolated diverse fungi from a range of substrates found in marine habitats, including microalgae and sailfin eggs. High enzymatic activity was present in many of these fungi (Lee et al., 2019; Park et al. 2015a, 2019). It was anticipated that fungi isolated from plastic would be able to break down plastic substrates because these investigations showed that fungi could break down complicated organic substances. Numerous fungi were found to be able to thrive on plastics that had been taken from the sea floor (De Tender et al. 2017) and seawater (Lacerda et al. 2020, Davidov et al. 2020), according to a metabarcoding investigation.

1.2 Aim and Objectives

Aim

To examine the variety of fungal species associated with plastic debris collected from mangroves along the west coast of India and determine their potential to produce enzymes involved in plastic degradation.

Objectives

1. To isolate and identify fungi associated with plastic debris from mangroves.
2. To analyze the ability of fungal isolates to produce enzymes involved in plastic degradation.

1.3 Hypothesis

According to our hypothesis, a variety of fungus can live in plastic trash, and the majority of them actively contribute to the decomposition of plastic.

1.4 Scope

The results of the work provide baseline data about the Diversity of Marine fungi associated with plastic from Mangrove Environment, and their potential role in the enzymatic degradation of plastic polymers.

CHAPTER 2: LITERATURE REVIEW

In order to characterize the varied microbial population adhered to plastic surfaces and differentiable from the environment, Zettler et al., (2013) coined the term "plastisphere." The most popular primary approaches for studying the plastisphere are environmental sampling and laboratory incubation techniques. However, because sampling is expensive and challenging, there aren't many reports on benthic aquatic ecosystems. Studies using artificial laboratory setups to incubate polymers have been carried out. Such tests are often suitable for investigating the enzymatic activities and degradation potential of microorganisms. Prior research on the plastisphere employed scanning electron microscopy (SEM) to identify distinct species morphologically.

Early scanning electron micrographs of biofilms developed on plastics have reported on the population diversity of bacteria within the plastisphere (Stanier et al.,1975). According to Flemming et al., (2016) "biofilms" are defined as collections of cells that are either adhered to or detached from a substrate and develop inside a matrix made of extracellular polymeric substances (EPS). A certain group of genes involved in adhesion, chemotaxis, communication, and substrate transport are used during the creation of biofilms. This process allows for the formation of matrix and fluorescent channels, which are used to transfer nutrients between cells (Dey et al.,2022).

Any substance that ends up in the water, whether it is man-made or natural (like plastic litter), is quickly colonized by microorganisms. From a microbiological standpoint, there are benefits to switching from a pelagic to a particle-attached lifestyle, such as improved nutrition availability, defense against UV rays and protection from grazing (de Carvalho,

2018). The makeup of microbial communities that reside on plastic materials in various marine environments has been the subject of numerous studies (Zettler et al., 2013; Amaral-Zettler et al., 2015; Eich et al., 2015; Oberbeckmann et al., 2016; Debroas et al., 2017; Dussud et al., 2018; Ogonowski et al., 2018; Miao et al., 2019; Dudek et al., 2020; Krause et al., 2020; Vaksmaa et al., 2021b).

Similar to other biofilms, EPS (extracellular polymeric substances) secretion, adhesion, and proliferation are the typical steps in the creation of a plastisphere (Zettler et al.,2013). In the early phases of colonization, plastics' physical characteristics are crucial for selection, and the choices made by these pioneers later influence the choices made by other populations within the plastisphere (Rummel et al.,2017). Numerous environmental parameters, including temperature, pH, nutrient availability, and hydrodynamic conditions, have an impact on microbial adhesion (Oder et al.,2017). These factors have the ability to change the MP surface's physicochemical characteristics, including charge, hydrophobicity, and texture (Pagán et al.,2015). If flow velocity, water temperature, or nutrient concentration do not exceed threshold values, these factors can enhance microbial adhesion in aquatic environments (Prakash et al.,2003).

When comparing the bacteria in biofilms on plastics to those in the surrounding saltwater or those affixed to natural surfaces, a few of them observed a variation in community organisation. Wright et al., (2020) compilation provides a thorough synopsis of this issue. The majority of research, however, used amplicon sequencing of the 16S rRNA gene and concentrated on the communities of bacteria and archaea that were adhering to the plastic waste in biofilms. However, there hasn't been much research done on the composition of fungi on plastic in the marine environment up until now.

The very small number of studies focusing on fungi on marine plastic debris (MPD) is indicative of the general under researched status of marine fungi (Jacquin et al., 2019). A seasonal comparison of microbial communities, comprising both bacteria and fungi, on submerged PET bottles, glass slides, and seawater in the North Sea was carried out by Oberbeckmann et al. (2016). Three taxa that represent fungal communities were Ascomycota, Basidiomycota, and Chytridiomycota. According to the study, the PET-attached eukaryotic communities—fungi included—varied greatly depending on the time of year and the region.

Kettner et al. (2017, 2019) studied fungal colonization of PE and PS plastic surfaces by conducting exposure experiments in the Baltic Sea and the Warnow River. On both plastic types at both locations, the fungus genus *Chytridium* and fungal-like *Rhinosporideacae, Rhizidiomyces*, and *Pythium* taxa exhibited a high read count. Nonetheless, a sizable portion of the genomes were identified as unclassified fungus. Location had a substantial impact on the composition of the fungal community, while polymer type had little effect. In addition, PE and PS had far less alpha diversity than the nearby water and wood particles.

PE plastic sheets and dolly ropes were weighed down near to the sediment at a harbour and an offshore location in the North Sea throughout the course of a 44-week experiment by De Tender et al. (2017). In line with research conducted in the Baltic Sea by Kettner et al. (2017, 2019), they discovered that Ascomycota were the most prevalent group, with a lesser proportion of Basidiomycota and Mucoromycota. Additionally, *Physconia, Candelidella*, and *Caloplaca*—three minor fractions of Ascomycota members belonging

to the *Lecanoromycetes* class—were identified. Research on the beta diversity of the composition of the fungal community revealed statistically significant impacts of exposure period, environment, and sample type (natural substrate vs. plastic polymers). A few of the species found have been recognised as possible degraders of PE in terrestrial settings before: *Fusarium redolens* and *Cladosporium cladosporioides* (Bonhomme et al., 2003; Albertsson, 1980; Albertsson and Karlsson, 1990).

Synthetic substances such as insecticides (Pinto et al., 2012), polycyclic aromatic hydrocarbons (PAHs) (Cerniglia and Sutherland, 2001), persistent organic pollutants (POPs) (Singleton, 2001), and BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) (Buswell, 2001) can all be broken down by fungi. The capacity of fungi to break down complex substances and their metabolic diversity suggest that some fungi may have the ability to biodegrade plastics in the environment (Vaksmaa et al., 2021).

Aspergillus, *Fusarium*, and *Penicillium* are also members of the Ascomycete phylum, which includes some of the plastic-degrading fungi that have been identified thus far ((Tachibana et al., 2010; Raghavendra et al., 2016). Furthermore, several strains belonging to *Penicillium* are considered as potential plastic degraders. *P. chrysogenum, P. oxalicum, P. simplicissimum* isolated from soil (Yamada-Onodera et al., 2001; Sowmya et al.,2012; Ojha et al., 2017) and Penicillium sp. isolated from seawater (Alshehrei, 2017) showed potential to degrade PE. Similar to *Penicillium,* different species belonging to the genus *Aspergillus* were found to be potential plastic degraders as well.

A. flavus isolated from soil (Deepika and Madhuri, 2015) and a marine environment (Sangeetha Devi et al., 2015; Alshehrei, 2017) exhibited the potential to degrade PE and A. niger isolated from soil (Deepika and Madhuri, 2015; Raghavendra et al., 2016) and seawater (Alshehrei, 2017) showed potential to degrade PE and PU. Also, *A. terreus* isolated from soil (Zahra et al., 2010), mangrove sediments (Ameen et al., 2015; Sangale et al., 2019) and seawater (Alshehrei, 2017) were potentially able to degrade PE. In addition to these species, several other types of Aspergillus strains were considered as potential PE and PU degraders. Many of the plastic degrading species that were isolated from terrestrial environments are also found in marine habitats.

Hydrophobin-induced attachment to a plastic surfac. Prior research indicates that the adhesion of a fungal cell to the polymer surface is the first step in the breakdown of plastic. The primary mediator of the event is hydrophobin, a tiny, globular protein family with a low molecular weight (≤20 kDa) released by filamentous fungus. According to Berger and Sallada (2019), they are distinguished by eight cysteine residues arranged in a conserved array with four disulphide bridges and a typical amphipathic nature with both hydrophilic and hydrophobic portions.

They consist of 70–350 amino acids, including a signal peptide sequence that facilitates the protein's translocation across the endoplasmic reticulum before being broken down by certain peptidases (Khalesi et al., 2015). Since the proteins are essential to fungal growth and development, survival, and adaptation in all environments, they are deposited on the surface of mycelia, spores, and aerial sections of fungi (Linder et al. 2005). According to Scholtmeijer et al. (2005), they mediate the attachment of hyphae to

hydrophobic solid supports and are involved in the creation of hydrophobic aerial structures. Therefore, as demonstrated by various researchers in the case of LDPE deterioration by fungi (Santacruz-Juárez et al. 2021; Zahra et al. 2010), hydrophobins also aid in anchoring mycelia on the polymer surface, paving the way for biodegradation. This ability makes the organism superior to bacteria in the degradation of plastic (Ghatge et al. 2020). According to Han et al. (2020), the attachment stage gradually increases the hydrophilicity of the plastic film surface, facilitating subsequent microbial colonization.

Numerous extracellular and membrane-bound enzymes that are capable of rupturing the chemical bonds in plastic polymers are produced by fungi. These proteins fall into the hydrolase and oxidase classes, which are the two main groups (Atanasova et al. 2021; Ren et al. 2021). The class of enzymes known as hydrolases (EC 3.1.x) is responsible for catalysing the cleavage of chemical bonds in the presence of water, which breaks bigger molecules into smaller ones. The hydrophobic gap that is located close to the enzyme's active site can accept hydrophobic groups found in the polymer, making the enzyme more accessible to plastic (Kaushal et al. 2021). Esterases and lipases (EC 3.1.1.X), which are mostly active on aliphatic polyesters and have the ability to cleave ester bonds in the carbon chain, are two examples of hydrolases.

Extracellular enzymes secreted by fungi usually target carbon polymers derived from plants in order to get nutrients for growth (Chen et al., 2020). The hypothesis that fungi have evolved their carbohydrate-active enzyme (CAZyme) systems to allow them to utilise and thrive on refractory substances, particularly while growing under settings with inadequate carbon resources, was alluded to by Chen et al. (2020) and Hage and Rosso (2021). Hydrolytic enzymes like esterase, cutinase, lipase, protease, and urease, or

oxidative fungal enzymes like peroxidase, laccase, and monooxygenase, are typically involved in the decomposition of plastic polymers (Srikanth et al., 2022).

The breakdown of different polymers into pieces that can be absorbed and mineralized in the mycelial cells is frequently started by the oxidase and hydrolase enzymes released by fungal hyphae (Lucas et al., 2008; Urbanek et al., 2020). However, due to their compact molecular structure, polyolefins are resistant to the majority of fungal enzymes and frequently require aided breakdown through chemical and heat oxidation. However, it has been reported that oxidoreductase enzymes are produced by fungi like *Fusarium falciforme, Purpureocillum lilacinum* (Spina et al., 2021), *Zelererionmaritimum* (Paço et al., 2017), *Psychotria flavida, Humboldtia brunonis, Paecilomyces lilacinum, and Lasiodiplodia theobromae* (Sheikh et al., 2015). These enzymes allow for the depolymerization of polyolefins, primarily plastic, rubber, and PVC.

Oxidative enzymes, specifically peroxidases, laccases, and oxygenases, can aid in the hydrolysis of polyolefins (Sivan, 2011). Oxidative stress is typically formed in the presence of synthetic polymers and restricted carbon sources, resulting in the creation of reactive oxygen species (ROS), hydrogen peroxide, and oxidative enzymes. Polymer disintegration results from the enzymes' oxidation of polyolefin polymers, which alters their shape, molecular weight, tensile strength, and composition of terminal groups (Saravanan et al., 2021). Laccase and peroxidase, in contrast to fungal hydrolases, function similarly against synthetic polymers by initiating redox reactions that produce ROS, which oxidise the polymer and form radicals and parent polymer structures with terminal groups (Albertsson et al., 1987).

