

EVALUATION OF ANTIFUNGAL PROPERTIES OF ACTINOBACTERIA

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of
Bachelor of science degree in Microbiology

By

K. Parkavi

Rejith R.S. Kumar (41300034)



DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

CATEGORY - 1 UNIVERSITY BY UGC

Accredited with Grade “A++” by NAAC | 12B Status by UGC | Approved by AICTE
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

APRIL – 2024



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY-I UNIVERSITY BY UGC

Accredited "A++" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

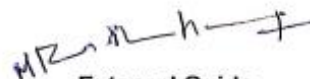
DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of **K. Parkavi (41300026)** & **Rejith R.S. Kumar (41300034)** & who carried out the project entitled "Evaluation of antifungal properties of actinobacteria" under my supervision from September 2023 to April 2024.


Internal Guide

Dr. S. USHA NANDHINI


External Guide

Dr. M. RADHAKRISHNAN

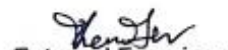


Head of the Department

Dr. V. RAMESH KUMAR

Submitted for Viva-voce Examination held on 26/09/2024


Internal Examiner

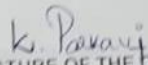

External Examiner

DECLARATION

We, **K. Parkavi (41300026)** and **Rejith R.S. Kumar(41300034)** and declare that the Project Report entitled "**EVALUATION OF ANTIFUNGAL PROPERTIES OF ACTINOBACTERIA**" done by us under the guidance of **Dr. S. USHA NANDHINI (Internal)** and **DR. M. RADHAKRISHNAN (External)** at Centre for Drug Discovery and Development, Sathyabama Research Park, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Microbiology.

DATE: 26/4/24

PLACE: CHENNAI


SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. REKHA CHAKRAVARTHI, Dean, School of sciences and humanities** and **Dr. V. RAMESHKUMAR, Head of the Department, and Department of Biotechnology** for providing me with the necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guides **Dr. M. RADHAKRISHNAN (External Guide)** and **Dr. S. USHA NANDHINI (Internal Guide)** for their valuable guidance, suggestions, and constant encouragement that paved the way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Microbiology and Centre for Drug Discovery and Development, Sathyabama Research Park, who were helpful in many ways for the completion of the project.

ABSTRACT

The study explores the potential of Actinobacteria, sourced from the Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, to produce bioactive metabolites with antifungal properties. Twenty isolates were cultivated under selective conditions, and strains BM-I A6 and BM-I A2 exhibited significant antifungal activity against pathogenic *Candida* species. Following isolation in the ISP2 broth medium, metabolic activity was observed using the Well diffusion technique. Both strains showed optimal growth in most ISP2 media, with notable inhibition against *Candida krusei* and *Candida Pelliculosa*. After conducting additional optimization tests, it was definitively determined that utilizing Mannitol as a carbon source, soybean as a nitrogen source, and CaCl_2 as a mineral source led to a noteworthy boost in metabolite production. This was evidenced by the presence of notably larger inhibitory zones. The strains demonstrated strong antifungal activity against *Candida krusei* and *Candida Pelliculosa*, with a maximum inhibitory zone of approximately 20mm. A time point assay technique indicated peak metabolite production on day 10. Further processing involved centrifugation and solvent extraction using methanol, ethyl acetate, chloroform, n-hexane, and dichloromethane (DCM). Extracts were concentrated and tested for antifungal activity against specific *Candida* species. The minimum inhibitory concentration of the extracted metabolites was determined using the triplicate micro broth dilution method. For partial purification of the metabolite, thin-layer chromatography was utilized, and its characterization was done through HPLC. The 16srrna sequencing technique was employed to identify and characterize the actinobacterial species, using previously extracted genomic DNA. This comprehensive study suggests promising avenues for the utilization of Actinobacteria-derived metabolites in clinical and medical applications, particularly in combating pathogenic fungi.

Keywords: Actinobacteria, antifungal activity, candida, metabolite production, extraction

TABLE OF CONTENT

S.NO	CONTENTS	Pg. No
	ABSTRACT	
	LIST OF TABLES	
	LIST OF FIGURES	
	LIST OF ABBREVIATIONS	
1	INTRODUCTION	
1.1	Candida	
1.2	Actinobacteria	
2	REVIEW LITERATURE	11
2.1	Candida infection	16
2.2	Anti candidal compounds	18
2.2.1	Plants based compounds	19
2.2.2	Nanoparticle-based anti-candidal compounds	20
2.2.3	Phenolic compounds	21
2.3	Significance of actinobacteria	25
2.4	Impact of actinobacteria against candidal infections	26
3	AIM AND OBJECTIVES	29
3.1	Aim	
3.2	Objectives	
4	MATERIALS AND METHODS	30
4.1	Description of Actinobacteria	30
4.2	Characterization of Actinobacterial strains	30
4.3	Production of Bioactive metabolites by Agar surface Fermentation	30
4.3.1	Inoculum preparation	30
4.3.2	Production of bioactive compounds	30
4.3.3	Testing for antifungal properties	30

4.4	Effect of fermentation methods on antifungal compound production	31
4.5	Characterization and Identification of Potential	32
4.5.1	Submerged fermentation	32
4.6	Antifungal activity by micro broth dilution method (minimum inhibitory concentration- MIC)	32
4.7	Purification of extracts	32
4.7.1	Thin layer chromatography (TLC)	33
4.7.2	High-performance liquid chromatography (HPLC)	33
4.8	Characterization and identification of potential strain	33
4.8.1	Micromorphology	33
4.8.2	Cultural Characterization	33
4.8.3	Physiological Characterization	33
4.8.3.1	Assimilation of carbon source	33
4.8.3.2	Assimilation of Nitrogen Source	33
4.8.3.3	Assimilation of Mineral Source	33
4.8.3.4	Effect of pH	33
4.8.4	Molecular characterization of potential strain	33
4.8.4.1	16s rRNA sequencing	34
4.8.4.2	Phylogenetic analysis	34
4.9	Statistical analysis	34
5	RESULTS AND DISCUSSION	35
5.1	Description and morphological characterization of selected actinobacterial strain	35
5.2	Characterization of collected Actinobacteria	35
5.3	Primary screening of selected actinobacterial cultures against microbial pathogens	37
5.4	Effect of fermentation method on bioactive compound production	37
5.5	Production of bioactive compounds by submerged fermentation	38

5.6	Antifungal activity by microbroth dilution method (minimum inhibitory concentration- MIC)	39
5.7	Partial purification and bioassay of crude extracts	40
5.7.1	Thin layer chromatography (TLC)	40
5.8	Characterization and taxonomy of potential actinobacterial strain	41
5.8.1	micromorphology	41
5.8.2	Cultural characteristics	41
5.8.3	Physiological characteristics	42
6	SUMMARY AND CONCLUSION	45
7	REFERENCE	46
8	APPENDICES	56

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
2.3	Comprehensive Analysis of Secondary Metabolites Profiles Across Actinobacterial Species	24
5.2	Morphological characteristics of actinobacterial cultures	35
5.3	Antifungal activity of actinobacterial culture in agar plug Method	37
5.5	Antifungal activity of extracted metabolites	37
5.6	Minimum inhibitory concentration	39
5.7.1	Calculation of the R _f value of the band formed by MOH-EA (1:0.5)	40
5.8.2	Culture Characteristic of strain BMI-A6 on different media	41
5.8.3	Effect of culture conditions on antifungal activity of potential actinobacteria strain BMI-A6	43

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
5.2	Actinobacterial culture from different ecosystem	36
5.3	Antifungal activity of actinobacterial culture in agar plug Method	37
5.4	Effect of fermentation method on bioactive compound production against <i>Candida krusei</i> and <i>Candida pelliculosa</i> .	38
5.6	Minimal inhibitory concentration of antifungal extracts on microtiter plates	40
5.7	Observation of solvent-metabolite movement of silica sheet under UV light	41
5.8.1	Microscopic observation of strain BMI-A6	41
5.8.2	Growth of potential actinobacterial strain BMI-A6 on ISP2 medium	42
5.8.3-A	Examination of effect of culture condition of potential culture BMI-A6	42
5.8.3-B	Effect of culture condition on antifungal activity of potential actinobacteria strain BMI-A6	43
5.9.2	Characterization of genomic DNA using agar gel electrophoresis technique	44

LIST OF ABBREVIATIONS

ATCC	- American Tissue Cell Culture
ISP 2	- International Streptomyces Project 2
MIC	- Minimum Inhibitory Concentration
ZOI	- Zone of Inhibition
MHA	- Muller Hinton Agar
OD	- Optimal Density
NaCl	- Sodium Chloride
MgSO ₄	- Magnesium Sulphate
FeSO ₄	- Ferrous Sulphate
KNO ₃	- Potassium Nitrate
SCA	- Starch Casein Agar
AIA	- Actinomycetes Isolation Agar
Rpm	- Revolutions per minute
Mg	- Milligram
µg	- Micro
DCM	- dichloromethane
UV	- Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 CANDIDA

There are only approximately 400 identified human disease-causing species among the estimated 1.5–5.1 million fungi on Earth. Around one billion infections and over 1.6 million fatalities are caused by this pathogenic fungus each year globally. Fungal infections are a growing category of nosocomial bloodstream infections; they are especially prevalent in patients in critical and intensive care, as well as in patients recovering from abdominal surgery or solid malignancies (Pongracz *et al.*, 2015). Around 1.7 million people die annually as a result of invasive fungal infections, which are a global concern. They are prevalent in immunocompromised individuals, as evidenced by their organ transplantation, acquired immune deficiency syndrome, and chemotherapy. More than 300,000 instances of invasive aspergillosis, 750,000 cases of candidiasis, and 10,000 cases of mucormycosis are reported each year, respectively. After accounting for Indian data estimations, the annual incidence of mucormycosis may surpass 900,000 cases. Moreover, a high death rate is linked to these illnesses. Invasive fungal infection epidemiology typically concentrates on particular regions. The fatality rates for invasive aspergillosis and candidiasis, for example, vary widely due to a lack of global data, ranging from 30% to 95% and 46% to 75%, respectively (Houst 2020).

In recent times, there has been a growing recognition that opportunistic fungal infections are a significant contributor to human illnesses, particularly in patients with compromised immune systems, including those undergoing transplants, HIV/AIDS patients, and cancer patients (Li *et al.*, 2015). Over 300 million people worldwide suffer from a serious fungal infection, and 25 million more could lose their lives or become blind as a result. Invasive fungal infections are the most prevalent and potentially fatal, including aspergillosis, pneumocystis, candidiasis, and cryptococcosis (Lacey, 2013). The past 30 years have seen a sharp increase in these infections; in certain hospital settings, the rate of invasive aspergillosis, for example, has increased by over 14 times. More than a million new cases of cryptococcosis will occur annually among

AIDS patients worldwide, according to the Centre for Disease Control and Prevention (CDC), and 600,000 people will pass away from the infection (Park, 2009). This is a significant increase given that, before the mid-1950s, the medical literature only contained reports of less than 300 cases of cryptococcosis (Sorrell *et al*, 2011).

Fungi can enter the body through certain medical devices, like catheters, and colonize the skin and mucosa. According to Leading International Fungal Education (LIFE), disseminated *aspergillosis* is another invasive fungal infection that is steadily rising in immunocompromised patients (Lass-Flörl, 2011). Its mortality rate ranges from 40% to 90% (Brown *et al.*, 2012), with an estimated 450,000 deaths annually. According to an estimate of 100,000 deaths per year, *Aspergillus* spp. is also the cause of severe asthma by fungal sensitization (SAFS) (Denning, 2012). Kauffman, Carol A, 2006. Approximately 70% of people have an opportunistic fungal pathogen called *Candida albicans* in their gastrointestinal and genitourinary tracts as a harmless commensal and 75% of women have had an infection at some point in their lives. But for immunocompromised patients, certain immunocompromised people, or even healthy people, it becomes an opportunistic pathogen. The general term for the infection brought on by *Candida albicans* is candidiasis. Depending on how severe the condition is, there are two classifications for candidiasis. The most well-known mucosal infection in the first category is thrush, which is indicated by white spots on the infected membranes. The mucosa of the vagina, oropharynx, and gastrointestinal epithelial cells are typically affected by these illnesses. In addition, vulvovaginal candidiasis (VVC) is a common infection among women, and some of them get recurrent cases of the infection, also referred to as RVVC. Nonetheless, it puts critically sick patients at risk for fatal systemic infections, with a 30% death rate. Immunocompromised people, such as HIV-positive patients, transplant recipients, chemotherapy patients, and low-birth-weight babies, frequently get systemic candida infections. Although some non-*albicans* species like *Candida glabrata*, *Candida krusei*, *Candida dubliniensis*, *Candida parapsilosis*, and *Candida tropicalis* are recovered from infected individuals, *C. albicans* remains a major infectious fungal agent. Historically, *C. albicans* has been known to us since 400 BC when the renowned Greek physician, Hippocrates, identified a microbial infection and named it “thrush,” which is caused by this pathogen. However, it was not studied like any other model organisms until the

late twentieth century, and early studies were mainly confined to identifying *C. albicans* strains (Kabir 2012).

Candida species have been associated with human beings for quite a long time as harmless commensals. They are commonly found on the mucosal surfaces of gastrointestinal and genitourinary tracts and the skin of humans. However, they become opportunistic pathogens in immunologically weak and immunocompromised patients. As opportunistic pathogens, they can cause local mucosal infections and sometimes, systemic infections in which *Candida* species can spread to all major organs and colonize these organs. Systemic infections can be life-threatening among individuals having severely paralyzed immune systems such as AIDS patients, people undergoing chemotherapy and radiotherapy treatment for cancers, and patients undergoing organ transplants. As the number of immunocompromised patients is increasing worldwide due to changes in lifestyle and improvements in medical facilities, infections caused by *Candida* species and mainly by *Candida albicans* have increased dramatically in the last two decades. This has posed a serious and daunting challenge to the effective management of candidiasis and cost has been increased manifold. It is estimated that in the United States itself, the excess cost due to candidemia is between \$1 and \$2 billion per year (Kabir & Ahmad 2013). According to (Liankis *et al.* (2014), systemic infection resulting from candida species is the fourth most common cause of nosocomial bloodstream infection in contemporary hospitals. A difficult clinical issue is presented by the rising rate of non-*Candida albicans* isolation and the quickly spreading resistance of *Candida* species. There are limited classes of antifungal drugs that are accessible, including polyenes, azoles, allylamines, echinocandins, and miazines. Their methods of action are limited to blocking DNA synthesis and targeting the cell envelope, which includes the wall and plasma membrane (Odds *et al.*, 2003).

