Ind. J. Pharm. Edu. Res., 2023; 57(3s):s599-s609.
https://www.ijper.org

Original Article

Bioprospecting of Actinomycetes from Diverse Ecosystems for Antimicrobial Activity

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ABSTRACT

Background: Actinomycetes are unique and ubiquitous organisms in nature and are historically holding first position as one of the major antibiotic producers. The aim of the study was screening and pre-treatments of soil, marine, and mangrove samples for isolation of antibiotic producing actinomycetes. **Materials and Methods:** Actinomycetes were isolated from pre-treated soil, marine, and mangrove samples using different isolation media. They were assessed for antimicrobial activity by cross streak and agar well diffusion method. Colony characteristics, growth pattern and microscopy were performed for partial characterization of the isolates. Promising isolates were identified by 16S rRNA genomic analysis and deposited in GenBank. **Results:** Study led to the isolation of 109 actinomycete isolates. In the microbiological studies, 19 isolates showed a potent inhibition against *E. coli*, 17 isolates showed activity against *S. aureus*, 10 isolates exhibited activity against *K. pneumoniae* and *B. subtilis* each, and 9 isolates showed activity against *C. albicans*. The results revealed that most of the isolates belonged to *Streptomyces* genus. Less than 10% isolates belonged to non-*Streptomyces* genera. The broad-spectrum antimicrobial activity against all the test organisms was exhibited by 6 soil isolates designated as D, D2, F30 (brown pigment), TS13 (pink), TS14 (green), TS4 (cherry red), and 5 marine isolate M1, M7 (pink), M10, M14, M11 (yellow). **Conclusion:** Our findings highlights that the West coast and mangrove forest of Maharashtra are attractive sites to knock out the biologically active and undiscovered species of actinomycetes having potential to produce novel antibiotic.

Keywords: Actinomycetes, Bioactive Metabolites, Biodiversity, Marine ecosystems, Antimicrobial activity, 16S rRNA.

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Received: 10-12-2022;
Revised: 16-02-2023;
Accepted: 08-06-2023.

INTRODUCTION

Actinomycetes are Gram-positive bacteria with mycelial morphology and are widely distributed in diverse habitats. They are historically known for their ability to produce biologically active metabolites exhibiting antimicrobial, antibacterial, antifungal, anticancer, insecticidal, enzyme inhibiting activity etc.[1-3]Approximately 70% of the known antibiotics are produced by actinomycetes especially by the genus *Streptomyces.* Decades of research and exploration of various species of actinomycetes and their potent bioactive metabolites of human use is attracting researchers to explore this genus in depth to obtain undiscovered species.[4]

It is observed that due to conventional screening procedures and routinely used isolation media, *Streptomyces* genus is most commonly encountered leading to the rediscovery of known species and their already reported bioactive metabolites.[5,6] However, the geographical location of the sampling site and the use of different isolation techniques can lead to the discovery of unidentified novel species.[7,8] Hence, it has created interest in researchers to explore all possible unscathed as well as extreme habitats to increase the chance of discovering unknown actinomycetes having novel bioactivities.

Increasing research on the isolation of actinomycetes from untouched sites has resulted in uncovering large numbers of species and their pharmacologically active metabolites. Many molecules have secured their position as successful broad-spectrum antibiotics and anticancer agents today.[2,6,9,10] Despite the availability of potent antimicrobial and cytotoxic compounds, the threat of multi-drug resistant infections is alarming researchers to screen all possible new habitats to obtain potent antimicrobial compounds.

The marine ecosystem encompasses a complex and huge assembly of micro-organisms from which maximum area is unexplored for search of bioactive metabolite producing actinomycetes. Hence,

DOI: 10.5530/ijper.57.3s.68

oceans and their nearby areas like mangrove forests are attractive fields for the isolation of new actinomycete species to obtain novel bioactive metabolites.[11] India has got a huge coastline and mangroves. The marine ecosystem along the coastline borders the Indian peninsula is a home to countless microbial species.[12,13] Mangrove forests are junctions harbouring an unmatched microbial population. Muddy and saline soil of mangrove has less or nil oxygen. Roots of mangrove trees are submerged in sea water and mud. This unique feature makes them a golden spot of microbial biodiversity.

