

ANTI-TUBERCULOSIS ACTIVITY FROM MANGROVE ASSOCIATED MICROBES

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

Naviya R (38230022)
Madhumitha S (38230020)



DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL
ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of R. Naviya (38230022), S. Madhumitha (38230020) who carried out the project entitled "Antituberculosis Activity from Mangrove associated microbes" under my supervision from Dec,2021 to April,2022

Internal Guide

Dr.M.Masilamani Selvam

External Guide

Head of the Department

Submitted for Viva voce Examination held on _____

Internal Examiner

External Examiner

DECLARATION

We Naviya R, Madhumitha S hereby that the Project Report entitled Antituberculosis Activity from Mangrove associated Microbes done by us under the guidance of Dr. M. MASILAMANI SELVAM, Ph.D., and Dr. M.MASILAMANI SELVAM, Ph.D. is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology

DATE:

PLACE:

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Anti-tuberculosis activity from Actinomycetes isolated from the Marine Ecosystem. Actinomycetes isolated from the Marine Environment currently received considerable attention due to the Structural Diversity and Unique Biological activity of their Secondary Metabolites. Actinomycetes were isolated in the Soil sample of Mangroves from the Coastal Areas around Chennai, Tamil Nādu, India. The soil Sample was diluted by using the Serial Dilution Method. This Study will focus on the Isolation, Identification, Characterization, and Activity of Actinomycetes against Mycobacterium tuberculosis. In Pure Culture, we got 5 isolated strains of Actinomycetes. Starch casein agar is used in pure culture .5 strains were characterized morphologically based on the difference in the Appearance and Growth Mediums (Yeast Extract- Malt Extract Agar, Inorganic salts - Starch Agar, Glycerol Asparagine Agar).2 strains got contaminated during Mass Culture Production. The remaining 3 strains were carried on to the Extraction process. The process is carried out in 2 step process. First, the Separating Funnel Method is used to separate the Crude Secondary Metabolites from the unwanted biomolecules such as Lipids, Fats, etc. It is extracted by using the organic solvent, Ethyl Acetate. It pulls up the lipids from the secondary metabolites. Second, the Fractional Distillation method is used to remove the small amount of solvent present in the crude. The Crude Extract of Actinomycetes was first screened by using 3 test Bacterial species such as staphylococcus aureus, Bacillus subtilis, and Escherichia coli. Mueller Hinton agar is used. 3 isolates were partially sequenced by using 16s rRNA Sequencing .BN01, BN13, BN14 Strains were identified as Streptomyces rochei, Streptomyces sp., Streptomyces sp. respectively. DNA Sequences of 3 isolates was submitted to the GenBank, NCBI, USA and its accession number BN01(ON042751), BN13(ON042452), BN14(ON042463) the GenBank, NCBI, USA and its Accession Number BN01(ON042751), BN13(ON042452), BN14(ON042463) and BLASTN tool is used to construct the Blast tree, pairwise Alignment. The present study is to investigate whether Actinomycetes from mangrove ecosystems are potential against Mycobacterium Tuberculosis.

TABLE OF THE CONTENTS

S.NO	TITLE	PAGE NO
1	INTRODUCTION	12
1.1	TWO TYPES OF TUBERCULOSIS	12
1.1.1	TREATMENT AND SYMPTOMS FOR TUBERCULOSIS	13
1.1.2	CURRENT RESEARCH ON TB	13
1.2	MARINE ACTINOBACTERIA	14
1.3	MANGROVE ACTINOBACTERIA	17
2	LITERATURE SURVEY	19
3	AIM & SCOPE OF THE INVESTIGATION	28
3.1	AIM	28
3.2	OBJECTIVES	28
4	MATERIALS & METHODS	29
4.1	ISOLATION OF ACTINOMYCETES FROM SOIL SAMPLE	29
4.2	SUBCULTURING	30
4.3	METHODS FOR GENOMIC DNA ISOLATION	30
4.3.1	CTAB	30
4.3.2	AGAROSE GEL ELECTROPHORESIS	31
4.3.3	PCR	33
4.4	MASS PRODUCTION AND EXTRACTION	34
4.4.1	PREPARATION OF ISP-2	34

4.4.2	PREPARATION OF PRODUCTION MEDIUM	35
4.5	EXTRACTION OF CRUDE SECONDARY METABOLITES	37
4.5.1	SEPARATING FUNNEL METHOD	37
4.5.2	FRACTIONAL DISTILLATION METHOD	39
4.6	AGAR – WELL DIFFUSION METHOD	41
5	RESULTS & DISSCUSSION	42
5.1	MORPHOLOGICAL CHARACTERIZATION	42
5.1.1	COMPOSITION OF THE MEDIUM	42
5.2	PRIMARY SCREENING	49
5.3	LIGHT MICROSCOPE	50
5.4	DNA SEQUENCE DETERMINATION	51
5.5	DATABASE SEARCHING	56
5.6	ANTIBACTERIAL ACTIVITY USING DISC METHOD	58
6	SUMMARY & CONCLUSION	59

LIST OF ABBREVIATIONS

HCl	Hydrogen Chloride
SCA	Starch casein Agar
μL	Micro Litre
°C	<i>Celsius</i>
XDR	Extensively drug-resistant
MDR	Multi drug resistant
Tb	Tuberculosis
G	Gram
mg	Milligram
BCG	Bacille-Calmette Guerin
G+	Gram positive bacteria
NaCl	Sodium Chloride
PH	Potential of hydrogen
%	Percentage
SDS	Sodium Dodecyl Sulphate
EDTA	Ethylene diamine tetra acetic acid
Mm	Milli Molar
ml	Milli litre
Ctab	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
sp	species
bp	Base pairs
ISP	International Streptomyces Project
mins	minutes
TE	Tris- EDTA
TAE	Tris acetate EDTA
V	Volt
EtBr	Ethidium bromide
PCR	Polymerase Chain Reaction
NaOH	Sodium Hydroxide
YEME	Yeast extract malt extract
BLAST	Basic Local Alignment Search Tool
NCBI	National Centre for Biotechnology Information
Dil	dilute
M2	Yeast extract malt extract agar
M4	Starch agar
M5	Glycerol- asparagine agar

LIST OF FIGURES

S.NO	TITLE	PAGE NO
4.1	The above figure represents the pure culture of the Actinomycetes (a)BN14, (b)BN15, (c)BN13, (d)BN01	29
4.2	The above figure represents the Separation of the DNA Fragments of the Sample (BN01, BN11, BN13, BN14, B N15) from the gel based on molecular weight	32
4.3	The above picture represents the Confirmation of Amplified DNA of Actinomycetes, running it with DNA Ladder by using Agarose Gel Electrophoresis	33
4.4	The above figure represents the culture broth of the three isolates of actinomycetes (BN01, BN13, and BN14)	35
4.5	The above figure is the Mass culture Production in a of 3 isolates was cultured in a ISP-2 broth	37
4.6	The above picture shows the separating funnel – separation of the secondary metabolite from other biomolecules like fats, lipids	38
4.7	The above picture is the Supernatant of 3 Actinomycetes isolates (BN01, BN13, and BN14) from Mass Production	39
4.8	The above picture represents the separation of ethyl acetate from the extract	39
4.9	The above figure shows that the antibiotic crude extracts of 3 isolates is kept in the glass bowl for Air Drying	40

4.10	The above picture is the crude extract of 3 actinomycetes samples (BN01, BN13, BN14) in the Eppendorf tubes.	41
5.1	The above figure represents the Growth of the Actinomycetes sample (BN01, BN12) on M2, M4, M5	46
5.2	The above figure represents the Growth of the Actinomycetes sample (BN13, BN14) on M2, M4, M5	47
5.3	The above figure represents the Growth of the Actinomycetes sample (BN15) on M2, M4, M5	48
5.4	The above figure shows that the crude extract of BN01, BN13, and BN14 doesn't have potential against a Staphylococcus aureus, Bacillus.subtilis, Escherichia coli	50
5.5	The above picture is the microscopic view of 5 Actinomycetes strains a) BN01 b) BN11c) BN13 d) BN14 e) BN15	51
5.6	The above picture represents the DNA Sequence of BN01 isolates was deposited in the GenBank, NCBI	53
5.7	The above picture represents the DNA Sequence of the BN13 isolate was deposited in the GenBank, NCBI	54
5.8	The above picture shows the DNA Sequence of the BN14 isolate was deposited in the GenBank, NCBI	55
5.9	the above picture represents the Blast tree of the BN01 isolate	56
5.10	The above the picture shows the BLAST Tree of the BN13isolate	57
5.11	The above picture shows the BLAST Tree of the BN14 isolate	57

LIST OF TABLES

S.NO	TITLE	PAGE NO
5.1	the below table list the composition of m2 medium	43
5.2	The below table list the composition of M4 Medium	44
5.3	The below table list the composition of M5 Medium	45
5.4	the comparison of 5 actinomycetes samples with the difference in the color and growth on the three different media; Maxi. Growth+++, Medium growth++	49
5.5	DNA sequence data of 3 isolates deposited in the GenBank, NCBI	51

CHAPTER 1

INTRODUCTION

Mycobacterium tuberculosis is an Aerobic Bacillus, Rod-Shaped, Non-Spore-Forming, Non-Motile, very Slow-Growing Bacteria, Acid-Fast Bacilli. It is also known as Tubercle Bacilli. It was first discovered by the Robert Koch in 1882. It comes under the order of Mycobacteriales, which are comprised of a large number of well-characterized species, which are associated with Human and Animal Diseases. It is a causative agent of Tuberculosis. *Mycobacterium tuberculosis* is present as an airborne particle in the air with a size in diameter of 1-5 microns. Transmission of TB from person to person through air droplets. The probability of transmission of *Mycobacterium tuberculosis* is determined by the 4 factors are Susceptibility, Infectiousness, Environment, Exposure.