It was suggested that extended exposure of the polymer to the radicals and enzymes would break the parent structure, resulting in small, fragmented particles with polar functional groups. Furthermore, oxidative enzymes interact with functional groups to produce intermediate molecules that hydrolyze further into simple carbon monomers that can be absorbed (Corti et al., 2010). Ellouze and Sayadi (2016) and Iiyoshi et al. (1998) claim that there is a close relationship between the enzyme oxidation methods used by fungi to break down plastics and the catabolism of lignin. Analogous to the hydrolysis of lignin, Dăassi et al. (2016) suggested the use of laccase and peroxidase to facilitate the redox reaction-based hydrolysis of hydrophobic plastic.

Fungal species that secrete oxidase enzymes that can discolorise and break down synthetic polymers include Phanerochaete chrysosporium (Bhardwaj et al., 2013), *Penicillium simplicissimum* (Sowmya et al., 2015), *Trichoderma harzianum* (Sowmya et al., 2014), *Pleurotus ostreatus, Trametes versicolor, and Penicillium chrysogenum* (Nayanashree and Thippeswamy, 2015).

Extracellular esterase enzymes have been identified in the majority of plastic biodegradation investigations as remarkable hydrolases that facilitate the fungal degradation of polyester-based polymers (Gricajeva et al., 2022). Most fungi have esterases, which have been demonstrated to assist the fungi in locating and splitting ester links present in various polymer substrates to produce acid- and alcohol-based compounds with polar groups (Lisboa et al., 2013). PU, PET, PES, PBS, polycaprolactone (PCL), polyethylene succinate (PES), organic polylactic acid (PLA), bis(hydroxyethyl)terephthalate (BHET), synthetic fertilisers, and pesticides are examples of polyester plastic polymers in which cutinase and lipase break down through the

cleaving of ester bonds (Tan et al., 2021; Thirunavukarasu et al., 2016; V¨azquez-Alcantara et al., 2021).

The enzyme PET hydrolase (PETase), which selectively targets and hydrolyzes ester bonds in PET polymers, is present in certain fungal species, including *Pestalotiopsis microspora*. The fungi secrete PETase, an enzyme with active sites that binds to PET polymer and cleaves it into MHET, BHET, and terephthalic acid (TPA) intermediates. The final product of the enzymatic reaction is ethylene glycol (EG), which is produced in the presence of PET (Guard and Rugby School, 2020; Han et al., 2017). Furthermore, it has been shown that urethane and amide linkages, which are frequently found in polyamides (PAs) and polymeric units (PUs), are hydrolyzed by protease, amidase, and urease enzymes produced by a variety of basidiomycete and ascomycete fungus species (Temporiti et al., 2022; Tokiwa et al., 2009).

Studies are examining the effectiveness, mechanisms, and viability of these enzymes from diverse species in hydrolyzing plastics made of polar bonds, such as carbonyl, carboxyl, hydroxyl, amide, amine, and urethane (Magnin et al., 2021; Wei and Zimmermann, 2017). Cutinase, lipase, protease, and other hydrolases can cleave polar bonds of various types. Actually, more than 2000 homolog proteins found in various microorganisms have the ability to independently act on PET and PU, among other synthetic polymers derived from fossil fuels, according to Buchholz et al. (2021). This calls for additional research on the separation of fungal enzymes, with a particular emphasis on characterizing proteins that may be involved in the breakdown of plastics.

The breakdown of lipids is catalyzed by lipases, which are an enzyme subclass belonging to the esterase family. The poly (butylene succinate-cohexamethylene succinate)

copolymer was being broken down by a few well-known fungal species that are known to produce lipases and are involved in the degradation of plastics, such as *Rhizopus delemer, Candida antarctica* (Vertommen et al. 2005), *Termomyces lanuginosus* (Eberl et al. 2009)*, and Candida rugosa*. (Pereira and others, 2001). After a 24-hour response, *Rhizopus delemer* lipase broke down 53% of the polyester type-polyurethanes (ES-PU) film (Tokiwa and Calabia 2009). According to tirunavukarasua et al. (2008), a lipase enzyme isolated from the yeast *Cryptococcus sp*. demonstrated hydrolysis of polybutylene succinate (PBS) and polybutylene succinate-co-adipate (PBSA). Lipase FE-01 from *Terminomyces languinosus* demonstrated the enzymatic breakdown of electrospun polycaprolactone fibre, while lipase B from *Candida antarctica* was effective in hydrolyzing PET to TPA (Carniel et al. 2017). (Furukawa et al. 2019).

Multi-copper oxidases called laccases catalyse the oxidation of phenolic substances. It generates water and byproducts while using molecular oxygen as a co-substrate (Nunes and Kunamneni 2018). These laccases have a unique property in that they may oxidise lignin, which contributes to its degradation (Osma et al. 2010). According to Sivan (2011), laccase may also be involved in the oxidation of polyethylene's hydrocarbon backbone. Laccases are being used by a specific fungus called *Cochliobolus sp.* to break down PVC (Sumathi et al. 2016). According to Bo Ram Kang (2019), *Bjerkandera adusta* TBB-03 was identified due to its capacity to break down HDPE when treated with lignocellulose substrate through the synthesis of laccase. Laccase, which breaks down polyethylene, is produced by *Trametes versicolor, Pleurotus ostreatus, Streptomyces, P. ostreatus,* and *T. pubescens* (Osma et al. 2010). Phenol oxidase (laccase), heme peroxidases, lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile

peroxidase (VP) are the general members of the liginolytic enzyme family (Dashtban et al. 2010). It was discovered that two proteolytic enzymes, papain and urease, hydrolyze urethane and urea bonds to break down medical polyester polyurethane and produce free amine and hydroxyl groups (Phua et al. 1987). PE breakdown may be facilitated by laccase produced from *Penicillium* (Abd El-Rehim et al. 2004).

CHAPTER 3: METHODOLOGY

3.1. Site Description

Two mangrove areas were selected for sampling i.e., Ribandar and Chorao.

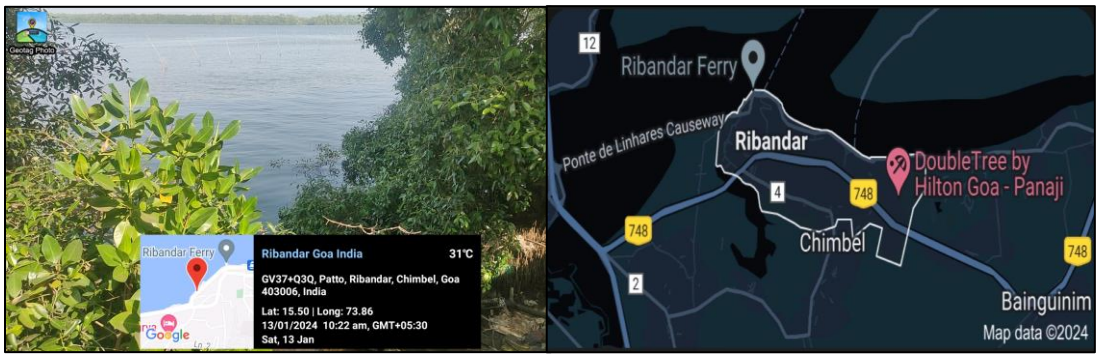


Fig. 3.1: Ribandar mangroves

Ribandar is a very serene and beautiful village nestled between the main city of Panjim and Old Goa in the district of Ilhas or Tiswadi.

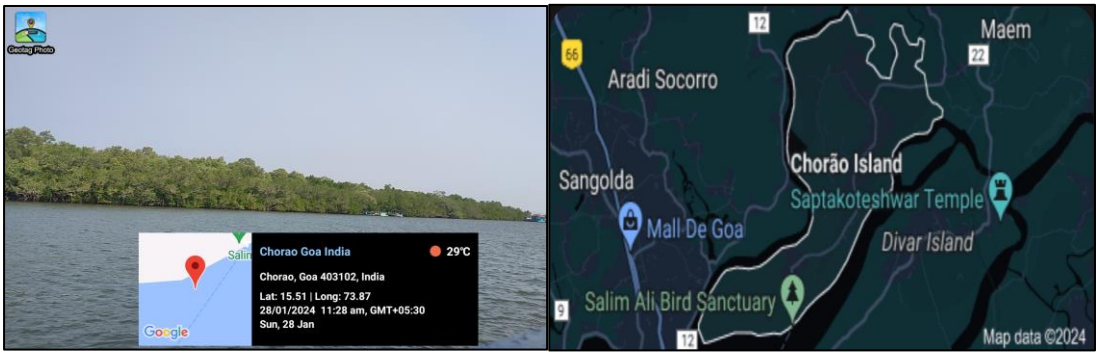


Fig. 3.2: Chorao mangroves

Chorao is an island on the Mandovi River in North Goa. It is the largest of the 17 Goa islands. The Chorao Island is 9.5 kilometers from Panaji.

3.1.1 **Sampling Details**

Sampling was done on 7th January 2024 and 28th January 2024 at Ribandar and Chorao mangroves respectively.

3.2 Collection of plastic samples

Five plastic samples each were collected from Ribandar and Chorao mangroves (Figs. 3 & 4) using sterile forceps and were washed with sterile seawater. Next the plastic samples were wrapped in aluminium foil and brought back to laboratory in ice.

Fig. 3.4: Sample collection from Ribandar mangroves.

Fig. 3. 4: Sample collection from Chorao mangroves.

3.2.1 Supporting Parameters analyzed:

Temperature, Salinity and pH of the mangrove waters were analysed.

Temperature: The surface water was collected in a beaker and the thermometer was dipped into it. Readings (temperature) were noted down in (^{o}C).

Salinity: The water sample was collected in a beaker. The water was taken out using a dropper, and 2-3 drops were put onto the refractometer. The lid was closed, ensuring no air bubbles were trapped in and viewed through the eyepiece. Salinity reading was noted

pH: Water sample was collected in a beaker and the pH paper was dipped into it. pH reading was noted.

3.4 Isolation of fungi on various media

Each of the 5 samples from both study areas were cut into pieces using surface sterilized scissors rinsed with sterile seawater 5 times to remove the loosely adhered soil particles and fungi, and placed on 4 different sterile media plates: Czapek Dox Agar (CDA), 2% Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) and Sabouraud's Dextrose Agar (SDA), containing 50 mg/L chloramphenicol. After incubation at Room Temperature for upto 5 days, morphologically different colonies were purified and maintained on CDA petriplates at 4 °C.

3.4.1 Purification of Fungal isolates

This was done using the Spot Inoculation method, using different procedures for sporulating and non-sporulating fungal isolates.

Sporulating fungi

The sterile nichrome loop was moistened by touching the agar in a sterile plate. Then, the growing sporulating colony was touched and spot inoculated on a fresh media plate. Incubation was for 3-5 days at room temperature. Colony characters were noted.

Non-sporulating fungi

Using surface-sterilized forceps or sterile nichrome loop, a piece of agar (~0.25 cm^2) on which non-sporulating fungus was growing, was picked and placed on the surface of the plate. Incubation was for 3-5 days at room temperature. Colony characters were noted.

3.5 Identification of fungal isolates by Lactophenol Cotton Blue Stain (LCBS) method

A drop of 10% Lactophenol cotton blue stain was added on grease free slide. A portion of the isolated fungal colony containing mycelial fibers was transferred onto a drop of LCBS on the slide using forceps. The fibres were teased with a needle and covered with a clean coverslip. The prepared slide was then observed under a microscope at 10x and 40x objectives to study the morphology of the fungal hyphae for identification based on standard taxonomic keys.

3.7 Characterization of fungal isolates by detection of enzymes

3.7.1 Qualitative detection of lipase enzyme

Tween 80 medium was prepared and poured into petriplates. Next, all fungal cultures were spot inoculated onto it. Plates were incubated at room temperature for 24 hours, and checked for precipitation around cultures.

3.7.2 Qualitative detection of laccase enzyme

PDA medium with 0.02% Guaiacol was prepared in dim conditions (since guaiacol is a light-sensitive compound) and poured into petriplates. The fungal cultures were spot inoculated onto it. Plates were incubated for 3 days in dark at room temperature, and checked for brown zones around cultures.

3.8 Scanning Electron Microscopy

Selected fungal cultures were selected for SEM analyses based on the results of the enzyme detection. Active fungal cultures were grown in CDA broth along with plastic polymer (polythene) pieces measuring approximately 1cm². Incubation was at room temperature for 48 hours. Then, the plastic pieces were picked from broth, washed with sterile seawater and fixed in 4% paraformaldehyde for 2-23 hours. Subsequently, they were transferred to 50% ethanol in Phosphate Buffered Saline (PBS) and stored at -20^{o}C, till analysis. Then, the plastic samples were dehydrated on ice through an ethanol series – 50%, 70%, 85%, 95% (10 mins in each concentration) followed by 3 cycles (15 mins each) in 100% ethanol; air dried in dessicator, sputter coated with gold, and observed with a Scanning Electron Microscope.