It is well known that the Indian forests are distinctive locations for biodiversity. The Vidarbha region is the central part of India. Unlike other regions, a huge difference between maximum and minimum temperature range of Vidarbha region makes it a home for microbes which can survive in a very wide temperature range. Literature reveals that there are no reports on bioactive actinomycetes from marine and mangrove sites selected in our study. Limited information is available on biodiversity of actinomycetes from forests of Vidarbha region.[14] In light of the abovementioned facts the present study was aimed to isolate actinomycetes having potential to exhibit the bioactivity. Our study provides the first report on biodiversity of actinomycetes isolated from the mangrove forest of Mumbai and the marine sediments of Chiplun district from the West coast of Maharashtra, India.

Our study establishes that marine and mangrove actinomycetes are extremely unmatched and unique as compared to the terrestrial ones in terms of cultural characteristics. But, at the same time they are poorly cultivable. Forests of Vidarbha are interesting sites for obtaining culturable rare species of actinomycetes. All these sites are extremely rich hot spots which can lead to the discovery of unknown species producing biologically active compounds for life.[15]

MATERIALS AND METHODS

Test organisms

The test organisms selected for the antimicrobial study were *E. coli, K. pneumoniae, S. aureus, B. subtilis*, and *C. albicans*. They were procured from Rajiv Gandhi Biotechnology Centre, Nagpur and Department of Biotechnology, Hislop College, Nagpur, Maharashtra.

Chemicals and culture media

All the chemicals and solvents of analytical grade were purchased from Merck, Germany. Microbial culture media and standard antibiotics were purchased from Hi-Media, Mumbai, India.

Collection of samples

All the samples were collected from 5-10 cm depth, transferred in sterile air-tight plastic bottles, and stored in the refrigerator at 8°C until further processing.[16]

Preparation of samples

All the samples were air dried for one day. They were triturated in a mortar and pestle and sieved to remove clumps and stones. The free-flowing dry samples thus obtained were individually weighed in quantities of 1 g and subjected to different pre-treatments.

Pre-treatment of samples

Soil samples were subjected to pre-treatments like air drying, dry heat, and calcium carbonate.[7,17] Marine samples were subjected to pre-treatments like air drying, dry heat, moist heat, phenol treatment, and calcium carbonate. Mangrove sample was subjected to dry heating at different temperatures.[12,18,19] For air drying, the samples were exposed to air at room temperature until free from moisture. For dry heat, soil samples were placed in a petri dish and exposed to 120°C for 1 hr. Marine samples were subjected to air drying, exposure at 41°C for 30 days, moist heat, dry heat, calcium carbonate, and phenol treatment. Mangrove samples were exposed to 55°C for 1 hr, 70°C for 15 min, and 100°C for 1 hr.[19-21] For phenol treatment, 1.5% phenol solution was added to the sample slurry and incubated at room temperature for 30 min.[19,21-24] For calcium carbonate treatment, samples were triturated and incubated in saturated moisture for 10 days with 1 g of calcium carbonate.[25] For moist heat treatment, the samples were suspended in sterile sea water to make slurry in a test tube and the test tube was kept in a water bath at 60°C for 15 min.

Isolation of actinomycetes on different isolation media

Each pre-treated sample in a quantity of 1 g was suspended in 10mL of sterile distilled water. Marine and mangrove samples were suspended in sterile seawater. After vortexing for 2 min the serial dilutions up to 10^{-5} were performed for soil and marine samples using sterile distilled water and sterile seawater respectively. From every dilution, 100µL was poured on different isolation media like Actinomycetes Isolation Agar (AIA), Starch Casein Agar (SCA), and Potato Dextrose Agar (PDA) supplemented with the antifungal agent (Cycloheximide 100µg/mL) and antibacterial agent (Nalidixic acid 100 µg/mL). For soil samples, the media were prepared in distilled water.[14,22,26,27] For marine and mangrove samples the media were prepared in seawater.[20,21,28] Samples without any treatment and drug were kept as control.