- Susceptibility is the status of the individual based on immune.
- Infectiousness is related to how much the persons are infected by the tubercle bacilli.
- Environment is the concentrations of *Mycobacterium tuberculosis* in the environment.
- Exposure - Persons Duration and Frequency Proximity of *Mycobacterium tuberculosis*.

1.1 TWO TYPES OF TUBERCULOSIS

- Latent TB - The germs were present in the body but the immune system in the body is stronger it will keep them from spreading. It is in the sleeping stage or in the active Stage. This stage doesn't show any symptoms but later it turns into the active stage.
- Active TB - The presence of germs is higher it will multiply and make persons sick .in this stage it will spread to Persons. Above 90%of actives, cases came from the first type of infection, Latent.

1.1.1 Treatment and symptoms for tuberculosis

- Anti-Tuberculosis (Rifampicin, Ethambutanol, Pyrazinamide, INAH)
- Multidrug-resistant Tuberculosis (Isoniazid and Rifampin)

As per WHO, 2020 MDR has a slow treatment success rate of 52%, when Isoniazid and Rifampicin resistance are tightly linked, with 78% of the reported Rifampicin-resistant TB cases in 2019 being resistant to Isoniazid as well

Extensively drug-resistant (XDR) is characterized as resistance to both isoniazid and Rifampicin. This would annoy the public health crisis. Therefore, the development of alternative therapeutic measures must be taken priorly

- Diagnosis for tb
- Tuberculin skin test
- Acid-fast stain
- Culture
- Polymerase chain reaction

Symptoms of Tuberculosis are Chest pain, coughing up blood, feeling tired, night sweats, chills, fever, loss of appetite, weight loss, cough for more than 21 days.

According to WHO, TB is the 13th leading cause of death. Worldwide in 2020, 10 million people fell ill with Tb and 1.5 million people have died, including patients with HIV were estimated.

1.1.2 Current Research on TB

- An integrated computational framework to design a multi-epitope vaccine against mycobacterium tuberculosis
- M.TB-specific subunit vaccine that provides synergistic immunity upon co-administration with BCG

- Robust IgM Responses following IV vaccination with BCG associated with Mycobacterium tuberculosis infection with macaques.
- At Clinical Development, Phase 1 (TBA-354), Phase 3 (Bedaquiline (TMC-207) with OBR for MDR-TB)

Still, there is a lack of work on Mycobacterium Tuberculosis in the novel aspects of medical. As a result, new antibiotics are urgently needed to combat emerging bacterial diseases. Natural products and microbial metabolites, in particular, are a prominent source of bioactive scaffolds that serve as the foundation for the creation of life-saving antibiotics, despite the use of massive synthetic combinatorial libraries of compounds to produce new medications. (Challinor V.L., Bode H.B. 2015)

1.2 MARINE ACTINOBACTERIA

Gram-positive (G+) bacteria with a variety of Morphological, Physiological, and Metabolic Characteristics, as well as DNA with a high Guanine + Cytosine (G+C) composition, make up the phylum Actinobacteria. The majority of this phylum's genera are Free-living Saprophytes that may be found in both Aquatic and Terrestrial habitats. Actinobacteria are ecologically and commercially significant prokaryotes with an unrivalled capacity to synthesise new compounds. This phylum also contains pathogens like Mycobacterium, Corynebacterium, Nocardia, Propionibacterium, and Tropheryma, as well as soil dwellers like Streptomyces spp. and Micromonospora spp., Plant Commensals like Frankia spp., and Gastrointestinal Commensals like Bifidobacterium spp. Actinobacteria come in a range of shapes and sizes, from rod-coccoid (e.g., Arthrobacter spp.) to coccoid (e.g., Micrococcus spp.) to highly distinct Mycelium (e.g., Streptomyces spp.) to fragmented Hyphal forms (e.g., Nocardia spp.) In 2016 Streptomyces, an Actinobacterial genus, dominates soil microbial communities in general. The majority of Actinobacterial genera are Mesophilic, which means they thrive at temperatures between 25 and 30 degrees Celsius and a neutral pH, with the optimum growing pH between 6 and 9. Actinobacteria's growth is influenced by factors such as pH, Temperature, and Moisture Content. Due to their Eco physiological conditions and immense Microbial Diversity, Mangroves are the most fruitful and

productive marine habitat. Many Unique Actinobacteria have been discovered in Mangrove habitats across the world and are being studied for their bioprospecting potential. Actinobacteria from selected mangrove environments have been studied by Indian microbiologists over the past five decades, with a focus on culturable diversity and Antibacterial, Antimycobacterial, Anticancer, and Enzymatic Activity. Around 22 uncommon Actinobacterial Taxa have been discovered in Indian Mangrove Habitats thus far. Several additional New Actinobacteria with Intriguing Bio prospecting Potential, on the other side, still have to be investigated (R.Manikkam, et al 2019)

Approximately 70,000 of the 500,000 natural chemicals known from biological sources are microbially generated (both from bacteria and fungus), with actinomycetes accounting for 29 percent. Between 1950 and 1970, over 60% of antibiotics were identified from actinomycetes, exclusively from the genus *Streptomyces*. Rare actinomycetes are actinomycetes strains that are less frequently isolated than 'typically' isolated *Streptomyces* spp., despite the fact that they may not be rare in the environment.

Microbial diversity provides an inexhaustible source of unique chemistry, making it a rich resource for biotechnology innovation. According to estimations in 1995, the culturability of microorganisms in coastal sediments (0.25 percent) and, in particular, saltwater (0.001–0.10 percent), is significantly lower than in soil (0.30 percent). Although efforts in this field have dwindled since the late 1980s due to the perception that this resource has been extensively investigated, Terrestrial Microorganisms have produced a lot of useful Antibiotics and Metabolites (99 percent of known microbial chemicals). In this regard, researchers have shifted their focus to new habitats for the development of innovative medicinal substances and the battling of human infections. The ocean floor has lately been discovered to contain an ecosystem with many different types of actinomycetes. Actinomycetes are the most prolific makers of Secondary Metabolites among all microorganisms. Currently known microbial metabolites 45 percent (10,000 compounds) from different Actinomycetales were identified (Ramesh Subramani, et al 2012)

As the frequency of Novel Bioactive Compounds obtained from Terrestrial Actinobacteria decreases with time, Actinobacteria from diverse environments have been increasingly screened for their ability to produce New Secondary Metabolites.

In recent years, Terrestrial and Marine Microorganisms have been known for Antimicrobial, Antiviral, Antitumor, Anticoagulant, Antidiabetic, and Cardioactive Properties. The Antibiotic effect of Actinomycetes has been used in many fields including Agriculture, Veterinary, and Pharmaceutical Industries.

The genus *Streptomyces* are in the fact known as the major source of Bioactive Natural Product. The Intensive Screening for Secondary Metabolites in the last decade has also focused on the minor group of Actinomycetes species that are difficult to culture and isolate and those that are grown in extreme conditions (Alkaline and acidic conditions) (A.Basilo, et al 2003).

For a long time, it was believed that there was not much difference between Terrestrial and Marine Actinomycetes. recent realization of the fact that the Marine Environment can be a source for Novel Actinomycetes with yet unexploited potential for biosynthesis for the natural product. Notably, most of the bioactive molecules from the marine actinomycetes have been discovered in the representative of *Streptomyces* sp. this reflects the fact that marine *Streptomyces* like their terrestrial relative are easier to culture and induce the synthesis of secondary metabolites compared to other Marine Actinomycetes (Sergey B. Zotchev 2012)

Actinomycetes are the widely known groups of filamentous bacteria especially for their metabolic versatility. These bacteria's bioactive potential allows them to survive even in difficult and unfavorable environmental settings. The main focus on the Actinomycetes is the special issues in the multitude of Primary and Secondary metabolites produced by them. Enzymes from Actinomycetes have an capability to replace an Chemical Catalyst. The Commercialization of these enzymes is a essential step towards changing "Green Technology." Enzymes such as Endoglucanases

produced from the *Streptomyces* sp leads to the cost reduction for the Enzyme Production. (Neelu nawani, et al 2013)

1.2 MANGROVE ACTINOBACTERIA

Actinobacteria are abundant in soil, where they play an important part in the Biogeochemical Cycling of Complex Polysaccharides such as Starch, Cellulose, Chitin, Pectin, and Xylan. Some resistant compounds, such as Insecticides, may also be degraded by them (Goodfellow M, Haynes, 1984).

Microorganisms produce Primary Metabolites during the Active Growth Phase (exponential/trophophase), when the growth media has required nutrients or substrates. Almost all microbes create primary metabolites because they are required for their development and proliferation. Primary metabolites, such as Nucleotides and Amino acids, are either the end or intermediate products of anabolic reactions, or they are converted into Co-enzymes, such as vitamins. Other main metabolites (such as acetic acid, citric acid, and ethanol) are produced by Catabolic processes and are not employed to create cellular components. However, their Synthesis is linked to Energy Generation, and Substrate Consumption is required for development (R. Manikkam et al 2019).

Marine microorganisms appeared to have adapted excellently to the marine environment, for this reason gaining their numerous Metabolic and Genetic Abilities. With increasingly more chemically interesting and biologically sizeable metabolites isolated from marine microorganisms, it is now believed that marine microorganisms are rich resources of novel compounds with numerous organic sports. Among the Microorganisms, the Actinomycetes group remains a prolific source of Bioactive Compounds. It turned into as soon as assumed that Marine Actinomycetes were simply of Terrestrial starting place. However, extra data of studies have shown that indigenous Marine Actinomycetes indeed exist in the oceans. Although the Exploitation of marine Actinomycetes as a source for the invention of novel secondary metabolites continues

to be at an early degree, the invention rate of novel secondary metabolites from marine Actinomycetes has these days surpassed that of their terrestrial counterparts (Caixia Chen, et al 2013).