Furthermore, the majority of these medications have major adverse effects on the host, such as amphotericin B's nephrotoxicity, voriconazole's visual abnormalities (Bayhan *et al.*, 2015), and itraconazole's congestive heart failure (Vollenbroich *et al.*, 2014). Furthermore, because of their excessive costs, some of these medications like echinocandins have limited clinical application. The emergence of various *Candida* species that are resistant to many widely used antifungal medications, particularly fluconazole, is particularly significant nowadays (Pfaller *et al.*, 2012). The problems of

drug resistance, patient sensitivity, and the inability to control infectious diseases have given real impetus to the ongoing global search for new antibiotics due to the exponential emergence of *Candida albicans* becoming resistant to antifungal antibiotics (Chopra *et al.*, 1997). This circumstance emphasizes the necessity of developing new, safe, and efficient antifungal medications. (Nickerson *et al.*, 2024). They may create biofilms, which would increase their resistance to antifungal medications (Chowdhary *et al.*, 2017).

1.2 ACTINOBACTERIA

According to Barka *et al.* (2016), Actinobacteria are Gram-positive bacteria with a linear genome rich in G+C that have the powerful metabolic ability to create secondary metabolites with a wide range of structural variety and commercial significance. Rahlwes *et al.* (2019), most of this species' members have a complex mycelial lifestyle with distinctive coloring and sporulation. Actinobacteria colonize an insect's exterior or interior and defend the host and its food supplies from infection (Kaltenpoth, 2009). According to Kaltenpoth (2009), actinobacteria colonize the exterior or inside of insects and defend them against pathogen attacks by supplying sustenance. Additionally, they foster nutrient cycling under challenging circumstances and can endure severe environments, such as the deep ocean (Bull and Goodfellow, 2019). Additionally, they propel nutrient cycling in challenging circumstances and endure in severe habitats, such as the deep ocean (Jose, 2021). Because of their ability to adapt to a wide range of environments and the interactions that accompany them, actinobacteria have evolved distinct biosynthetic potentials, allowing them to produce a broad range of secondary metabolites (Jose, 2021b).

According to Nejad *et al.* (2017), and other notable agricultural microorganisms, plant pathogenic fungi are responsible for severe illnesses and significant financial losses worldwide. Rot, necrosis, wilt, and other symptoms are typically present in plants infected with phytopathogenic fungus. Chemical insecticides are frequently used to prevent and treat a wide range of agricultural diseases because of their distinguishing qualities, which include high efficiency and ease of use. However, extensive and heavy use of fungicides has led to ecological degradation and food and environmental contamination, in addition to developing plant resistance to these chemicals (Xue *et al.*, 2013, Soltanzadeh *et al.*, 2016).

A variety of sustainable and alternative approaches were employed to reduce reliance on chemical fungicides for disease management (Kamil *et al.*, 2018). In addition to lessening the likelihood that other organisms will contract the disease, biological control can decrease the viability and activity of pathogens. It might be possible to effectively use natural enemies to lower or eliminate pathogen populations. Recently, there has been a lot of focus on increasing plant resistance to infections. Controlling pathogen invasion can also be accomplished by engineering transgenic plants, which are known to introduce genes to promote innate plant defense. Thus, it is imperative to identify novel compounds with potential bioactivity against a variety of phytopathogenic fungi to create innovative antifungal scaffolds that are as environmentally friendly and low-toxic as possible.

A type of potential resource to prevent plant fungal diseases, actinobacteria are a diverse group of filamentous Gram-positive soil bacteria with a high GC content. They are widely distributed in the environment (soil, freshwater, seawater, etc.) and have potent biological activities against most phytopathogens, including fungi and oomycetes (Trabelsi *et al.*, 2016). The most common actinobacterium genus, *Streptomyces*, is capable of producing a wide range of useful antibiotics, industrial enzymes, and bioactive secondary metabolites, such as fungicides, herbicides, and antibacterials (Ruanpanun and Chamswarnng, 2016). Since it is getting harder to find actinobacteria with a high potential for producing novel antibiotics (Bredholdt *et al.*, 2007), infectious diseases linked to multidrug resistance are spreading quickly all over the world. Over the past 20 years, the scientific community and researchers have become increasingly interested in the isolation of novel actinobacteria from extreme environments (Tanvir *et al.*, 2016). According to research, these actinobacteria from harsh environments have a distinct source of newly discovered biologically active substances.

Actinobacteria that are rare in nature possess the capacity to generate active metabolites, which are characterized by their diverse, singular, unparalleled, and sometimes complex compounds that are typically low in toxicity. Inner Mongolia has

an abundance of desert and calcareous soil due to its high altitude and variable climate, which creates a variety of harsh environments and an abundance of microorganisms with a broad range of environmental adaptation and resilience. These microorganisms are more resilient to harsh environmental conditions than other types of bacteria and fungi (Saeed *et al.*, 2017). Furthermore, there aren't many reports on Inner Mongolian soil actinobacteria's antibacterial activity. Inner Mongolia's actinobacteria may possess distinctive features due to its distinct ecological environment. Therefore, it's essential to assess the isolated actinobacteria's agricultural potential.

CHAPTER 2

REVIEW LITERATURE

2.1. CANDIDAL INFECTIONS

In recent decades, the nature of infectious diseases has undergone significant changes. Fungi that were once considered non-pathogenic or less virulent are now recognized as the primary cause of morbidity and mortality in severely ill or immunocompromised patients. Among the most common fungal pathogens are *Candida spp.* Previously, *Candida albicans* was the predominant cause of candidiasis. However, a recent shift towards non-*albicans* *Candida* species has been observed. These non-*albicans* *Candida* species demonstrate reduced susceptibility to commonly used antifungal drugs (Andes *et al.*, 2016).

Candida albicans is a type of yeast that can form biofilms, which are clusters of cells that are held together by an extracellular matrix (ECM). The ECM plays an important role in the development of biofilm-related infectious diseases and can be targeted to combat these diseases. In addition, extracellular DNA (eDNA) also plays a crucial role in the formation and structural integrity of *Candida* biofilms, as well as inducing the transition from yeast to hyphal growth form during biofilm development. Various factors, including systemic, local, hereditary, and environmental factors, can lead to disturbances in *Candida*'s normal homeostasis, resulting in a transition from normal flora to pathogenic and opportunistic infections. *Candida*'s virulence traits also contribute to the development of candidiasis. Oral candidiasis is a type of candidiasis that has a wide range of clinical manifestations, which can be classified into primary and secondary candidiasis. The main supply of *C. albicans* in the body is located in the gastrointestinal tract, and the development of infections occurs due to dysbiosis of the residential microbiota, immune dysfunction, and damage to the mucosal barrier (Patil *et al.*, 2015).

A recent study investigated the prevalence of non-*albicans* *Candida spp.* among *Candida* isolates from various clinical specimens and analyzed their virulence factors and antifungal susceptibility profile. A total of 523 *Candida spp.* was isolated from various clinical specimens, with non-*albicans* *Candida* species being the predominant

pathogens isolated. Non-albicans *Candida* species also demonstrated the production of virulence factors once attributed to *Candida albicans*. Moreover, non-albicans *Candida* demonstrated high resistance to the azole group of antifungal agents. Hence, non-albicans *Candida* species have emerged as an important cause of infections, and their isolation from clinical specimens can no longer be ignored as a non-pathogenic isolate nor dismissed as a contaminant (Arendrup & Patterson, 2017).

Candidiasis frequently occurs in newborns, immune-deficient people like AIDS patients, and people being treated with broad-spectrum antibiotics. It is mainly caused by *C. albicans*, while other species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* are increasingly being isolated. For effective treatment of local candidiasis, over-the-counter antifungal dosage forms such as creams and gels can be used. However, to prevent the spread of the disease to deeper vital organs, candidiasis antifungal chemotherapy is preferred. The use of probiotics and the development of novel vaccines are advanced approaches for the prevention of candidiasis (Aslam & Rotstein, 2019). *Candida* infections can result in serious health issues including candidemia and invasive candidiasis leading to high morbidity and mortality rates. Early detection and treatment are crucial in reducing the risk of death. Initially, echinocandin is recommended for both candidemia and invasive candidiasis. However, when the patient stabilizes and the organism is susceptible, transitioning to oral therapy is advisable. Targeted *Candida* prophylaxis should be considered for high-risk patients such as liver, small bowel, and pancreas transplant recipients (Deorukhkar *et al.*, 2014).

After confirming the diagnosis, treatment focuses on identifying and eliminating underlying causes such as poorly fitting oral appliances, medication history (antibiotics, corticosteroids, etc.), immunological and endocrine disorders, nutritional deficiencies, and prolonged hospitalization. Treatment using appropriate topical antifungal agents such as amphotericin, nystatin, or miconazole is typically effective in resolving superficial infection symptoms. In some cases, systemic antifungal agents may be necessary in immunocompromised patients. The selection of systemic antifungal agents should be based on the patient's history of recent azole exposure, intolerance to an antifungal agent, the dominant *Candida* species, and current susceptibility data (Hani *et al.*, 2015).

Antifungal use, subtherapeutic drug levels at infection sites, drug sequestration in the biofilm matrix, and suboptimal infection control are the main drivers of antifungal resistance in *Candida* infections. Recent studies suggest that DNA mismatch repair gene mutations may facilitate the acquisition of resistance mutations in *C. glabrata* specifically. Therefore, prompt diagnosis of antifungal-resistant *Candida* infections is crucial for the successful management of patients with these infections (Hirota *et al.*, 2016).

From 2006 to 2008, a study was conducted in 72 intensive care units (ICUs) across 14 European countries. The study observed 779 surgical patients with invasive candidiasis (IC). Of the patients, 62.5% were male and had a median age of 63 years. The study found that the median rate of candidemia was 9 per 1000 admissions. In 10.8% of the cases, the infection was already present at the time of admission to the ICU. This study identified *Candida albicans* as the most common species, accounting for 54% of the isolates. *Candida parapsilosis* accounted for 18.5%, *Candida glabrata* for 13.8%, *Candida tropicalis* for 6%, *Candida krusei* for 2.5%, and other species for 5.3%. Infections caused by *C. krusei* and *C. glabrata* had the highest crude mortality rates at 57.9% and 43.6%, respectively. The most common preceding surgery was abdominal (51.5%), followed by thoracic (20%) and neurosurgery (8.2%). *C. glabrata* was more often isolated after abdominal surgery in patients over 60 years old, while *C. parapsilosis* was more often isolated in neurosurgery and multiple trauma patients as well as children under 1 year old. The most common first-line treatment was fluconazole (60%), followed by caspofungin (18.7%), liposomal amphotericin B (13%), voriconazole (4.8%), and other drugs (3.5%). The mortality rate for surgical patients with IC in ICU was 38.8%. Multivariate analysis showed that the factors independently associated with mortality were patient age over 60 years (hazard ratio (HR) 1.9, p 0.001), central venous catheter (HR 1.8, p 0.05), corticosteroids (HR 1.5, p 0.03), not receiving systemic antifungal treatment for IC (HR 2.8, p <0.0001), and not removing intravascular lines (HR 1.6, p 0.02) (Lockhart, 2014).

A study identified 639 cases of invasive candidiasis (IC), with *C. albicans* being the most common species, followed by *Candida glabrata* and *Candida parapsilosis*. The most common site of infection was the bloodstream, followed by the intra-abdominal

area. The most frequently affected allograft groups were the liver and kidney. All-cause mortality at 90 days was 26.5% for all species, with *Candida tropicalis* and *C. parapsilosis* having the highest mortality rates. Non-white race and female gender were more commonly associated with non-*albicans* species. A high rate of breakthrough IC was seen in patients receiving antifungal prophylaxis, and factors associated with mortality include organ dysfunction, lung transplant, and treatment with a polyene antifungal. The only modifiable factor identified was the choice of antifungal drug class based on the infecting *Candida* species (Talapko *et al.*, 2023)

Candida albicans is still the predominant species causing candidemia, but it is causing fewer than 50% of U.S. cases. An increasing number of infections are being caused by *C. glabrata*, which now accounts for almost 30% of U.S. cases, as well as *C. parapsilosis* and other non-*C. albicans* species. In other countries, the predominant species varies by region. While pediatric candidemia rates are decreasing in the U.S., the cause of this decline is not yet fully understood. Although overall resistance to antifungal agents remains low among *Candida* species, there is concern about echinocandin resistance and multidrug resistance in *C. glabrata*. Although some aspects of candidemia and candidiasis remain the same, the rates, species, and susceptibility are constantly changing (Klingspor *et al.*, 2015).

2.2 Anti-candidal compounds

2.2.1 Plants-based compounds

Candida albicans is an organism that can cause both acute and chronic infections. It is hazardous for immune-compromised patients and can lead to nosocomial infections. In one study, researchers investigated the use of *Myrtus communis* extracts to develop new anti-candida agents using Accelerated Solvent Extraction (ASE). They also identified Gallic, Ferulic, and Quercetin phenolic compounds, which are known to have antimicrobial activity, using High-performance Liquid Chromatography (HPLC). Gallic acid and Quercetin were found in high amounts in Acetone and Methanol extracts, while Ferulic was recorded in low amounts in Methanol, Ethanol, and ethyl acetate extracts. The Methanol, Acetone, Ethanol, and Ethyl acetate extracts showed good anti-candida activity against *C. albicans*, with Minimum Inhibitory Concentration (MIC) ranging from 0.187 to 1.5 mg/mL, and Minimum Fungicidal Concentration (MFC) ranging from 0.375 to 3 mg/mL (Erdogan *et al.*, 2014).

In another study, researchers aimed to biosynthesize silver nanoparticles (AgNPs) for the first time using *Lotus lalambensis* Schweinf leaf extract (L-AgNPs). They investigated their anti-candidal potency alone or in combination with the leaf extract of *L. lalambensis* (L-AgNPs/LL) against *C. albicans*. The biosynthesized L-AgNPs were characterized by imaging techniques such as transmission electron microscopy (TEM), UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The results showed that L-AgNPs/LL had potent synergistic anti-candidal activity (24 mm inhibition zone). L-AgNPs/LL completely inhibited the morphogenesis of *C. albicans* and suppressed the adhesion and formation of biofilm by 82.5% and 78.7%, respectively. Further, L-AgNPs/LL inhibited the production of antioxidant enzymes of *C. albicans* by 80%. Scanning electron microscopy (SEM) and TEM revealed deteriorations in the cell wall ultrastructure in L-AgNPs/LL-treated *C. albicans*. Interestingly, L-AgNPs/LL showed less than 5% cytotoxicity when examined with either primary bone marrow-derived mesenchymal stem cells (BMSCs) or the MCF-7 cell line at MIC values of L-AgNPs/LL. The researchers identified L-AgNPs/LL as a potential drug for oral candidiasis in humans and animals (Abdallah & Ali, 2021). In a similar study, the essential oil and major bioactive components of *Mentha piperita* L. were tested for their anti-Candida activity and mode of action. Mint EO, carvone, menthol, and menthone were tested and showed varying degrees of anti-Candida activity. Mint EO showed the most potent activity. At their respective minimal inhibitory concentrations, these compounds also exhibited synergy with fluconazole. The anti-Candida activity was attributed to the compounds' ability to reduce ergosterol levels and inhibit PM-ATPase leading to intracellular acidification and ultimately cell death (Samber *et al.*, 2015).

