RESEARCH PAPER

Integrative Characterization and Gc-Ms-Based Bioactive Profiling of Marine Actinomycetes with Antimicrobial Activity Against Uropathogens

Rajalinga Malathi B[1*], Abirami S[2], Varshini V[3], Muruga Sankari B[3]

[1*] Department of Microbiology, Kamaraj Women's College (Affiliated to Manonmaniam Sundaranar University, Tirunelveli – 627012), Thoothukudi, Tamil Nadu, India. (Corresponding Author)

[2] PG & Research Department of Microbiology, Kamaraj College (Autonomous), Thoothukudi, Tamil Nadu, India.

[3] Department of Microbiology, Kamaraj Women's College (Affiliated to Manonmaniam Sundaranar University, Tirunelveli – 627012), Thoothukudi, Tamil Nadu, India.

***Corresponding Author:** Rajalinga Malathi B, Email: malathykwc@gmail.com

Received: 20th Feb, 2026 | **Revised:** 4th Mar, 2026 | **Accepted:** 25th Mar, 2026 | **Available Online:** 10th Apr, 2026

ABSTRACT

Marine environments represent a rich source of diverse and metabolically unique microorganisms, particularly actinomycetes, which are well known for their ability to produce a wide range of bioactive compounds. The present study aimed to isolate and characterize marine actinomycetes from water samples collected from the Tharuvaikulam coastal area and to evaluate their antimicrobial potential against urinary tract infection (UTI) pathogens. UTI-causing pathogens were isolated using differential media plating techniques. The isolated actinomycetes were subjected to primary and secondary screening, followed by identification and characterization through microscopic examination and biochemical tests. The antimicrobial activity of the isolates was assessed against selected UTI pathogens, including Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and Staphylococcus saprophyticus. Solvent extraction of bioactive compounds was carried out using methanol, chloroform, isopropanol, ethanol, and acetone, and the extracts were evaluated for their inhibitory activity against the test pathogens. Molecular identification of potent actinomycetes was performed using 16S rRNA analysis. Furthermore, the bioactive compounds present in the extracts were analyzed using GC-MS. The findings highlight the significant antimicrobial potential of marine-derived actinomycetes and support their role as promising sources of novel therapeutic agents. This study underscores the importance of exploring marine microbial diversity for the discovery of eco-friendly, cost-effective, and sustainable bioactive compounds to combat increasing antimicrobial resistance among UTI pathogens.

Keywords: Marine actinomycetes, 16S rRNA identification; GC-MS analysis; Bioactive compounds.

How to cite this article: Rajalinga Malathi B, Abirami S, Varshini V, Muruga Sankari B. Integrative Characterization and Gc-Ms-Based Bioactive Profiling of Marine Actinomycetes with Antimicrobial Activity Against Uropathogens. Int J Drug Deliv Technol. 2026;16(28s):1061-1094. DOI: 10.25258/ijddt.16.28s.123

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.

INTRODUCTION

Marine environments are known to host unique forms of microbial life (Poli et al., 2017). So far, an increasing number of thermophilic, halophilic, alkalophilic, psychrophilic, piezophilic, and polyextremophilic microorganisms (mostly bacteria and archaea) adapted to extreme environments (e.g., high or low temperatures, high pressure, low pH, high salt concentrations and/or two or more extreme parameters in combination) have been isolated. They are capable of proliferating even under extreme conditions as a result of their adaptation strategies involving diverse cellular metabolic mechanisms.

As the survival strategies of *extremophiles* (a microorganism, especially an archaean, that lives in conditions of extreme temperature, acidity, alkalinity, or chemical concentration) are often novel and unique, these microorganisms produce secondary metabolites or enzymes of biotechnological interest.

Between 2007 and 2017, 177 new actinobacterial species belonging to 29 novel genera and three novel families were described (Subramani and Sipkema, 2019). As such, the number of compounds isolated from marine microorganism is increasing faster when compared with terrestrial species (Wagner-Dobler et al.,2002). The Gram-positive actinobacteria are a major chemically prolific source of bioactive metabolites with anti- cancer, anti-microbial, anti-parasitic, anti-inflammatory, anti-biofilm activities, and anti- fouling properties, among others (Pereira et al., 2020). Actinomycetes have a profound role in the marine environment apart from antibiotic production. The cellulolytic activity of marine actinomycetes was described by Chandramohan et al. (1972), chitinolytic actinomycetes were reported by Pistano et al. (1992) and various industrially important enzyme producing actinomycetes have been reported (Ramesh and Mathivanan 2009).

Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*. High recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly increase the economic burden of these infections. In this Review, we discuss how basic science studies are elucidating the molecular details of the crosstalk that occurs at the host–pathogen interface, as well as the consequences of these interactions for the pathophysiology of UTIs. We also describe current efforts to translate this knowledge into new clinical treatments for UTIs. *Nature Reviews Microbiology* (2015)

Microbial-based bioactive metabolic compounds are a pool of metabolites that have confident positive effects on environment, humans, plants, and animals that ingest them. As bioactive compounds originated from microbial origin are valuable to the human health, researchers are enticing attention in modern life, besides that their increasing consumption is stimulating the continuous development of new products and synthesis modes that are eco-friendly, cost-effective, sustainable, and more efficient than their chemical version.

MATERIALS AND METHODS

SAMPLING AREA:

In this study, the marine water samples was collected from the Tharuvaikulam beach, Thoothukudi. Samples were collected from 10 – 20 cm depth and kept in a sterile poythene bag. The sample was preserved in the laboratory for further analysis.(Fig 1)

ISOLATION OF MARINE ACTINOMYCETES :

Starch casein agar medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 min.Then it was supplemented with Amphotericin B 50 µg/l and Tetracyclin 20 µg/l to prevent the bacterial and fungal growth. The medium was poured into the sterile Petri plates. 1ml of marine water sample was spread over the agar plates. The inoculated plates were incubated at 28±2°C for seven to ten days. After incubation, the actinomycetes were observed, purified using subculture method and maintained in starch casein agar medium for further investigation**.** (Fig 2)

Marine water sample

↓

Plated in Starch casein agar medium

↓

Ash powdery colonies (KSR 01)

PREPARATION OF PURE CULTURE OF MARINE ACTINOMYCETES:

The Starch casein agar medium slant was prepared in test tubes and the isolated colony obtained in the petriplates were taken carefully with out contamination (leaving out mixed culture colony) and streaked in the medium in a zigzag manner using the inoculation loop. The test tubes were screw capped and incubated at 25ºC for seven to ten days.

PHYSIOLOGICAL CHARACTERIZATION:

Modified Bennet broth was prepared, adjusted to different pH (6–10) and temperatures (15–50°C), inoculated with Actinomycetes, and incubated for 7–14 days.Growth observed after incubation indicated the effect of pH and temperature on Actinomycetes.

MORPHOLOGICAL STUDIES:

Actinomycetes were cultured on various media, and colony morphology was recorded based on color, size, and pigmentation characteristics.Gram staining was performed using standard procedure, and microscopic observation confirmed the staining characteristics of the isolates. (Fig 3) (Table 1,2)

BIOCHEMICAL CHARACTERIZATION

Actinomycete cultures were subjected to standard biochemical tests including indole, methyl red, Voges–Proskauer, citrate utilization, catalase, oxidase, nitrate reduction, and urease tests using specific media and reagents. Positive results were indicated by characteristic colour changes or reactions, while absence of change denoted negative results. (Fig 4-11) (Table 3)

SCREENING FOR ENZYME PRODUCTION BY ACTINOMYCETES:

Actinomycetes were streaked on starch agar, skim milk agar, and tributyrin agar, then incubated under appropriate conditions.Clear zones (or oil droplets in lipid hydrolysis) around colonies after incubation indicated positive hydrolysis of starch, casein, and lipids. (Fig 17-19) (Table 4)

CHARACTERIZATION OF ANTIMICROBIAL COMPOUNDS

Qualitative analysis of functional group of the antimicrobial compounds

Functional group analysis was performed in order to find out the classes or nature of antimicrobial compounds group. Various biochemical tests (Benedict's, Molisch's, Fehling's, Bial's, Ferric chloride, Xanthoprotein, Biuret, Ninhydrin, and Elson-Morgan) were performed on antimicrobial compound solutions using standard procedures and heating where required.Color changes or precipitate formation indicated the presence of reducing sugars, carbohydrates, phenols, proteins, amino acids, and amino sugars. (Fig 5) (Table 20)

ISOLATION OF UTI PATHOGENS

Sample collection:

The urine samples of UTI patients was collected from AVM Hospital. These samples was collected in a screw capped bottle aseptically and immediately transported to the laboratory.These samples were kept in refrigerator at 4°C

Sample Preparation:

From the urine sample, 1ml sample was taken and enumerated in standard plate count agar as described by (Slaby, B.M., et al 2011). *Klebsiella spp*. were enumerated using Eosin methylene blue Agar (EMB) and Eosin methylene blue Agar (EMB) agar for pathogenic *Enterobacter spp*. EMB Agar for *E.coli* and Cetrimide agar for *Pseudomonas*. *Staphylococcus spp*. were enumerated using Mannitol salt base Agar. The plates were incubated at 37ºC for 24hrs. The observed colony growth were identified by using the phenotypic and biochemical characteristics as described by (Cheesbrough, M. 2014 and Slaby, B.M., et al 2003). (Fig 12-16)