Till date, more than 10,000 Antibiotics have been isolated from actinomycetes. For a long time in the past, this Identification of such numbers of Antimicrobial has brought about a misconception that the invention of Bioactive Metabolites from Actinomycetes may reach its excessive restriction. However, with the advent of subsequent Generation Sequencing and sophisticated Bioinformatics tools, wide variety of entire Genome sequences of model Actinomycetes have become to be had and analysis of these genome sequences found out identity of wide variety of rare Actinomycetes. In addition, characterization of these new isolates has result in Isolation and Identification of novel bioactive compounds with massive Therapeutic Capability. In line with a Mathematical Modelling, most effective three % of all Antibacterial agents synthesized via Streptomyces. In concomitant with this information and rising hassle of Multi Drug Resistance (MDR) and new Phyto -Pathogens, Actinomycetes regained its role because the of Interest and Isolation of Actinomycetes from notably unexplored vicinity is once more popularized (Sohan Sengupta, et al 2015).

The present study focused on the Screening of Marine Actinomycetes and their Antibacterial activity against Mycobacterium tuberculosis

CHAPTER 2

LITERATURE SURVEY

- Jeffrey L. S. H, (2008), In this study 62 isolates have been isolated, isolates are isolated from 7 different soil samples. They were Purified and All 62 isolates were then subjected to antimicrobial test using selected phytopathogens as test strains and it was observed that 3, 25, 35 and 37 of the isolates showed Antagonistic Reaction with *Fusarium palmivora*, *Bacillus subtilis*, *Pantoea dispersa* and *Ralstonia solanacearum* respectively. All isolates were identified as *Streptomyces* spp by using 16s RNA sequence.[11]
- A.Basilio, et al (2003), The results of this study support the idea that species of *Actinomycetes* isolated in alternative selective conditions of pH and Salinity present a significant capacity to produce compounds with Antibacterial or Antifungal activity. The best group of isolates in terms of production of Active Secondary Metabolites was the one isolated in Saline conditions [1].
- V. Vimal, Benita et al (2009), This study was to isolate and to identify the *Actinomycetes* having Antagonistic activity. An *Actinomycetes* strain isolated from Marine Sediment samples collected at the Puducherry coast of India, showed Antibacterial activity against selected Microbial pathogens. Based on Morphological, Biochemical, Physiological and Phylogenetic Characterization, the strain was identified as *Nocardiopsis* sp. VITSVK5 (FJ973467). In conclusion the isolated strain has broad spectrum of Antagonistic activity against Gram positive and Gram-negative bacteria and *Aspergillus* sp. [38].

- Caixia Chen, et al (2013), In the course of a screening program for bioactive compounds from a marine natural product library a newly isolated Actinomycetes strain, designated as MS100061, exhibited strong anti-*Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) activity. The strain belongs to the genus *Streptomyces* according to its morphological and 16S rDNA Phylogenetic Analysis [3].
- Sohan Sengupta, et al (2015), Rare bioactive actinomycetes is isolated from the unexplored sites. The antimicrobial compound has also been identified and purified which works against a wide range of pathogens. Fifty-four actinomycetes are classified and analyzed Anti-bacterial activity against fifteen experiments comprising three phytopathogens, among these nine isolates three specific strains were found to have notably higher degree of antimicrobial potential effective in a broader range including Phyto-pathogenic fungus [34].
- T Janaki, et al (2016), Totally 25 actinomycetes were isolated, from the soil sample that was collected nearer to the root region of the mangrove *Avicennia marina* from the back water area, Ariyankuppam, Puducherry (UT). All the 25 actinomycetes were subjected for primary screening against the 10-gram negative bacteria, 2-gram positive bacteria. Totally 20 (80%) of actinomycetes showed antibacterial activity towards any one of the tested bacteria, 5 (20%) actinomycetes showed no Antagonistic activity [36].
- Usha Kiranmayi Mangamuri, et al (2012), have identified and demonstrated antimicrobial actinomycetes from the Mangrove ecosystems of Nizampatnam located in the southern coastal region of Andhra Pradesh, India, which have been instrumental in the discovery of effective bioactive novel metabolites for a wide

variety of pathogens. The 16S rRNA sequence was performed and revealed that the species is closely related to *Pseudonocardia endophytica* and bioactive metabolites produced by isolate inhibited Gram positive, Gram negative and Fungi [37].

- Zhonghui Zheng, et al (2000), the purpose of this work was to investigate actinomycetes with antitumor or antimicrobial activities, which were isolated from the surface of epidermis and intestines of marine plants and animals collected in Taiwan Strait, China. Of all the marine actinomycetes, the *Micromonospora* genus has the highest level of inducing activity. However, most antimicrobial activity was found in the *Streptomyces* genus. These results suggest that marine organism associated with actinomycetes may be a promising source of antitumor and antimicrobial bioactive agents [40].
- Gayathri, et al (2013), In this study endophytic Actinomycetes are isolated from the roots, stem and leaf of two mangrove species such as *Rhizophora apiculata* and *Avicennia marina* for their antibacterial activity. six endophytic actinomycetes were found in all samples. At the top of these 6,1 isolate the endophytic actinomycetes isolate (MAR1) shows good activity against all isolated sites. Based on the above factors Mar1 was identified as *Streptomyces* sp. and species may be *Streptomyces coelicolor* [23].
- Hotam S. Chaudhary, et al (2012), The main objective of this study was the Separation, Purification, and Separation of Actinomycetes from soil samples, which have antimicrobial activity against 12 selected pathogenic species. Soil samples were taken from various niche areas of Sheopur district, Madhya Pradesh, India. These samples were filtered sequentially and placed in Actinomycetes Isolation

Agar Media. A total of 31 Actinomycetes isolate tested for Antibodies against 12 pathogenic microorganisms. Isolates AS14, AS27, and AS28 were highly active, while AS1 showed little activity against pathogenic microorganisms. Isolate AS7 showed very high antagonistic activity against *Bacillus cereus* (24 mm) and AS16 showed very high activity against *Enterococcus faecalis* (21 mm) [10].

- Sergey B. Zotchev, (2012), The review highlights achievements and challenges in the isolation of marine actinomycetes, some examples of bioactive metabolites identified by conventional screening, and presents new developments in the field of genome mining and heterologous expression of biosynthetic gene clusters leading to the discovery of novel compounds. Actinomycetes from terrestrial sources have been studied that they yield many important anti-infective and anti-cancer drugs [31].
- Selvakumar Dharmaraj, (2010), this review is made on the present state of studies on marine Streptomyces and its perspectives. The highlights consist of the production of metabolites inclusive of antibiotics, anticancer compounds, enzymes, enzyme inhibitors and pigments by way of marine Streptomyces and their application as single cell protein and as probiotics in aquaculture. With growing advancement in technology and generation, there would be greater needs in future for new bioactive compounds synthesized by Streptomyces from numerous marine sources [30].
- Xiaochun Gaoa, et al (2012) A marine actinomycete, was isolated from a sediment sample collected in the Arctic Ocean, , designated strain BM-17, Based on morphological, cultural, physiological, biochemical characteristics, along with the cell wall analysis and 16S rDNA gene sequence analysis, The strain was identified

as *Nocardia dassonvillei*, A new secondary metabolite (1), N-(2-hydroxyphenyl)-2-phenazinamine (NHP), and six known antibiotics have been isolated from the saline culture broth. The new compound showed significant antifungal activity against *Candida albicans*, with a MIC of 64 g/ml and high cancer cell cytotoxicity against HepG2, A549, HCT-116 and COC1 cells [39].

- Sunil Laxman Attimarad¹ et al (2012), Marine residues, collected in the coastal areas of Gokharna and Muradeshwara in the state of Karnataka, the screening was done seventeen isolates were found in starch-casein agar media by soil dilution techniques. Only six isolates namely ACT-A2, ACT-A3, ACT-A4, ACT-A5, ACTA7 and ACT-A15 showed significant antibacterial activity against both gram-positive and gram-negative viruses. Morphological, cultural and biochemical characterization have shown that isolate is part of the *Streptomyces* genus of Actinomycetes. [38]
- Sheng Qin, et al (2009). In this work, they have studied the diversity of actinobacteria isolated from medicinal plants collected from tropical rain forests in Xishuangbanna, A total of 2,174 actinobacteria were isolated by the use of different selective isolation media and methods. On the basis of their morphologies on different media Forty-six isolates were selected and were further characterized by 16S rRNA gene sequencing. The results showed diversity, with 32 different genera, 46 isolates were tested for antimicrobial activity and were screened for the presence of genes encoding polyketide synthetases and non-ribosomal peptide synthetases. The results confirm that the medicinal plants of Xishuangbanna represent an extremely rich reservoir for the isolation of a significant diversity of actinobacteria, including novel species, that are potential sources for the discovery of biologically active compounds.[32]

- Baskaran, R et al (2011). for the isolation of bioactive actinomycetes from mangrove species in the Andaman and Nicobar Islands, India, various pre-treatment methods and three different media were used. The fossils were collected from four different parts of the mangrove forest, and 22 species were found to be producers of antibacterial metabolites against experimental bacteria, among the 42 isolates tested. Of these, A107 was identified as *Streptomyces* spp. [2]
- Ramesh Subramani, et al (2012) they have concluded that the Role of Actinomycetes from marine environment, a number of novel Secondary Metabolites produced by Marine Actinomycetes, how to grow a rare Actinomycetes and their Selective Isolation and Molecular Approaches.[28]
- Nanjwade Basavaraj, et al (2010) they have investigated a strain called A4, is an Actinomycete out of 9 isolates of actinomycetes of soil sample from Belgaum, Karnataka, India.[21]
- Mukesh Sharma, et al (2014) in this paper they have described about the growth of Actinomycetes from different soil samples with suitable growth medium and how the isolates were characterized by physiological, biochemical and morphological methods.[20]
- Hanki lee, et al (2016) in this paper they have investigated that the natural products from actinomycetes such as Cyclomarin A, Lassomycin, and Ecumicin which are New Anti – tuberculosis Lead compounds produced by the actinomycetes have shown that potent anti- tb activity against MDR and XDR[8].