CHAPTER 4: ANALYSIS AND CONCLUSION

4.1 RESULTS

4.1.1 Plastic-associated fungi from Ribandar and Chorao mangroves

Fifty-six plastic-associated fungal isolates were recovered from Ribandar and Chorao mangroves. 28 from Ribandar and 28 from Chorao respectively (Table 1). Parameter values were similar at both sites (Table 2). Anthropogenic activity is higher at Panjim city due to casino, which results in higher influx of potential plastic substrates into the water, which could lead to large number of plastisphere communities. Both these sites are close to Panjim city.

Table 4.1: Selected Location Sites and no. of fungal isolates recovered from there.

Sr. No.	Location site	No. of Fungal Isolates
1	Ribandar	28
2	Chorao	28

4.1.2 Environmental parameters.

Table 4.2: Parameters measured at Study sites.

Sr. No.	Study Site	Temperature	Salinity	pH
1	Ribandar	26^{o} C	32	7
2	Chorao	25^{o} C	29	7

4.1.3 Fungal diversity on different media

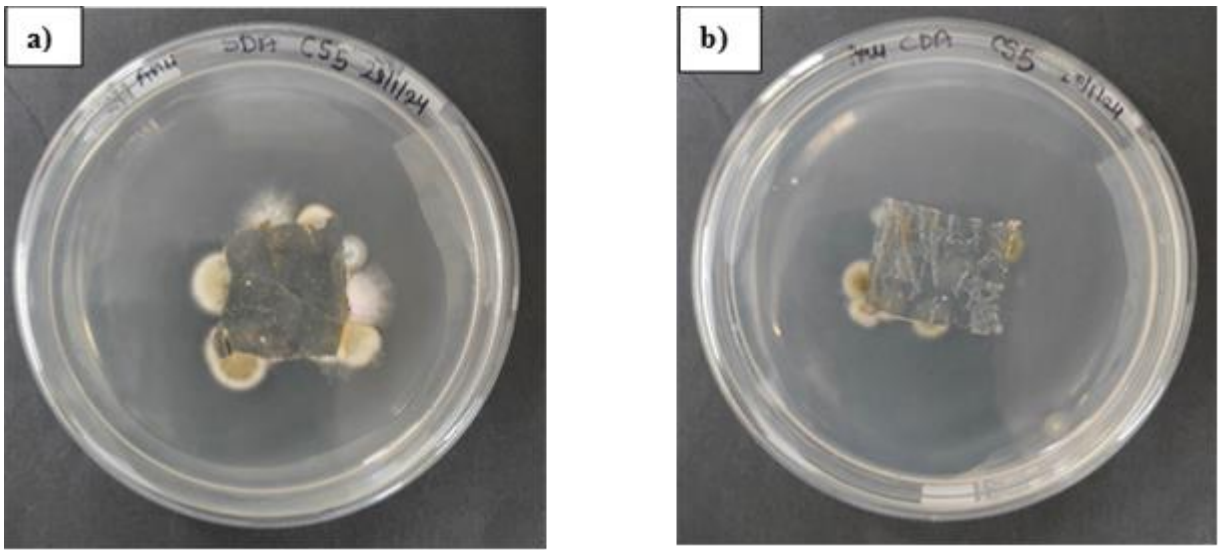


Fig 4.1: Fungal isolates growing attached to plastic samples on a) Sabourauds Dextrose Agar; b) Czapek Dox Agar

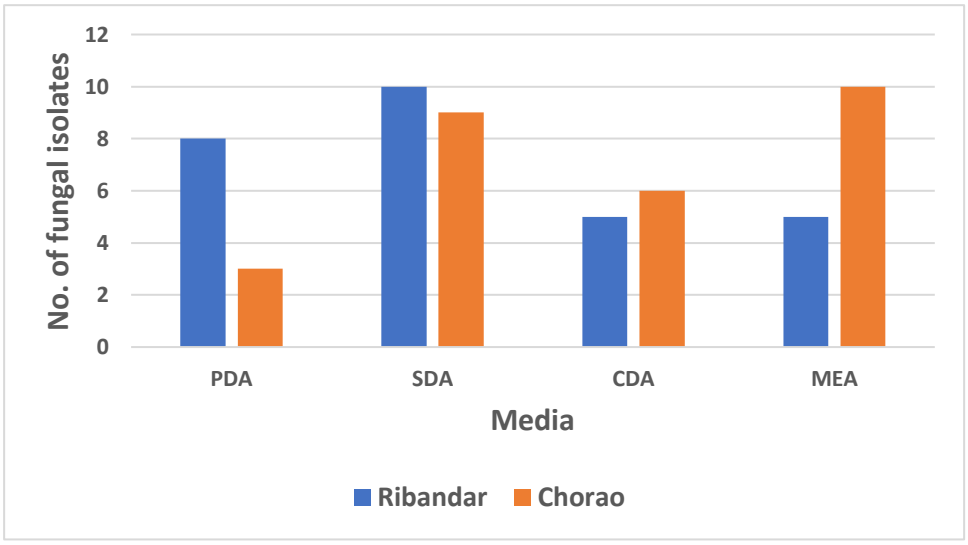


Fig 4.2: Number of fungal isolates on different media.

Fungal colonies were observed growing attached to the edges of the plastic debris samples (Fig. 4.1). Fungal isolates were recovered from all the 4 media (Fig. 4.2). In the

Ribandar samples, the highest number of fungal isolates were recovered on SDA plate followed by PDA, CDA and MEA. In the Chorao samples, the highest numbers of fungal isolates were recovered from MEA plate, followed by SDA, CDA and PDA (Fig. 4.2). Details of the fungal isolates are given in Tables 4.3 and 4.4.

Table 4.3: Details of plastisphere fungal isolates from Ribandar mangroves.

Sr. No.	Culture code	Location	Media
1	RS1C2	Ribandar	CDA
2	RS2C1	Ribandar	CDA
3	RS3C1	Ribandar	CDA
4	RS3C2	Ribandar	CDA
5	RS5C3	Ribandar	CDA
6	RS2M1	Ribandar	MEA
7	RS2M2	Ribandar	MEA
8	RS4M1	Ribandar	MEA
9	RS5M1	Ribandar	MEA
10	RS5M2	Ribandar	MEA
11	RS1P1	Ribandar	PDA
12	RS1P2	Ribandar	PDA
13	RS1P3	Ribandar	PDA
14	RS1P4	Ribandar	PDA
15	RS1P5	Ribandar	PDA
16	RS4P1	Ribandar	PDA
17	RS5P2	Ribandar	PDA
18	RS5P3	Ribandar	PDA
19	RS1S1	Ribandar	SDA
20	RS2S1	Ribandar	SDA
21	RS2S2	Ribandar	SDA
22	RS2S3	Ribandar	SDA
23	RS3S1	Ribandar	SDA
24	RS4S1	Ribandar	SDA
25	RS4S2	Ribandar	SDA
26	RS4S3	Ribandar	SDA
27	RS5S1	Ribandar	SDA
28	RS5S2	Ribandar	SDA

Table 4.4: Details of plastisphere fungal isolates from Chorao mangroves.

Sr. No.	Culture code	Location	Media
29	CS1C1	Chorao	CDA
30	CS3C1	Chorao	CDA
31	CS4C1	Chorao	CDA
32	CS5C1	Chorao	CDA
33	CS5C2	Chorao	CDA
34	CS5C3	Chorao	CDA
35	CS1M2	Chorao	MEA
36	CS2M1	Chorao	MEA
37	CS2M2	Chorao	MEA
38	CS4M1	Chorao	MEA
39	CS4M2	Chorao	MEA
40	CS5M1	Chorao	MEA
41	CS5M3	Chorao	MEA
42	CS5M4	Chorao	MEA
43	CS5M5	Chorao	MEA
44	CS5M6	Chorao	MEA
45	CS3P1	Chorao	PDA
46	CS4P1	Chorao	PDA
47	CS5P1	Chorao	PDA
48	CS1S1	Chorao	SDA
49	CS3S1	Chorao	SDA
50	CS4S2	Chorao	SDA
51	CS4S3	Chorao	SDA
52	CS4S1	Chorao	SDA
53	CS5S1	Chorao	SDA
54	CS5S2	Chorao	SDA
55	CS5S3	Chorao	SDA
56	CS5S4	Chorao	SDA

4.1.4. Identification of fungal isolates

In Ribandar mangroves, Aspergillus fungi was most dominant (18 out of 28) and 1 *Circinella* sp. were also identified, 9 remained unidentified (Table 4.5). In Chorao mangroves, *Aspergillus* sp., *Fusarium* sp., *Circinella* sp., *Penicillium* sp. and *Cladosporium* sp. were identified. However, 21 fungal isolates were unidentified (Table 4.6).

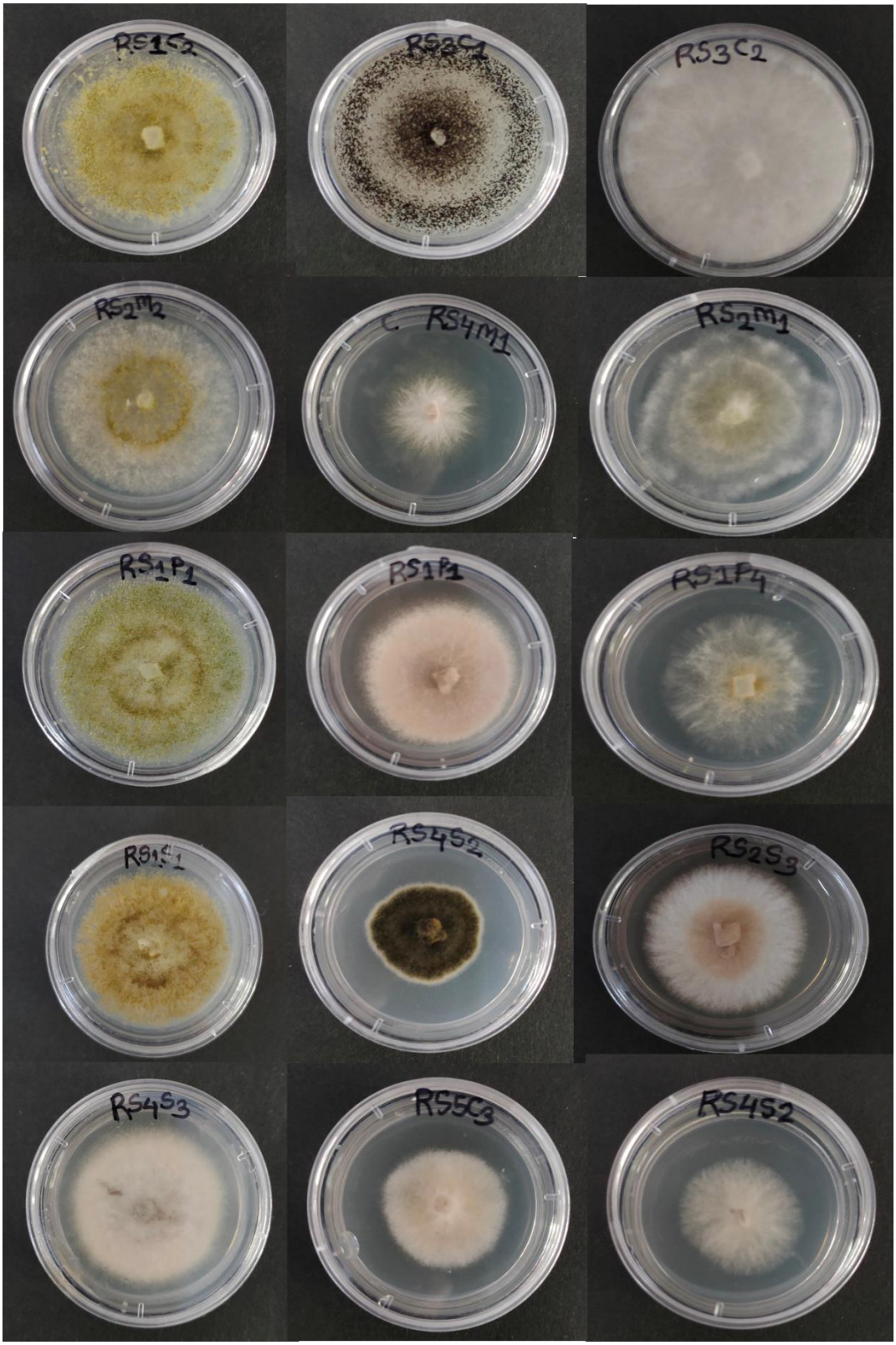


Fig. 4.3 Morphological diversity of plastic-associated fungal colonies from Ribandar mangroves