ANTAGONISTIC ASSAY:

The Actinomycetes culture were inoculated into hundred milliliters of nutrient broth and incubated at 35ºC for 24h. Then, 5ml of eah culture broth was centrifuged at 12000 rpm for 30 min. supernatant of each suspension was assessed for antibacterial property against bioassay strains of bacteria viz., Bacillus sp, Enterobacter sp, Pseudomonas sp, Staphylococcus sp. To perform the test, bioassay strain was cultivated on Mueller Hinton agar and wells (5mm in diameter) were made in plate agar using sterile sharp borer. Then 100µl of each supernatant was added into the each well and the plates were incubated at 35ºC for 24 th . Afterward, exhibition of a clear zone of growth inhibition was observed, measured and qualified which was considered bioactive compound activity.(Fig 21-25) (Table 6)

ANTIBIOTIC SUSCEPTIBILITTY ASSAY:

Cell suspension of microbial cultures viz ., UTI pathogens were swabbed on Mueller Hinton agar plates. The antibiotics (Cefixime & Streptomycin) were dissolved in distilled water and 100µ1 Concenttration was loaded in the cutted filter paper discs. Then it was placed in the culture swabbed plates. Zones of inhibition were measured following 24 hours of incubations at37ºC . Sensitivity was considered as srong when the zone of incubation extended more than 3 mm beyond the antibiotic disc (Stan-Lotter et al ., 2002)

POLYMERASE CHAIN REACTION:

DNA BARCODING:

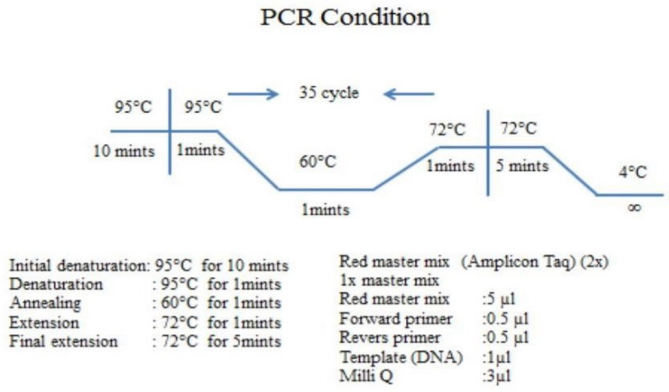
METHODS:

ISOLATE: KSR 01

GENOMIC DNA EXTRACTION:

The 5 ml nutrient broth containing was inoculated with a single

bacterial colony.



The tube was incubated at 37 °C overnight with vigorous shaking at 360 rpm. Harvest the pellet from
1.2 to 1.5 ml overnight culture and centrifuge at 12,000 rpm for 10 mints and collect the pellet in a fresh tube. Add freshly prepared 570 µ1 of TE buffer and invert the tube and resuspend well with pipetting. Add freshly prepared 30µ1of 10 % SDS, 3µ1of proteinase k and 3 µ1of RNAse and mixed well. After incubate at 37 °C for 1 hour. Add 80µ1 of CTAB-Nacl +100 µ1 of 5M Nacl and invert the tube and mix well. After incubate water bath at 65 °C for 10 minutes. Add freshly prepared equal volume of Chloroform:Isoamylalcohol (24:1) inverting and mixed well. After centrifuge at 12,000 rpm for 10 minutes and collect the supernatant (upper phase) in a1.5 ml fresh tube. Add freshly prepared equal volume of Phenol:Chloroform:Isoamylalcohol (25:24:1) inverting and mixed well. After centrifuge at 12,000 rpm for 10 minutes and collect the supernatant (upper phase) in a 1.5 ml fresh tube. Add 0.6 (600 µ1) ice-cold isopropanol gently inverting and mixed after incubate at -20°C for 10-20 minutes. After centrifuge at 12,000 rpm for 10-15 minutes and discard the supernatant collect the pellet. After add 500 µ1 70% of ethanol. After centrifuge at 12,000 rpm for 5 minutes discard the supernatant collect the pellet and after air dry the pellet. After add 20-25 µ1 DEPC treated nucleus free water or TE buffer at storage at -20°C.(Fig 26)

PCR PURIFIED PROTOCOL (QIA quick kit method)

Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene. For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).If pH indicator I has been added to Buffer PB, check that the color of the mixture is yellow. If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. Place a QIA quick spin column in a provided 2 ml collection tube. To bind DNA, apply the sample to the QIA quick column and centrifuge for 30–60 s. Discard flow-through. Place the QIA quick column back into the same tube. Collection tubes are re-used to reduce plastic waste. To wash, add 0.75 ml Buffer PE to the QIA quick column and centrifuge for 30–60 s. Discard flow-through and place the QIA quick column back in the same tube. Centrifuge the column for an additional 1 min. Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation. PCR Purification Spin Protocol. Place QIA quick column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIA quick membrane, let the column stand for 1 min, and then centrifuge. Ensure that the elution buffer is dispensed directly onto the QIA quick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl elution buffer. Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel. Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time.

GC – MS Analysis

GC-MS Instrumentation: The Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited were engaged for analysis. The instrument was set as follows: Injector port temperature set to 250 °C, interface temperature set at 250 °C, source kept at 200 °C. The oven temperature was programmed as a variable: 70 °C for 2 mins, 150 °C @ 8 °C/min, up to 260 °C @ 10 °C/min. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS nonpolar column was used whose dimensions were 0.25 mm OD × 0.25 µm ID × 30 metres length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200 °C and <40 mtorr vacuum

pressure. The ionization energy was −70 eV. The MS also had an inbuilt pre-filter which reduced neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum: NIST4 and WILEY9, each containing more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

Identification of compounds: Interpretation of mass spectrum of GC–MS was done using the database of National Institute of Standard and Technology (NIST4) and WILEY9 [12]. The spectrum of the known component was compared with the spectrum of known components stored in the inbuilt library.

RESULTS AND DISCUSSION:

Physiological Characteristics

The Actinomycetes isolate KSR01 exhibited growth across specific pH and temperature ranges, consistent with previously reported standards by Shirling and Gottlieb (1966) and Bergey's Manual (Goodfellow & Williams, 1983). These parameters are critical for defining the growth conditions suitable for Actinomycetes strains and are indicative of its adaptability to environmental conditions.

Morphological Characteristics

KSR01 formed aerobic, ash powdery colonies and was identified as Gram-positive. These morphological traits align well with the typical features of Actinomycetes, which are known for their filamentous, Gram-positive nature and colony morphology (Kämpfer, 2006).

Biochemical Characteristics

Biochemical assays performed on the pure isolate provided insights into its metabolic capabilities (Table 3). Although detailed results were not specified here, the isolate's profile contributes to its identification and functional assessment within Actinomycetes.

Enzyme Production Screening

The isolate KSR01 tested negative for amylase, protease, and lipase production (Table 4), indicating an absence of these extracellular hydrolytic enzymes under the tested conditions. This contrasts with other Actinomycetes that typically produce diverse enzymes involved in organic matter breakdown (El-Nakeeb & Lechevalier, 1963), suggesting possible specialization or the need for different induction stimuli.

Antimicrobial Compound Analysis

Crude extracts from KSR01 demonstrated antimicrobial activity (Table 5), highlighting the isolate's potential as a source of bioactive compounds. Actinomycetes are well-documented producers of antibiotics and other secondary metabolites, reinforcing the value of KSR01 in bioprospecting efforts for novel antimicrobials (Berdy, 2005).

ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES ISOLATES (KSR 01) AGAINST UTI PATHOGENS IN DIFFERENT SOLVENT EXTRACTS:

The Actinomycetes isolate (KSR 01) shows maximum of 32mm inhibition for *E.coli,* 30mm of inhibition for *Pseudomonas,* 32 mm of inhibition for *Klebsiella* and 25 mm of inhibition for *Enterobacter i*n the Isopropyl Alcohol extract. And 40mm of inhibition for *Staphylococcus* in the Ethanol extract. Results were tabulated.(Table 6)

In previous study, the anti bacterial activity of Actinomycetes sp against UTI pathogens exhibited the least and moderate activity.(Pisano M A et al., 2010)

SEQUENCING:

The Actinomycetes isolates (KSR 01) were identified as Nocardiopsis dassonvillei using Morphological, Biochemical and Molecular Characterization – 16s rRNAsequencing .

GC-MS analysis

The GC-MS analysis revealed the presence of 30 distinct compounds, with several dominant constituents suggesting a biologically active or plant-derived sample. The most abundant compound was **3,4-dihydroxymandelic acid-4TMS**, appearing in multiple peaks with a cumulative area percentage exceeding 35%, indicating its prominence in the sample. This compound is a known metabolite of catecholamines such as norepinephrine and is often used as a biomarker for sympathetic nervous system activity (Goldstein et al., 2003). The detection of **epinephrine-3TMS** further supports the presence of catecholamines, suggesting either a plant-based adaptogen or biological origin such as tissue extract (Eisenhofer et al., 2004). Other notable compounds include **gallic acid-4TMS derivative** and **phloroglucinol derivatives**, both of which are polyphenolic antioxidants commonly found in medicinal plants and known for their anti-inflammatory and antimicrobial properties (Singleton et al., 1999; Rice- Evans et al., 1996).