- Loh Teng-Hern Tan, et al (2018) in this study they have explored the antioxidant potentials of Streptomyces bacteria derived from mangrove soil. Strain MUM292 was identified as the genus Streptomyces by using 16S rRNA phylogenetic analysis. Finally, they have demonstrated that the Streptomyces MUM292 strain can be potent antioxidant resource which is useful for Food and Pharma Industries [17].
- Gebreselema Gebreyohannes, et al (2013) this study revealed the isolation, evaluation and characterization of potential antibiotic producing actinomycetes. They were isolated from freshwater and sediments of Lake Tana, Ethiopia. They have isolated 31 actinomycetes strains and tested against gram positive and gram-negative bacteria as a primary screening. Among these 11 were identified and subjected to crude extracts by the solid state and submerged fermentation methods. The biomass was further processed by solvent extraction method and tested against bacterial strains using agar well diffusion method and disc method. They have concluded that agar well diffusion is better than the other method. The crude extract of actinomycetes against gram positive bacteria is good than gram negative strains. Therefore, they revealed that actinomycetes from freshwater sediments from Lake Tana have better for an antibacterial compound [6].
- Kornelia Smalla, et al (1997) In this study they have developed group specific primer F243 (positions from 226 to 243 E. coli numbering) by gene encoding 16S rRNA or 16S rDNA sequences for the actinomycetes detections with the PCR and temperature or denaturing gradient gel electrophoresis. TGGE or DGGE Method both shows similar results. To study the natural microbial community they have used 2 strategies. First, for direct analysis of the products from denaturing gradient they

have use selective amplification of Actinomycetes (E. coli positions from 226 to 528). Second, Nested PCR for producing Actinomycete specific fragments (E. coli positions 226to 1401) were used as templates for PCR. Mainly they focused on the actinomycetes from the soil. they revealed that Actinomycetes diversity were vary in the potato Rhizosphere and they investigated from the different soils [33].

- Jinhua Zhang, et al (2011) In this study they have isolated actinomycetes from the soil samples and they comparing differences of effects of actinomycetes in different isolation medium sand they found easier medium to isolate rare actinomycetes with rich diversity than the traditional medium such as Gause`s No.1, HV and ISP5; CHV is for micromonospora, ZSSE was easier to isolate rare actinomycetes with rich diversity, and could replace routine agars such as Gause`s No.1, HV and ISP5; CHV was easier to isolate micromonospora.[12]
- M.S Degani, et al (2017) this study explores the Aegiceras corniculatum (a mangrove plant) leaf extract against Mycobacterium Tuberculosis and some different micro-organisms. They have used different extraction techniques to check the antituberculosis activity. Microwave assisted extraction showed an MIC of 19.53 µg/ml against M.tb H37Rv strain in vitro using Resazurin Microtiter Assay (REMA), soxhlet 39.06 µg/ml and in maceration technique 1250 µg/ml. on further process the crude extract were fractionated by using different solvents. then it is testes invitro for antituberculosis activity [18].
- Panchanathan Manivasagana, et al (2014) this paper explains about the active secondary metabolites of actinomycetes from marine environment. This study is on the marine actinomycetes contains more secondary bioactive metabolites which is unique in structural diversity comparatively than the Terrestrial Actinomycetes [25].

- Pachaiyappan Saravana Kumar, et al (2012) In this paper they have isolated novel actinomycetes and to find the antimicrobial activity. three soil samples were isolated from the Vengodu village, Kanchipuram district, Tamil Nadu, India. Total of 35 isolates were obtained on the basis of colony characterization. All the isolates were screened for antibacterial activity by cross streak method. Isolation of bioactive compounds is done. The most active VAS 10 is identified as Actinobacterium Loyola. the hexane, ethyl acetate, dichloromethane and butanol extract were tested against test bacteria, in which the dichloromethane and ethyl acetate shows maximum zone of inhibition against Enterococcus Durans [24].
- L.Ashokkumar et al, (2012) This paper investigation is based on the five different soil samples of Actinomycetes have been collected and collected in Salem, Tamilnadu. These are then tested for their antimicrobial activity. All five of the Actinomycetes showed Antagonistic activity. The Zone of Inhibition was between 11-25 mm. Among the 5 isolates of Actinomycetes A5 isolate has a high Anti-Bacterial Activity against S.aureus and E.coli. Pseudomonas aeruginosa was strongly suppressed by actinomycetes followed by E. coli. On day 14 of incubation a significant antibacterial activity was observed on. The results of the main tests indicate that most of the active actinomycetes are active against gram-positive bacteria (S.aureus) rather than gram-negative bacteria.[15]
- K.Selvam, et al (2011). Fifty-six species of actinomycete were isolated from marine fossils in the coastal region of southern India in India. Isolate has been identified as actinomycete by morphological studies. varieties 1, 3, 51 and 12, 29, 49 Amylolytic and cellulolytic activities were exhibited 3, 10, 28 exhibited Lipolytic activity [13].

CHAPTER 3

AIM AND SCOPE OF THE PROJECT

3.1 AIM

Aim of the Study is to investigate the Antituberculosis Activity from Mangrove Associated microbes.

3.2 OBJECTIVES

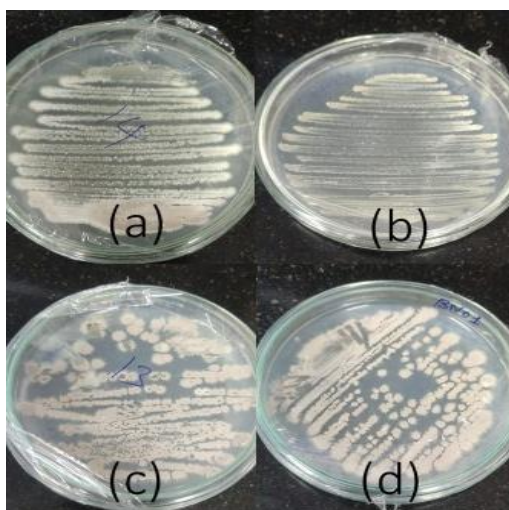
- Isolation of Actinomycetes Species from Mangrove Environment
- Screening of Potential Actinomycetes strain against Mycobacterium tuberculosis
- The characterization of potent Actinomycetes.
- DNA Sequencing was performed
- The DNA Sequences were submitted to GenBank, NCBI
- DNA sequences were aligned
- BLAST Tree was Constructed

CHAPTER 4

MATERIALS AND METHODS

4.1 ISOLATION OF ACTINOMYCETES FROM SOIL SAMPLE

Soil samples were collected from Mangrove Ecosystems, coastal areas of Chennai, Tamil Nadu, and India. Samples were collected in a sterile plastic bag and it's transported to the laboratory in aseptic condition. Soil samples were air dried at room temperature for 1week prior isolation. This helps in decreasing the population of gram-negative bacteria, where 1 g of the soil sample were taken and suspended with 9ml of sterile double distilled water. Up to 10^{-5} Dilutions were carried out. Aliquots (0.1ml) of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} were spread on the isolation agar. Plates were incubated for 10 days at 30° C. Based on the morphology actinomycetes culture were selected and purified on ISP-2(International Streptomyces Project-2) Medium.



**Fig: 4.1: The above figure represents the pure culture of the Actinomycetes
(a)BN14, (b)BN15, (c)BN13, (d)BN01**

4.2 SUBCULTURING

The petri plates and the conical flask are washed and dried. 250 ml of SCA agar is prepared and the PH of 7.0 is maintained. The petri plates and the agar is autoclaved. The agar is poured in the petri plates and it is allowed to solidify. parent culture plate is selected. the inoculating loop is sterilized. Touch the loop to the surface of selected discrete colonies. The inoculum is transferred from the parent plate to fresh plate. The culture is incubated at 37 °C for 4-5 days.

4.3 METHODS FOR GENOMIC DNA ISOLATION

- CTAB - Cetyl Trimethylammonium Bromide
- Agarose gel electrophoresis.
- PCR-Polymerase chain reaction

4.3.1 CTAB - Cetyl Trimethylammonium Bromide

MATERIALS REQUIRED:

ISP-2 broth culture, Eppendorf tube, TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA, Solution 1 [(1%, w/v) Sarkosyl, 0.5 M NaCl, (1%, w/v) SDS], phenol: chloroform: isoamyl alcohol; 25:24:1). sodium acetate (3 M, pH 5.2) isopropanol, deionized water, 70% ethanol,

PROTOCOL:

- ISP-2 broth is prepared and the cultured is inoculated. The broth is kept in the shaker and kept undisturbed for 2-4 days
- Set up a 4-ml microbial culture and pellet the cells in Eppendorf tubes (2.0 ml) by centrifugation at 10,000g or 10 min at 37 °C (the cell pellets can be stored for 1 week at -20 °C to continue later).
- Resuspend the cell pellet in 300 µl of TE solution.