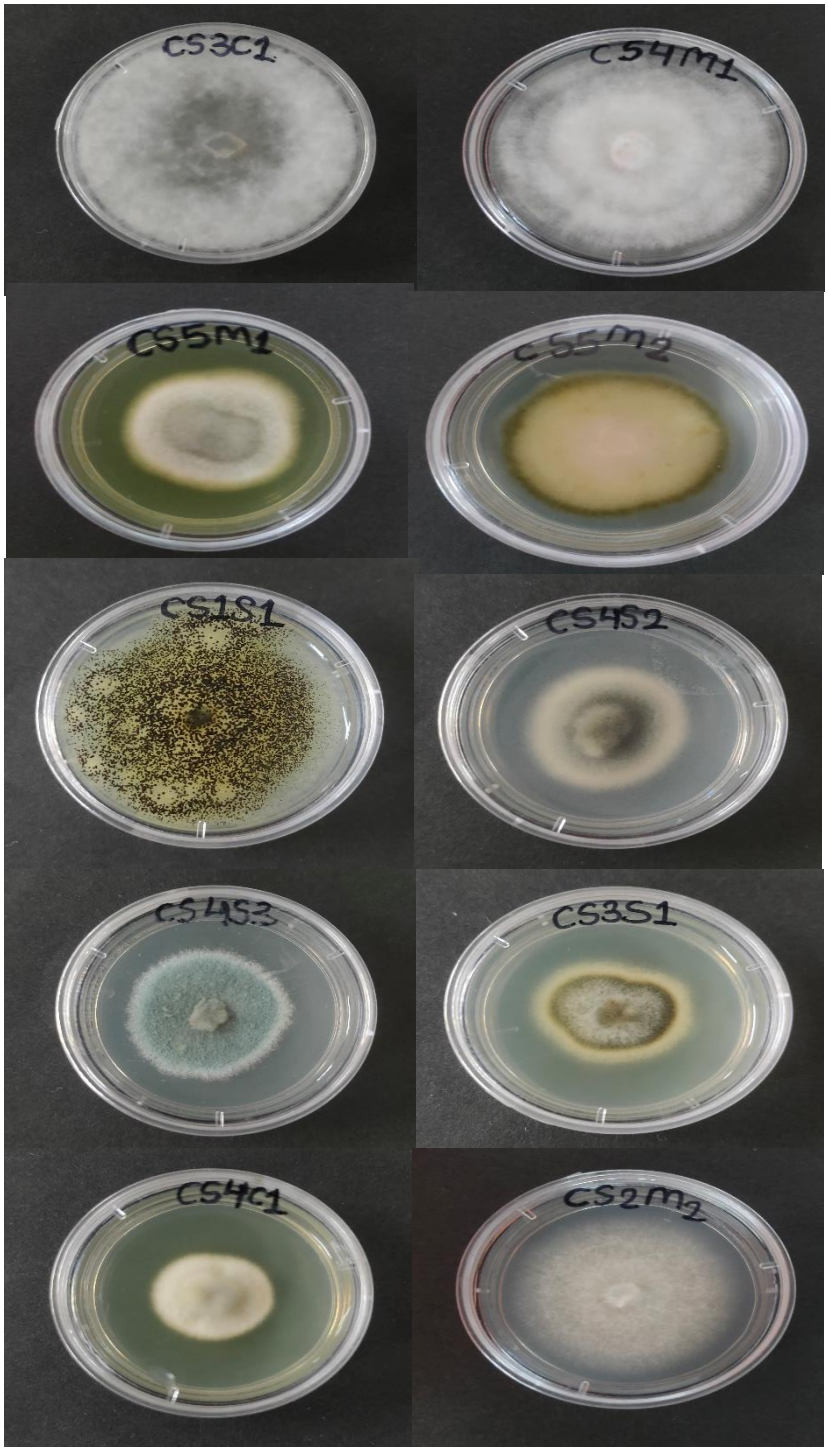


Fig. 4.4 Morphological diversity of plastic-associated fungal colonies from Chorao mangroves.

4.1.5 Microphotographic view of isolated fungi

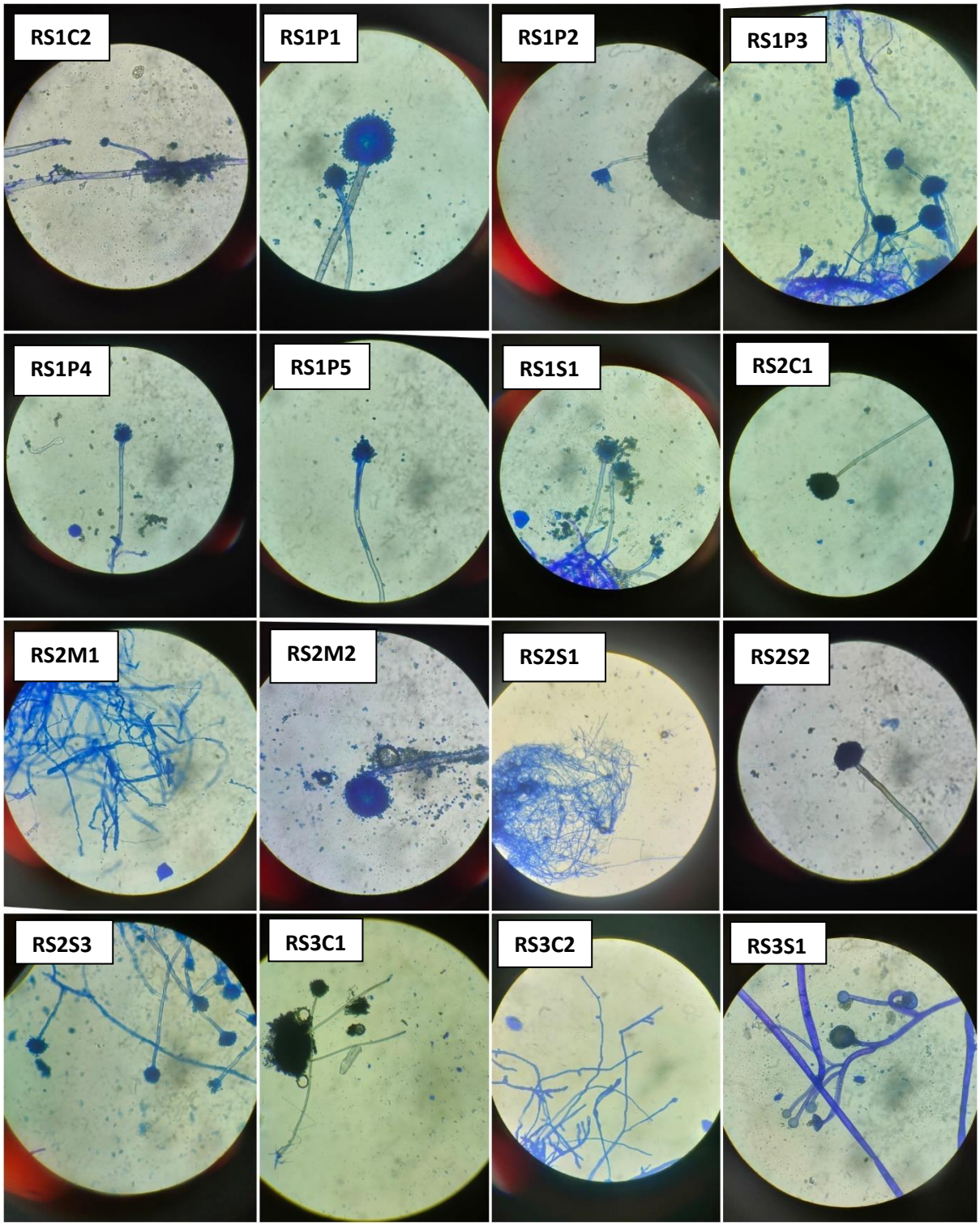


Fig. 4.5: Microscopic images of fungal isolates from Ribandar mangroves.

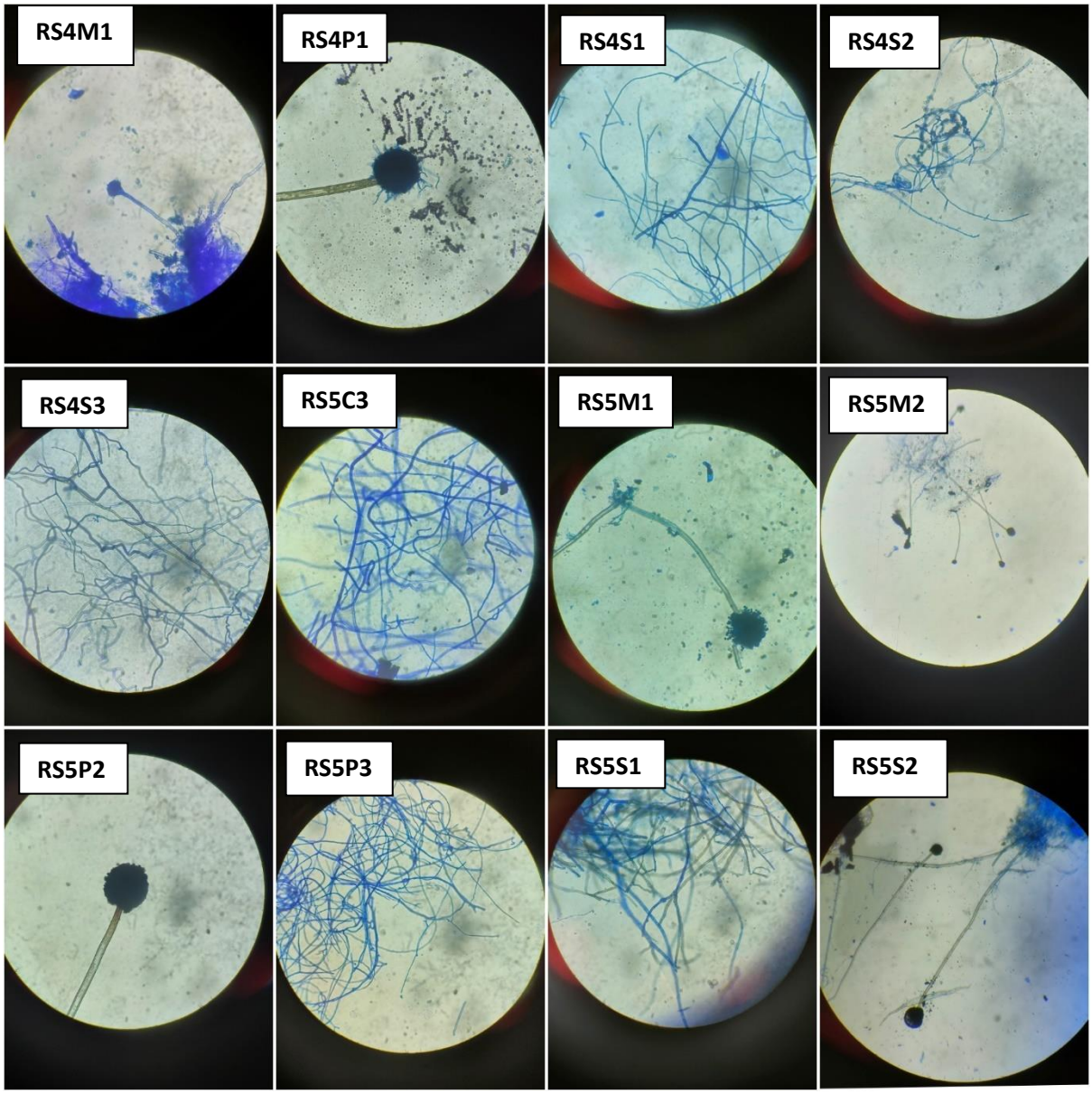


Fig. 4.5 (continued): Microscopic images of fungal isolates from Ribandar mangroves.