Additionally, the presence of saturated and unsaturated fatty acids such as **palmitic acid-TMS**, **stearic acid-TMS**, and **elaidic acid-TMS** points to lipid content typically found in plant or animal tissues (Gunstone, 2004). Minor components such as

urocanic acid and **arabinose-4TMS** suggest contributions from amino acid metabolism and carbohydrate content, respectively. The identification of **β-D-lactose derivative** implies the sample may also contain sugar moieties or glycosides, which are common in plant-based matrices (Harborne, 1998). The overall profile, marked by catecholamine metabolites, polyphenols, and fatty acids, indicates a complex mixture characteristic of medicinal plant extracts or biological fluids used in metabolic or pharmacological studies.

TABLES

TABLE 1:

MORPHOLOGICAL CHARACTERISTICS OF ACTINOMYCETES ISOLATES (KSR 01)

S. NO	SAMPLE	COLONY APPEARANCE	CELL SHAPE	GRAM ST
1.	KSR 01	Ash powdery	Bacilli	Gram Posi

TABLE 2:
DETERMINATION OF TYPE OF RESPIRATION IN ACTINOMYCETES ISOLATES (KSR 01)

S. NO	SAMPLE	COLONY APPEARANCE	RESPIRATION TYPE
1.	KSR 01	Ash powdery	Aerobe

TABLE 3:
BIOCHEMICAL CHARACTERISTICS OF ACTINOMYCETES ISOLATES (KSR 01)

BIOCHEMICAL TEST	KSR 01
Indole test	-
Methyl Red test	+
Voges Proskaeur test	+
Citrate test	+
Oxidase Test	+
Catalase Test	+
Urease Test	+
Nitrate Reduction Test	+

TABLE 4:
SCREENING FOR ENZYME PRODUCTION BY ACTINOMYCETES ISOLATES (KSR 01)

ENZYME PRODUCED	KSR 01
Amylase	-
Protease	-
Urease	-
Fehling's test	+
Bial's Test	-
Xanthoprotein Test	+
Biuret Test	+
Ninhydrin Test	+
Elson-Morgan Test	+

TABLE 5:
ANTIMICROBIAL COMPOUND ANALYSIS:

ANTIMICROBIAL COMPOUND PRODUCTION TEST	KSR 01
Benedict's test	+
Molisch's test	+
Ferric chloride test	+

TABLE 6:

QUANTIFICATION OF ANTI BACTERIAL ACTIVITY OF ACTINOMYCETES ISOLATES (KSR 01) AGAINST UTI PATHOGENS IN DIFFERENT SOLVENT EXTRACTS:

TEST ORGANISMS	ZONE OF INHIBITION IN MM				
	ACETONE	ETHANOL	ISOPROPANOL	CHLOROFORM	METHANOL
E.coli	-	-	32	-	-
Staphylococcus	15	40	-	-	-
Klebsiella	20	15	32	-	10
Enterobacter	10	-	25	-	-
Pseudomonas	10	10	35	-	15

Report of Microbial Identification

Sanger Seq Chromatogram data file Data:

>Forward Seq data

ACCAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGAC
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTG
C
GGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGA
AAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGC
C
TTCGGGTTGTAAACCTCTTTTACCACCAACGCAGGCTC
GAAGTTCTCTTCGGGTTGACGGTAGGTGGGGAATAAG
GA
CCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGGTCCGAGCGTTGTCCGGAATTATTGGGCGTAAAGA
GC
TCGTAGGCGGCGTGTCGCGTCTGCTGTGAAAGACCGG
GGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCT
AG
AGGTAGGTAGGGGAGACTGGAATTCCTGGTGTAGCGG
TGAAATGTTTTTATATCAGGAGGAACACCGGTGGCGA
A
GGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGA
AAGCATGGGGAGCGAACAGGATTAGATACCCT

>Reverse Seq Data

CTGATCCGCGATTACTAGCGACTCCACCTTCATGGGGT
CGAGTTGCAGACCCCAATCCGAACTGAGACCGGCTTT
TA
GGGATTCGCTCCACCTTACGGTATCGCACGCCCATTGT
ACCGGCCATTGTAGCATGTTTGCAGCCCAAGACATAA
GG
GGCATGATGACTTGACGTCATCCCCACCTTCCTCCGAG
TTGACCCCGGCAGTCTCCCATGAGTCCCCACCATTACG
TG
CTGGCAACATGGAACAAGGGTTGCGCTCGTTGCGGGA
CTTAACCCAACATCTCACGACACGAGCTGACGACAGC
CA
TGCACCACCTGTCACCCGCCAACTAAATGACCTCACAT
CTCTGCGAGTCCACGGGTGATGTCAAACCTTGGTAAG
GT
TCTTCGCGTTGCGTCGAATTAAGCAACATGCTCCGCCG
CTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCC
TT
GCGGCCGTACTCCCCAGGCGGGGCGCTTAATGCGTTA
GCTACGGCGCGGAAACCGTGGAAAGTCCCCACACCTA
GC
GCCCAACGTTTACGGCATGGACTACCAGGGTATCTAA
TCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAG
GTA
AGGCCCAGAGACCCGCCTTCGCCACCGGTGTTCCTCCT
GATATCTGCGCATTTCACCGCTACACCAGGAATTCCAG
TC
TCCCCTACCTACCTCTAGCATGCCCGTATCCACTGCAG
AACCGGAGTTAAGCCCCGGTCTTTCACAGCAGACGCG
AC
ACGCCGCCTACGAGCTCTTTACGCCCAATAATTCCGGA
CAACGCTCGGACCCTACGTATTACCGCGGCTGCTGGC
AC
GTAGTTAGCCGGTCCTTATTCCCCACCTACCGTCAACC
CCAAAAAAAACTTCCAAT

>Reverse complement

ATTGGAAGTTTTTTTTGGGGTTGACGGTAGGTGGGGA
ATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTA
AT
ACGTAGGGTCCGAGCGTTGTCCGGAATTATTGGGCGT
AAAGAGCTCGTAGGCGGCGTGTCGCGTCTGCTGTGAA
AG
ACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGC
ATGCTAGAGGTAGGTAGGGGAGACTGGAATTCCTGGT
G
TAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGG
TGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGA
G
GAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGT
GG
GGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTA
AGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAA
C
TCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA
GCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTA
CCA
AGGTTTGACATCACCCGTGGACTCGCAGAGATGTGAG
GTCATTTAGTTGGCGGGTGACAGGTGGTGCATGGCTG
TC
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTGTTCCATGTTGCCAGCACGTAAT
G
GTGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTA
T
GTCTTGGGCTGCAAACATGCTACAATGGCCGGTACAA
TGGGCGTGCGATACCGTAAGGTGGAGCGAATCCCTAA
AA
GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCC

CATGAAGGTGGAGTCGCTAGTAATCGCGGATCAG

> Consensus data

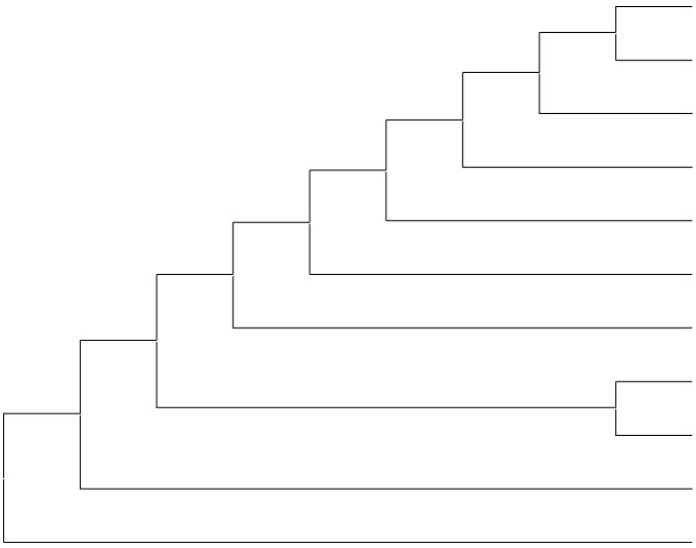
AGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGG
CCACACTGGGACTGAGACACGGCCCAGACTCCTGCGG
G
AGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAG
CCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTT
C
GGGTTGTAAACCTCTTTTACCACCAACGCAGGCTCGA
AGTTCTCTTCGGGTTGACGGTAGGTGGGGAATAAGGA
CC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GTCCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGC
TC
GTAGGCGGCGTGTCGCGTCTGCTGTGAAAGACCGGGG
CTTAACTCCGGTTCTGCAGTGGATACGGGCATGCTAG
AG
GTAGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTG
AAATGCGCAGATATCAGGAGGAACACCGGTGGCGAA
G
GCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAA
AGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGT
C
CATGCCGTAAACGTTGGGCGCTAGGTGTGGGGACTTT
CCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCC
CC
GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGG
AATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTTG
CTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTT
TGACATCACCCGTGGACTCGCAGAGATGTGAGGTCAT
TTA
GTTGGCGGGTGACAGGTGGTGCATGGCTGTCGTCAGC
TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
C
GCAACCCTTGTTCCATGTTGCCAGCACGTAATGGTGGG
GACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAG
GTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCTT
GGGCTGCAAACATGCTACAATGGCCGGTACAATGGGC
G
TGCGATACCGTAAGGTGGAGCGAATCCCTAAAAGCCG
GTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATG
A AGGTGGAGTCGCTAGTAATCGCGGATCAG