- Add 400 µl of Solution I. Mix well and keep the tubes for 10 min at 37 °C with intermittent shaking after every 5 min (do not vortex to avoid breaking DNA).
- Immediately add an equal volume of PCI 900 µl. Mix by inversion.
- Centrifuge at 10,000g for 10min at 37 °C and carefully transfer the supernatant into a new Eppendorf tube.
- Add 0.1 volume of sodium acetate and 0.6 volume of isopropanol.
- Mix gently by inverting the tubes four to six times and leave it overnight.
- Centrifuge for 5 min at 10,000g at 37 °C. The DNA will be precipitated in the pellet.
- Wash the pellet with 1 ml of 70% ethanol and centrifuge at 10,000g for 3 min at 37 °C.
- Remove the supernatant and air-dry the pellet.
- Resuspend the DNA pellet in 100 µl of sterile deionized water. The suspension can be stored at -20 ° C for further use.
- Check the integrity of DNA on 0.8% agarose gel electrophoresis.

4.3.2 Agarose gel electrophoresis

MATERIALS REQUIRED:

DNA sample, comb, boat, TAE (Tris Acetic Acid EDTA) buffer, agarose, EtBr, loading dye, power supply.

PROTOCOL:

- The boat and comb is sterilized using Acetone and placed
- TAE (Tris Acetic Acid EDTA) buffer is used as tank buffer. 1ml of TAE is mixed with 50ml of distilled water.
- 0.8% of agarose is prepared using TAE buffer (0.4g in 50ml buffer)
- 5 µl of EtBr is added.
- Casting- the comb is placed in boat and pour the prepared agarose. Allow the agarose to solidify (30 mins).
- Place the boat in the tank, then remove the comb.

- Buffer is poured in the tank until the gel is immersed.
- Add the sample and loading dye in 2:1 ratio (10 :5 µl)
- Switch on the power. Set the power to 50V.
- Quality and quantity of DNA can be checked by uv - transilluminator

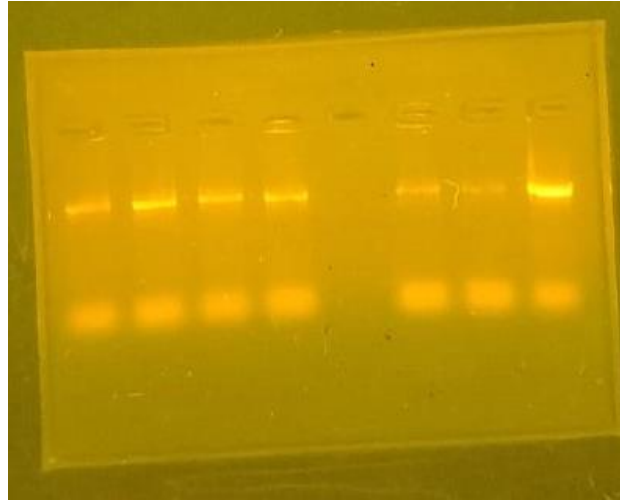


Fig: 4.2: The above figure represents the Separation of the DNA Fragments of the Sample (BN01, BN11, BN13, BN14, B N15) from the gel based on molecular weight

4.3.3 PCR

It is used for amplification of DNA i.e, to make a number of copies

MATERIALS REQUIRED:

PCR Tube with the sample DNA solution, primer, forward primer, reverse primer, master mix, water

PROTOCOL:

- Add 1 µl of primer to the PCR tube with the presence of DNA, followed by adding 1 µl of forward primer, 1 µl of reverse primer, 1 µl of DNA Solution, 12.5 µl of Master Mix, 9 µl of distilled water

- Total amount present in the PCR tube is 25.5 μ l. Add primer- 1 μ l, forward primer- 1 μ l, reverse primer-1 μ l, DNA solution- 1 μ l, master mix-12.5 μ l, water- 9 μ l.
- place the 3 tubes in the PCR. Then start running the PCR. It runs in the 5 steps first step is Initial denaturation, - set the temperature at 98°C and run it for 5 mins then followed by the second step, Denaturation-set the temperature at 94°C for 1 min then the third step Annealing, set the temperature at 55°C for 1 min. the Fourth step is Extension, set the temperature at 72°C for 2 mins.
- Total cycles- 30 cycles
- The Last step is the Final extension that first Hold, set the temperature at 72°C for 5 min, then followed second Hold, set the temperature at 4°C for 30 mins
- Check the integrity of DNA on 1.2% agarose gel electrophoresis

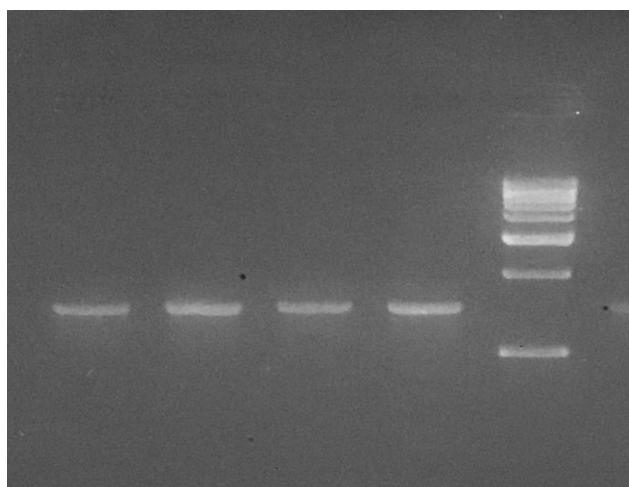


Fig. 4.3: The above picture represents the Confirmation of Amplified DNA of Actinomycetes, running it with DNA Ladder by using Agarose Gel Electrophoresis

4.4 MASS PRODUCTION AND EXTRACTION

4.4.1 Preparation of production media (isp-2)

Material required for ISP-2 are

- Yeast extract- 1g
- Malt extract- 2.5g

- Dextrose-1g
- Nacl-6.25g
- Distilled water-250ml
- Dil. NaOH and dil.HCL (PH adjustment)
- Inoculum Culture

EQUIPMENTS REQUIRED

Beaker(500ml), conical flask (100 ml), glass rod, PH meter, autoclave, laminar air flow, inoculating loop, shaker, cotton plug, weighing machine, spatula, burner, tissue paper, 70%Ethanol, thread.

PROTOCOL OF PREPARATION OF ISP-2

- Take 250ml of distilled water in 500ml beaker.
- Add all the nutrients one by one
- Check the level of PH using PH meter and adjust the PH to 7.3, if the solution acidic add diluted NaOH, if it is basic add diluted HCl
- Transfer the media into 50ml conical flask and autoclave the flask.
- Allow the broth to cool down to room temperature.
- Transfer the inoculum into the broth.
- Keep the culture broth in the shaker and leave it for 3-4 days without disturbing it.

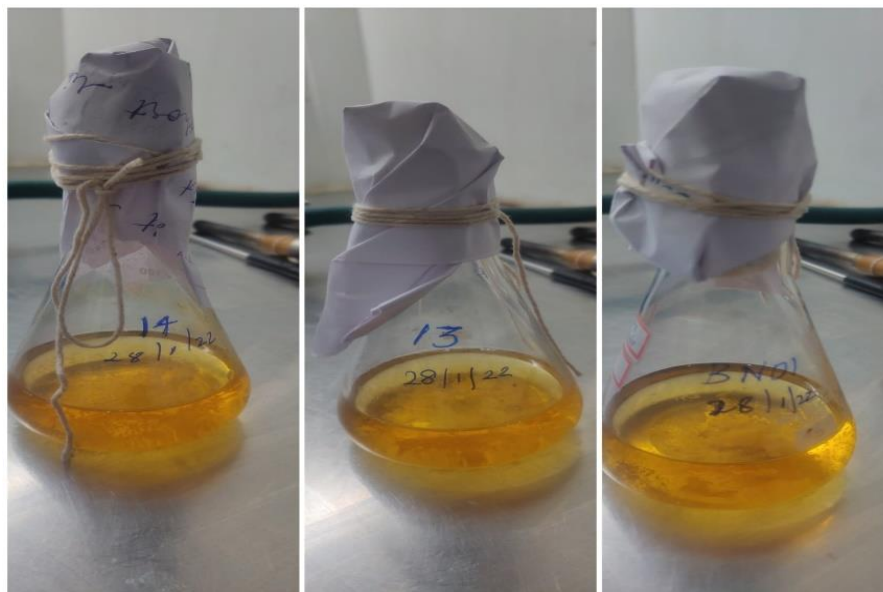


Fig. 4.4: The above figure represents the culture broth of the three isolates of actinomycetes (BN01, BN13, and BN14)

4.4.2 PRODUCTION MEDIA:

Production media is used to mass culturing of particular Actinomycetes species .Media used is International Streptomyces Project (ISP-2)

MATERIALS REQUIRED:

- Glycerol-52.5ml
- Glucose-22.5g
- Beef.extract-22.5g
- Peptone-6g
- Sodium nitrate- 1.5g
- MgSo4-0.075g
- CaCo3-2.25g
- K2HPo4-1.875
- KH4Po4-1.875

- PH-7.0
- Distilled Water-750ml
- Diluted NaOH AND HCL (PH adjustment)
- ISP-2 culture broth
- 70%Ethanol

EQUIPMENTS REQUIRED:

Beaker(1000ml), conical flask (250 ml), glass rod, PH meter, autoclave, laminar air flow, shaker, cotton plug, weighing machine, spatula, burner, tissue paper, thread.