2.2.2 Nanoparticle-based anti-candidal compounds

A study on silver nanoparticles concluded that the MIC values of ScAgNPs ranged from 0.125-0.250 mg/mL, whereas the MBCs and MFCs were 0.250 and 0.500 mg/mL, respectively. ScAgNPs significantly inhibited the production of phospholipases, proteinases, lipase, and hemolysin in *C. albicans*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, and *C. krusei*, respectively, at 500 µg/mL. ScAgNPs also inhibited germ tube formation in *C. albicans* by up to 97.1% at 0.25 mg/mL. LIVE/DEAD staining results showed that ScAgNPs almost completely inhibited biofilm formation in *C.*

albicans. To summarize, the biosynthesized ScAgNPs strongly suppressed the multiplication, germ tube, and biofilm formation, and most importantly, secretion of hydrolytic enzymes (phospholipases, proteinases, lipases, and hemolysin) by *Candida spp.* (Jalal *et al.*, 2019).

In another study, ZnS-ZnO nanocomposites were found to be effective against yeast species *Candida albicans*, *Candida tropicalis*, and *Saccharomyces cerevisiae*. These nanocomposites were prepared using a simple precipitation technique. Their antifungal activity was significantly higher when exposed to indoor light as compared to dark conditions. This was due to the inhibitory role of reactive oxygen species (ROS) generated by the photocatalytic nanocomposites. The generation of ROS was further confirmed by flow cytometry results and membrane permeabilization studies. However, antifungal activity was reduced under dark conditions due to the Zn²⁺ efflux in the solution (Suyana *et al.*, 2015)—a novel ketone derivative extracted from the *Diaporthe sp.* ED2, an endophytic fungus found in the medicinal herb *Orthosiphon stammers Benth*, has been studied for its anti-candidal properties. The compound, 3-hydroxy-5-methoxyhex-5-ene-2,4-dione, was found to be fungicidal against *C. albicans*, with a minimal inhibitory concentration of 3.1 µg/ml. However, its anti-candidal activity was affected at pH 2 and above 70°C (Tong *et al.*, 2017).

2.2.3 Phenolic compounds

Eighteen N-(4-aryl/cyclohexyl)-2-(pyridine-4-yl carbonyl) hydrazinecarbothioamide derivatives were synthesized and tested against ten clinical isolates of *Candida spp.* When comparing the results with itraconazole it was discovered that introducing p-chloro (2c), p-iodo (2q), m-chloro (2l), and o-nitro (2r) substitutions at the phenyl ring of thiosemicarbazide enhanced the anti-Candida activity. Compound (2c) bearing a p-chlorophenyl ring was the most effective against *Candida albicans* ATCC 66027, *Candida spp.* 12810 (blood), and *Candida spp.* 178 (HVS) with a MIC value of 0.09–0.78 lg/mL. Itraconazole showed inhibitory activity with a MIC value of 0.04–1.56 mg/mL against all tested strains. There is a correlation between anti-candidal activity and p-chloro substitution at the phenyl ring of thiosemicarbazide. The active compounds 2c, 2r, and 2a were further investigated for their cytotoxic effects on three cancer cell lines; HT1080 (skin), HepG2 (liver), and A549 (lung). The active compounds showed minimal cytotoxic activity against the non-cancer cell line and all

three cancer cell lines. Compound 2c displayed better activity against *C. albicans* ATCC66027 and *Candida spp.* [blood] compared to the reference drug (itraconazole), representing a good lead for the development of newer, potent, and broad-spectrum anti-Candidal agents (Bhat *et al.*, 2014).

New pyrazoline derivatives were synthesized from chalcones with cyclohexyl/phenyl thiosemicarbazide and were tested for anti-fungal activity against various strains of *Candida* species. Compound 7 was found to be the most potent antifungal agent against *Candida* strains, with para-methoxy substitution at one phenyl ring and meta-methoxy substitution at the other phenyl ring of pyrazoline moiety contributing significantly to the anti-candidal activity (Bhat *et al.*, n.d). New heterocycles containing disubstituted tetrazole derivatives were synthesized. O6 was found to be the best derivative that inhibited the growth of *Candida guilliermondii* with an average zone of inhibition of 14.0 mm, while O9 had the lowest zone of inhibition at 7.3 mm (Suyana *et al.*, 2015).

Similarly, three novel series of 20,40-difluoro-4-hydroxybiphenyl-3-carboxylic acid derivatives, namely 4-substituted-1,2,4-triazoline-3-thiones (4aeg); 2-substituted-1,3,4-thiadiazoles (5aeg), and 2-substituted-1,3,4-oxadiazoles (6aeg) were synthesized and tested twenty-one of the newly synthesized compounds against various bacteria, fungi, yeast species, and viruses. In addition, the carboxylic acid group of diflunisal was replaced with heterocycles and concluded the anti-inflammatory activity of heterocycles. Compound (5d) showed activity against *Escherichia coli* A1 and *Streptococcus pyogenes* ATCC-176 at a concentration of 31.25 mg/mL, whereas cefepime, the drug used as standard, was less active against the mentioned bacteria. Compound (4b) exhibited activity against *Aspergillus varicolor* and *Trichophyton rubrum* at a concentration of 31.25 and 15.25 mg/mL, respectively. In contrast, Amphotericin B, the drug used as standard, was less active against the mentioned yeast and fungi. The 1,3,4-thiadiazole derivative (5a) having a methyl group at the 2nd position showed the highest antiviral activity against Sindbis virus at 9.6 mg/mL. Compound (4c) exhibited the highest anti-inflammatory activity (73.03%), whereas diflunisal, the drug used as standard, was less active (24.16%). Compound (5f) presented similar antinociceptive activity to the standard drug (paw

withdrawal latency was 19.21 S compared to that of diflunisal which was 19.14 S in the hot plate test) (Küçükgüzel *et al.*, 2007).

Recent reports have shown that *Candida* is involved in the malignant transformation of oral fibrosis. This study aimed to isolate and identify an anticandidal compound from soil *Streptomyces sp. VITPK9*. This strain was isolated from a brine spring located in Thoubal district, Manipur, India—the ethyl acetate extract from the culture supernatant of *Streptomyces sp. VITPK9* was purified using silica gel column chromatography and HPLC. The purified compound was identified using ¹H and ¹³C NMR spectral data and based on its similarity index with reference compounds available in the mass spectra library of the National Institute for Standards and Technology. It was identified as pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-. The antifungal activity of the purified compound was tested against *Candida* strains following the guidelines of the National Committee for Clinical Laboratory Standards. The results showed that the MIC₅₀ value ranged from 0.78 to 2.00 µg/mL. These findings suggest that *Streptomyces sp. VITPK9* is a potential source of dithiopiperazine-type anticandidal compounds (Sanjenbam *et al.*, 2014).

2.3 SIGNIFICANCE OF ACTINOBACTERIA

Actinobacteria is a large group of microorganisms found in diverse environments including aquatic and terrestrial environments, and even within plants. They are known for their ability to produce bioactive compounds and act as biocontrol agents, especially against harmful fungi and bacteria. Additionally, some Actinobacteria can promote plant growth and development through various mechanisms such as producing phytohormones, siderophores, and solubilizing phosphate (Bull *et al.*, 2005).

Actinobacteria also play important ecological roles such as recycling substances, breaking down complex polymers, and producing bioactive molecules. They have been shown to have biotechnological potential for removing both organic and inorganic pollutants from the environment. This makes them promising candidates for bioremediation, which is becoming increasingly important due to the widespread release of contaminants into the environment. In particular, Actinobacteria have been studied for their ability to clean up areas contaminated with pesticides and heavy

metals. Various strategies such as bioaugmentation, biostimulation, cell immobilization, production of biosurfactants, and the use of plant-microbe systems have been developed to enhance their bioremediation capabilities (Álvarez *et al.*, 2017).

Actinobacteria are known for producing powdery colonies on agar surfaces due to their ability to create hyphae, sporangia, or conidia in culture media. Actinobacteria are also a valuable source of bioactive secondary metabolites that have important applications in pharmacology and other industries. They produce enzymes, enzyme inhibitors, and antibiotics, including aureomycin and streptomycin. Additionally, they are crucial in the decomposition of organic substances such as proteins, fats, cellulose, and chitin (Chaturvedi & Khurana, 2019). Most actinobacteria are obligate aerobes, which means that they need oxygen to survive, but this also exposes them to reactive oxygen species that can cause harm. They have to withstand oxidative attack by phagocytes when they cause infections. Actinobacteria can also survive for long periods under low oxygen tension, such as *Mycobacterium tuberculosis*, which can persist in the host for years in a latent, non-replicative state. This ability is due to the regulatory switches that control the actinobacterial response to peroxide stress, disulfide stress, and low oxygen tension. Actinobacteria use a unique pseudodisaccharide called mycothiol as their main low-molecular-weight thiol buffer. They also have WhiB-like proteins containing [4Fe-4S] which play important roles in their biology, although their biochemical role is still being studied. Various actinobacterial genera have the potential to produce commercially important bioactive natural products. Among them, *Streptomyces* is the most prolific producer of bioactive natural products (Hengst & Buttner, 2008).

Marine actinobacteria are unique in that they exhibit different biological properties, including antimicrobial, anticancer, antiviral, insecticidal, and enzyme-inhibitory activities. In the natural environment, antimicrobial compounds limit the growth of competitors and provide a selective advantage to the producer, especially when nutrients are limited, and the developmental program leading to spores begins. Understanding the control of this secondary metabolism provides insights into how it integrates with a complex lifecycle that takes multiple cues from the environment and

primary metabolism. This information can be used to devise laboratory screening conditions to identify new or improved clinical compounds (Hazarika & Thakur, 2020).

Actinobacteria have resistance mechanisms, including superoxide dismutase, efflux transporters, and metal-binding proteins that help to produce them. They can cause degradation of herbicides, pesticides (organochlorine-lindane), heavy metals, chromium (VI), petrochemicals, nitroaromatics, 2,4,6 trinitrotoluene (TNT), tannery wastes, and aromatic compounds. Bioremediation is an appropriate remedy that depends on the nature of the contaminants. If the contaminants are susceptible to biodegradation by the site organisms (without the need for human intervention), it is known as intrinsic bioremediation. Alternatively, relevant organisms can be added, i.e., Engineered bioremediation. In both cases, the rate of bioremediation depends on the type and concentration of contaminant, the microbial community, and the subsurface hydrogeochemical conditions (Menéndez & Carro, 2019)

Actinobacteria exhibit several mechanisms of pathogen suppression and plant growth promotion. To utilize these activities of actinobacteria in sustainable agriculture for crop protection and production, it is necessary to formulate the actinobacteria. Formulation is the process of mixing microbes or microbial products such as antibiotic secondary metabolites and cell wall-degrading enzymes with inert materials amended with nutritional supplements (in the case of live microbes) to enhance their activity and viability during storage and field application. Several researchers attributed the inconsistency and failure of biocontrol agents under field conditions to the lack of proper formulation of the biocontrol agents. The type of formulation depends on the nature of the biocontrol agent (either live microbe or microbial products), site of application (soil, seed treatment, or foliar application), stability, and delivery at the site of action and the target pathogen. Traditionally, biocontrol formulations were prepared as liquids or powders. Dry formulations (granules or powders) are generally preferred because of extended shelf life, easy transportation, and storage, which can be suspended in liquid (oil, water, or oil in water emulsion) at the time of application (Manivasagan *et al.*, 2014). In a study of cucumber plants, researchers found that healthy plants had more antifungal actinomycetes than diseased plants. They identified a *Streptomyces* strain HAAG3-15, that showed strong antifungal activity against *F. oxysporum* f. sp. *cucumerinum* and promoted growth. The researchers

isolated azalomycin B as the bioactive compound of HAAG3-15. They believe it could be developed as a potential biocontrol agent for use in organic agriculture. The study suggests that healthy roots near infected plants are a good source of biocontrol and plant growth-promoting endophytes (Cao *et al.*, 2020).

Actinobacteria presents a unique biotechnology resource that has a vast range of potential applications, including antimicrobials, biopharmaceuticals, agrichemicals, and biocatalysts. It is important to note that this resource is not yet fully exploited, and recent screening efforts have focused on marine actinobacteria, revealing numerous new chemical entities and bioactive. The discovery rate of these new compounds surpasses that of terrestrial actinobacteria, which underlines the immense potential of marine actinobacteria for biotechnology innovation (Van Der Heul *et al.*, 2018).

Table 2.3: Comprehensive Analysis of Secondary Metabolites Profiles Across Actinobacterial Species

Sl.no	Species	Antifungal compound	Fungal Pathogen	References
1.	<i>Sceliphron caementarium</i>	sceliphrolactam	amphotericin B-resistant <i>C. albicans</i>	(Jakubiec-Krzeński et al., 2018)
2.	<i>S.lavenduligriseus</i>	15-glycidylfilipin III	<i>Candida albicans</i>	
3.	<i>Streptomyces</i> sp.	enduspeptides A, B, and C	<i>Candida glabrata</i> ATCC 90030	
4.	<i>Streptomyces</i> sp. SNM55	Mohangamides A and B	<i>Candida albicans</i> isocitrate lyase (ICL)	
5.	<i>Actinoalloteichus</i> sp. NPS702	Neomacclafungin A	<i>Trichophyton mentagrophytes</i> ATCC9533	
6.	<i>S.albidoflavus</i> NBRC13010T	Antimycin A19	<i>Aspergillus niger</i>	
7.	<i>S. amritsarensis</i> V31	6-amino-5-nitrosopyrimidine-2,4-diol	<i>R. solani</i> , <i>A. flavus</i> , <i>F. oxysporum</i> , <i>S. oryzae</i> , and <i>S. sclerotiorum</i>	(Shahid et al., 2021)
8.	<i>Streptomyces zhaozhouensis</i> CA-185989	Ikarugamycin derivatives	<i>Aspergillus fumigatus</i> , <i>Candida albicans</i>	(Ahmed et al., 2018)
9.	<i>Kribella</i> MI481-42F6	Kribelloses	<i>Saccharomyces cerevisiae</i>	(Lacret et al., 2014)
10.	<i>Streptomyces puniceus</i>	Diketopiperazines	Isocitrate Lyase of <i>Candida albicans</i>	(Igarashi et al., 2017)
11.	<i>Streptomyces</i> sp.	n-hexadecanoic acid, tetradecanoic acid, and pentadecanoic acid	<i>Talaromyces marneffe</i>	(Kim et al., 2019)

12.	<i>Streptomyces xinghaiensis</i> SCSIOS15077	tunicamycin E	<i>Candida albicans</i> ATCC96901	(Sangkanu et al., 2020)
13.	<i>Streptomyces</i> sp. ZZ741	Streptoglutirimides A-J	<i>Candida albicans</i>	(Zhang et al., 2019)
14.	<i>Streptomyces</i> sp. TK-VL_333	indole-3-carboxylic acid	<i>Candida albicans</i>	(Kavitha et al., 2010)
15.	<i>Pseudonocardia alni</i>	glycopeptide antibiotic	<i>Trichophyton mentagrophyte</i>	(R Omran & MF Kadhem, 2016)

2.4 IMPACT OF ACTINOBACTERIA AGAINST CANDIDAL INFECTIONS

Actinobacteria are a group of microorganisms known for their ability to produce a large number of secondary metabolites, which has made them a subject of interest for their potential to be used as a source of novel antifungal agents. Fungal infections, especially those caused by *Candida* species, are a significant burden on global health, and the emergence of antifungal resistance has created a need for alternative therapeutic options. Actinobacteria, with their diverse secondary metabolite repertoire, offer promising avenues for discovering novel antifungal compounds. Actinobacteria are extensively studied for their potential use in producing antibiotics and other metabolites, and selective media and cultivation techniques like serial dilution and spread plate methods are commonly used to obtain pure cultures. Among the actinomycetes, *Streptomyces* species constitute 50% of the terrestrial population and are being screened for their therapeutic potential. Incorporating essential nutrients in the cultivation medium can significantly improve the production yield. After obtaining pure cultures, actinobacterial extracts or purified compounds are tested against a panel of clinically relevant fungal pathogens, including *Candida* species, for their antifungal activity (Assou *et al.*, 2023b).