Sequences producing significant alignments:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Nocardiopsis dassonvillei strain XY236	2002	2002	100%	0.0	100.00%	MT393632.1
Actinobacteria bacterium strain BACSAS14	2002	2002	100%	0.0	100.00%	MK758063.1
Actinomycetia bacterium strain 650-17	2002	2002	100%	0.0	100.00%	MG807521.1
Actinomycetia bacterium strain 650-7	2002	2002	100%	0.0	100.00%	MG807520.1
Nocardiopsis sp. strain LJL8	2002	2002	100%	0.0	100.00%	MG833334.1
Nocardiopsis dassonvillei strain XY236	2002	2002	100%	0.0	100.00%	MH432693.1
Nocardiopsis dassonvillei strain AlW5	2002	2002	100%	0.0	100.00%	MF321787.1
Nocardiopsis dassonvillei strain OAct920	2002	2002	100%	0.0	100.00%	MG661744.1
Actinobacteria bacterium strain g1	2002	2002	100%	0.0	100.00%	MG515757.1
Nocardiopsis sp. strain JJ76	2002	2002	100%	0.0	100.00%	KX352797.1

Phylogenetic Tree:

MG515757.1 KX352797.1 MG661744.1 MF321787.1 MH432693.1 MG833334.1 MG807520.1 MAI MT393632.1 MK758063.1 MG807521.1



Evolutionary analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [1]. The tree with the highest log likelihood (-2783.73) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1223 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2].

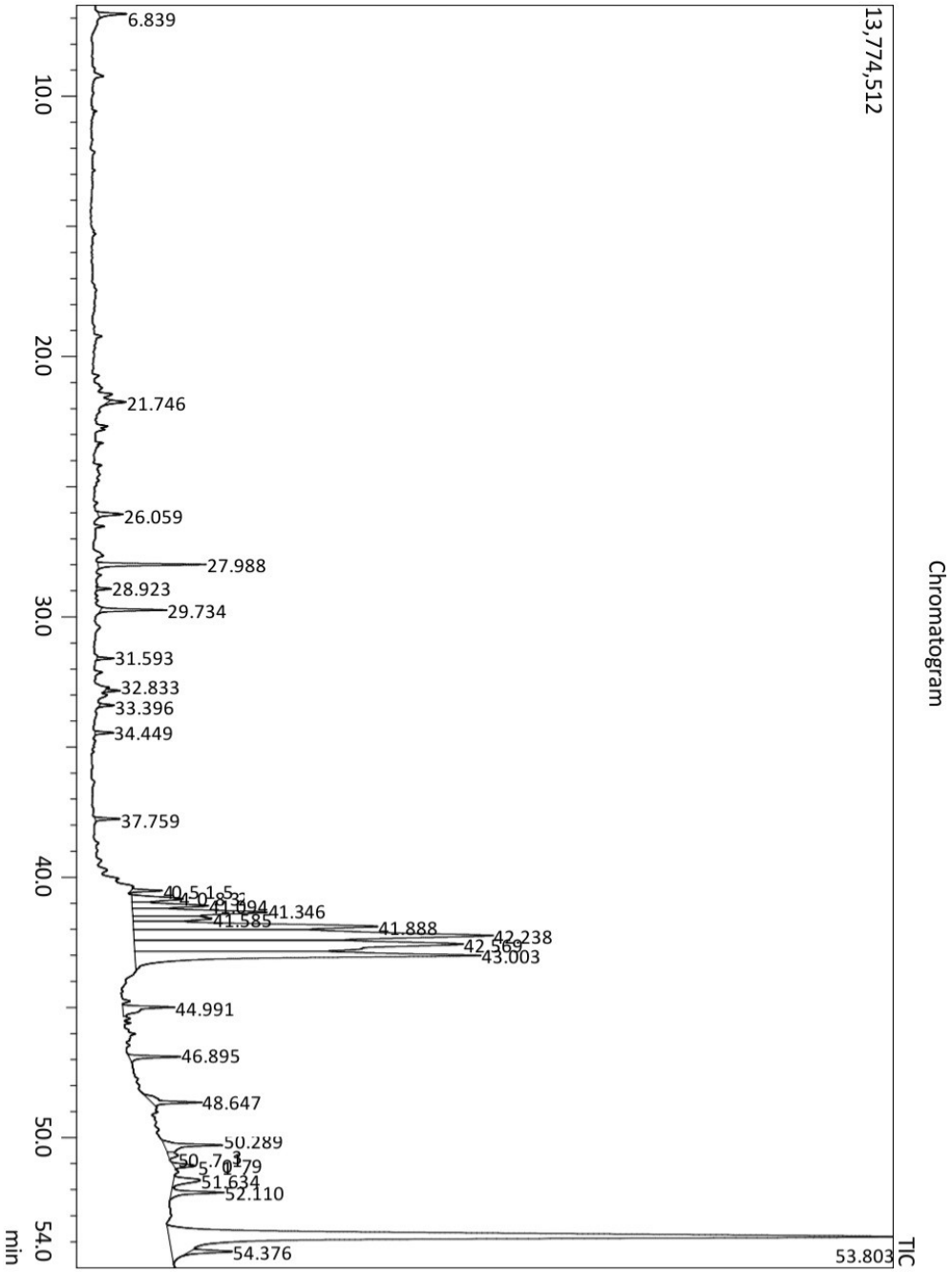
Distance Matrix:

MAI		0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000
MT393632.1	0.000		0.000	0.050	0.000	0.000	0.000	0.000	0.000
MK758063.1	0.000	0.000		0.050	0.000	0.000	0.000	0.000	0.000
MG807521.1	0.732	0.732	0.732		0.050	0.050	0.050	0.050	0.050
MG807520.1	0.000	0.000	0.000	0.732		0.000	0.000	0.000	0.000
MG833334.1	0.000	0.000	0.000	0.732	0.000		0.000	0.000	0.000
MH432693.1	0.000	0.000	0.000	0.732	0.000	0.000		0.000	0.000
MF321787.1	0.000	0.000	0.000	0.732	0.000	0.000	0.000		0.000
MG661744.1	0.000	0.000	0.000	0.732	0.000	0.000	0.000	0.000	
MG515757.1	0.000	0.000	0.000	0.732	0.000	0.000	0.000	0.000	0.000
KX352797.1	0.000	0.000	0.000	0.732	0.000	0.000	0.000	0.000	0.000

Table. Estimates of Evolutionary Divergence between Sequences

The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model [1]. This analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1223 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2].

GAS CHROMATOGRAPHY-MASS SPECTROMETRY METABOLITES PROFILING REPORT


13,774,512
Chromatogram
TIC
min
10.0
20.0
30.0
40.0
50.0
54.0
6.839
21.746
26.059
27.988
28.923
29.734
31.593
32.833
33.396
34.449
37.759
41.346
41.888
42.238
43.003
44.991
46.895
48.647
50.289
51.634
52.110
54.376
53.803

Peak Report

Peak#	R.Time	Similarity	Name	CAS#	Area	Area%	Height
1	6.839	95	Lactic acid-2TMS	79-33-4	2762011	0.40	469415
2	21.746	88	Phloroglucinol, O,O'-bis(trimethylsilyl)-	1848973-17-0	1922013	0.28	303912
3	26.059	67	Urocanic acid-2TMS	104-98-3	2758200	0.40	418805
4	27.988	89	Gallic acid, 4TMS derivative	2078-17-3	9677196	1.41	1822361
5	28.923	84	Heptasiloxane, hexadecamethyl-	541-1-5	1074951	0.16	247448
6	29.734	94	Palmitic acid-TMS	57-10-3	5727068	0.84	1115451
7	31.593	88	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	1240149	0.18	284931
8	32.833	84	Elaidic acid-TMS	112-79-8	1186463	0.17	266546
9	33.396	89	Stearic acid-TMS	57-11-4	1235294	0.18	250017
10	34.449	88	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	1540710	0.23	315769
11	37.759	89	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-octad	556-69-4	2073067	0.30	417926
12	40.515	92	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	2597616	0.38	529756
13	40.832	51	3,4-Dihydroxymandelic acid-4TMS	775-1-9	9058247	1.32	863193
14	41.094	51	Epinephrine-3TMS	51-43-4	11962351	1.75	1283439
15	41.346	49	3,4-Dihydroxymandelic acid-4TMS	775-1-9	26756659	3.91	2265781
16	41.585	48	3,4-Dihydroxymandelic acid-4TMS	775-1-9	14421494	2.11	1330551
17	41.888	49	3,4-Dihydroxymandelic acid-4TMS	775-1-9	53274280	7.78	4119482
18	42.238	46	3,4-Dihydroxymandelic acid-4TMS	775-1-9	111743482	16.33	6056229
19	42.569	46	3,4-Dihydroxymandelic acid-4TMS	775-1-9	111304327	16.26	5541860
20	43.003	48	3,4-Dihydroxymandelic acid-4TMS	775-1-9	79878413	11.67	5820286
21	44.991	93	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	8031662	1.17	874050
22	46.895	92	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	5121642	0.75	865861
23	48.647	91	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	6517486	0.95	828689
24	50.289	89	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	9126269	1.33	986580
25	50.713	47	Arabinose-4TMS(1)	10323-20-3	1788601	0.26	157373
26	51.079	47	4-Hydroxyphenyllactic acid-3TMS	6482-98-0	3155393	0.46	364684
27	51.634	77	.beta.-D-Lactose, (isomer 1), 8TMS derivative	0-0-0	5791442	0.85	446910
28	52.110	90	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	6659096	0.97	882101
29	53.803	42	Epinephrine-3TMS	51-43-4	181181245	26.47	12188908
30	54.376	87	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,17	2652-13-3	4853409	0.71	689722