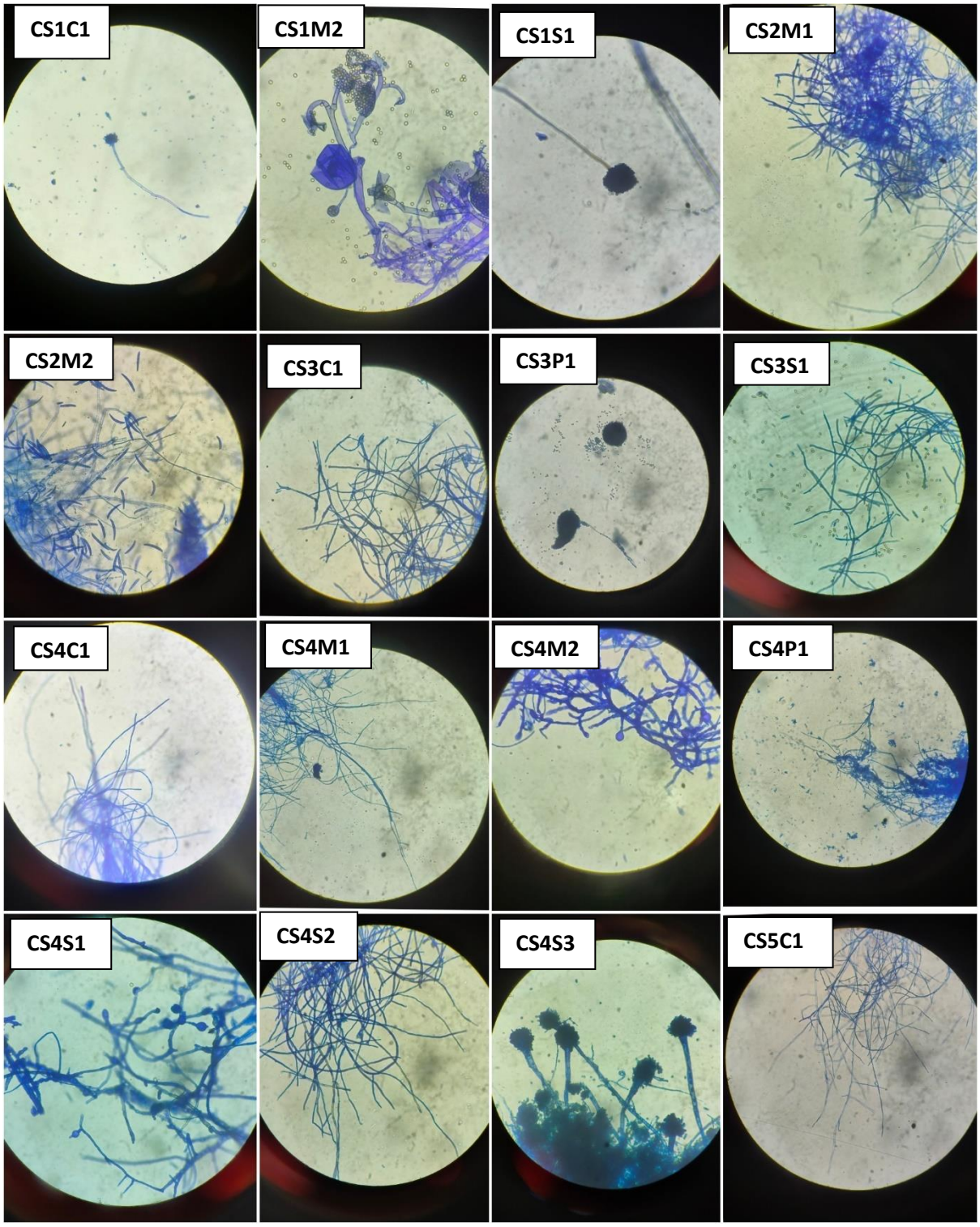


Fig. 4.6: Microscopic images of fungal isolates from Chorao mangroves.

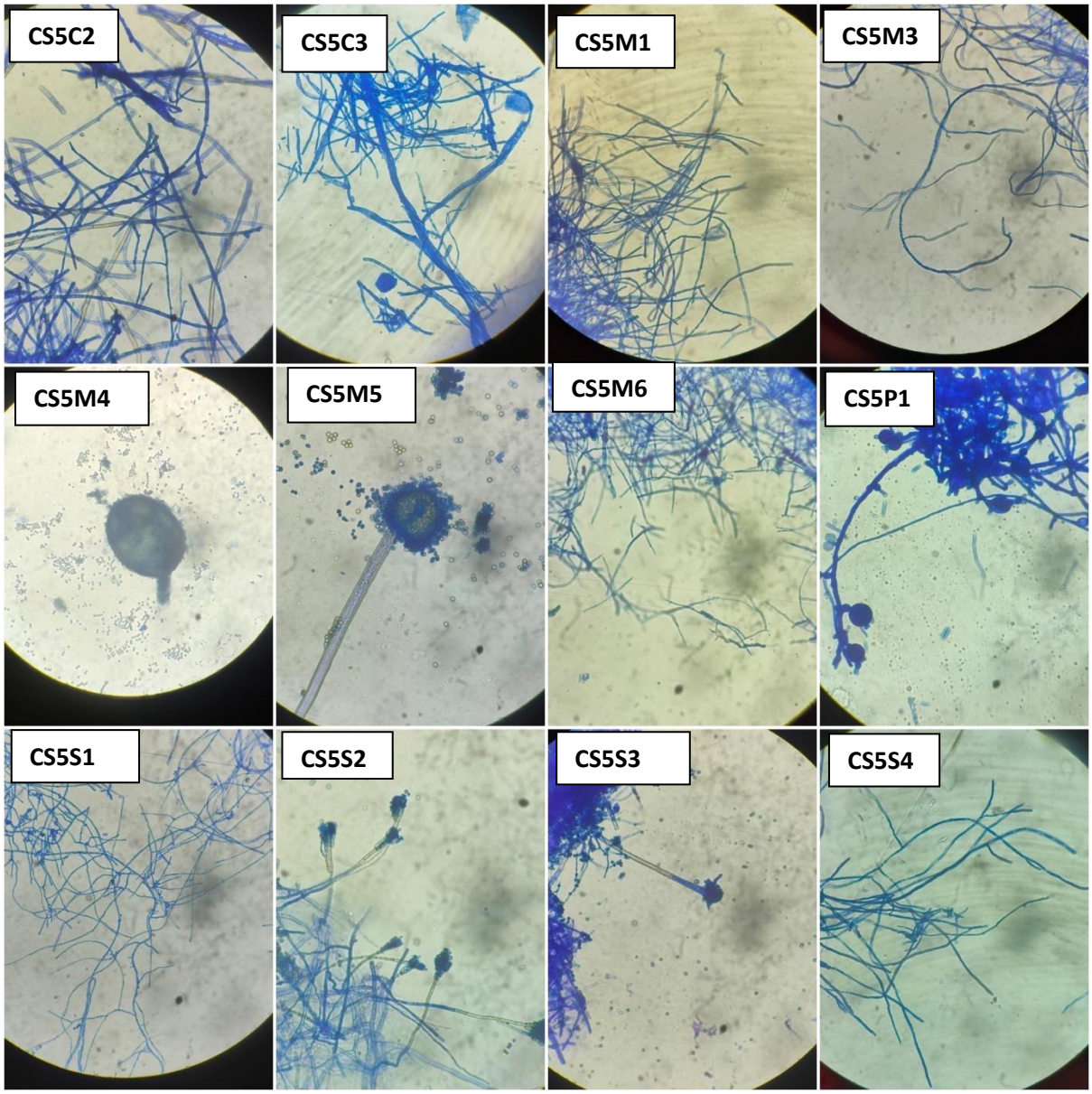


Fig. 4.6 (continued): Microscopic images of fungal isolates from Chorao mangroves.

Table 4.5: Details of fungal isolates from plastic marine debris from Ribandar mangroves.

Sr. No.	Culture code	Colony colour	S/NS	Identified as
1	RS1C2	Yellow	S	*Aspergillus sp.*
2	RS2C1	Black	S	*Aspergillus sp.*
3	RS3C1	Black	S	*Aspergillus sp.*
4	RS3C2	White	NS	Unidentified fungus
5	RS5C3	White	NS	*Aspergillus sp.*
6	RS2M1	White with yellow center	NS	Unidentified fungus
7	RS2M2	Yellowish brown	S	*Aspergillus sp.*
8	RS4M1	White	NS	Unidentified fungus
9	RS5M1	Black	S	*Aspergillus sp.*
10	RS5M2	Black	S	Unidentified fungus
11	RS1P1	Yellow	S	*Aspergillus sp.*
12	RS1P2	Black	S	Unidentified fungus
13	RS1P3	Pink	S	*Aspergillus sp.*
14	RS1P4	White with yellow center	S	*Aspergillus sp.*
15	RS1P5	Black	S	*Aspergillus sp.*
16	RS4P1	Black	S	*Aspergillus sp.*
17	RS5P2	Black	S	*Aspergillus sp.*
18	RS5P3	Yellow	NS	Unidentified fungus
19	RS1S1	Brown	S	*Aspergillus sp.*
20	RS2S1	White (Brown spores)	S	*Aspergillus sp.*
21	RS2S2	Black	S	*Aspergillus sp.*
22	RS2S3	Pink center with white border	S	*Aspergillus sp.*
23	RS3S1	Transparent	NS	*Circinella sp.*
24	RS4S1	White	NS	Unidentified fungus
25	RS4S2	Green	S	*Aspergillus sp.*
26	RS4S3	White	NS	Unidentified fungus
27	RS5S1	White	NS	Unidentified fungus
28	RS5S2	Black	S	*Aspergillus sp.*

Table 4.6: Details of fungal isolates from plastic marine debris from Chorao mangroves.

Sr. No.	Culture code	Colony colour	S/NS	Identified as
1	CS1C1	Brown	S	*Aspergillus sp.*
2	CS3C1	White	NS	Unidentified fungus
3	CS4C1	White	NS	Unidentified fungus
4	CS5C1	Green	NS	Unidentified fungus
5	CS5C2	White	NS	Unidentified fungus
6	CS5C3	Green	NS	Unidentified fungus
7	CS1M2	Transparent	NS	*Circinella sp.*
8	CS2M1	White	NS	Unidentified fungus
9	CS2M2	White	NS	*Fusarium sp.*
10	CS4M1	White	NS	Unidentified fungus
11	CS4M2	White	NS	Unidentified Ascomycetes fungus
12	CS5M1	White	NS	Unidentified fungus
13	CS5M3	White	NS	Unidentified fungus
14	CS5M4	Green with white border	NS	Unidentified fungus
15	CS5M5	White with green centre	S	*Aspergillus sp.*
16	CS5M6	White	NS	Unidentified fungus
17	CS3P1	White	NS	Unidentified fungus
18	CS4P1	White	NS	Unidentified fungus
19	CS5P1	White	NS	Unidentified fungus
20	CS1S1	Black	S	*Aspergillus sp*.
21	CS3S1	Green	NS	*Cladosporium sp.*
22	CS4S1	White	NS	Unidentified Ascomycetes fungus
23	CS4S2	White with green centre	NS	Unidentified fungus
24	CS4S3	Green	NS	Unidentified fungus
25	CS5S1	White	NS	Unidentified fungus
26	CS5S2	Green with white border	NS	*Penicillium sp.*
27	CS5S3	Brown with white something	NS	Unidentified fungus
28	CS5S4	White	NS	Unidentified fungus

4.1.6. Detection of Enzymes

In Ribandar, lipase was detected in only 1 culture RS4S2 and Laccase was negative for all cultures (Table no. 4.6). In Chorao samples, lipase was positive for 6 cultures out of 28 and laccase however was negative for all cultures (Table no. 4.7). Strong positive results for lipase were seen as precipitation around cultures (Fig 4.1.9 a-e).

Table 4.7: Enzymes detected in plastic-associated fungal isolates from Ribandar.