One such experiment has concluded that marine-derived actinomycete extracts are potent against *T. marneffe*, one of the most important thermally dimorphic pathogenic fungi. Actinobacterial cultures are isolated and incubated under the conditions

provided, which involves adopting plating techniques, especially the spread plate technique and streak plate technique (Chandrashekar *et al.*, 2019).

To prepare the media for the study, distilled water is commonly used, but in rare cases, seawater is used as an alternative. Sediment samples are suspended in sterile distilled water, mixed, and serially diluted up to 10^5 . Aliquots from the dilutions are spread over agar plates and incubated at 28°C for one month, ensuring quality growth of isolated in the respective media (Ranjita *et al.*, 2018).

When studying actinobacterial isolates, it is important to conduct a thorough phenotypic characterization. This involves closely observing the unique features of the bacteria, such as the pigmentation of colonies, the presence of diffuse and melanoid pigments, and the color of aerial and substrate mycelia. Additionally, the arrangement of spore chains and aerial hyphae must be examined. To provide further characterization of the isolates, chemotaxonomic and classical biochemical tests are conducted (Siddharth *et al.*, 2020).

One method of studying the morphological characteristics of actinomycetal isolates is through the coverslip culture method. This involves growing the isolates on glycerol asparagine agar and examining them through a microscope to observe the structure of the mycelium and the arrangement of conidiospores. This allows for a more in-depth understanding of the unique features of the actinobacterial isolates and can aid in their identification and classification (Silambarasan, 2012b).

Actinobacteria are tested for their ability to produce metabolites that can combat specific species of microorganisms to confirm their antimicrobial properties. A study investigated the production of antifungal compounds by a particular strain using solid-state and submerged fermentation. Agar plates and broth-containing flasks were incubated for 23 days at a temperature of 28°C. Samples of the agar plug and cell-free supernatant were collected every 24 hours to test their effectiveness against *C. albicans* (Wang *et al.*, 2018). Metabolites responsible for antimicrobial properties are extracted via various techniques, and actinobacterial isolates are fermented for mass production of these metabolites. In a study, pure isolates were subcultured in Tryptone Yeast Extract broth as the seed medium (ISP-1 medium, Himedia, India) at 28°C for 2

weeks. After fermentation, the cell-free supernatant from each flask was extracted thrice with ethyl acetate. The crude concentrate was dried and suspended in methanol before bioactivity screening assays (Gulve & Deshmukh, 2012a).

The stability of the bioactive metabolite in the crude supernatant was tested under various conditions, such as subjected to temperatures ranging from -20 °C to 100 °C for 1 hour, as well as autoclaving at 121 °C/15 lbs pressure for 20 minutes. Additionally, the stability of the compound was tested by adjusting the pH ranges from 2 to 10. Finally, all samples were tested for their antagonism effect against *C. albicans* (Palla et al., 2018). A comparative study was conducted to examine the liability and antimicrobial properties of an extract. This was done by testing its ability to inhibit the growth of pathogens as compared to standard antifungal agents—the following study aimed to identify the antifungal properties of actinobacteria obtained from isolated areas. Twenty-four actinobacterial isolates with a diameter of inhibition of ≥ 25 mm against the MTCC strains were further tested using the disc diffusion assay. The crude culture extracts were obtained by mixing the cell-free culture supernatant with ethyl acetate and evaporated under reduced pressure. These extracts were then dissolved in 10% DMSO to a final concentration of 1mg/ml and loaded onto sterile filter paper discs. The MHA plates were seeded separately with test organisms and loaded with sterile discs impregnated with crude extracts. Finally, the diameters of the zone of inhibition were evaluated (Pazhanimurugan, 2012).

In the preliminary screening, the bacterial and fungal cultures were tested. To experiment, sterile cotton swabs were used to inoculate the cultures into freshly prepared NA plates (for bacterial strains) and SDA plates (for fungal strains). After that, wells were made on all the inoculated plates using a well cutter, each about 5mm in diameter. Then, 100 μ l of cell-free culture supernatant was loaded into each well. The plates containing bacterial strains were incubated at 37°C for 24 hours, while the ones containing fungal strains were incubated at room temperature for 4-5 days. After the incubation period, the diameter of the zone of inhibition was measured and expressed in millimeters (Radhakrishnan *et al.*, 2016). To determine the best solvent system for the separation of the compound, different solvents were used, including methanol, chloroform, acetic acid, n-butanol, n-hexane, and water in various proportions. After separation, the sheet was left at room temperature for complete drying, and the spots

were visualized in an iodine chamber. The R_f value of each spot on the TLC plate was determined (Subramani & Mathivanan, 2009). GCMS analysis was used to screen for bioactive or medicinally important compounds in *Actinomycetes* sp. The crude extract was separated using TLC and analyzed via column chromatography. GCMS analysis was used to determine the eluted individual compounds. The compounds were then categorized into three types: polyketides, peptides, and heterocyclic compounds. Their physical and chemical properties were also examined (Sasidhar *et al.*, 2021).

Actinobacterial metabolites exhibit diverse mechanisms of antifungal action, including disruption of cell membrane integrity, inhibition of cell wall synthesis, and interference with essential cellular processes. Elucidating these mechanisms enhances our understanding of their therapeutic potential. Promising actinobacterial-derived antifungal compounds undergo rigorous preclinical evaluation, including toxicity testing and efficacy studies in animal models of fungal infections. Advancements in formulation techniques aim to enhance the bioavailability and stability of these compounds for clinical use.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To evaluate the Antifungal properties of Actinobacteria

3.2 OBJECTIVES

- Culturing and screening of actinobacteria against *Candidal spp.* by agar plug method.
- Production of metabolites from active cultures in solid and liquid state fermentation.
- Optimization and purification of potential strains for metabolite production.
- Characterization and identification of potential strains

CHAPTER 4

MATERIAL AND METHODS

4.1 DESCRIPTION OF ACTINOBACTERIA

For this present study, about 20 actinobacterial cultures were obtained from the Actinobacterial Research Lab, Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu. All the cultures were previously isolated from terrestrial samples collected from different less explored ecosystems in India and maintained in International Streptomyces Project-2 (ISP-2) Agar slants as well as in 30% glycerol broth.

Table 4.1: List of Actinobacterial strains used for the anti-fungal stud

S.NO	STRAIN NAME	SOURCE	ECOSYSTEM
1.	BM 1-A2	Coal mine - NLC, Neyveli	Terrestrial
2.	BM 1-A5	Coal mine - NLC, Neyveli	Terrestrial
3.	BM 1-A6	Coal mine - NLC, Neyveli	Terrestrial
4.	BM 1-A7	Coal mine - NLC, Neyveli	Terrestrial
5.	BM 1-A8	Coal mine - NLC, Neyveli	Terrestrial
6.	BM 1-A15	Coal mine - NLC, Neyveli	Terrestrial
7.	BM 1-A16	Coal mine - NLC, Neyveli	Terrestrial
8.	PRA -11	Pahalgam River, Kashmir	Terrestrial
9.	PRA -14	Pahalgam River, Kashmir	Terrestrial
10.	PRA -15	Pahalgam River, Kashmir	Terrestrial
11.	PRA -16	Pahalgam River, Kashmir	Terrestrial
12.	PRA -17	Pahalgam River, Kashmir	Terrestrial
13.	SRA -3	Sind River, Kashmir	Terrestrial
14.	SRA -8	Sind River, Kashmir	Terrestrial
15.	AKA -11	Karungadu	Mangrove
16.	AKA -12	Karungadu	Mangrove
17.	MNA -1	Noney, Manipur	Terrestrial
18.	MNA -2	Noney, Manipur	Terrestrial
19.	MNA -3	Noney, Manipur	Terrestrial
20.	MNA -4	Noney, Manipur	Terrestrial

4.2 CHARACTERIZATION OF ACTINOBACTERIAL STRAINS

Cultural characterization of actinobacterial strains was studied using yeast extract malt extract agar (YEME) or International Streptomyces Project- 2 (ISP-2) medium. ISP-2 agar was prepared and sterilized by autoclaving at 120 °C for 15 minutes poured into sterile petri plates under aseptic conditions and allowed to solidify. Then all the actinobacterial cultures were inoculated into ISP-2 agar plates and incubated at 28 °C for 7-14 days. After incubation, all the plates were observed for growth, consistency, aerial mycelia color, reverse side pigment, and soluble pigment production (Shirling & Gottlieb 1966).

4.3 PRIMARY SCREENING OF ACTINOBACTERIA AGAINST CANDIDA SPECIES

4.3.1 Inoculum preparation

The fungal pathogens Viz., *Candida albicans* (C1), *Candida krusei* (C10), *Candida pelliculosa* (B169), and *Candida rugosa* (B19) used for this study were subcultured into Sabouraud dextrose agar (SDA) and incubated for 48 hours at 30 °C. Then the fresh single colonies were inoculated into Sabouraud dextrose broth (SDB) and incubated at 30 °C for 48 hours. To prepare standardized inoculum, the broth inoculum was adjusted to a final concentration of about 1x10⁶ CFU/ mL (OD₆₀₀ =0.08 to 0.1) by comparing with 0.5 McFarland standard.

4.3.2 Production of bioactive compounds

Bioactive compound from actinobacteria cultures was produced by agar surface fermentation (Radhakrishnan *et al.*, 2014). All the actinobacterial cultures were inoculated into yeast extract malt extract (YEME) agar plates and incubated at 28 °C for 10 days for the production of secondary metabolites. During incubation, the extracellular metabolites are secreted into the agar medium.

4.3.3 Testing for antifungal activity

The antifungal activity of actinobacterial cultures was tested by adopting the agar plug method (Radhakrishnan *et al.*, 2014). Actinobacterial cultures grown on ISP-2 agar plates were taken. Standardized test pathogens were inoculated into SDA plates using a sterile cotton swab. Agar plugs with 7mm diameter were cut from the ISP-2 agar grown with actinobacterial cultures were placed over an SDA plate seeded with test

pathogens. All the plates were incubated at 28 °C for 48 hours. The zone of inhibition was expressed in millimeters (mm) in diameter.

4.4 EFFECT OF FERMENTATION METHOD ON ANTIFUNGAL COMPOUND PRODUCTION

The effect of agar surface and submerged fermentation on antifungal compound production by the strain BM 1-A6 was investigated (Thenmozhi et al., 2023). Spores of the actinobacterial strain BM 1-A6 were inoculated into ISP -2 agar plates and broth was incubated at 28 °C for 10 days. ISP 2 broth was incubated in a rotary shaker with 110 RPM for 10 days. For every 24 hours, agar plugs from ISP-2 agar plates were taken and tested against *Candida krusei* (C10) and *Candida pelliculosa* (B169). Each 2 ml of ISP-2 broth was taken and centrifuged at 10,000 rpm for 10 minutes. The cell-free supernatant was tested against *Candida* species using the well diffusion method. Thus, the activity was checked for every 24 hours till the 10 days of incubation.

4.5 PRODUCTION OF BIOACTIVE COMPOUND BY SUBMERGED FERMENTATION USING SOLVENT EXTRACTION

Since the strain BM 1-A6 showed activity on both the agar plug and well diffusion methods, the actinobacterial compounds were produced in both solid and liquid cultures till the 10th day of fermentation. Hence, for further bulk production of the antibacterial compound from strain BM 1-A6, submerged fermentation methods were carried out (Thenmozhi et al., 2023).

4.5.1 Submerged fermentation

The potential BM 1-A6 strain was inoculated in an ISP-2 broth and then incubated for 10 days at 28 °C in a rotary shaker with 110 rpm. After incubation, the broth was centrifuged at 10,000 rpm for 15 minutes, then the supernatant was collected and an equal volume of different solvents which included ethyl acetate, methanol, n-hexane, Dichloromethane (DCM), and chloroform was added, mixed and kept undisturbed. The solvent portion was collected and concentrated using a Concentrator plus (Eppendorf) and quantified. Quantified crude extract was screened for activity against test pathogens by the well diffusion method. The crude extract was dissolved in 10% DMSO and made up to the working concentration at 100 ug/ml. The standard fungal inoculum was prepared and swabbed over SDA plates using sterile cotton. The extract

at working concentration was loaded into well-made SDA plates. The zone of inhibition was measured after 48 hours of incubation at 30 °C and expressed in millimeters (mm) in diameter.