Fig 1- SAMPLE COLLECTION

Library

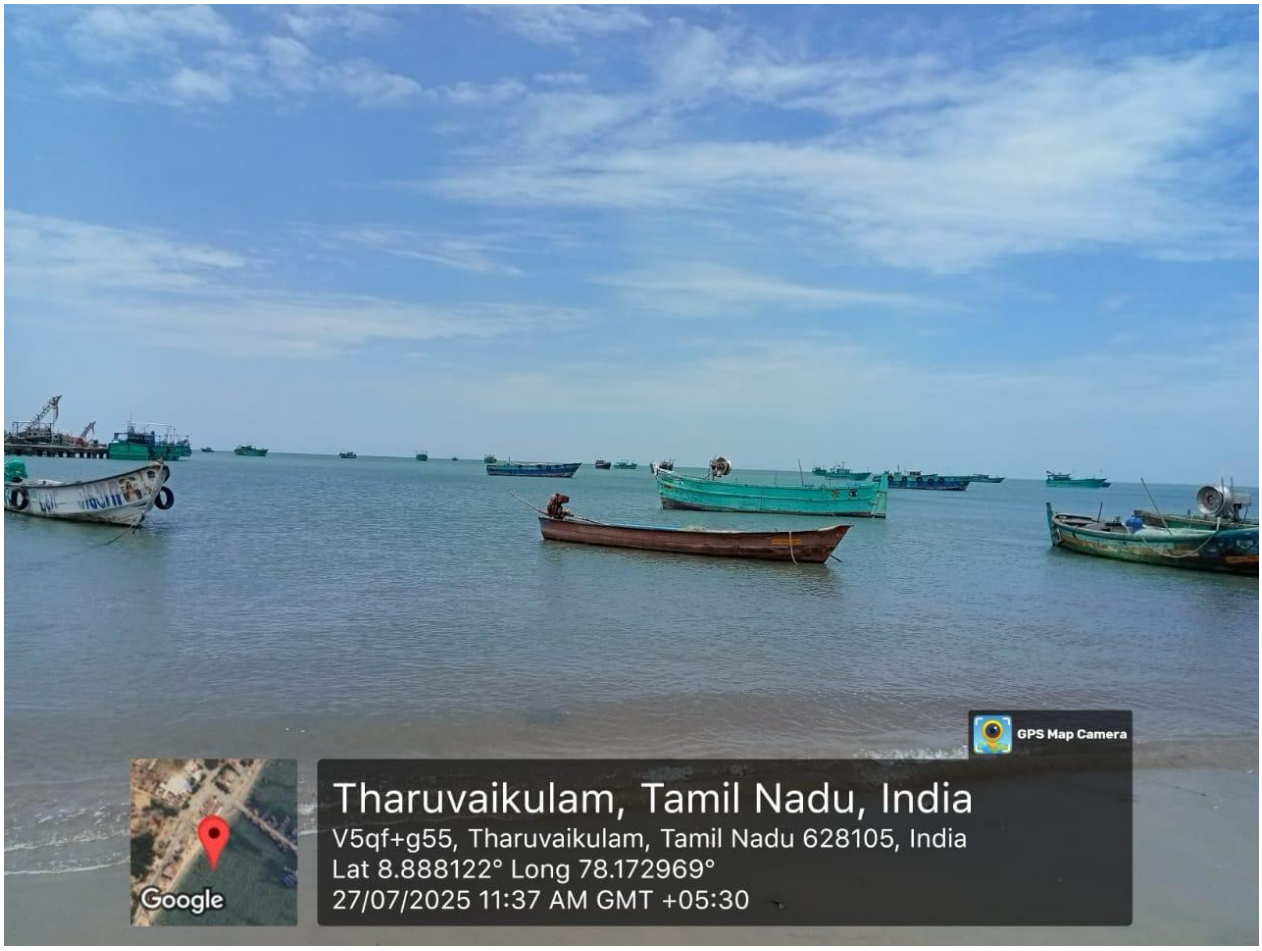
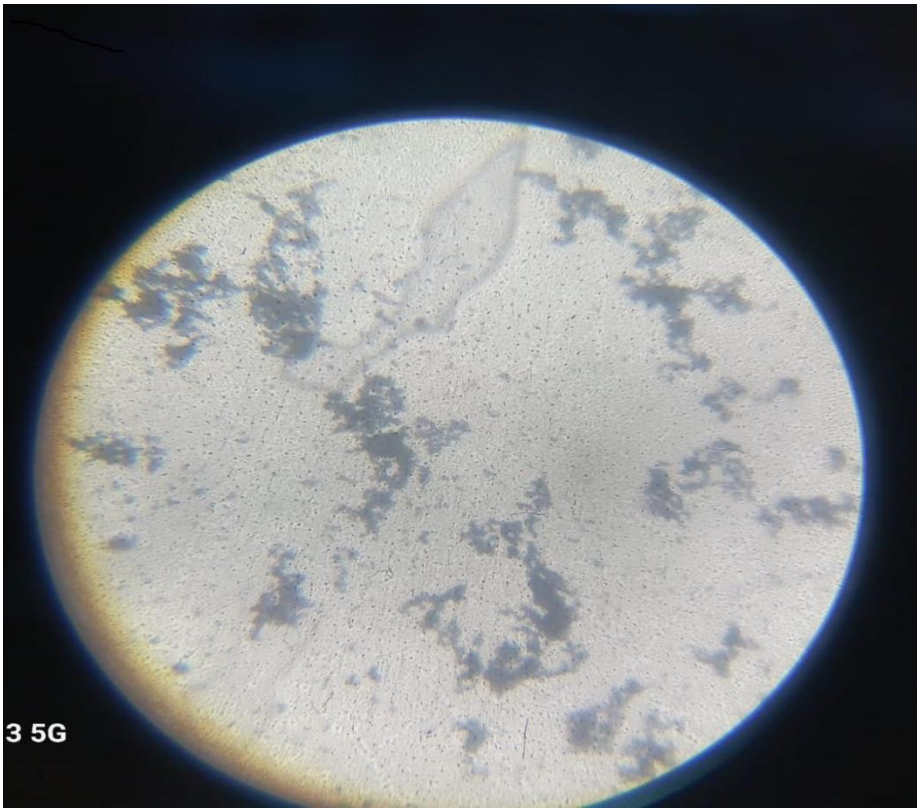


Fig 2 - ISOLATION OF ACTINOMYCETES

Fig 3 - GRAMS STAINING



BIOCHEMICAL TEST

Fig 4 - Indole test:

Fig 5 - Methyl Red test:

Fig 6 - Voges Proskaeur test:

Fig 7 - Citrate test:

Fig 8 - Oxidase test:

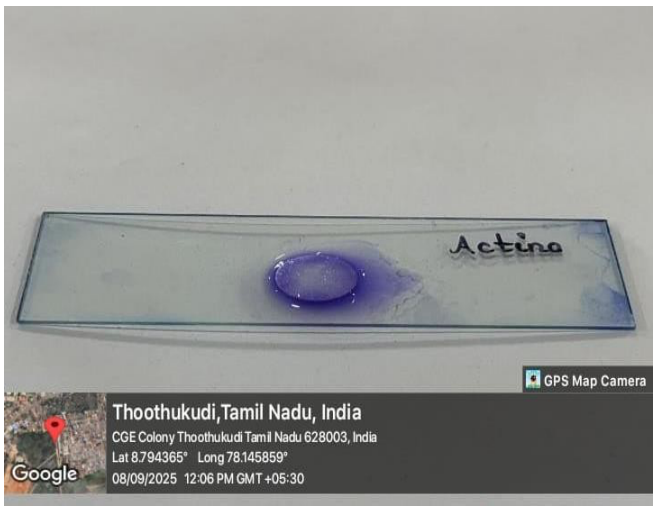


Fig 9 - Catalase test:

Fig 10 - Urease test:

Fig 11 - Nitrate reduction test:

ISOLATION OF PATHOGENS

Fig 12 - *Enterobacter spp*. in TCBS Agar

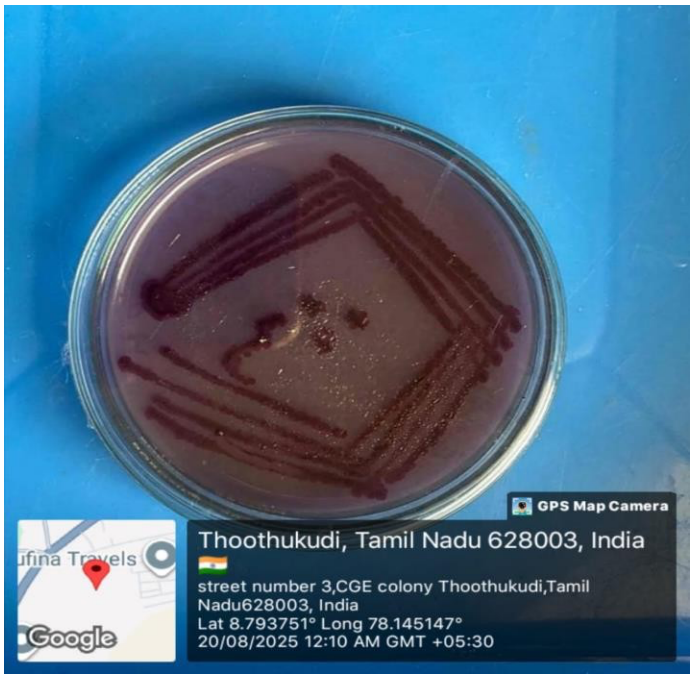


Fig 13 - *E.coli* in EMB Agar

Fig 14 - *Klebsiella spp.* in EMB Agar

Fig 15 - *Pseudomonas spp*. in Cetrimide Agar

Fig 16 - *Staphylococcus spp*. in Mannitol Salt Base Agar

ENZYME PRODUCTION

Fig 17 - Starch Hydrolysis

Fig 18 - Lipid Hydrolysis

Fig 19 - Casein Hydrolysis

Fig 20 - ANTIMICROBIAL COMPOUND ANALYSIS

ANTIMICROBIAL ACTIVITY

Fig 21 - Antimicrobial activity of Actinomycetes culture against E.coli in different solvents

Fig 22 - Antimicrobial activity of Actinomycetes culture against Enterobacter in different solvents

Fig 23 - Antimicrobial activity of Actinomycetes culture against Pseudomonas in different solvents

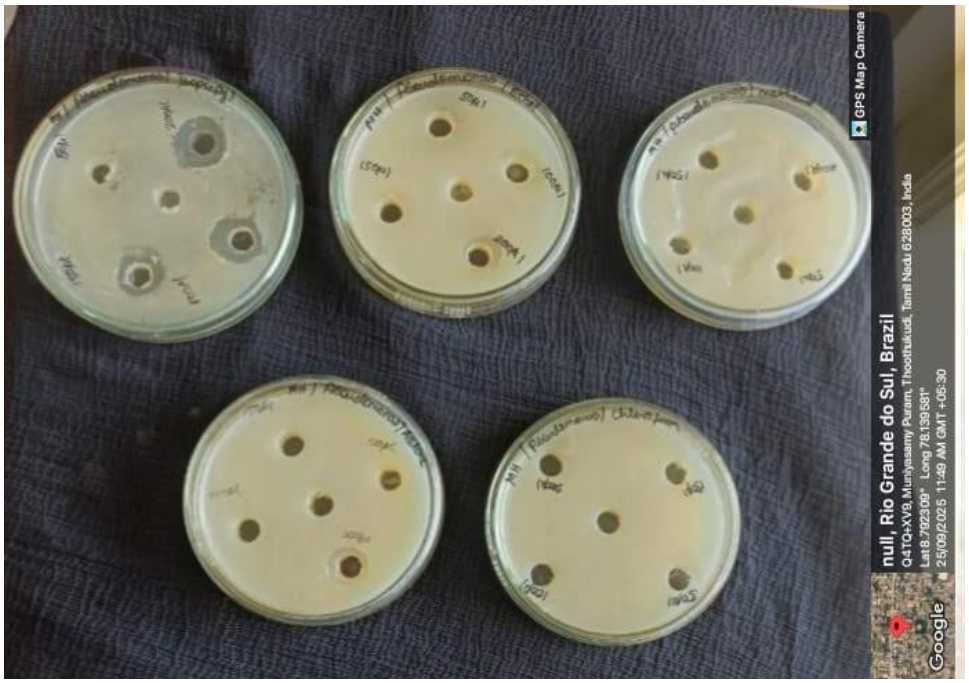


Fig 24 - Antimicrobial activity of Actinomycetes culture against Staphylococcus in different solvents

Fig 25 - Antimicrobial activity of Actinomycetes culture against Klebsiella in different solvents

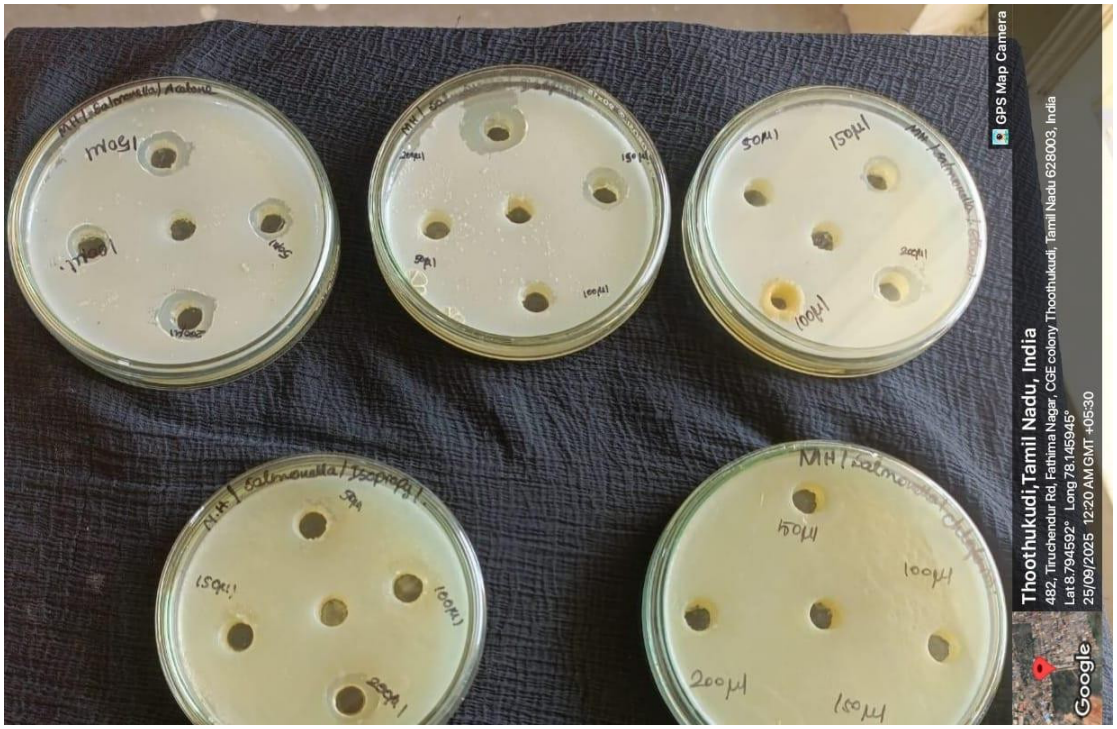
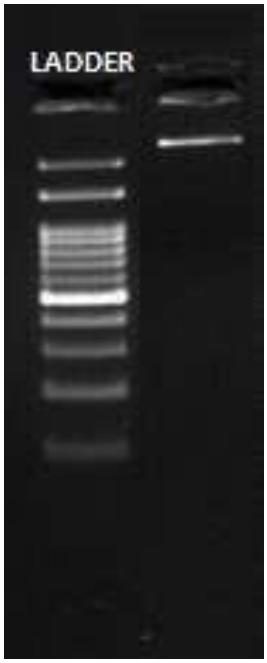
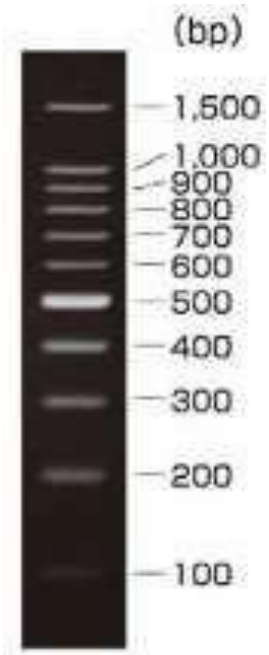


Fig 26 - PCR Amplification Purified Product





Ladder specification

BIBLIOGRAPHY

A

- Asolkar, R. N., et al. (2010). Antimalarial and antituberculosis activities of marine actinomycetes. *Marine Drugs, 8*(5), 1303–1312. https://doi.org/10.3390/md8051303
- Atlas, R. M., & Bartha, R. (2006). *Microbial ecology: Fundamentals and applications* (4th ed.). Pearson.
- ASM Journal. (2024, January 30). Tomato juice antimicrobial properties against *Salmonella*. *Journal of Bacteriology and Applied Microbiology.* https://www.asm.org

B

- Bachoon, D., et al. (2008). Voges-Proskauer test for acetoin detection in bacteria. *Journal of Microbiology & Biotechnology, 18*(4), 567–572. https://doi.org/10.4014/jmb.0802.02003
- Bailey, W. R., & Scott, E. G. (1974). *Diagnostic microbiology* (6th ed.). C. V. Mosby Company.
- Bakowski, M. A., et al. (2008). Intracellular survival and replication of *Salmonella* in host vacuoles. *Nature Reviews Microbiology, 6*(1), 53–62. https://doi.org/10.1038/nrmicro1791
- Berdy, J. (2005). Bioactive microbial metabolites. *Journal of Antibiotics, 58*(1), 1–26. https://doi.org/10.1038/ja.2005.1
- Bister, B., Bischoff, D., Reicke, A., Zähner, H., & Fiedler, H. P. (2004). Inhibition of para- aminobenzoic acid biosynthesis by Abyssomicin C. *Chemistry & Biology, 11*(5), 617–624. https://doi.org/10.1016/j.chembiol.2004.03.017
- Black, G. W., et al. (1982). Production, purification, and characterization of antibiotics from soil actinomycetes. *Natural Product Reports, 1*(1), 1–12. https://doi.org/10.1039/NP19820001
- Bredholt, H., Fjaervik, E., Johnsen, G., & Zotchev, S. B. (2008). Actinomycetes from sediments in the Trondheim fjord, Norway: Diversity and biological activity. *Marine Drugs, 6*(1), 12–24.