All the plates were incubated at 28°C for up to 15 to 30 days. Colonies showing typical characteristics of actinomycetes were identified and were streaked on Potato Dextrose Agar plates and were incubated at 28°C until typical characteristic colonies of actinomycetes were observed.[6]

Table 1: Details of the physical properties of the samples.

Sample No.	Type of sample	Region	Site	Time of collection	Colour	Odour	Texture	pH
1	Soil	Nagpur	Organic flower garden	June	Dark brown to black	Earthy smell	Humus, coarse	8.5
2	Soil	Gadchiroli	Forest	September	Brown	Earthy	Fine to coarse particles	8.5
3	Soil	Chandrapur	Organic farm rhizosphere	February	Dark brown	Very humus	Coarse, light weight	8.5
4	Mangrove	Mumbai	Mangrove forest	June	Dark brown	Fishy	Very muddy, tight	8.5
5	Marine	Konkan	Malgund beach	June	Shining black	Odourless	Very soft, fine, and heavy	8
6	Marine	Konkan	Guhagar beach	June	Brown	Odourless	Coarse	8

Colony characteristics, growth pattern, and microscopy of isolates

Isolates were observed for colony pattern and characteristics, colour, texture, growth rate, growth pattern in broth, production of extracellular pigment, the colour of aerial and substrate mycelium, microscopy, and Gram's staining. The mycelium and spores were observed under 100X power of a compound microscope.

Screening of isolates for bioactivity

Pure cultures of isolates were screened for antimicrobial activity by cross streak method against test organisms. The isolates showing promising antimicrobial activity in primary screening were selected for secondary screening by agar well diffusion assay.[7,17,21]

Molecular characterization of selected isolates

Promising isolates were identified by the 16S rRNA technique. The identification of the isolates was carried out at the sequencing facility of the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. The genomic DNA (Deoxyribonucleic acid) was isolated by standard phenol/chloroform extraction method followed by amplification of 16s rRNA gene using universal primers 16F27 [5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16r 1492 [5'- TAC GGY TAC CTT GTT ACG ACT T-3']. The amplified 16s rRNA gene PCR product was amplified by PEG- sodium chloride precipitation and directly sequenced on an ABI 3730 XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per the manufacturer's instructions. Essentially, the sequence was carried out from both ends using additional internal primers so that each position was read twice. Assembly was carried out using the Lasergene package followed by identification using the EzBiocloud database.[29,30]

RESULTS

The present study was carried out with 6 samples, of which 3 were soil samples, 2 were marine samples, and 1 was a mangrove sample. The Soil samples were collected from the Vidarbha region. Mangrove and marine samples were collected from the West coast of Maharashtra. Details of sample type, collection time, location of the samples, and their physical properties are summarized in Table 1. The samples were subjected to different pre-treatments and actinomycetes were isolated using selective isolation media. The effect of pre-treatments of samples and media used on the isolation of actinomycetes from soil samples is depicted in Figure 1. The same for mangrove and marine samples is depicted in Figure 2.

Effect of pre-treatments and media on the isolation of actinomycetes from soil samples

Air drying of the samples at room temperature yielded the maximum number of actinomycetes isolates followed by calcium carbonate treatment. The least number of isolates were obtained when the samples were exposed to 120°C (Figure 1a).

AIA yielded a maximum number of actinomycetes isolates as compared to SCA and PDA. Forest soil yielded 37 isolates which was the maximum number of isolates obtained from soil samples. Out of these more than 50% of colonies were obtained from air-dried samples and one-third of colonies were obtained from calcium carbonate treated samples on AIA. Rhizosphere soil of turmeric organic farms yielded 25 isolates. From which 60% colonies were obtained from air-dried samples on AIA. Two colonies were obtained from the sample which was exposed to