Sr. No.	Fungal isolates	Lipase	Laccase
1	RS1C2	-	-
2	RS2C1	-	-
3	RS3C1	-	-
4	RS3C2	-	-
5	RS5C3	-	-
6	RS2M1	-	-
7	RS2M2	-	-
8	RS4M1	-	-
9	RS5M1	-	-
10	RS5M2	-	-
11	RS1P1	-	-
12	RS1P2	-	-
13	RS1P3	-	-
14	RS1P4	-	-
15	RS1P5	-	-
16	RS4P1	-	-
17	RS5P2	-	-
18	RS5P3	-	-
19	RS1S1	-	-
20	RS2S1	-	-
21	RS2S2	-	-
22	RS2S3	-	-
23	RS3S1	-	-
24	RS4S1	-	-
25	RS4S2	+	-
26	RS4S3	-	-
27	RS5S1	-	-
28	RS5S2	-	-

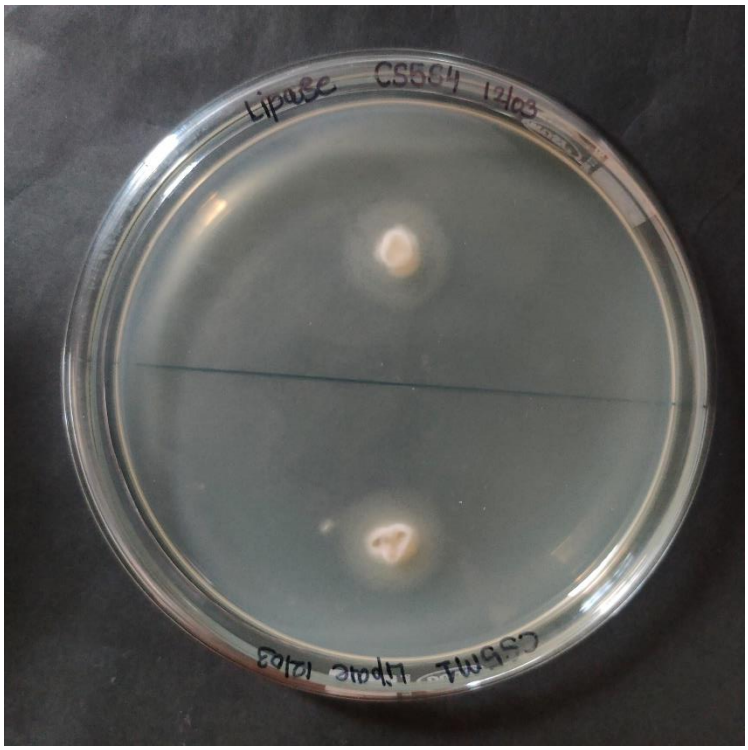
Table 4.8: Enzymes detected in plastic-associated fungal isolates from Chorao.

Sr. No.	Fungal isolates	Lipase	Laccase
1	CS1C1	-	-
2	CS3C1	-	-
3	CS4C1	+	-
4	CS5C1	-	-
5	CS5C2	-	-
6	CS5C3	-	-
7	CS1M2	-	-
8	CS2M1	-	-
9	CS2M2	-	-
10	CS4M1	-	-
11	CS4M2	-	-
12	CS5M1	+	-
13	CS5M3	+	-
14	CS5M4	-	-
15	CS5M5	-	-
16	CS5M6	-	-
17	CS3P1	-	-
18	CS4P1	-	-
19	CS5P1	-	-
20	CS1S1	-	-
21	CS3S1	+	-
22	CS4S1	-	-
23	CS4S2	+	-
24	CS4S3	-	-
25	CS5S1	-	-
26	CS5S2	-	-
27	CS5S3	-	-
28	CS5S4	+	-

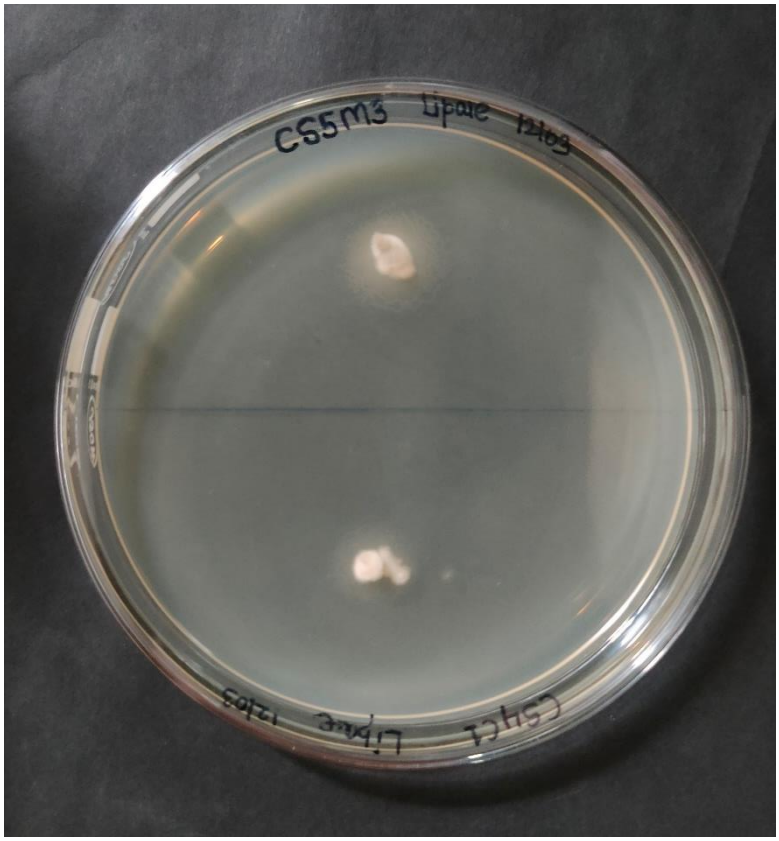
a)


Lipase RS4S2
Precipitation around fungal isolate

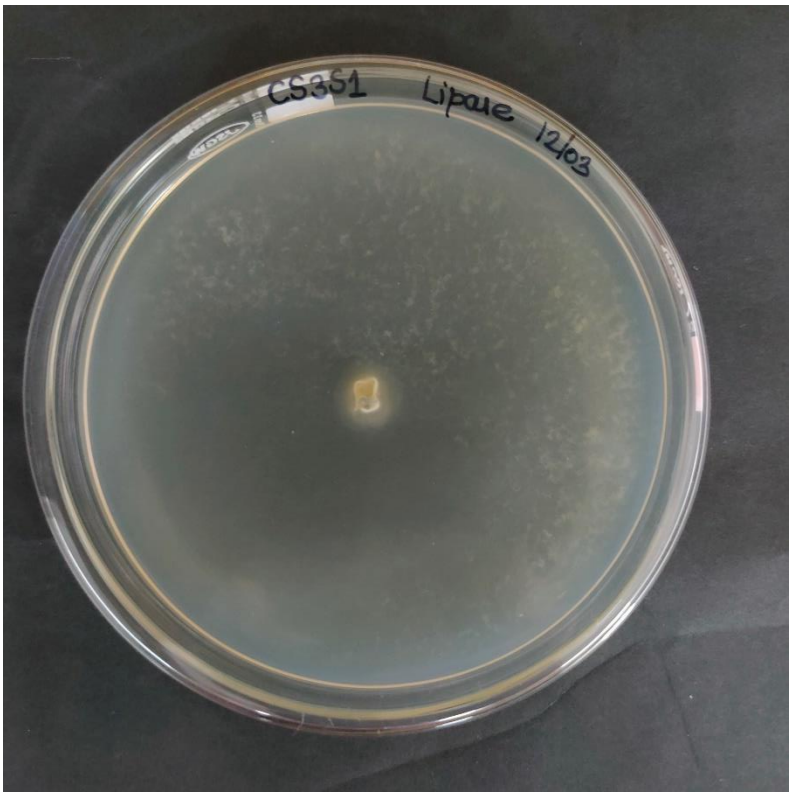
b)


Lipase CS5S4

c)



d)



e)

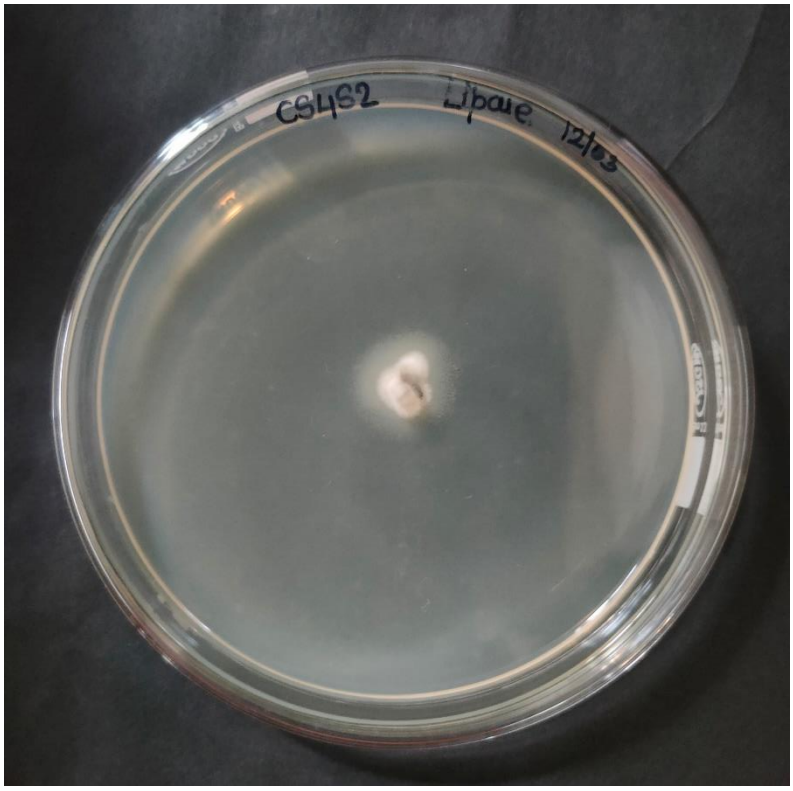
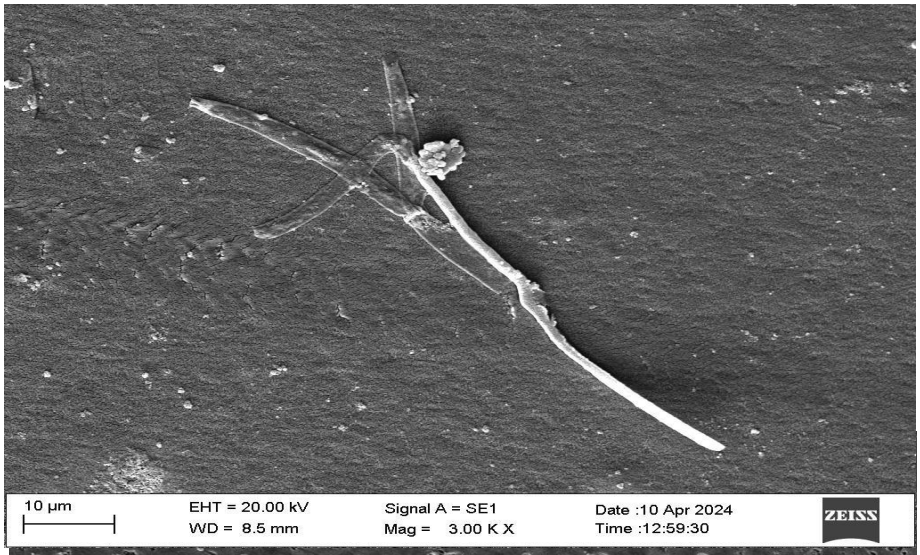


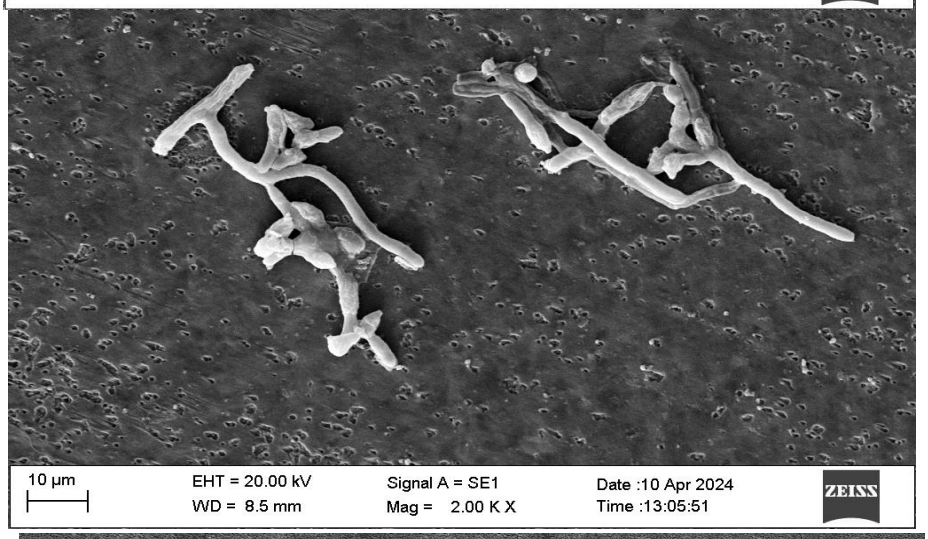
Fig. 4.7.: Fungal isolates showing positive result for Lipase activity; a) RS4S2; b) CS5S4 and CS5M1; c) CS5M3 and CS4C1; d) CS3S1; and e) CS4S2.