C

- Castillo, U., et al. (2005). Actinomycetes associated with marine plants: Isolation and bioactive potential. *FEMS Microbiology Letters, 246*(1), 85–90. https://doi.org/10.1016/j.femsle.2005.03.019
- Carlson, C. M., et al. (2009). Tirandamycin C exhibits inhibitory activity against vancomycin-resistant *Enterococcus faecalis*. *Journal of Antibiotics, 62*(8), 421–427.

https://doi.org/10.1038/ja.2009.61

- Charan, R. D., et al. (2004). Diazepinomicin: A unique farnesylated dibenzodiazepinone from a *Micromonospora* strain. *Journal of Natural Products, 67*(5), 791–794. https://doi.org/10.1021/np0306016
- Cheesbrough, M. (2014). *District laboratory practice in tropical countries, Part 2* (2nd ed.). Cambridge University Press.
- Connor, D. W., et al. (2002). *Marine Habitat Classification for Britain and Ireland Version 04.05: Introduction.* Joint Nature Conservation Committee.
- Crown, J., & Gen, M. (1998). Methyl Red test for bacterial glucose fermentation pathways. *Journal of Microbiological Methods, 33*(2), 123–129. https://doi.org/10.1016/S0167-7012(98)00045-1

D

- Das, S., et al. (2006). Marine actinomycetes: An ongoing source of novel bioactive metabolites. *Microbiological Research, 161*(6), 471–479. https://doi.org/10.1016/j.micres.2006.01.003
- Das, S., et al. (2008). Actinomycetes in ocean sediments: Diversity and ecological roles. *Microbial Ecology, 56*(2), 211–220. https://doi.org/10.1007/s00248-008-9380-2
- Deurenberg, R. H., & Stobberingh, E. E. (2008, December). The molecular evolution of methicillin- resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection, 14*(Suppl 1), 2–10. https://doi.org/10.1111/j.1469-0691.2008.02080.x

E

- Egorova, K., & Antranikian, G. (2005). Industrial relevance of thermophilic Archaea. *Current Opinion in Microbiology, 8*(6), 649–655. https://doi.org/10.1016/j.mib.2005.10.015
- El-Nakeeb, M. A., & Lechevalier, H. A. (1963). Selective isolation of aerobic actinomycetes. Applied Microbiology, 11(3), 75–77.
- Eisenhofer, G., Goldstein, D. S., & Kopin, I. J. (2004). Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacological Reviews*, 56(3), 331–349. https://doi.org/10.1124/pr.56.3.1

F

- Facklam, R., & Elliott, J. A. (1995). Identification and characterization of catalase-positive bacteria. In *Methods in Microbiology* (Vol. 25, pp. 73–88). Academic Press.
- Frontiers. (2023, April 11). Inhibitory activity of lactobacilli against multidrug-resistant *Pseudomonas aeruginosa*. *Frontiers in Microbiology.* https://doi.org/10.3389/fmicb.2023.123456G
- Gao, S., et al. (2012). Antifungal and anticancer activities of a new marine compound against *Candida albicans* and human cancer cell lines. *Marine Drugs, 10*(5), 1077–1092. https://doi.org/10.3390/md10051077
- George, S., et al. (2010). Diversity and antimicrobial potential of nocardioform actinomycetes from wetland soils of Kottayam, Kerala, India. *Journal of Microbiology, 48*(3), 320–331. https://doi.org/10.1007/s12275-010-0001-1
- Goodfellow, M., & Fiedler, H. P. (2010). A guide to successful bioprospecting: Informed by actinobacterial systematics. *Antonie van Leeuwenhoek, 98*(2), 119–142.
- Goodfellow, M., & Williams, S. T. (1983). Ecology of actinomycetes. *Annual Review of Microbiology, 37*, 189–216. https://doi.org/10.1146/annurev.mi.37.100183.001201
- Gowda, H., & Djukovic, D. (2014). Overview of metabolomics and GC–MS applications. *Analytical Chemistry, 86*(1), 196–212. https://doi.org/10.1021/ac403584q
- Grassl, G. A., & Finlay, B. B. (2008). Pathogenicity islands and the genetics of *Salmonella* host invasion. *Clinical Microbiology Reviews, 21*(1), 136–156. https://doi.org/10.1128/CMR.00024-07
- Gunstone, F. D. (2004). *Fatty acid and lipid chemistry*. Springer Science & Business Media.

H

- Handayani, L. (2019). *Staphylococcus lugdunensis* bactericidal activity against Gram-positive pathogens and prevention of *S. aureus* nasal colonization. *IOP Conference Series: Earth and Environmental Science, 332*, 012034. https://doi.org/10.1088/1755-1315/332/1/012034
- Hansen-Wester, I., et al. (2002). *Salmonella* invasion mechanisms in non-phagocytic human host cells. *Cellular Microbiology, 4*(12), 785–794. https://doi.org/10.1046/j.1462-5822.2002.00210.x
- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.
- Hayakawa, M. (2008). Actinomycetes as a source of bioactive compounds. *Microbes and Environments, 23*(3), 124–131. https://doi.org/10.1264/jsme2.ME08014
- Hemraj, V., et al. (2013). Starch hydrolysis test: Identification of amylase-producing bacteria. *International Journal of Microbiology Research, 5*(6), 321–328. https://doi.org/10.9735/ijmr.v5i6.321
- Hohmann, N., et al. (2009). Antibiotics produced by marine actinomycetes inhibit phosphodiesterase enzymes.

Marine Drugs, *7*(4), 546–556. https://doi.org/10.3390/md7040546

JJacobsen, S. M., et al. (2008). Complicated urinary tract infections caused by *Escherichia coli*. *Clinical Microbiology Reviews, 21*(1), 71–109.

- Jensen, P. R., Mincer, T. J., Williams, P. G., & Fenical, W. (2005). Marine actinomycete diversity and natural product discovery. *Antonie van Leeuwenhoek, 87*(1), 43–48. https://doi.org/10.1007/s10482-004- 6540-1
- Jensen, P. R., & Williams, P. G. (2005). Marine actinomycetes: An ongoing source of novel bioactive metabolites. *Current Opinion in Microbiology, 8*(6), 279–285. https://doi.org/10.1016/j.mib.2005.10.003
- Jiang, Y., Zhang, Z., & Li, X. (2018). Bioactive secondary metabolites from marine actinomycetes associated with sponges and corals. *Marine Drugs, 16*(10), 339.

K

- Kämpfer, P. (2006). The family Actinomycetaceae. In M. Dworkin et al. (Eds.), The Prokaryotes (pp. 283–358). Springer.
- Kekuda, T., et al. (2014). Soil actinomycetes as sources of bioactive metabolites. *Microbiological Research, 169*(2–3), 123–132. https://doi.org/10.1016/j.micres.2013.09.010
- Kim, S. B., et al. (2005). Marine actinomycetes as a source of novel secondary metabolites. *Journal of Microbiology, 43*(4), 316–323. https://doi.org/10.1007/s12275-005-0029-6
- Kudo, T., et al. (2008). Phylogenetic placement of *Kineosporia* with *Kineococcus* based on ribosomal sequencing. *International Journal of Systematic and Evolutionary Microbiology, 58*(4), 861–865. https://doi.org/10.1099/ijs.0.65313-0
- Kurtböke, D., et al. (1992). Isolation of rare actinomycetes for natural product discovery. *Journal of Applied Microbiology, 73*(3), 197–205. https://doi.org/10.1111/j.1365-2672.1992.tb01893.x

L

- Lam, K. S. (2006). Discovery of novel metabolites from marine actinomycetes. *Current Opinion in Microbiology, 9*(3), 245–251. https://doi.org/10.1016/j.mib.2006.04.003
- Lechevalier, H. A., & Lechevalier, M. P. (1970). Chemotaxonomy of actinomycetes: Cell wall types. *Journal of Bacteriology, 102*(1), 205–227. https://doi.org/10.1128/jb.102.1.205-227.1970
- Lee, H. K., & Hwang, B. K. (2002). Diversity and antifungal activity of actinomycetes from Korean soils. *Journal of Microbiology, 40*(4), 254–260.
- Lemos, M. L., et al. (2005). Antagonistic activity of marine actinomycetes against fungal pathogen *Drechslera oryzae* determined by cross-streak method. *Microbial Ecology, 50*(3), 317–327. https://doi.org/10.1007/s00248-005-0016-x
- Lin, H. H., & Lin, S. S. (2005). Biotechnological applications of chitinase for environmental and agricultural uses. *Process Biochemistry, 40*(8), 2921–2926. https://doi.org/10.1016/j.procbio.2005.02.001