PROTOCOL:

- Take 1000ml beaker and add 750ml of distilled water
- Add all the nutrients one by one
- Check the level of PH using PH meter and adjust the PH to 7.0, if the solution acidic add diluted NaOH, if it is basic add diluted HCl
- Transfer into 250ml conical flask
- Autoclave the ISP broth.
- Allow the production media to cool down
- Pour the ISP-2 broth culture into production media in the laminar air flow.
- Keep the production media the shaker incubator and leave undisturbed for 5-7 days

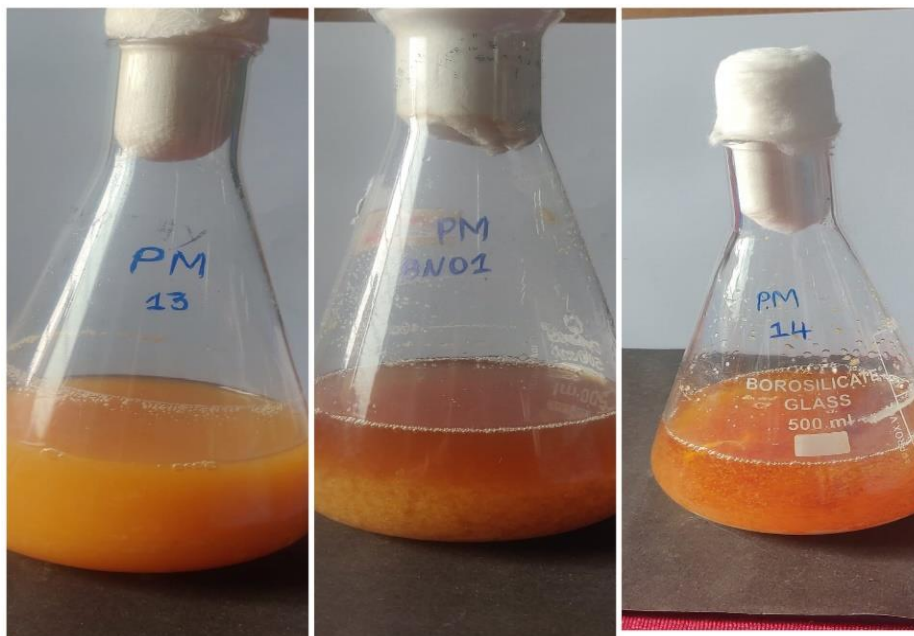


Fig. 4.5: The above figure is the Mass culture Production in a of 3 isolates was cultured in a ISP-2 broth

4.5 EXTRACTION OF CRUDE SECONDARY METABOLITES

MATERIAL REQUIRED

Production media, ethyl acetate, ethanol, distilled water

EQUIPMENT REQUIRED

Centrifuge, Conical flask, 13ml Eppendorf tube, separating funnel, fractional distillation, glass bowl, 2ml Eppendorf tube, incubator, water, ice cubes, 1 ml pipette, weighing machine, round bottom flask, heating mantle, receiving flask, condenser tube

4.5.1 Separating funnel

It is used to separate the secondary metabolites from other biomolecule compounds like fat lipids. Ethyl acetate is used as a solvent for extraction in the ratio of (1:3)



Fig. 4.6: The above picture shows the separating funnel –separation of the secondary metabolite from other biomolecules like fats, lipids

PROTOCOL

- The production media of 01 is taken and poured into a 13ml Eppendorf tube.
- The Eppendorf tube is centrifuged for 20mins.
- The pellet is discarded and the supernatant is collected in the conical flask.
- Same procedure is repeated for 13 and 14 production media.
- The supernatant from the conical flask is poured into the separating funnel.
- Equal volume of ethyl acetate is added to the supernatant in the funnel with an interval of 30mins
- The step 6 was repeated twice, till the ratio of 1:3 achieved
- The separating flask is shaken for 2mins for every 5mins totally for 30mins.
- Lipids and fatty acids are separated from the secondary metabolites present in the extract by the force created by shaking the ethyl acetate with supernatant in the separating funnel.
- The ethyl acetate is collected in a separate 500ml conical flask.
- Lipids were collected in a separate 500ml conical flask.

- Separated extract contains a small amount of the organic solvent
- Therefore 3 Separated extracts were collected in 3 different 500ml conical flask



Fig. 4.7: The above picture is the Supernatant of 3 Actinomycetes isolates (BN01, BN13, and BN14) from Mass Production

4.5.2 Fractional distillation (liquid-liquid separation):

This method is used to remove the organic solvent from the separated extract



Fig. 4.8: The above picture represents the separation of ethyl acetate from the extract

PROTOCOL:

- First Pour the 150ml of separated extract into the round bottom flask and it is placed it on the heating mantle
- Round bottom flask is connected to the condenser tube with the help of the distillation head
- The condenser tube is supplied with the cold water to convert the vapor into water droplets and to reduce the heat generated from the motor
- Heating mantle is set on to the temperature of 50°C
- Ethyl acetate is collected from the condenser to the receiving flask
- The process is carried on till the solvent is separated from the crude extract
- Crude which remains in the round bottom flask is collected in the sterilized glass bowl
- The crude is placed in the incubator and it is allowed to air dry



Fig. 4.9: The above figure shows that the antibiotic crude extracts of 3 isolates is kept in the glass bowl for Air Drying

- weigh the 2 ml Eppendorf tube before the collection of crude extract
- then the crude extract is collected in a 2ml Eppendorf tube with the help of a pipette
- after collection of crude extract weigh the sample again

- to calculate the amount of the sample by subtracting the before and after weight of the Eppendorf tube
- the above procedure is carried out for the remaining 2 samples
- the crude extract was screened against *Mycobacterium tuberculosis*



Fig. 4.10: The above picture is the crude extract of 3 actinomycetes samples (BN01, BN13, BN14) in the Eppendorf tubes.

4.6 AGAR WELL DIFFUSION METHOD

Anti-bacterial activity was carried out by agar well diffusion method. Petri plates were prepared, 20ml of sterile MHA (muller Hinton agar). The microbes were swabbed on top the solidified media. Create a well by using puncher in the solidified agar load 5mg per concentration for each crude extract in the well. The plates were incubated at 37°C for 24 hours and zone of inhibition were recorded.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 MORPHOLOGICAL CHARACTERIZATION

Colonies of Actinomycetes were characterized by following methods given in the ISP (International Streptomyces Project). Strains were observed under light microscope for micromorphological studies. (Growth, Aerial Mycelium Color, Substrate Mycelium color). Three different Media is used to culture Marine Actinomycetes for comparing the difference in the colour and growth in specific Media. Media used for the studies are Yeast Extract Malt Extract Agar is called M2, Inorganic salts -Starch Agar is called M4, Glycerol Asparagine Agar is called M5.

5.1.1Composition of the medium

M2-Yeast Extract Malt Extract agar

- Take 150ml of distilled water in 500ml beaker.
- Add all the nutrients one by one
- Check the level of PH using PH meter and adjust the PH to 7.3, if the solution acidic add diluted NaOH, if it is basic add diluted HCl
- Transfer the media into 250ml conical flask and autoclave the flask.
- Agar of 3g is weighed and added.
- The conical flask is autoclaved along with the petri plates
- The agar is poured in the petri plates inside the chamber.
- The agar is allowed to solidified.
- The inoculating loop is sterilized, the loop is touched with selected colony.
- The culture is transferred to fresh plates.
- The plates are wrapped with parafilm
- The plates are placed in the incubator for 4-5 days
- The substrate and arial mycelium color is recorded.

Table 5.1: The below table list the composition of M2 Medium

Yeast extract	0.6g
Malt extract	1.5g
Dextrose	0.6g
Distilled water	150ml
PH	7.3
Nacl	3g
Agar	20g

M4-Starch agar

- Take 150ml of distilled water in 500ml beaker.
- Add all the nutrients one by one
- Check the level of PH using PH meter and adjust the PH to 7.0-7.4, if the solution acidic add diluted NaOH, if it is basic add diluted HCl
- Transfer the media into 250ml conical flask and autoclave the flask.
- Agar of 3g is weighed and added.
- The conical flask is autoclaved along with the petri plates
- The agar is poured in the petri plates inside the chamber.
- The agar is allowed to solidified.
- The inoculating loop is sterilized, the loop is touched with selected colony.
- The culture is transferred to fresh plates.
- The plates are wrapped with parafilm
- The plates are placed in the incubator for 4-5 days.
- The substrate and arial mycelium color is recorded

Table 5.2: The below table list the composition of M4 Medium

DipotassiumHydrogenPhosphate(K_2HPO_4)	0.3g
Magnesium sulfate($MgSO_4 \cdot 7H_2O$)	0.3g
Nacl	2.7g
Ammonium sulfate($(NH_4)_2SO_4$)	0.6g
Calcium carbonate($CaCO_3$)	0.6g
Distilled water	150ml
Ph	7.0&7.4
Trace salt solution	1.0ml
Agar	3g

M5-Glycerol-Asparagine agar

- Take 150ml of distilled water in 500ml beaker.
- Add all the nutrients one by one
- Check the level of PH using PH meter and adjust the PH to 7.0-7.4, if the solution acidic add diluted NaOH, if it is basic add diluted HCl
- Transfer the media into 250ml conical flask and autoclave the flask.
- Agar of 3g is weighed and added.
- The conical flask is autoclaved along with the petri plates
- The agar is poured in the petri plates inside the chamber.
- The agar is allowed to solidified.

- The inoculating loop is sterilized, the loop is touched with selected colony.
- The culture is transferred to fresh plates.
- The plates are wrapped with parafilm
- The plates are placed in the incubator for 4-5 days.