4.6 ANTIFUNGAL ACTIVITY BY MICROBROTH DILUTION METHOD (MINIMUM INHIBITORY CONCENTRATION -MIC)

100µl of SDB was added in 96 well plates, and then 100 µl of the desired concentration of extract (500 µg/ml) was added to 1st well. The two-fold dilution was done until the final deserved concentration was achieved. Then, 50 µl of standard inoculum was added to all the wells. The positive well contains broth, pathogens, and fluconazole; the negative control contains broth, and pathogen and only broth serves as blank. The optical density at 600 nm was taken and incubated at 30°C for 48 hours. After incubation, the OD reading at 600 nm was again taken and compared with the negative control value. The percentage of antifungal activity was calculated by using the formula (Thenmozhi et al.,2023);

$$\text{Percentage of antifungal activity (\%)} = \frac{\text{OD}_{600} \text{ of NC} - \text{OD}_{600} \text{ of Test}}{\text{OD}_{600} \text{ of NC}} \times 100$$

4.7 PURIFICATION OF CRUDE EXTRACT

4.7.1 Thin Layer Chromatography Analysis

A TLC sheet (Merck) was taken which is generally coated with silica gel, it was cut into small strips. The extract was diluted methanol was spotted on the TLC sheet using a capillary tube. The extract was usually spotted near the bottom of the sheet. The TLC sheet was then placed in the beaker containing the mixture of the solvents. Chromatogram was run by using different solvent systems with different ratios such as methanol, DCM, ethyl acetate, DMSO, n-hexane, and chloroform. The solvent moves up the sheet by capillary action carrying the extract with it. The solvent front was allowed to move to few centimetres off the top of the sheet. The TLC sheet was removed and allowed to air dry. The separated spots were then visualized by placing the sheet in UV @254nm and also by placing them in an iodine powder. The Retention factor (Rf) value was calculated using the below formula.

$$\text{Rf} = \frac{\text{Distance travelled by component}}{\text{Distance travelled by liquid}}$$

4.8 CHARACTERIZATION AND IDENTIFICATION OF POTENTIAL STRAIN

4.8.1 Micromorphology

Micromorphological characteristics of the potential strain were studied by adopting the slide culture method (Thenmozhi et al., 2022). 2 ml of melted ISP- 2 agar medium was mixed with the spores of actinobacterial strains BM 1-A6. It was poured on the surface of the sterile microscopic slide as a thin layer and kept in a sterile Petri plate for incubation at 28⁰ C for 7 days. The slide was observed under a bright field microscope at 45X magnification. Gram stain was performed and observed under 100X oil immersion.

4.8.2 Cultural characterization

Culture characteristics were studied by inoculating the Actinobacterial strains into Trypton yeast extract agar (ISP-1), Oatmeal agar (ISP-3), Glycerol Asparagine Agar (ISP-5), Tyrosin Agar (ISP-7), Starch Casein Agar (SCA), Actinomycete Isolation Agar (AIA), Glucose Yeast Malt Agar (GYM), Czapek Dox Agar (CDA) and Nutrient Agar (NA). The plates were incubated for 7 days at 28⁰C.

4.8.3 Physiological characterization

The selected isolate BM 1-A6 was optimized at different criteria and the suitable media for maximum metabolite production. The composition of the fermentation media used for optimization is Yeast extract 0.4%, NaCl 1%, Glucose 0.4%, and Agar-agar 2%, with pH maintained at 7 (Shirling & Gottlieb 1966).

4.8.3.1 Assimilation of carbon source

To study the effect of carbon source the selected isolate was streaked on individual petri plates containing fermentation media with different 1% carbon sources namely Glucose, Lactose, Xylose, Starch, Mannitol, Inositol, and Raffinose, Cellulose, Starch, Sucrose. The plates were further incubated, at 30⁰ C for 7 days and checked for antibacterial activity. All sources were filter sterilized in a 0.4 micro filter.

4.8.3.2 Assimilation of nitrogen source

The effect of the nitrogen source on the selected isolate was streaked on Petri plates containing fermentation media and replaced with the nitrogen source alone. The nitrogen sources used are soybean, Malt, Peptone, Yeast, and Potassium nitrate (KNO₃). The plates were further incubated at a room temperature of 28 ⁰C for 7 days.

4.8.3.3 Assimilation of mineral source

The effect of the mineral source on the selected isolate was streaked on Petri plates containing fermentation media and replaced with the mineral source alone. The mineral sources used are Sodium chloride (NaCl), Magnesium sulfate (MgSO₄), Ferrous sulfate (FeSO₄), Manganese chloride (MnCl₂), and Calcium chloride (CaCl₂). The plates were further incubated at room temperature.

4.8.3.4 Effect of pH

To study the effect of pH the isolate was streaked on a fermentation medium and the pH was adjusted to 4, 5, 6, 7, 8, and 9 and incubated to identify the optimum pH required for metabolite production. The plates were further incubated at room temperature.

4.8.3.5 Effect of NaCl tolerance

To study the NaCl tolerance the isolate was streaked on a medium with different concentrations of NaCl. (i.e.,) 0.1%, 0.5%, 1%, 2%, 3%, 4% and 5% of NaCl. The plates were further incubated at room temperature.

4.8.4 Molecular characterization

4.8.4.1 16s RNA sequencing

The potential Actinobacterial, strain BM 1-A6 was subjected to 16s rRNA sequencing. The 16s rRNA gene was amplified by PCR using universal primers 27F (5'AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' TACGGNTACCTTGTTACGACTT 3').

4.8.4.2 Phylogenetic analysis

16s rRNA gene sequences of the isolate were compared with 16s rRNA sequences available in the BLAST search in NCBI, Gene bank database. The 16s rRNA sequences of potential strains BM 1-A6 were deposited in the GenBank database.

4.9 STATISTICAL ANALYSIS

All values represent the mean values (n=3).

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 DESCRIPTION AND MORPHOLOGICAL CHARACTERIZATION OF SELECTED ACTINOBACTERIAL STRAINS

A total of 20 actinobacterial cultures were obtained from the Actinobacterial Research Lab at the Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu. These cultures were isolated from soil samples collected from different ecosystems in India and maintained in ISP2 agar slants and 30% glycerol broth.

5.2 CHARACTERIZATION OF COLLECTED ACTINOBACTERIA

All the 20 actinobacterial cultures showed optimal growth on the ISP2 agar medium. About 50% of the culture produced white color aerial mycelial growth on ISP2 agar. All the plates were observed for growth, consistency, aerial mycelia, reverse side pigment, and soluble pigment production which are listed below in Table 5.2, Figure 5.2.

Table 5.2: Morphological characteristics of actinobacterial cultures

Sl. No	Strain	Media	Growth	Consistency	Aerial mycelium	Reverse pigment	Soluble pigments
1.	BM 1-A2	ISP-2	Good	Rough and powdery	Half white	Yellowish brown	-
2.	BM 1-A5	CDA	Good	Powdery	Grey	Pale brown	-
3.	BM 1-A6	CDA	Good	Powdery	Sandal	yellow	-
4.	BM 1-A7	AIA	Good	Powdery	White	Pink orange	-
5.	BM 1-A8	AIA	Good	Powdery	Grey	Half white	-
6.	BM 1-A15	AIA	Good	Powdery	White	Pale grey	

7.	BM 1-A16	AIA	Good	rough and powdery	White	Pinkish orange	-
8.	PRA -11	AIA	Good	Leathery	White	White	
9.	PRA -14	SCA	Good	Powdery	Grey	Purple	+
10.	PRA -15	SCA	Good	Powdery	Green	Purple	-
11.	PRA -16	SCA	Good	Powdery	Half white	Half white	
12.	PRA -17	SCA	Good	Powdery	Greenish grey	Blackish green	+
13.	SRA -3	NA	Moderate	Rough	White	White	-
14.	SRA -8	SCA	Good	Soft	Pale pink	-	-
15.	AKA -11	NZ-A	Good	Powdery	Grey	Black	Black
16.	AKA -12	NZ-A	Good	Powdery	Grey	Black	Black
17.	MNA -1	ISP-2	Good	Soft	Pale orange	Pale yellow	-
18.	MNA -2	ISP-2	Poor	Soft	Pale brown	Half white	-
19.	MNA -3	ISP-2	Moderate	Leathery	Pale white	Half white	-
20.	MNA -4	ISP-2	Good	soft	Pale orange	sandal	-



Figure 5.2: Actinobacterial cultures from different ecosystem

5.3 PRIMARY SCREENING OF SELECTED ACTINOBACTERIAL CULTURES AGAINST MICROBIAL PATHOGENS

In this study, all 20 actinobacterial strains were screened for antifungal activity against the fungal pathogens viz, *Candida albicans* and non-albican species. Of the 20 tested five actinobacterial cultures showed promising results in which BMI-A6 showed a maximum zone of inhibition against all pathogens (7.5- 15 mm) (Figure 5.3 & Table 5.3).

Table 5.3: Antifungal activity of actinobacterial culture in agar plug Method

S. No	Strain	Zone of Inhibition in diameter (in mm)			
		C I	C 10	B 19	B 169
1	BMI-A2	12	15	12.5	12.5
2	BMI-A5	9	7.5	12.5	9.5
3	BMI-A6	15	14	7.5	15
4	BMI-A16	9	7.5	15	10
5	SRA -3	9	7.5	9	8.5

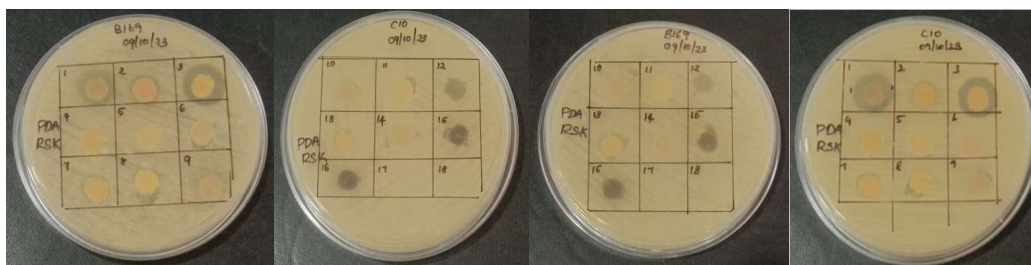
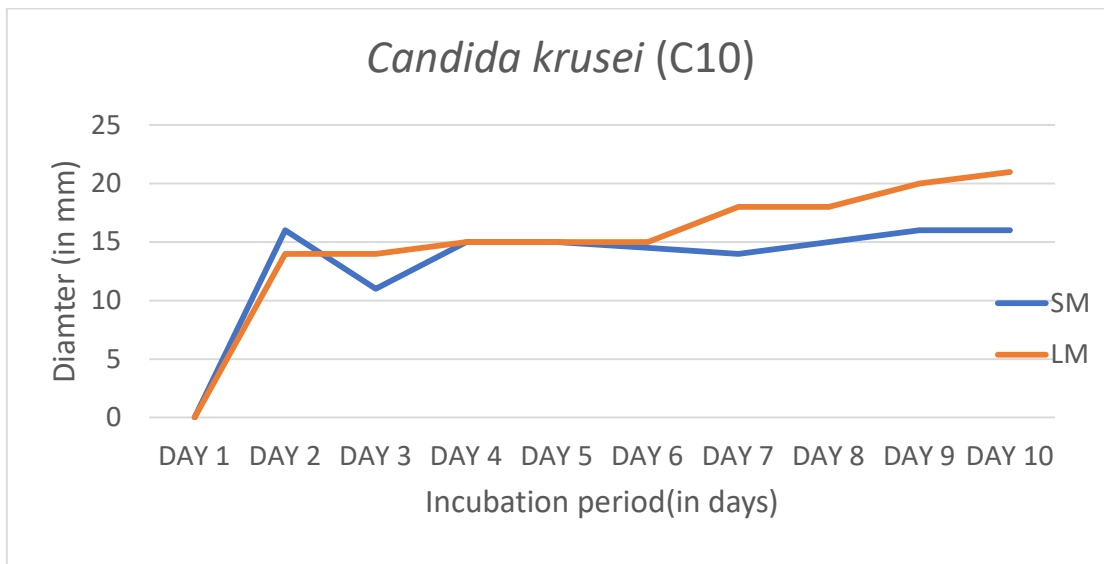


Figure 5.3. The agar plug method was performed to examine the antifungal properties of actinobacterial cultures against different *Candida* spp.

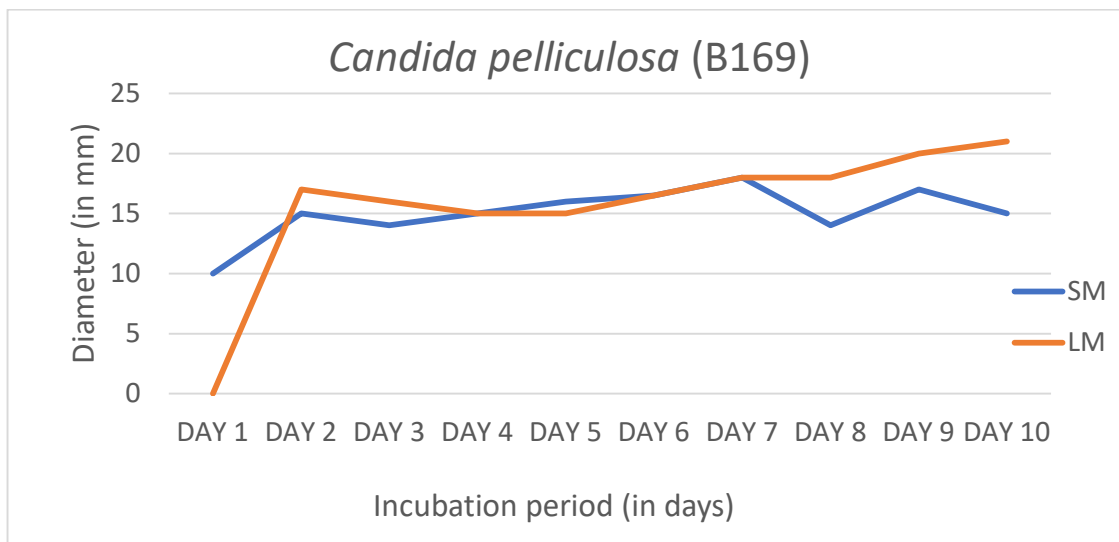
5.4 EFFECT OF FERMENTATION METHOD ON ANTIFUNGAL COMPOUND PRODUCTION

The effect of the fermentation method on Antifungal compound production from strain BMI-A6 is given in graphs 5.4.1 & 5.4.2. The result showed that strain BMI-A6 was found to produce Antifungal metabolites from the second day of fermentation in both agar surface and submerged fermentation, after comparing the results in different

fermentation methods, submerged fermentation showed promising Antifungal activity on the eighth day.



Graph 5.4.1: Effect of fermentation method on bioactive compound production against *Candida krusei*.



Graph 5.4.2: Effect of fermentation method on bioactive compound production against *Candida pelliculosa*.

5.5 PRODUCTION OF BIOACTIVE COMPOUND BY SUBMERGED FERMENTATION

Actinobacterial strain BMI-A6 showed optimal growth on the ISP2 broth medium. The broth medium was exposed to different solvents such as methanol, ethyl acetate, dichloromethane (DCM), chloroform, and n-hexane. The final product was

concentrated and dissolved in 10% DMSO for antifungal activity (table 5.5). The methanol extract of the strain showed inhibition for about 14mm and 13mm against *Candida pelliculosa* and *Candida krusei*.