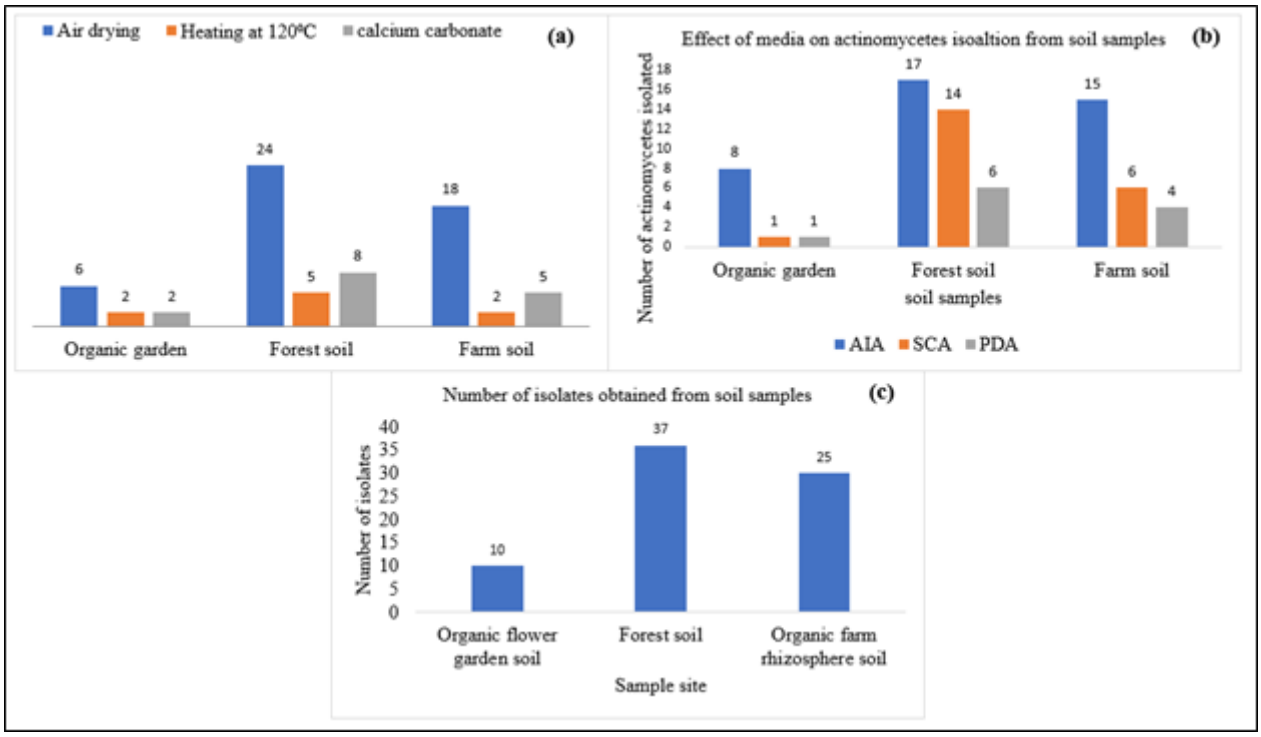

Figure 1: (a) Effect of pre-treatments on the isolation of actinomycetes from soil; (b) Number of actinomycetes isolated from different soil samples; (c) Details of actinomycetes isolates obtained from different soil samples.