The substrate and arial mycelium color is recorded

Table 5.3: The below table list the composition of M5 Medium

Asparagine	0.015g
Glycerol	1.5g
DipotassiumHydrogenPhosphate(K_2HPO_4)	0.015g
Trace salt solution	0.015 MI
Ph	7.0-7.4
Agar	3g
Distilled water	150ml

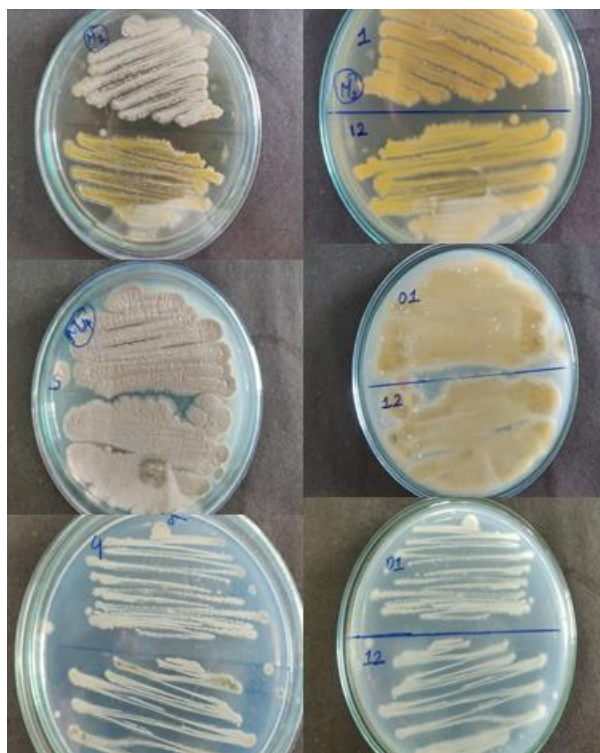


Fig. 5.1: The above figure represents the Growth of the Actinomycetes sample (BN01, BN12) on M2, M4, M5



Fig. 5.2: The above figure represents the Growth of the Actinomycetes sample (BN13, BN14) on M2, M4, M5

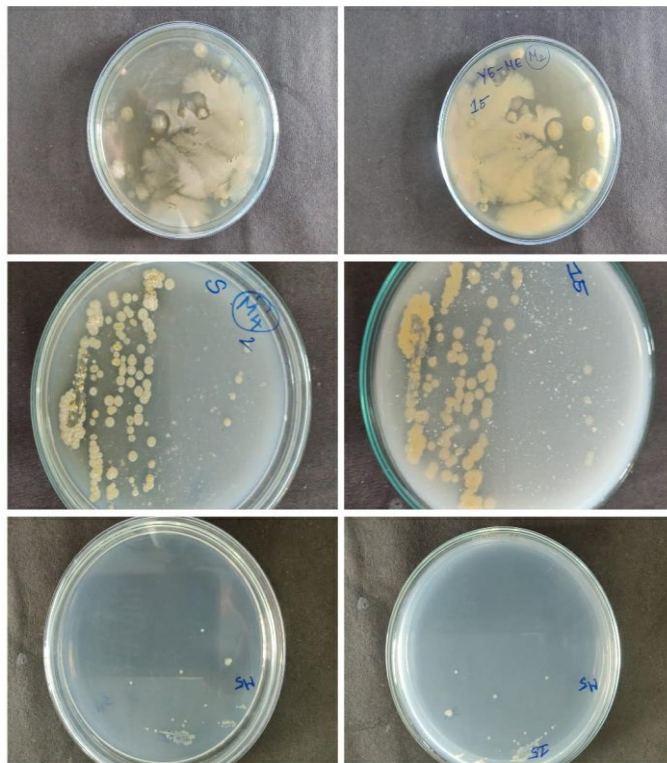


Fig. 5.3: The above figure represents the Growth of the Actinomycetes sample (BN15) on M2, M4, M5

- The growth period for BN01, BN11, BN13, BN14 is about 5-10 days. Within this time period the maximum growth of Actinomycetes was reached.
- For BN15 was slow growing isolate it took long period about 15-20 days for attaining minimum growth

The below table is the comparison of all 5 actinomycetes samples with the difference in the color and growth on three different media M2,M4,M5

Table 5.4: the comparison of 5 actinomycetes samples with the difference in the color and growth on the three different media; Maxi.growth+++, Medium growth++

NAME OF THE CULTURE	NAME OF THE MEDIUMS	AERIAL MYCELIUM	SUBSTRATE MYCELIUM	GROWTH
BN01	M2	GREY	YELLOW TO BROWN	+++
	M4	GREY	YELLOW TO BROWN	+++
	M5	GREY	GREY	++
BN13	M2	GREY	YELLOW	+++
	M4	DARK GREY	YELLOW	+++
	M5	GREY	GREY	++
BN14	M2	GREY	YELLOW	+++
	M4	GREY	YELLOW TO BROWN	+++
	M5	GREY	GREY	++

5.2 PRIMARY SCREENING

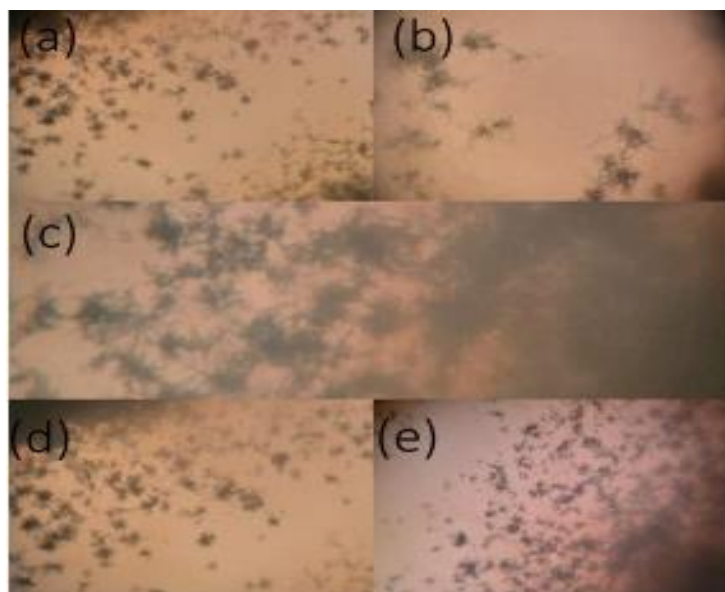
The Crude Extract of the 3 Actinomycetes sample was screened against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The Medium used for primary screening is Mueller Hinton Agar. Agar well diffusion method is used to find actinomycetes activity against test organisms used in primary screening. No activity was found against test organisms.



Fig. 5.4: The above figure shows that the crude extract of BN01, BN13, and BN14 doesn't have potential against a *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*

5.3 LIGHT MICROSCOPE

With the help of a light microscope, 5 stains of Actinomycetes were isolated from the plate. Medium used for plating is Starch Casein Agar. Using spread plate technique it is cultured and it is incubated at 37 degree Celsius for 10 to 15 days. After 10 days the Actinomycetes are fully Grown. Then remove the plastic wrapper from the cultured the petri plates. Then place it in the microscope and view the plates.



**Fig. 5.5: The above picture is the microscopic view of 5 Actinomycetes strains
a) BN01 b) BN11c) BN13 d) BN14 e) BN15**

5.4 DNA SEQUENCE DETERMINATION

For DNA sequencing, the Sanger dideoxy sequencing method is used to identify the specific actinomycetes species. the following data is about the specific information of each 3 samples by using 16s Ribosomal RNA gene.

The 16s rRNA sequence of 3 different actinomycetes strains were submitted to GenBank, NCBI, USA and its Accession numbers for the nucleotide sequence of 3 strains are listed below,

Table 5.5 DNA sequence data of 3 isolates deposited in the GenBank ncbi

NAME OF THE STRAIN	ACCESSION NUMBER	NAME OF THE SPECIES	BASE PAIRS	TYPE OF THE GENOMIC DNA
BN01	ON042751	Streptomyces rochei	1021bp	Linear

BN13	ON042452	Streptomyces sp.	1438bp	Linear
BN14	ON042463	Streptomyces sp.	1410bp	Linear

Streptomyces rochei strain BN01 16S ribosomal RNA gene, partial sequence

GenBank: ON042751.1

[FASTA](#) [Graphics](#)

[Go to:](#) [☺](#)

LOCUS ON042751 1021 bp DNA linear BCT 27-MAR-2022
DEFINITION Streptomyces rochei strain BN01 16S ribosomal RNA gene, partial sequence.
ACCESSION ON042751
VERSION ON042751.1
KEYWORDS .
SOURCE Streptomyces rochei
ORGANISM [Streptomyces rochei](#)
Bacteria; Actinobacteria; Streptomycetales; Streptomycetaceae;
Streptomyces; Streptomyces rochei group.
REFERENCE 1 (bases 1 to 1021)
AUTHORS Kumaravel,M., Suresh,M., Ramasamy,N., Masilamani,M., Kalimuthu,K.,
Halder,D. and Perumal,A.
TITLE Direct Submission
JOURNAL Submitted (22-MAR-2022) Biotechnology, Karunya Institute of
Technology and Sciences, Karunya Nagar, Coimbatore, Tamil Nadu
641114, India
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Sequencing Technology :: Sanger dideoxy sequencing
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1021 a

Fig. 5.6: The above picture represents the DNA Sequence of BN01 isolates was deposited in the GenBank, NCBI

Streptomyces sp. strain BN13 16S ribosomal RNA gene, partial sequence

GenBank: ON042452.1

[FASTA](#) [Graphics](#)

Go to: 

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ORGANISM [Streptomyces sp.](#)
Bacteria; Actinobacteria; Streptomycetales; Streptomycetaceae; Streptomyces.
REFERENCE 1 (bases 1 to 1438)
AUTHORS Kumaravel,M., Suresh,M., Ramasamy,N., Masilamani,M., Kalimuthu,K., Halder,D. and Perumal,A.
TITLE Direct Submission
JOURNAL Submitted (22-MAR-2022) Biotechnology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore, Tamil Nadu 641114, India
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Fig. 5.7: The above picture represents the DNA Sequence of the BN13 isolate was deposited in the GenBank, NCBI

Streptomyces sp. strain BN14 16S ribosomal RNA gene, partial sequence

GenBank: ON042463.1

[FASTA](#) [Graphics](#)

[Goto: ☺](#)

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AUTHORS Kumaravel,M., Suresh,M., Ramasamy,N., Masilamani,M., Kalimuthu,K., Halder,D. and Perumal,A.
TITLE Direct Submission
JOURNAL Submitted (22-MAR-2022) Biotechnology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore, Tamil Nadu 641114, India
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Fig. 5.8: The above picture shows the DNA Sequence of the BN14 isolate was deposited in the Genbank, NCBI

5.5 DATABASE SEARCHING

The Sequences was compared for similarity with the reference species of bacteria contained in genomic Database banks using NCBI, (BLASTN Tool). The DNA Sequences were aligned and BLAST Tree was constructed by using Fast Minimum Evolution Method produced by BLAST Pairwise Alignment.