Table 5.5: Antifungal activity of extracted metabolites

Sl.no	Sample	Antifungal activity (in mm)	
		<i>Candida krusei</i> (C10)	<i>Candida pelliculosa</i> (B169)
1.	Ethyl acetate	9 mm	9mm
2.	Chloroform	-	-
3.	Dichloromethane (DCM)	9mm	9mm
4.	Methanol	13mm	12mm
5.	n-hexane	-	-
6.	Dimethyl sulfoxide (DMSO)	-	-

5.6 ANTIBACTERIAL ACTIVITY BY MICROBROTH DILUTION METHOD (MINIMUM INHIBITORY CONCENTRATION -MIC)

To measure the MIC value, two-fold serial dilutions of 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 1.9 ug/mL of the extract were tested in the presence of fluconazole as a control system. 50 % of growth was inhibited at 125 ug/mL and 250 ug/mL concentration of extract against *C. krusei* and *C. pelliculosa* (table 5.6).

Table 5.6: Minimum inhibitory concentration

Sample	<i>Candida krusei</i> (C10)		<i>Candida pelliculosa</i> (B169)	
	Concentration (in µg/ml)	Percentage (in %)	Concentration (in µg/ml)	Percentage (in %)

A6	500	72.4	500	69.6
	250	67.9	250	51.6
	125	52.3	125	43.8
	62.5	46.7	62.5	36
	31.25	30.1	31.25	25.7
	15.62	17.5	15.62	13.3
	7.81	9.1	7.81	5.2
	3.90	3.3	3.90	0.7
	1.95	0.4	1.95	0.1

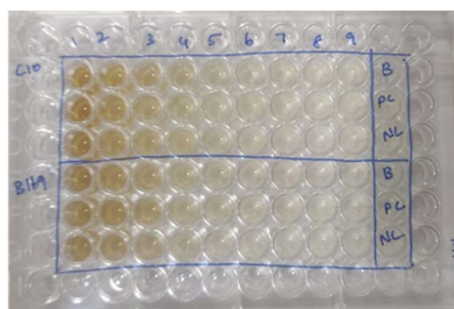


Figure 5.6 Minimal inhibitory concentration of antifungal extracts on microtiter plates.

5.7. PURIFICATION OF CRUDE EXTRACT

5.7.1 Thin Layer Chromatography Analysis

Thin-layer chromatography (TLC) was performed on a crude extract of BMI-A6 using a TLC silica sheet. The targeted metabolites were obtained and observed in the conserved combination of methanol and ethyl acetate at a ratio of 1:0.5. The spots were observed under fluorescent UV (ultraviolet) light. The R_f values of the bands were calculated.

Table 5.7.1. calculation of the R_f value of the band formed by MOH-EA (1:0.5)

Sl.no	Band	R _f value
1.	A	0.84
2.	B	0.89

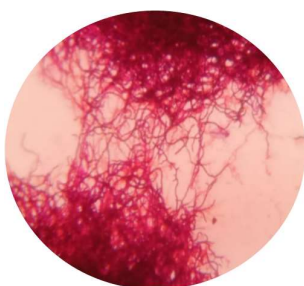


Figure 5.7. Observation of solvent-metabolite movement of silica sheet under UV light.

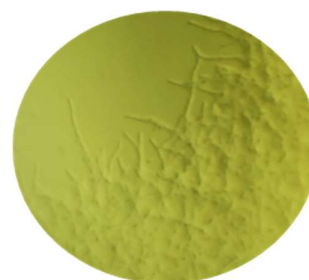
5.8. CHARACTERIZATION AND TAXONOMY OF POTENTIAL ACTINOBACTERIAL STRAIN

5.8.1 Micromorphology

Under bright-field microscopic observation, strain BMI-A6 appeared as Gram-positive filamentous bacteria with the presence of aerial and substrate mycelium.



Gram staining under 100 X oil immersions



Substrate mycelium of BMI-A6 under 45 X objective

Figure 5.8.1: Microscopic observation of strain BMI-A6.

5.8.2 Cultural characteristic

The cultural morphology of strain BMI-A6 is given in Table 5.8.2. The strain exhibited robust growth and produced powdery, white aerial mycelium on the ISP2 medium (figure 5.8.2).

Table 5.8.2: Culture Characteristic of strain BMI-A6 on different media

Media	Growth	Consistency	Aerial mycelium	Reverse pigment	Soluble pigment
ISP1	Moderate	No sporulation	None	None	-
ISP 2	Good	Powdery	White	White	-
ISP 3	Moderate	No sporulation	None	None	-
ISP 5	Moderate	No sporulation	None	None	-
ISP 7	Good	Powdery	White	White	-
AIA	Good	Powdery	White	White	-
GYM	Poor	No sporulation	None	None	-
CDA	Moderate	No sporulation	None	None	-
NA	Good	Powdery	White	White	-
SCA	Good	Powdery	White	White	-



Figure 5.8.2: Growth of potential actinobacterial strain BMI-A6 on ISP2 medium.

5.8.3 Physiological characteristics

Strain BMI-A6 grew well and examined for growth and sporulation (figure 5.8.3-A). It utilized a wide range of carbon sources, nitrogen sources, mineral sources, and also in different pH (table 5.8.3). Following thorough optimization tests, it has been established that incorporating Mannitol as a carbon source, soybean as a nitrogen source, and CaCl₂ as a mineral source resulted in a significant increase in metabolite production. This was supported by the observation of significantly larger inhibitory

zones. The strains exhibited robust antifungal properties against *Candida krusei* and *Candida Pelliculosa*, with an impressive maximum inhibitory zone of approximately 20mm (figure 5.8.3-B).

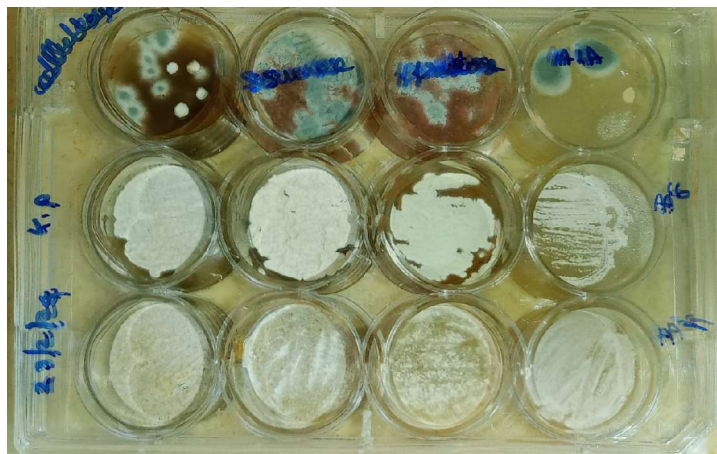


Figure 5.8.3-A: Examination of effect of culture condition of potential culture BMI-A6



Figure 5.8.3-B: Effect of culture condition on antifungal activity of potential actinobacteria strain BMI-A6.

Table 5.8.3: Effect of culture conditions on antifungal activity of potential actinobacteria strain BMI-A6.

Variables		Growth	Antifungal activity (diameters in mm)	
			C10	B169
Carbon source	Glucose	Good	17mm	12mm
	Sucrose	Good	15mm	13mm
	Xylose	Good	16mm	17mm
	Inositol	Good	17mm	16mm
	Mannitol	Good	16mm	22mm
	Fructose	Good	11mm	12mm
	Raffinose	Good	16mm	14mm
	Lactose	Good	18mm	15mm
	Starch	Good	15mm	16mm
	cellulose	Good	12mm	13mm
Nitrogen source	Malt	Good	13mm	NI
	Yeast	Good	10mm	10mm
	Peptone	Good	12mm	15mm
	Soybean meal	Good	13mm	19mm
	KNO ₃	moderate	NI	10mm
Mineral source	NaCl	Good	14mm	NI
	MgSO ₄	Good	11mm	NI
	MnCl ₂	poor	NI	NI
	FeSO ₄	moderate	9mm	NI
	KCL ₂	Good	NI	NI
	CaCl ₂	Good	15mm	12mm
pH	4	Good	17mm	15mm
	5	Good	NI	NI
	6	Good	NI	NI
	7	Good	18mm	18mm
	8	Good	14mm	15mm

	9	Good	12mm	12mm
NaCl (in %)	0.1	Good	13mm	9mm
	0.5	Good	12mm	10mm
	1.0	Good	14mm	10mm
	2.0	Good	14mm	10mm
	3.0	Good	14mm	10mm
	4.0	Good	15mm	14mm
	5.0	Good	15mm	10mm
Different media	ISP1	Moderate	14mm	11mm
	ISP 2	Good	17mm	17mm
	ISP 3	Moderate	12mm	9mm
	ISP 5	Moderate	11mm	NI
	ISP 7	Good	13mm	NI
	AIA	Good	17mm	16mm
	GYM	Poor	12mm	11mm
	CDA	Moderate	14mm	15mm
	NA	Good	12mm	15mm
	SCA	Good	NI	NI

CHAPTER 6

SUMMARY AND CONCLUSION

The present study attempted to explore actinobacteria for antifungal activity against Candidal pathogens. Totally 20 actinobacterial colonies were procured from the Bioprospecting division of the Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu. The antifungal activity of all 20 strains was determined by the agar plug method. Strains BM-1-A6 showed promising activity (7.5 - 15 mm inhibition) against *Candida krusei* and *Candida pelliculosa*. Bioactive metabolites from the strain BM-1-A6 were produced by adopting agar surface fermentation and submerged fermentation using ISP2 agar and ISP2 broth, respectively. Antimicrobial Activity of agar plug from ISP2 agar and cell-free supernatant from ISP2 broth was tested against *Candida* by agar plug and well diffusion method, respectively for every 24 hours up to 10 days. Through the strains, BM-1-A6 produced antifungal compounds in both ISP2 broth and agar and submerged fermentation was selected for further studies. Crude antifungal extract from the strain BM-1-A6 was produced by adopting submerged fermentation and extracted using methanol. Strain BM-1-A6 produces 12mg of crude extract per 100 ml of ISP2 agar medium. The extract showed 12-14 mm inhibition against the fungal pathogens. The MIC study revealed that the growth of the pathogen was inhibited at 250 – 500 µg/ml concentration of crude extract. Microscopic, culture, and physiological characteristics of strains BM-1-A6 were studied by adopting standard procedures. Based on the studied phenotypic characteristics, actinobacterial strain BM-1-A6 was identified as *Streptomyces* sp. The effect of various medium components on antimicrobial compound production by the strain BM-1-A6 was studied by adopting the agar plug method. Various sugars nitrogen compounds and minerals were found to influence the antifungal compound production. The finding of the present study concluded that Strain BM-1-A6 will be a promising source for antifungal metabolites against *Candida* sps.

CHAPTER 7

REFERENCE

1. Abdallah, B. M., & Ali, E. M. (2021). Green Synthesis of Silver Nanoparticles Using the Lotus Lalambensis Aqueous Leaf Extract and Their Anti-Candidal Activity against Oral Candidiasis. *ACS Omega*, 6(12), 8151–8162. <https://doi.org/10.1021/acsomega.0c06009>
2. Alvarez, A., Saez, J. M., Davila Costa, J. S., Colin, V. L., Fuentes, M. S., Cuozzo, S. A., Benimeli, C. S., Polti, M. A., & Amoroso, M. J. (2017). Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals. In *Chemosphere* (Vol. 166, pp. 41–62). Elsevier Ltd. <https://doi.org/10.1016/j.chemosphere.2016.09.070>
3. Andes, D. R., Safdar, N., Baddley, J. W., Alexander, B., Brumble, L., Andes, D. R., Safdar, N., Baddley, J. W., Alexander, B., Brumble, L., Freifeld, A., Hadley, S., Herwaldt, L., Kauffman, C., Lyon, M., Morrison, G., Patterson, V., Perl, T., Walker, T., ... Chiller, T.; (n.d.). Public Health Resources Public Health Resources 2016 Public Health Resources (Vol. 509). <http://digitalcommons.unl.edu/publichealthresources/509>
4. Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant candida: Epidemiology, molecular mechanisms, and treatment. *Journal of Infectious Diseases*, 216, S445–S451. <https://doi.org/10.1093/infdis/jix131>
5. Arendrup, M.C.; Fursted, K.; Gahrn-Hansen, B.; Schønheyder, H.C.; Knudsen, J.D.; Jensen, I.M.; Bruun, B.; Christensen, J.J.; Johansen, H.K. Semi-national surveillance of fungemia in Denmark 2004–2006: Increasing incidence of fungemia and numbers of isolates with reduced azole susceptibility. *Clin. Microbiol. Infect.* 2008, 14, 487–494.
6. Arendrup, M.C.; Fursted, K.; Gahrn-Hansen, B.; Schønheyder, H.C.; Knudsen, J.D.; Jensen, I.M.; Bruun, B.; Christensen, J.J.; Johansen, H.K. Semi-national surveillance of fungemia in Denmark 2004–2006: Increasing incidence of fungemia and numbers of isolates with reduced azole susceptibility. *Clin. Microbiol. Infect.* 2008, 14, 487–494.