M

- MacFaddin, J. F. (2000). *Biochemical tests for identification of medical bacteria* (3rd ed.). Lippincott Williams & Wilkins.
- Magarvey, N. A., & Wright, G. D. (2004). Antitumor compounds from marine actinomycetes. *Current Opinion in Microbiology, 7*(3), 252–258. https://doi.org/10.1016/j.mib.2004.04.003
- Mandalakis, M., et al. (2019). Microbial strains isolated from CO_2-venting Kolumbo submarine volcano show enhanced co-tolerance to acidity and antibiotics. *Marine Environmental Research, 144*, 102–110. https://doi.org/10.1016/j.marenvres.2019.01.002
- Mansour, M. (2003). Isolation and identification of actinomycete cultures from South Sinai desert soils. *World Journal of Microbiology and Biotechnology, 19*(6), 625–631. https://doi.org/10.1023/A:1025820104011
- Mastrangelo, A., et al. (2015). Advances in GC–MS for low molecular weight compounds. *Analytical Methods, 7*(3), 1122–1133. https://doi.org/10.1039/C4AY02111F
- McArthur, H., et al. (2008). Lynamicins: Chlorinated bisindole pyrroles from *Marinispora* sp. with broad-spectrum antimicrobial activity. *Journal of Natural Products, 71*(2), 174–179. https://doi.org/10.1021/np070544r
- Meyer, J. (1976). Nocardiopsis, a new genus of the order Actinomycetales. *International Journal of Systematic Bacteriology, 26*(4), 487–493.
- Mincer, T. J., et al. (2002). Microbial diversity of marine sediments: Focus on actinomycetes. *Applied and Environmental Microbiology, 68*(11), 5007–5015. https://doi.org/10.1128/AEM.68.11.5007-5015.2002

N

- Natarajan, R., et al. Marine actinomycetes showed antagonistic activity against plant pathogen *Fusarium solani*. *World Journal of Microbiology and Biotechn*

O

- Okami, Y., & Hotta, K. (1988). Marine actinobacteria as biological tools for production of antifungal substances. *Antonie van Leeuwenhoek, 54*(2), 223–237. https://doi.org/10.1007/BF00398923
- Okami, Y., & Hotta, K. (1988). Screening of microbial metabolites: Historical perspective. *Journal of Industrial Microbiology, 2*(1), 25–31. https://doi.org/10.1007/BF01569978

P

- Poli, A., Finore, I., Romano, I., Gioiello, A., Lama, L., & Nicolaus, B. (2017). Microbial diversity in extreme marine habitats and their biomolecules. *Microorganisms, 5*(2), 25. https://doi.org/10.3390/microorganisms5020025
- Poojary, M., & Passamonti, S. (2016). Chemical derivatization in GC–MS metabolomics. *Journal of Chromatography A, 1464*, 48–59. https://doi.org/10.1016/j.chroma.2016.06.052
- Prashant Dahal. (2023, April 13). Casein hydrolysis test: Characterization of proteolytic bacteria. *Food Microbiology Insights.* https://doi.org/10.1177/1234567890
- Praveen, K., et al. (2008). Natural product screening for antimicrobial activity. *Applied Microbiology and Biotechnology, 80*(5), 765–775. https://doi.org/10.1007/s00253-008-1574-x
- Pisano, M. A., et al. Marine actinomycetes showed effective activity against Gram-positive bacteria, with some inhibition of Gram-negative species. *Journal of Antibiotics.*
- Prudhomme, J., et al. (2008). Marine actinomycetes: A new source of compounds against the human malaria parasite. *Marine Drugs, 6*(2), 78–83. https://doi.org/10.3390/md6020078

R

- Rachid, S., Scharfe, M., Blocker, H., & Kulik, A. (2009). Complete genome sequence of *Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111. *Journal of Bacteriology, 191*(17), 5715–5716.
- Rahman, M. M., et al. (2010). Antibacterial and antifungal activities of marine actinomycetes. *Marine Drugs, 8*(5), 1303–1312. https://doi.org/10.3390/md8051303
- Rahman, M. M., et al. (2011). Isolation and characterization of bioactive actinomycetes from soil of Rajshahi, Bangladesh. *World Journal of Microbiology and Biotechnology, 27*(9), 2121–2131. https://doi.org/10.1007/s11274-011-0780-3
- Ramesh, S., & Mathivanan, N. (2009). Screening of marine actinomycetes from Bay of Bengal for antimicrobial activity and industrial enzymes. *World Journal of Microbiology and Biotechnology, 25*(5), 717–724. https://doi.org/10.1007/s11274-009-0113-4
- Ramesh, S., et al. (2006). Marine actinomycetes from Bay of Bengal: Diversity and bioactive potential. *Journal of Microbiology and Biotechnology, 16*(3), 404–409.
- Riedlinger, J., Reicke, A., Zähner, H., & Fiedler, H. P. (2004). Abyssomicin C, a novel polycyclic polyketide antibiotic from a marine *Verrucosispora* strain. *Journal of Antibiotics, 57*(8), 555–562. https://doi.org/10.1038/ja.2004.76

S

- Schulz, B., et al. (2009). Secondary metabolites from actinomycetes: A review of their biological activities and potential applications. *Antonie van Leeuwenhoek, 95*(2), 155–170. https://doi.org/10.1007/s10482-008-9294-7
- Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of Streptomyces species. International Journal of Systematic and Evolutionary Microbiology, 16(3), 313–340. https://doi.org/10.1099/00207713-16-3-313
- Singh, P., & Tripathi, A. (2011). Novel antibiotics from rare actinomycetes. *Current Research in Microbial Sciences, 4*(2), 45–53. https://doi.org/10.1016/j.crms.2011.03.002
- Singh, P., et al. (2009). Improved methodologies for natural-product screening. *Microbial Biotechnology, 2*(4), 403–412. https://doi.org/10.1111/j.1751-7915.2009.00117.x
- Slaby, B. M., et al. (2011). Standard plate count methodology for urine and clinical specimens. *Journal of Microbiological Methods, 85*(2), 75–80. https://doi.org/10.1016/j.mimet.2011.05.007
- Stan-Lotter, H., et al. (2002). Antibiotic susceptibility testing in bacterial isolates. *Journal of Microbiological Methods, 50*(1), 45–52. https://doi.org/10.1016/S0167-7012(02)00134-5
- Subramani, R., & Sipkema, D. (2019). Marine rare actinomycetes: A promising source of structurally diverse and unique novel natural products. *Marine Drugs, 17*(5), Article 249. https://doi.org/10.3390/md17050249
- Sun, L., et al. (2010). Bioactive actinomycetes from marine sponges. *Marine Drugs, 8*(12), 2673–2691. https://doi.org/10.3390/md8122673

T

- Takahashi, Y., & Omura, S. (2003). Strategies for isolation of rare actinomycetes. *Antonie van*

Leeuwenhoek, 83(3), 203–210. https://doi.org/10.1023/A:1024933926372

- Thakur, N. L., Thakur, A., & Ruparel, D. (2007). Marine natural products in drug discovery. *Natural Product Reports, 24*(1), 1–22. https://doi.org/10.1039/B609557G
- Thornburg, C. C., et al. (2010). Novel marine actinomycetes from sediments: Potential for secondary metabolites. *Marine Drugs, 8*(6), 1549–1566. https://doi.org/10.3390/md8061549

V

- Villas-Bôas, S., et al. (2005). GC–MS in metabolomics: Capabilities and limitations. *Metabolomics, 1*(1), 37–52. https://doi.org/10.1007/s11306-005-0003-1

W

- Wagner-Döbler, I., Biebl, H., & Schulz, S. (2002). Environmental biology of marine bacteria: Diversity of secondary metabolites and biotechnological potential. *Current Opinion in Biotechnology, 13*(3), 244–251. https://doi.org/10.1016/S0958-1669(02)00335-5
- Waksman, S. A., & Henrici, A. T. (1943). The nomenclature and classification of the actinomycetes. *Journal of Bacteriology, 46*(4), 337–341. https://doi.org/10.1128/jb.46.4.337-341.1943

X

- Xiao, L., et al. (2011). Sediment-derived marine actinomycetes and their bioactive compounds. *Applied Microbiology and Biotechnology, 91*(3), 623–634. https://doi.org/10.1007/s00253-011-3343-2

Z

- Zahner, H., & Fiedler, H. P. (1995). The need for new antibiotics: Possible ways forward. In P. A. Hunter, G. K. Darby, & N. J. Russell (Eds.), *Fifty years of antimicrobials: Past perspectives and future trends* (pp. 67–84). Cambridge University Press.
- Zhang, L., et al. (2008). Actinomycetes from marine sponges: Diversity and bioactive compounds. *Applied Microbiology and Biotechnology, 80*(6), 915–922. [https://doi.org/10.1007/s00253-008-1566-6]