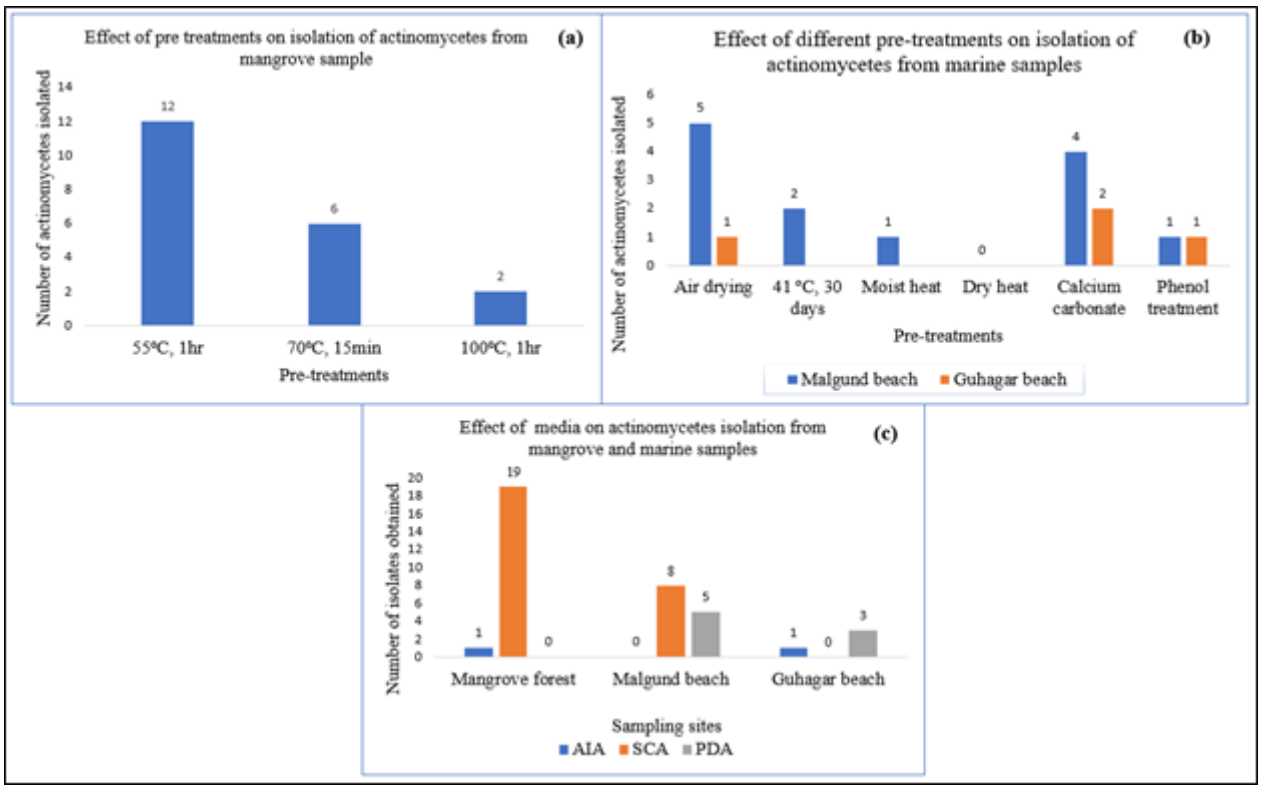

Figure 2: Effect of pre-treatments on the isolation of actinomycetes from mangrove sample; (b) Effect of pre-treatments on the isolation of actinomycetes from the marine sample; (c) Effect of media on actinomycetes isolation from mangrove and marine sample.

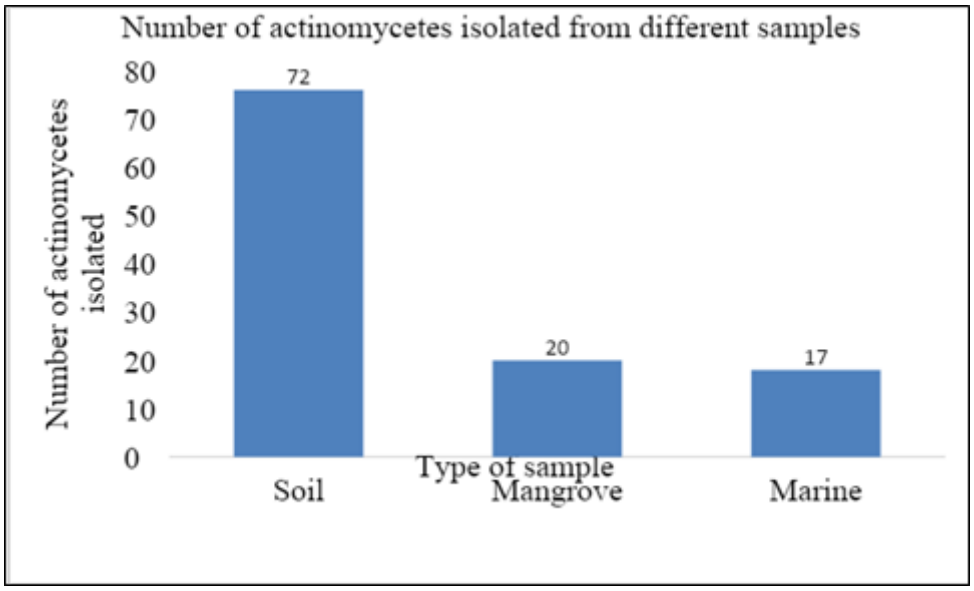

Figure 3: Details of actinomycetes isolated from soil, mangrove, and marine samples.