7. Aslam, S., & Rotstein, C. (2019). Candida infections in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clinical Transplantation*, 33(9). <https://doi.org/10.1111/ctr.13623>
8. Bhat, M. A., Arif Khan, A., Al-Omar, M. A., & Khan, A. A. (n.d.). Synthesis and anti-candidal activity of some new pyrazoline derivatives. www.biomedres.info
9. Bhat, M. A., Khan, A. A., Khan, S., Al-Omar, M. A., Parvez, M. K., Al-Dosari, M. S., & Al-Dhfyan, A. (2014). Synthesis and anti-Candidal activity of N-(4-aryl/cyclohexyl)-2-(pyridine- 4-yl carbonyl) hydrazinecarbothioamide. *Bioorganic and Medicinal Chemistry Letters*, 24(5), 1299–1302. <https://doi.org/10.1016/j.bmcl.2014.01.060>
10. Borg, B. L., Brainerd, S. M., Meier, T. J., & Prugh, L. R. (2014). Impacts of breeder loss on social structure, reproduction, and population growth in a social canid. *Journal of Animal Ecology*, 84(1), 177–187. <https://doi.org/10.1111/1365-2656.12256>
11. Bull, A. T., & Goodfellow, M. (2019). Dark, rare, and inspirational microbial matter in the extremobiosphere: 16 000 m of bioprospecting campaigns. *Microbiology*, 165(12), 1252–1264. <https://doi.org/10.1099/mic.0.000822>
12. Bull, A. T., Stach, J. E. M., Ward, A. C., & Goodfellow, M. (2005). Marine actinobacteria: Perspectives, challenges, future directions. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 87(1), 65–79. <https://doi.org/10.1007/s10482-004-6562-8>
13. Cao, P., Li, C., Wang, H., Yu, Z., Xu, X., Wang, X., Zhao, J., & Xiang, W. (2020). Community structures and antifungal activity of root-associated endophytic actinobacteria in healthy and diseased cucumber plants and streptomyces sp. HAAG3-15 is a promising biocontrol agent. *Microorganisms*, 8(2). <https://doi.org/10.3390/microorganisms8020236>
14. Chandrashekar, A., Muralidharan, A., Koteswara, A., Alex, A. T., & Subrahmanyam, V. M. (2019). Isolation and characterization of an actinomycete strain producing an antifungal metabolite effective against candida albicans. *Research Journal of Pharmacy and Technology*, 12(10), 4601–4606. <https://doi.org/10.5958/0974-360X.2019.00791.1>

15. Chaturvedi, S., & Khurana, S. M. P. (2019). Importance of actinobacteria for bioremediation. In *Plant Biotechnology: Progress in Genomic Era* (pp. 277–307). Springer Singapore. https://doi.org/10.1007/978-981-13-8499-8_13
16. Chowdhary, A.; Sharma, C.; Meis, J.F. *Candida auris*: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathog.* 2017, 13, e1006290.
17. Cohan, R. H., Ellis, J. H., & Garner, W. (1996). Extravasation of radiographic contrast material: recognition, prevention, and treatment. *Radiology*, 200(3), 593–604. <https://doi.org/10.1148/radiology.200.3.8756899>.
18. Cortegiani, A.; Misseri, G.; Chowdhary, A. What's new on emerging resistant *Candida* species? *Intensive Care Med.* 2019, 45, 512–515.
19. Das, R., Romi, W., Das, R., Sharma, H. K., & Thakur, D. (2018). Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of Northeast India. *BMC Microbiology*, 18(1). <https://doi.org/10.1186/s12866-018-1215-7>
20. De Graaf, M., & Fouchier, R. a. M. (2014). Role of receptor binding specificity in influenza A virus transmission and pathogenesis. *The EMBO Journal*, 33(8), 823–841. <https://doi.org/10.1002/embi.201387442>.
21. Deevey, E. S. (1947). Life tables for natural populations of animals. *The Quarterly Review of Biology*, 22(4), 283–314. <https://doi.org/10.1086/395888>.
22. den Hengst, C. D., & Buttner, M. J. (2008). Redox control in actinobacteria. In *Biochimica et Biophysica Acta - General Subjects* (Vol. 1780, Issue 11, pp. 1201–1216). <https://doi.org/10.1016/j.bbagen.2008.01.008>
23. Deorukhkar, S. C., Saini, S., & Mathew, S. (2014). Non- albicans *Candida* infection: An emerging threat. *Interdisciplinary Perspectives on Infectious Diseases*, 2014. <https://doi.org/10.1155/2014/615958>
24. Dupont, B.F.; Lortholary, O.; Ostrosky-Zeichner, L.; Stucker, F.; Yeldandi, V. Treatment of candidemia and invasive candidiasis in the intensive care unit: Post hoc analysis of a randomized, controlled trial comparing micafungin and liposomal amphotericin B. *Crit. Care* 2009, 13, 159.
25. Erdogan, E. A., Goksen, G., & Everest, A. (2014). Anti-candidal activities of some *Myrtus communis* L. extracts obtained using accelerated solvent extraction (ASE). *Journal of Applied Biology and Biotechnology*, 2(5), 012-014.

26. Fira, D., Dimkić, I., Berić, T., Lozo, J., & Stanković, S. (2018). Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology*, 285, 44–55. <https://doi.org/10.1016/j.jbiotec.2018.07.044>.
27. Garcia-Hermoso, D., Alanio, A., Lortholary, O., & Dromer, F. (2015). Agents of systemic and subcutaneous mucormycosis and entomophthoromycosis. In *ASM Press eBooks* (pp. 2087–2108). <https://doi.org/10.1128/9781555817381.ch121>.
28. Geetha Saravanan, T., Singaraj, R., Annamalai, K. K., Baskaran, A., Manikkam, R., & Venugopal, G. (2023). Antibacterial Activity of Postbiotics. In *Postbiotics* (pp. 187-194). New York, NY: Springer US.
29. Geissdoerfer, M., Savaget, P., Bocken, N., & Hultink, E. J. (2017). The Circular Economy – A New Sustainability Paradigm? *Journal of Cleaner Production*, 143, 757–768. <https://doi.org/10.1016/j.jclepro.2016.12.048>.
30. Geissdoerfer, M., Savaget, P., Bocken, N., & Hultink, E. J. (2017b). The Circular Economy – A New Sustainability Paradigm? *Journal of Cleaner Production*, 143, 757–768. <https://doi.org/10.1016/j.jclepro.2016.12.048>.
31. Gouda, S., Das, G., Sen, S. K., Shin, H., & Patra, J. K. (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.01538>.
32. Gulve, R. M., & Deshmukh, A. M. (n.d.). Antimicrobial activity of the marine actinomycetes. *International Multidisciplinary Research Journal*, 2012(3), 16–22. <http://irjs.info/>
33. Hani, U., Shivakumar, H., Vaghela, R., M. Osmani, R., & Shrivastava, A. (2015). Candidiasis: A Fungal Infection- Current Challenges and Progress in Prevention and Treatment. *Infectious Disorders - Drug Targets*, 15(1), 42–52. <https://doi.org/10.2174/1871526515666150320162036>
34. Hazarika, S. N., & Thakur, D. (2020). Actinobacteria. In *Beneficial Microbes in Agro-Ecology: Bacteria and Fungi* (pp. 443–476). Elsevier. <https://doi.org/10.1016/B978-0-12-823414-3.00021-6>
35. Hirota, K., Yumoto, H., Sapaar, B., Matsuo, T., Ichikawa, T., & Miyake, Y. (2017). Pathogenic factors in *Candida* biofilm-related infectious diseases. In *Journal of Applied Microbiology* (Vol. 122, Issue 2, pp. 321–330). <https://doi.org/10.1111/jam.13330>

36. Houšť, J., Spížek, J., & Havlíček, V. (2020). Antifungal drugs. *Metabolites*, 10(3), 106. <https://doi.org/10.3390/metabo10030106>.
37. Igarashi, M., Sawa, R., Yamasaki, M., Hayashi, C., Umekita, M., Hatano, M., ... & Nomoto, A. (2017). Kribellosides, novel RNA 5'-triphosphatase inhibitors from the rare actinomycete *Kribbella* sp. MI481-42F6. *The Journal of Antibiotics*, 70(5), 582-589.
38. Jakubiec-Krzesniak, K., Rajnisz-Mateusiak, A., Guspiel, A., Ziemska, J., & Solecka, J. (2018). Secondary metabolites of actinomycetes and their antibacterial, antifungal, and antiviral properties. *Polish journal of microbiology*, 67(3), 259-272.
39. Jalal, M., Ansari, M. A., Alzohairy, M. A., Ali, S. G., Khan, H. M., Almatroudi, A., & Siddiqui, M. I. (2019). Anticandidal activity of biosynthesized silver nanoparticles: Effect on growth, cell morphology, and key virulence attributes of *Candida* species. *International Journal of Nanomedicine*, 14, 4667–4679. <https://doi.org/10.2147/IJN.S210449>
40. Jose, P. A., Maharshi, A., & Jha, B. (2021). Actinobacteria in natural products research: Progress and prospects. *Microbiological Research*, 246, 126708. <https://doi.org/10.1016/j.micres.2021.126708>.
41. Kabir, M. A., & Ahmad, Z. (2013). *Candida* infections and their prevention. *ISRN Preventive Medicine*, 2013, 1–13. <https://doi.org/10.5402/2013/763628>.
42. Kabir, M. A., Hussain, M. A., & Ahmad, Z. (2012). *Candida albicans*: A Model Organism for Studying Fungal Pathogens. *ISRN Microbiology (Print)*, 2012, 1–15. <https://doi.org/10.5402/2012/538694>
43. Kajimura, S., Spiegelman, B. M., & Seale, P. (2015). Brown and Beige Fat: Physiological Roles beyond Heat Generation. *Cell Metabolism*, 22(4), 546–559. <https://doi.org/10.1016/j.cmet.2015.09.007>.
44. Kauffman, Carol A., and Sara A. Hedderwick. "Fungal infections." *Infection management for geriatrics in long-term care facilities* (2006): 479-498.
45. Kavitha, A., Prabhakar, P., Vijayalakshmi, M., & Venkateswarlu, Y. (2010). Purification and biological evaluation of the metabolites produced by *Streptomyces* sp. TK-VL_333. *Research in Microbiology*, 161(5), 335-345.
46. Keesstra, S., Pereira, P., Novara, A., Brevik, E. C., Azorín-Molina, C., Parras-Alcántara, L., Jordán, A., & Cerdà, A. (2016). Effects of soil management

- techniques on soil water erosion in apricot orchards. *Science of the Total Environment*, 551–552, 357–366. <https://doi.org/10.1016/j.scitotenv.2016.01.182>.
47. Kim, H., Hwang, J. Y., Shin, J., & Oh, K. B. (2019). Inhibitory effects of diketopiperazines from marine-derived *Streptomyces puniceus* on the isocitrate lyase of *Candida albicans*. *Molecules*, 24(11), 2111.
 48. Klingspor, L., Tortorano, A. M., Peman, J., Willinger, B., Hamal, P., Sendid, B., Velegraki, A., Kibbler, C., Meis, J. F., Sabino, R., Ruhnke, M., Arian-Akdagli, S., Salonen, J., & Dóczy, I. (2015). Invasive *Candida* infections in surgical patients in intensive care units: A prospective, multicentre survey initiated by the European Confederation of Medical Mycology (ECMM) (2006-2008). *Clinical Microbiology and Infection*, 21(1), 87.e1-87.e10. <https://doi.org/10.1016/j.cmi.2014.08.011>
 49. Küçükgülzel, Ş. G., Küçükgülzel, I., Tatar, E., Rollas, S., Şahin, F., Güllüce, M., de Clercq, E., & Kabasakal, L. (2007). Synthesis of some novel heterocyclic compounds derived from diflunisal hydrazide as potential anti-infective and anti-inflammatory agents. *European Journal of Medicinal Chemistry*, 42(7), 893–901. <https://doi.org/10.1016/j.ejmech.2006.12.038>
 50. Kullberg, B.J.; Verweij, P.E.; Akova, M.; Arendrup, M.C.; Bille, J.; Calandra, T.; Cuenca-Estrella, M.; Herbrecht, R.; Jacobs, J.; Kalin, M.; et al. European expert opinion on the management of invasive candidiasis in adults. *Clin. Microbiol. Infect.* 2011, 17, 1–1.
 51. Kullberg, B.J.; Verweij, P.E.; Akova, M.; Arendrup, M.C.; Bille, J.; Calandra, T.; Cuenca-Estrella, M.; Herbrecht, R.; Jacobs, J.; Kalin, M.; et al. European expert opinion on the management of invasive candidiasis in adults. *Clin. Microbiol. Infect.* 2011, 17, 1–12.
 52. Kullberg, B.J.; Verweij, P.E.; Akova, M.; Arendrup, M.C.; Bille, J.; Calandra, T.; Cuenca-Estrella, M.; Herbrecht, R.; Jacobs, J.; Kalin, M.; et al. European expert opinion on the management of invasive candidiasis in adults. *Clin. Microbiol. Infect.* 2011, 17, 1–12.
 53. Lacret, R., Oves-Costales, D., Gómez, C., Díaz, C., De la Cruz, M., Pérez-Victoria, I., ... & Reyes, F. (2014). New ikarugamycin derivatives with antifungal

- and antibacterial properties from *Streptomyces zhaozhouensis*. *Marine drugs*, 13(1), 128-140.
54. Landt, S. G., Marinov, G. K., Kundaje, A., Kheradpour, P., Pauli, F., Batzoglou, S., Bernstein, B., Bickel, P. J., Brown, J. B., Cayting, P., Chen, Y., DeSalvo, G., Epstein, C. B., Fisher-Aylor, K. I., Euskirchen, G., Gerstein, M., Gertz, J., Hartemink, A. J., Hoffman, M. M., . . . Snyder, M. (2012). ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. *Genome Research*, 22(9), 1813–1831. <https://doi.org/10.1101/gr.136184>.
 55. Lieberman, D. A., Rex, D. K., Winawer, S. J., Giardiello, F. M., Johnson, D. A., & Levin, T. R. (2012). Guidelines for colonoscopy surveillance after screening and polypectomy: A consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*, 143(3), 844–857. <https://doi.org/10.1053/j.gastro.2012.06.001>.
 56. Litjens, G., Kooi, T., Bejnordi, B. E., Setio, A. a. A., Ciompi, F., Ghafoorian, M., Van Der Laak, J., Van Ginneken, B., & Sánchez, C. I. (2017). A survey on deep learning in medical image analysis. *Medical Image Analysis*, 42, 60–88. <https://doi.org/10.1016/j.media.2017.07.005>.
 57. Lockhart, S. R. (2014). Current Epidemiology of Candida Infection. *Clinical Microbiology Newsletter*, 36(17), 131–136. <https://doi.org/10.1016/j.clinmicnews.2014.08.001>
 58. Malic, S.; Kuriyama, T.; Lewis, M.A. *Candida* biofilms and oral candidosis: Treatment and prevention. *Periodontology* 2011, 55, 250–265.
 59. Manivasagan, P., Kang, K. H., Sivakumar, K., Li-Chan, E. C. Y., Oh, H. M., & Kim, S. K. (2014). Marine actinobacteria: An important source of bioactive natural products. In *Environmental Toxicology and Pharmacology* (Vol. 38, Issue 1, pp. 172–188). Elsevier. <https://doi.org/10.1016/j.etap.2014.05.014>
 60. Menendez, E., & Carro, L. (2019). Actinobacteria and Their Role as Plant Probiotics (pp. 333–351). https://doi.org/10.1007/978-3-030-18933-4_15
 61. Moroni, F., Naya-Català, F., Piazzon, M. C., Rimoldi, S., Caldusch-Giner, J. À., Giardini, A., Martínez, I., Brambilla, F., Pérez-Sánchez, J., & Terova, G. (2021). The Effects of Nisin-Producing *Lactococcus lactis* Strain Used as Probiotic on Gilthead Sea Bream (*Sparus aurata*) Growth, Gut Microbiota, and