4.1.7. SEM Analysis of Fungal isolates on Plastic

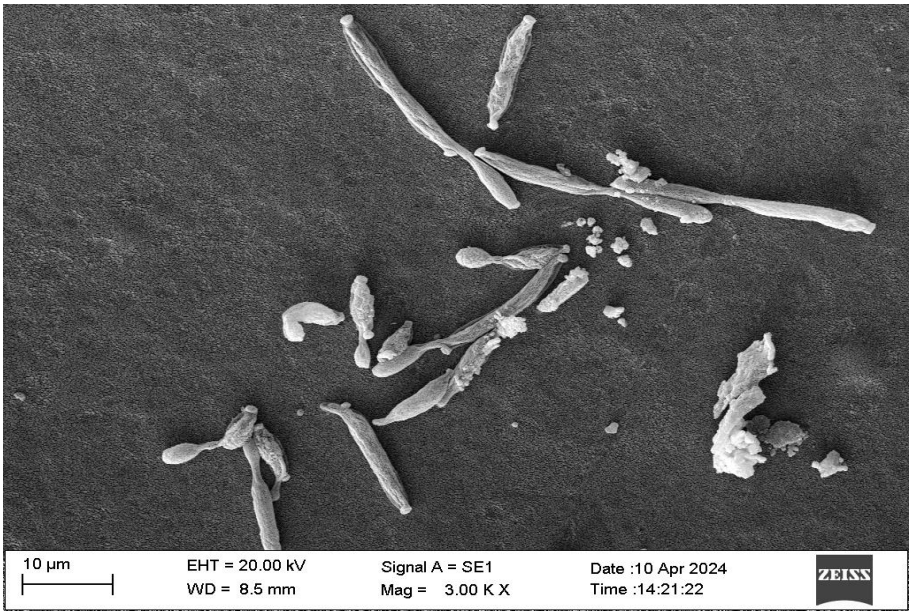
a)



b)



c)



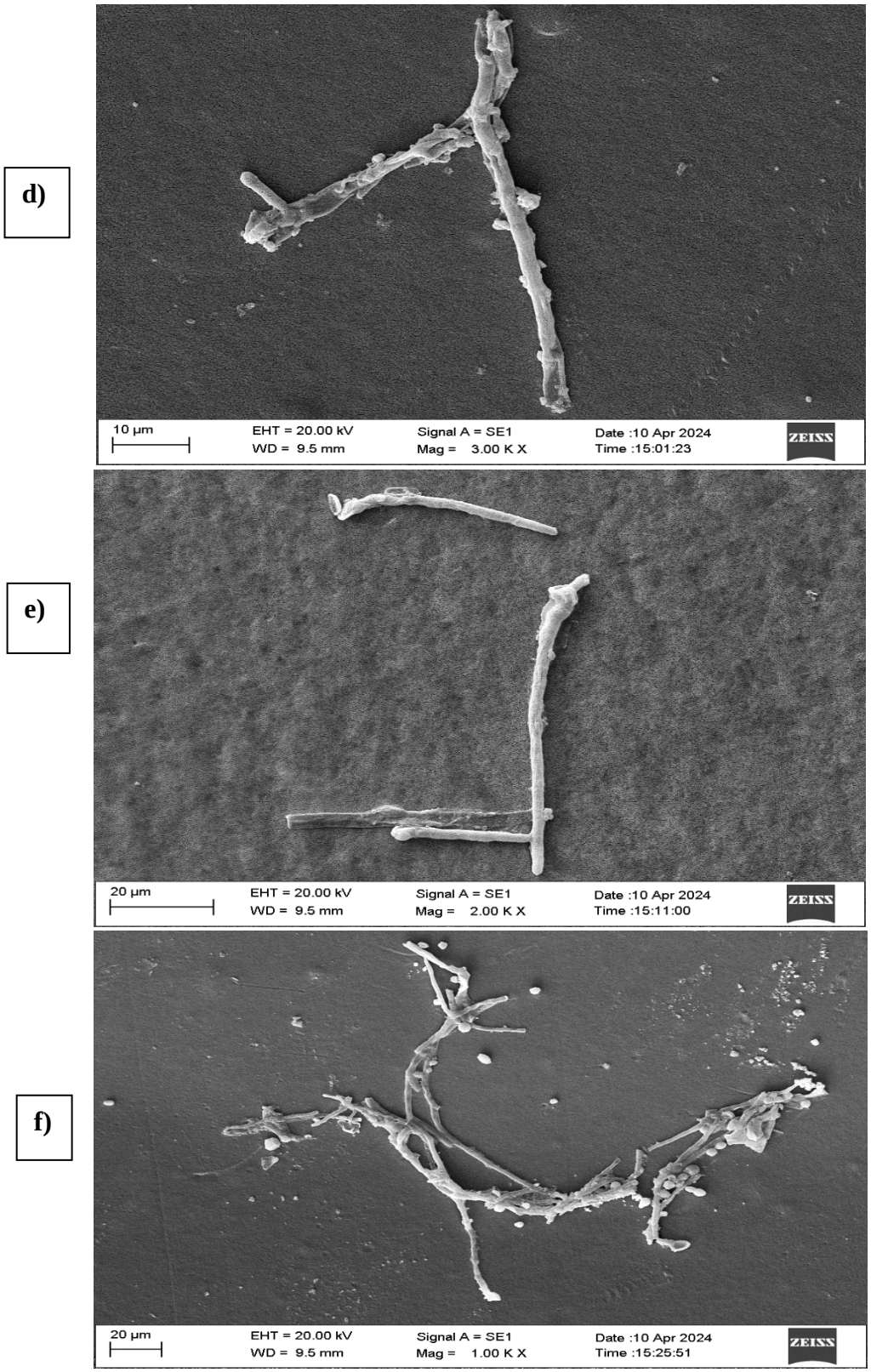


Fig 4.8: Scanning Electron Micrograph of fungal isolates on plastic; a) CS3S1; b) CS5S4; c) CS5M1; d) CS4C1; e) CS4S2; and f) RS4S2.

4.2 Discussion

In the present study, a total of 56 species were isolated from plastic samples from mangrove environment, 28 from Ribandar and 28 from Chorao mangroves, along the west coast of Goa, India. Aspergillus sp., Circinella sp., Penicillium sp., Fusarium sp. and Cladosporium sp, were identified using taxonomic keys and some remained unidentified. Sivakumar et al., (2006) reported that the distribution of fungi in Muthupettai mangroves along the East coast of Tamil Nadu, India was studied in terms of species diversity, seasonal variation, and frequency of occurrence in five sampling stations at two different seasons. A total of 118 species of fungi were isolated, of which maximum 94 species were from sediment samples followed by water with 83 species. Among the fungal isolates, *Aspergillus* was the common genus followed by *Penicillium*, *Curvualria* and *Alternaria*. Similarly, in the present study also *Aspergillus* was the predominant genus in Ribandar mangroves, which were more polluted with plastic debris compared to Chorao mangroves, though both these are on opposite sides of the same estuary (Mandovi estuary). The fungal community composition was significantly influenced by location but not polymer type (Zeghal et al.,2021). *Aspergillus* strains have been reported in several studies related to plastic degradation (Ameen et al. 2015, Sangale et al. 2019, Zeghal et al. 2021, Lacerda et al. 2020, Zahra et al. 2010, Raaman et al. 2021, Indumathi et al. 2016).

Further enzyme activity of fungal isolates such as Lipases and Laccases were checked using (Sadati et al., 2015) and (Zhou et al.,2014) protocols. Fungal isolates from Chorao showed 6 positive results and fungal isolates from Ribandar showed 1 positive result by *Aspergillus sp.* RS4S2. None of the fungal isolates from both sites showed positive results

for Laccase enzyme. Some fungal species that are well known to produce lipases and are involved in the degradation of plastics are *Rhizopus delemer, Candida antarctica* (Vertommen et al. 2005), *Termomyces lanuginosus* (Eberl et al. 2009). *Candida rugosa* were degrading poly (butylene succinate-co-hexamethylene succinate) copolymer. (Pereira et al. 2001). Lipase B from *Candida antarctica* was effectively hydrolyzing PET to TPA (Carniel et al. 2017). Lipase FE-01 from Termomyces languinosus showed enzymatic decomposition of electrospun polycaprolactone fiber (Furukawa et al. 2019). Elevated laccase, manganese peroxidase and lignin peroxidase activities were observed during PE degradation by a fungal consortium in a mangrove (Ameen et al., 2015). Bjerkandera adusta TBB-03 was identifed for its ability to degrade HDPE under lignocellulose sub-strate treatment by laccase production (Bo Ram Kang 2019). *Trametes versicolor, Pleurotus ostreatus, Streptomyces, P. ostreatus and T. pubescens* produce laccase that degrades polyethylene (Osma et al. 2010). In general, the ligninolytic enzyme families include phenol oxidase (laccase), heme peroxidases, lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP) (Dashtban et al. 2010). Papain and urease are the two proteolytic enzymes that were found to degrade medical polyester polyurethane by hydrolysis of urethane and urea linkages by producing free amine and hydroxyl groups (Phua et al. 1987). Penicillium-derived laccase potentially involves in PE breakdown (Abd El-Rehim et al. 2004). Most of the laccase activity were observed in macro fungi compare to micro fungi.

Scanning Electron microghraphs showed filaments on the surface of plastic polymers, although the cracks, pits and deformations on the plastic surface by the fungal isolates were not observed, probably because of the shorter incubation period of two days. The

study by Gkoutselis et al. (2021) on fungal colonisation of MP fragments visualised by SEM, showed fungi closely attached to the plastic surface presumably through a form of self-produced mucilage, numerous conidia lining a crack in the plastic surface, clumping of conidia in association with a hypha, mycelial meshwork, extensive intertwined hyphal laments adhering to the plastic surface via small peripheral bulges.

4.3 Conclusion

The results of this study reveal the diversity of fungi associated with plastic debris from two mangrove environments, and their potential to produce lipases, enzymes involved in plastic degradation. The mechanisms of adherence of these fungal isolates to plastic polymers need to be investigated in detail in future studies. This will offer promising insights into the fungal-mediated degradation of plastic wastes.

REFERENCES

- Al Mohaini, M., Farid, A., Muzammal, M., Ghazanfar, S., Dadrasnia, A., Alsalman, A. J., Al Hawaj, M. A., Alhashem, Y. N., & Ismail, S. (2022). Enhancing lipase production of bacillus salmalaya strain 139si using different carbon sources and surfactants. Applied Microbiology, 2(1), 237–247.
- Ali, S. S., Elsamahy, T., Al-Tohamy, R., Zhu, D., Mahmoud, Y. A.-G., Koutra, E., Metwally, M. A., Kornaros, M., & Sun, J. (2021). Plastic wastes biodegradation: Mechanisms, challenges and future prospects. Science of The Total Environment, 780, 146590.
- Amaral-Zettler, L. A., Ballerini, T., Zettler, E. R., Asbun, A. A., Adame, A., Casotti, R., Dumontet, B., Donnarumma, V., Engelmann, J. C., Frère, L., Mansui, J., Philippon, M., Pietrelli, L., & Sighicelli, M. (2021). Diversity and predicted inter- and intra-domain interactions in the Mediterranean Plastisphere. Environmental Pollution, 286, 117439.
- Amaral-Zettler, L. A., Zettler, E. R., & Mincer, T. J. (2020). Ecology of the plastisphere. Nature Reviews Microbiology, 18(3), 139–151.
- Arias-Andres, M., Klümper, U., Rojas-Jimenez, K., & Grossart, H.-P. (2018). Microplastic pollution increases gene exchange in aquatic ecosystems. Environmental Pollution, 237, 253–261.
- Beloe, C. J., Browne, M. A., & Johnston, E. L. (2022). Plastic debris as a vector for bacterial disease: An interdisciplinary systematic review. Environmental Science & Technology, 56(5), 2950–2958.