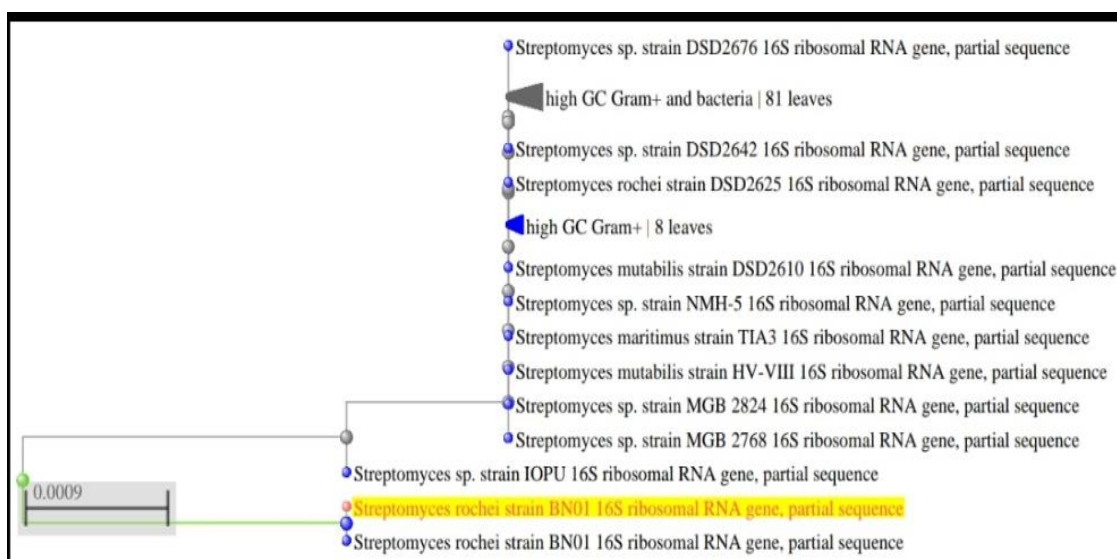


Fig. 5.9: the above picture represents the Blast tree of the BN01 isolate

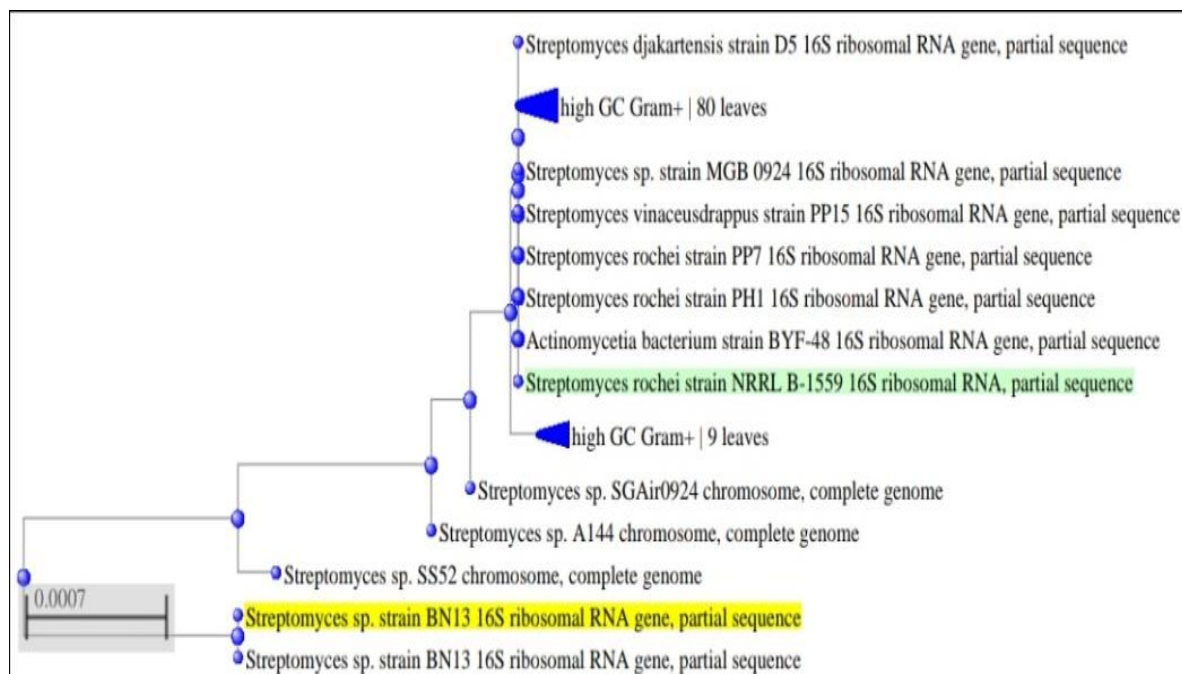


Fig. 5.10: The above the picture shows the BLAST Tree of the BN13 isolate

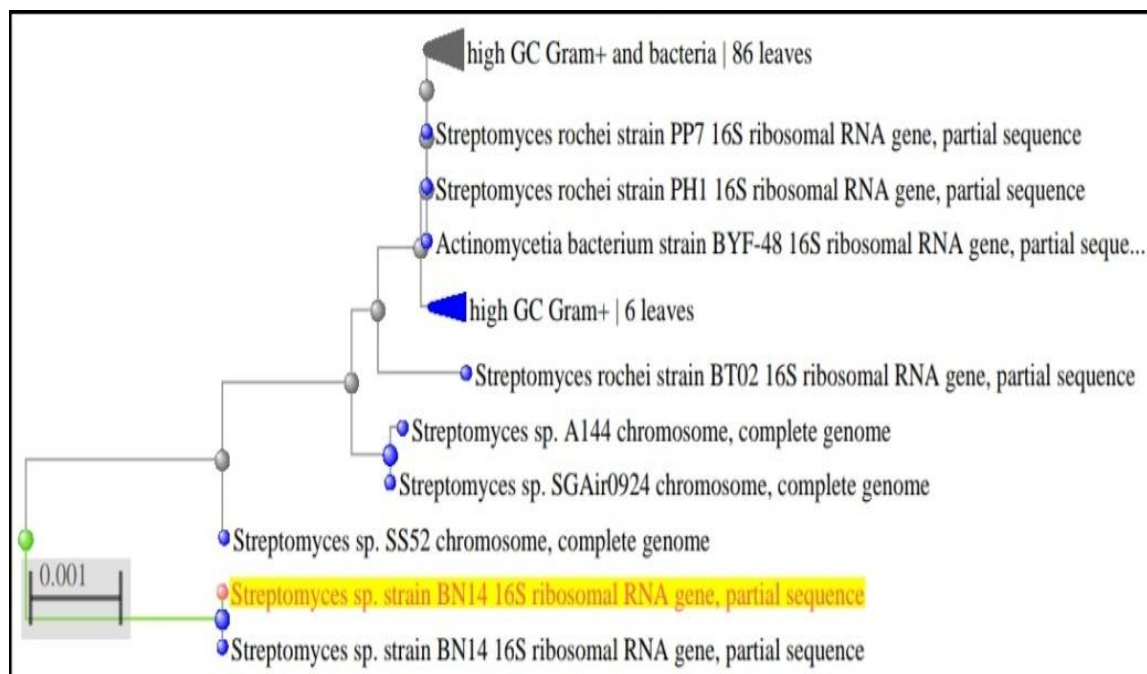


Fig. 5.11: The above picture shows the BLAST Tree of the BN14 isolate

5.6 ANTIBACTERIAL ACTIVITY USING DISC METHOD

The antibiotic crude extract of 3 isolates were screened against *Mycobacterium tuberculosis*. For screening, disc method is used. No zone was found. Hence, it shows that the extracts are not potential against *Mycobacterium tuberculosis*. The crude extract of actinomycetes, were found inactive against *Mycobacterium Tuberculosis*.

CHAPTER 6

SUMMARY AND CONCLUSION

The Main Purpose of the Study is to check the Actinomycete's activity against the Mycobacterium tuberculosis. At first, we confirmed that the 3 isolates are actinomycetes which are Streptomyces sp, DNA Sequence was submitted to the GenBank, NCBI. Blast Tress was constructed for 3 isolates by using Fast Minimum Evolution Method. To check whether it is potential or not potential against Mycobacterium tuberculosis. For this, we use the agar well diffusion method to check the Antibacterial activity.SCA Media is used for the Agar Well Diffusion method. No activity was found against Mycobacterium tuberculosis. We don't find activity in Primary Screening. More Intensive studies should be conducted to utilize the full potential.

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