62. Nafis, A., Elhidar, N., Oubaha, B., Samri, S. E., Niedermeyer, T., Ouhdouch, Y., ... & Barakate, M. (2018). Screening for non-polyenic antifungal produced by actinobacteria from Moroccan habitats: Assessment of antimycin A19 production by *Streptomyces albidoflavus* AS25. *International Journal of Molecular and Cellular Medicine*, 7(2), 133.
63. Nickerson, K. W., Gutzmann, D. J., Boone, C., Pathirana, R. U., & Atkin, A. L. (2024). Physiological adventures in *Candida albicans*: farnesol and ubiquinones. *Microbiology and Molecular Biology Reviews*. <https://doi.org/10.1128/membr.00081-22>
64. Nusse, R., & Varmus, H. (2012). Three decades of Wnts: a personal perspective on how a scientific field developed. *The EMBO Journal*, 31(12), 2670–2684. <https://doi.org/10.1038/emboj.2012.146>.
65. Omran, R. A. B. A. B., & Kadhem, M. F. (2016). Production, purification, and characterization of bioactive metabolites produced from rare actinobacteria *Pseudonocardia alni*. *Asian J Pharm Clin Res*, 9(3), 1.
66. Palla, M. S., Guntuku, G. S., Muthyala, M. K. K., Pingali, S., & Sahu, P. K. (2018). Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil. *Beni-Suef University Journal of Basic and Applied Sciences*, 7(2), 250–256. <https://doi.org/10.1016/j.bjbas.2018.02.006>
67. Patil, S., Rao, R. S., Majumdar, B., & Anil, S. (2015). Clinical appearance of oral *Candida* infection and therapeutic strategies. In *Frontiers in Microbiology* (Vol. 6, Issue DEC). Frontiers Media S.A. <https://doi.org/10.3389/fmicb.2015.01391>
68. Pazhanimurugan, R., Gopikrishnan, V., Shanmuga Sundaram, T., Radhakrishnan, M., & Balagurunathan, R. (2012). The bioactive potential of actinobacteria against drug-resistant pathogens. *Journal of Applied Pharmaceutical Science*, 2(5), 167–173. <https://doi.org/10.7324/JAPS.2012.2542>
69. Pranesh, V., Balasubramanian, S., Kumar, R., Sakthivel, R., Rajkumar, P., & Ravikumar, S. (2019). Kaolinite flakes and coal fines production in lignite core under ambient conditions: A Neyveli Lignite Field case study at Cauvery Basin,

- Southern India. *Journal of Natural Gas Science and Engineering*, 64, 72–80. <https://doi.org/10.1016/j.jngse.2019.01.023>.
70. Radhakrishnan, M., Vijayalakshmi, G., Gopikrishnan, V., & Jerrine, J. (2016). The bioactive potential of actinobacteria isolated from certain under-studied regions in India. *Journal of Applied Pharmaceutical Science*, 6(8), 151–155. <https://doi.org/10.7324/JAPS.2016.60823>
 71. Ramesh, S., & Mathivanan, N. (2009). Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. *World Journal of Microbiology and Biotechnology*, 25(12), 2103–2111. <https://doi.org/10.1007/s11274-009-0113-4>
 72. Ries, M., Steinmann, P., & Pfaller, S. (2020). Characterization of polystyrene under shear deformation using molecular dynamics. In *Advanced structured materials* (pp. 219–229). https://doi.org/10.1007/978-3-030-50460-1_14.
 73. Roth, B. L. (2016). DREADDs for neuroscientists. *Neuron*, 89(4), 683–694. <https://doi.org/10.1016/j.neuron.2016.01.040>.
 74. Samber, N., Khan, A., Varma, A., & Manzoor, N. (2015). Synergistic anti-candidal activity and mode of action of Mentha piperita essential oil and its major components. *Pharmaceutical Biology*, 53(10), 1496–1504. <https://doi.org/10.3109/13880209.2014.989623>
 75. Sangkanu, S., Rukachaisirikul, V., Suriyachadkun, C., & Phongpaichit, S. (2021). Antifungal activity of marine-derived actinomycetes against Talaromyces marneffeii. *Journal of Applied Microbiology*, 130(5), 1508-1522.
 76. Sanjenbam, P., Vinay Gopal, J., & Kannabiran, K. (2014). Isolation and identification of anticandidal compound from Streptomyces sp. VITPK9. *Applied Biochemistry and Microbiology*, 50(5), 492–499. <https://doi.org/10.1134/S0003683814050081>
 77. Saravanan, T. G., Loganathan, A., Rajendran, G., Vasanthakumar, R. K., & Ganapathy, K. (2022). Bioleaching of heavy metals by Streptomyces avermitilis–BBA4 isolate from coal mine soil of Tamil Nadu. *Current Microbiology*, 79(10), 303.
 78. Sasidhar, M., Masilamani, S., Baskaran, A., Kaari, M., & Manikkam, R. (2021). Antifungal activity of rare actinobacterium isolated from forest soil. *Research*

79. Shahid, M., Singh, B. N., Verma, S., Choudhary, P., Das, S., Chakdar, H., ... & Saxena, A. K. (2021). Bioactive antifungal metabolites produced by *Streptomyces amritsarensis* V31 help to control diverse phytopathogenic fungi. *Brazilian Journal of Microbiology*, 52, 1687-1699.
80. Shirling EB, Gottlieb D (1966) Methods for Characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16(3):313-340.
81. Siddharth, S., Ravishankar Rai, V., Wink, J., & Steinert, M. (2020). Diversity and bioactive potential of actinobacteria from unexplored regions of western Ghats, India. *Microorganisms*, 8(2). <https://doi.org/10.3390/microorganisms8020225>
82. Silambarasan, S., Praveen kumar, E., Murugan, T., Saravanan, D., & Balagurunathan, R. (2012). Antibacterial and antifungal activities of actinobacteria isolated from Rathnagiri hills. *Journal of Applied Pharmaceutical Science*, 2(10), 099–103. <https://doi.org/10.7324/JAPS.2012.21020>
83. Smarr, M. M., Kannan, K., & Louis, G. M. B. (2016). Endocrine disrupting chemicals and endometriosis. *Fertility and Sterility*, 106(4), 959–966. <https://doi.org/10.1016/j.fertnstert.2016.06.034>.
84. Suyana, P., Nishanth Kumar, S., Madhavan, N., Dileep Kumar, B. S., Nair, B. N., Mohamed, A. P., Warriar, K. G. K., & Hareesh, U. S. (2015). Reactive oxygen species (ROS) mediated enhanced anti-candidal activity of ZnS-ZnO nanocomposites with low inhibitory concentrations. *RSC Advances*, 5(94), 76718–76728. <https://doi.org/10.1039/c5ra13316e>
85. Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., & Škrlec, I. (2021). *Candida albicans*-the virulence factors and clinical manifestations of infection. *Journal of Fungi*, 7(2), 1–19. <https://doi.org/10.3390/jof7020079>
- Ait Assou, S., Anissi, J., Sendide, K., & el Hassouni, M. (2023). Diversity and Antimicrobial Activities of Actinobacteria Isolated from Mining Soils in Midelt Region, Morocco. *Scientific World Journal*, 2023. <https://doi.org/10.1155/2023/6106673>
86. Tawfeeq, H. M., Muslim, R. F., Abid, O. H., & Owaid, M. N. (2019). Synthesis and characterization of novel tetrazole derivatives and evaluation of their anti-

- candidal activity. *Acta Pharmaceutica Scientia*, 57(3), 45–63.
<https://doi.org/10.23893/1307-2080.APS.05717>
87. Thenmozhi, G. S., Sharmila, M., Radhakrishnan, M., Kishore, K. A., Manigundan, K & Anita, P. (2023). Distribution and anti-infective properties of culturable Actinobacteria from the Indian Himalayan region (IHR). *EVIS Bulletin Himalayan ecology*, 31, 82-90. ISSN: 2455-6815 (Online).
 88. Tong, Y., & Tang, J. (2017b). *Candida albicans* infection and intestinal immunity. *Microbiological Research*, 198, 27–35. <https://doi.org/10.1016/j.micres.2017.02.002>
 89. Tumvarello, M.; Posteraro, B.; Trecarichi, E.M.; Fiori, B.; Rossi, M.; Porta, R.; de Gaetano Donati, K.; La Sorda, M.; Spanu, T.; Fadda, G.; et al. Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with Candidemia. *J. Clin. Microbiol.* 2007, 45, 1843–1850.
 90. van der Heul, H. U., Bilyk, B. L., McDowall, K. J., Seipke, R. F., & van Wezel, G. P. (2018). Regulation of antibiotic production in Actinobacteria: New perspectives from the post-genomic era. In *Natural Product Reports* (Vol. 35, Issue 6, pp. 575–604). Royal Society of Chemistry. <https://doi.org/10.1039/c8np00012c>
 91. Vitale, R. G. (2021). Role of antifungal combinations in difficult-to-treat candida infections. *Journal of Fungi*, 7(9), 731. <https://doi.org/10.3390/jof7090731>.
 92. Wang, X., Zhang, M., Gao, J., Pu, T., Bilal, M., Wang, Y., & Zhang, X. (2018). Antifungal activity screening of soil actinobacteria isolated from Inner Mongolia, China. *Biological Control*, 127, 78–84. <https://doi.org/10.1016/j.biocontrol.2018.07.007>.
 93. Wang, X., Zhang, M., Gao, J., Pu, T., Bilal, M., Wang, Y., & Zhang, X. (2018). Antifungal activity screening of soil actinobacteria isolated from Inner Mongolia, China. *Biological Control*, 127, 78–84. <https://doi.org/10.1016/j.biocontrol.2018.07.007>
 94. Wink, J., Schumann, P., Atasayar, E., Klenk, H., Zaburannyi, N., Westermann, M., Martin, K., Glaeser, S. P., & Kämpfer, P. (2017). ‘*Streptomyces caelicus*’, an antibiotic-producing species of the genus *Streptomyces*, and *Streptomyces canchipurensis* Li et al. 2015 are later heterotypic synonyms of *Streptomyces*

- muensis Ningthoujam et al. 2014. *International Journal of Systematic and Evolutionary Microbiology*, 67(3), 548–556. <https://doi.org/10.1099/ijsem.0.001612>.
95. Wink, J., Schümann, P., Atasayar, E., Klenk, H., Zaburannyi, N., Westermann, M., Martin, K., Glaeser, S. P., & Kämpfer, P. (2017b). 'Streptomyces caelicus', an antibiotic-producing species of the genus Streptomyces, and Streptomyces canchipurensis Li et al. 2015 are later heterotypic synonyms of Streptomyces muensis Ningthoujam et al. 2014. *International Journal of Systematic and Evolutionary Microbiology*, 67(3), 548–556. <https://doi.org/10.1099/ijsem.0.001612>.
96. Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., & Wang, F. S. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine*, 8(4), 420–422. [https://doi.org/10.1016/s2213-2600\(20\)30076-x](https://doi.org/10.1016/s2213-2600(20)30076-x).
97. Yenn, T. W., Ring, L. C., Nee, T. W., Khairuddean, M., Zakaria, L., & Ibrahim, D. (2017). Endophytic Diaporthe sp. ED2 produces a novel anti-candidal ketone derivative. *Journal of Microbiology and Biotechnology*, 27(6), 1065–1070. <https://doi.org/10.4014/jmb.1612.12009>
98. Yuan, A., Rao, M. S., Veeranna, & Nixon, R. A. (2012). Neurofilaments at a glance. *Journal of Cell Science*, 125(14), 3257–3263. <https://doi.org/10.1242/jcs.104729>.
99. Yuan, B., & Moghanloo, R. G. (2018). Nanofluid precoating: an effective method to reduce fines migration in radial systems saturated with two mobile immiscible fluids. *Spe Journal*, 23(03), 998–1018. <https://doi.org/10.2118/189464>.
100. Zhang, D., Yi, W., Ge, H., Zhang, Z., & Wu, B. (2019). Bioactive streptoglutarimides A–J from the marine-derived Streptomyces sp. ZZ741. *Journal of natural products*, 82(10), 2800–2808.
101. Zhao, X., Zhang, X., Chen, Z., Zhen, W., Lu, Y., & Cheng, D. (2018). The Divergence in Bacterial Components Associated with Bactrocera Dorsalis across Developmental Stages. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.00114>.

APPENDICES

1. Starch Casein Agar (SCA)

REQUIREMENTS	AMOUNT (g/L)
Soluble starch	10
Casein (vitamin free)	0.3
Potassium nitrate	2
Magnesium sulphate	0.05
Ferrous sulphate	0.01
Dipotassium phosphate	2
Calcium carbonate	0.02
Sodium chloride	2
Agar Agar	20
pH	7

2. INTERNATIONAL STREPTOMYCES PROJECT – 1 (ISP-1)

REQUIREMENTS	AMOUNT (g/L)
Casein enzymic hydrolysate	5
Yeast extract	3
Agar Agar	20
pH	7

3. INTERNATIONAL STREPTOMYCES PROJECT – 3 (ISP -3)

REQUIREMENTS	AMOUNT (g/L)
Oatmeal agar	30 g
Agar	15 g

4. INTERNATIONAL STREPTOMYCES PROJECT -5 (ISP -5)

REQUIREMENTS	AMOUNT (g/L)
L- Asparagine	1
Glycerol	10
K ₂ HPO ₄	1
Agar	20

5. INTERNATIONAL STREPTOMYCES PROJECT -7 (ISP -7)

REQUIREMENTS	AMOUNT (g/L)
L- ASPARAGINE	1.00
L- TYROSINE	0.50
DIPOTASSIUM PHOSPHATE	0.50
MAGNESIUM SULPHATE	0.50
SODIUM CHLORIDE	0.50
TRACE SALT SODIUM	1.00
AGAR	20

6. NUTRIENT AGAR

REQUIREMENTS	AMOUNT (g/L)
BEEF EXTRACT	1.00
YEAST EXTRACT	2.00
PEPTONE	5.00
SODIUM CHLORIDE	5.00
AGAR	15.0

7. CZAPEX DOX AGAR (CDA)

REQUIREMENTS	AMOUNT (g/L)
SUCROSE	30.0

SODIUM NITRITE	2.00
DIPOTASSIUM PHOSPHATE	1.00
MAGNESIUM SULPHATE	0.05
POTASSIUM CHLORIDE	0.05
FERROUS SULPHATE	0.01

7. GLUCOSE YEAST MALT AGAR (GYM)

REQUIREMENTS	AMOUNT (g/L)
PEPTIC DIGEST OF ANIMAL TISSUE	5.00
YEAST EXTRACT	5.00
GLUCOSE	2.00
MONOPOTASSIUM PHOSPHATE	0.50
DIPOTASSIUM PHOSPHATE	0.50
MAGNESIUM SULPHATE	0.30
SODIUM CHLORIDE	0.01
MANGANESE SULPHATE	0.01
ZINC SULPHATE	0.0016
COPPER SULFATE	0.0016
COBALT SULFATE	0.0016
AGAR	15.00

8. ACTINOMYCETE ISOLATION AGAR (AIA)

REQUIREMENTS	AMOUNT (g/L)
AIA	22.00
GLYCEROL	5.00
AGAR	15.00