- Davidov, K., Iankelevich-Kounio, E., Yakovenko, I., Koucherov, Y., Rubin-Blum, M., & Oren, M. (2020). Identification of plastic-associated species in the Mediterranean Sea using DNA metabarcoding with Nanopore MinION. Scientific Reports, 10(1), 17533.
- De Tender, C., Devriese, L. I., Haegeman, A., Maes, S., Vangeyte, J., Cattrijsse, A., Dawyndt, P., & Ruttink, T. (2017). Temporal dynamics of bacterial and fungal colonization on plastic debris in the north sea. Environmental Science & Technology, 51(13), 7350–7360.
- Debroas, D., Mone, A., & Ter Halle, A. (2017). Plastics in the North Atlantic garbage patch: A boat-microbe for hitchhikers and plastic degraders. Science of The Total Environment, 599–600, 1222–1232.
- Dey, S., Rout, A. K., Behera, B. K., & Ghosh, K. (2022). Plastisphere community assemblage of aquatic environment: Plastic-microbe interaction, role in degradation and characterization technologies. Environmental Microbiome, 17(1), 32.
- Dodhia, M. S., Rogers, K. L., Fernández-Juárez, V., Carreres-Calabuig, J. A., Löscher, C. R., Tisserand, A. A., Keulen, N., Riemann, L., Shashoua, Y., & Posth, N. R. (2023). Microbe-mineral interactions in the Plastisphere: Coastal biogeochemistry and consequences for degradation of plastics. Frontiers in Marine Science, 10, 1134815.
- Du, Y., Liu, X., Dong, X., & Yin, Z. (2022). A review on marine plastisphere: Biodiversity, formation, and role in degradation. Computational and Structural Biotechnology Journal, 20, 975–988.

- Dudek, K. L., Cruz, B. N., Polidoro, B., & Neuer, S. (2020). Microbial colonization of microplastics in the Caribbean Sea. Limnology and Oceanography Letters, 5(1), 5–17.
- Dussud, C., Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., Delort, A.-M., Eyheraguibel, B., Meistertzheim, A.-L., Jacquin, J., Cheng, J., Callac, N., Odobel, C., Rabouille, S., & Ghiglione, J.-F. (2018). Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. Frontiers in Microbiology, 9, 1571.
- Eich, A., Mildenberger, T., Laforsch, C., & Weber, M. (2015). Biofilm and diatom succession on polyethylene (Pe) and biodegradable plastic bags in two marine habitats: Early signs of degradation in the pelagic and benthic zone? PLOS ONE, 10(9), e0137201.
- Hakkarainen, M., & Albertsson, A.-C. (2004). Environmental degradation of polyethylene. In A.-C. Albertsson (Ed.), Long Term Properties of Polyolefins (pp. 177–200). Springer.
- Harding, M. W., Marques, L. L. R., Howard, R. J., & Olson, M. E. (2009). Can filamentous fungi form biofilms? Trends in Microbiology, 17(11), 475–480.
- Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. Science, 347(6223), 768–771.
- Kettner, M. T., Oberbeckmann, S., Labrenz, M., & Grossart, H.-P. (2019). The eukaryotic life on microplastics in brackish ecosystems. Frontiers in Microbiology, 10, 538.

- Khatua, S., Simal-Gandara, J., & Acharya, K. (2024). Myco-remediation of plastic pollution: Current knowledge and future prospects. Biodegradation, 35(3), 249–279.
- Kim, S. H., Lee, J. W., Kim, J. S., Lee, W., Park, M. S., & Lim, Y. W. (2022). Plastic-inhabiting fungi in marine environments and PCL degradation activity. Antonie van Leeuwenhoek, 115(12), 1379–1392.
- Krause, S., Molari, M., Gorb, E. V., Gorb, S. N., Kossel, E., & Haeckel, M. (2020). Persistence of plastic debris and its colonization by bacterial communities after two decades on the abyssal seafloor. Scientific Reports, 10(1), 9484.
- Kumari, A., Chaudhary, D. R., & Jha, B. (2019). Destabilization of polyethylene and polyvinylchloride structure by marine bacterial strain. Environmental Science and Pollution Research, 26(2), 1507–1516.
- Lacerda, A. L. D. F., Proietti, M. C., Secchi, E. R., & Taylor, J. D. (2020). Diverse groups of fungi are associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula. Molecular Ecology, 29(10), 1903–1918.
- Lebreton, L. C. M., van der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. Nature Communications, 8(1), 15611.
- Lee, H.-J., Song, N.-S., Kim, J.-S., & Kim, S.-K. (2021). Variation and uncertainty of microplastics in commercial table salts: Critical review and validation. Journal of Hazardous Materials, 402, 123743.

- Li, M., Pan, Y., Hou, Z., Wu, Z., Zeng, Z., & Wang, B. (2023). Plastic or plastic-free life: From formation to removal. Science of The Total Environment, 890, 164359.
- Lin, Z., Jin, T., Zou, T., Xu, L., Xi, B., Xu, D., He, J., Xiong, L., Tang, C., Peng, J., Zhou, Y., & Fei, J. (2022). Current progress on plastic/microplastic degradation: Fact influences and mechanism. Environmental Pollution, 304, 119159.
- Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S., & Li, T. (2019). Distinct community structure and microbial functions of biofilms colonizing microplastics. Science of The Total Environment, 650, 2395–2402.
- Ndahebwa Muhonja, C., Magoma, G., Imbuga, M., & Makonde, H. M. (2018). Molecular characterization of low-density polyethene (Ldpe) degrading bacteria and fungi from dandora dumpsite, nairobi, kenya. International Journal of Microbiology, 2018, 1–10.
- Oberbeckmann, S., Osborn, A. M., & Duhaime, M. B. (2016). Microbes on a bottle: Substrate, season and geography influence community composition of microbes colonizing marine plastic debris. PLOS ONE, 11(8), e0159289.
- Oder, M., Fink, R., Bohinc, K., & Torkar, K. G. (2017). The influence of shear stress on the adhesion capacity of Legionella pneumophila. Arhiv Za Higijenu Rada I Toksikologiju, 68(2), 109–115.
- Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K. I., Bacsik, Z., & Gorokhova, E. (2018). Evidence for selective bacterial community structuring on microplastics. Environmental Microbiology, 20(8), 2796–2808.

- Okal, E. J., Heng, G., Magige, E. A., Khan, S., Wu, S., Ge, Z., Zhang, T., Mortimer, P. E., & Xu, J. (2023). Insights into the mechanisms involved in the fungal degradation of plastics. Ecotoxicology and Environmental Safety, 262, 115202.
- Prakash, B., Veeregowda, B. M., & Krishnappa, G. (2003). Biofilms: A survival strategy of bacteria. Current Science, 85(9), 1299–1307.
- Rummel, C. D., Lechtenfeld, O. J., Kallies, R., Benke, A., Herzsprung, P., Rynek, R., Wagner, S., Potthoff, A., Jahnke, A., & Schmitt-Jansen, M. (2021). Conditioning film and early biofilm succession on plastic surfaces. Environmental Science & Technology, 55(16), 11006–11018.
- Sadati, R., Barghi, A., & Abbasi Larki, R. (2015). Isolation and screening of lipolytic fungi from coastal waters of the southern caspian sea(North of iran). Jundishapur Journal of Microbiology, 8(4).
- Tamoor, M., Samak, N. A., Jia, Y., Mushtaq, M. U., Sher, H., Bibi, M., & Xing, J. (2021). Potential use of microbial enzymes for the conversion of plastic waste into value-added products: A viable solution. Frontiers in Microbiology, 12.
- Temporiti, M. E. E., Nicola, L., Nielsen, E., & Tosi, S. (2022). Fungal enzymes involved in plastics biodegradation. Microorganisms, 10(6), 1180.
- Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., & Russell, A. E. (2004). Lost at sea: Where is all the plastic? Science, 304(5672), 838–838.
- Vaksmaa, A., Knittel, K., Abdala Asbun, A., Goudriaan, M., Ellrott, A., Witte, H. J., Vollmer, I., Meirer, F., Lott, C., Weber, M., Engelmann, J. C., & Niemann, H.

(2021). Microbial communities on plastic polymers in the mediterranean sea. Frontiers in Microbiology, 12, 673553.

- Varshney, S., Gupta, V., Yadav, A. N., Rahi, R. K., Devki, D., & Neelam, D. K. (2022). An overview on role of fungi in systematic plastic degradation. Journal of Applied Biology & Biotechnology.

- Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. Environmental Pollution, 178, 483–492.
- Zeghal, E., Vaksmaa, A., Vielfaure, H., Boekhout, T., & Niemann, H. (2021). The potential role of marine fungi in plastic degradation – a review. Frontiers in Marine Science, 8, 738877.
- Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the "plastisphere": Microbial communities on plastic marine debris. Environmental Science & Technology, 47(13), 7137–7146.
- Zhou, P., Fu, C., Fu, S., & Zhan, H. (2014). Purification and characterization of white laccase from the white-rot fungus panus conchatus. BioResources, 9(2), 1964–1976

APPENDIX I – MEDIA, REAGENTS AND STAIN

1) Potato Dextrose Agar

Composition

Ingredients	**Gms / Litre**
Infusion from potatoes	200.000
Dextrose (Glucose)	20.000
Agar	15.000
pH	5.6±0.2

Directions

Suspend 39.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates or tubes as desired. In specific work, when pH 3.5 is required, acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml of sterile, cooled medium is approximately 1 ml. Do not heat the medium after addition of the acid.

2) Sabouraud Dextrose Agar

Composition

Ingredients	**Gms / Litre**
Dextrose (Glucose)	40.000
Mixture of Peptone and Tryptone (1:1)	10.000
Agar	15.000
pH after sterilization	5.6±0.2

Mixture of Peptic digest of animal tissue and Pancreatic digest of casein (1:1)#

Directions

Suspend 65.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

3) 2% malt extract agar

Composition

Ingredients	**Gms / Litre**
Malt extract	20.000
Agar	15.000
Final pH	5.5±0.2

Directions

Suspend 35.00 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates or distribute into tubes as desired.

4) Czapek Dox Agar, Granulated

Composition

Ingredients	**Gms / Litre**
Sucrose	30.000
Sodium nitrate	2.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.500
Potassium chloride	0.500
Ferrous sulphate	0.010
Agar	15.000
Final pH	7.3±0.2

Directions

Suspend 49.01 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

5) Czapek Dox Broth, Granulated

Composition

Ingredients	**Gms / Litre**
Sucrose	30.000
Sodium nitrate	3.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.500
Potassium chloride	0.500
Ferrous sulphate	0.010
Final pH	7.3±0.2

Directions

Suspend 35.01 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely.Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

6) Tween 80 medium

Composition

Ingredients	Gms/ Litre
Peptone	10.000
Calcium chloride	0.100
Agar	1.000
Tween 80	10 ml
Distilled water	1000 ml

7) Phosphate Buffered Saline (PBS) (1x)

Components	**g/L**
Sodium chloride (NaCl)	8
Potassium chloride (KCl)	0.2
Disodium hydrogen phosphate (Na_2HPO_4)	1.44
Potassium dihydrogen phosphate (KH_2PO_4)	0.24

Adjust pH to 7.4

50% ethanol in Phosphate Buffered Saline (PBS)

Add 50 mL of ethanol in 50 mL PBS

Ethanol series

50% - 50 mL ethanol and 50 mL distilled water.

75% -75 mL ethanol and 25 mL distilled water.

85% - 85 mL ethanol and 15 mL distilled water.

95% - 95 mL ethanol and 5 mL distilled water.

8) Lactophenol Cotton Blue

Composition

Ingredients	
Phenol crystals	20.0 gm
Cotton blue	0.05 gm
Lactic acid	20.0 ml
Glycerol	20.0 ml
Distilled water	20.0 ml

APPENDIX II – DIAMETER OF FUNGAL COLONIES

Fungal isolate	Diameter on day 2
RS1C2	0.6
RS2C1	3.8
RS3C1	4.0
RS3C2	-
RS5C3	2.0
RS2M1	2.3
RS2M2	3.7
RS4M1	2.5
RS5M1	4.0
RS5M2	4.5
RS1P1	3.8
RS1P2	4.9
RS1P3	2.8
RS1P4	-
RS1P5	-
RS4P1	4.6
RS5P2	4.8
RS5P3	2.5
RS1S1	3.5
RS2S1	2.8
RS2S2	4.5
RS2S3	3.0
RS3S1	5.2
RS4S1	1.8
RS4S2	1.2
RS4S3	-
RS5S1	7.5
RS5S2	4.0

Fungal isolate	Diameter on day 2
CS1C1	3.0
CS3C1	3.4
CS4C1	0.6
CS5C1	2.2
CS5C2	3.4
CS5C3	0.6
CS1M2	3.4
CS2M1	2.5
CS2M2	3.4
CS4M1	2.0
CS4M2	2.8
CS5M1	0.6
CS5M3	0.6
CS5M4	1.0
CS5M5	3.2
CS5M6	2.2
CS3P1	0.8
CS4P1	0.9
CS5P1	3.0
CS1S1	3.4
CS3S1	0.6
CS4S1	2.8
CS4S2	0.6
CS4S3	1.9
CS5S1	1.0
CS5S2	0.9
CS5S3	1.0
CS5S4	0.6