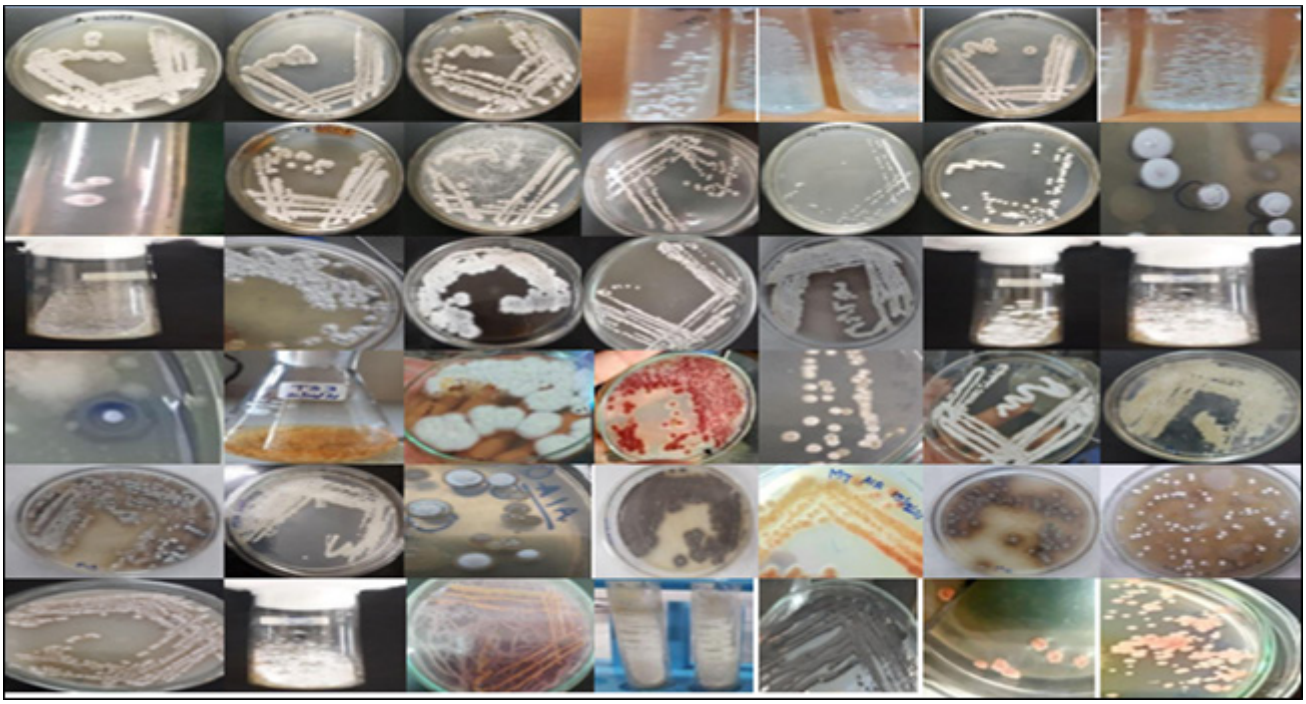

Figure 4: Summary of growth characteristics of selected isolates.

120°C of which one colony was obtained on AIA and the second colony was obtained on PDA. Flower garden soil yielded a minimum number of isolates. Amongst the three soil samples, maximum isolates were obtained from forest soil followed by rhizosphere farm soil. The least count was obtained from organic flower garden soil. Details are depicted in Figures 1b and 1c.

Exposure of a mangrove sample at 55°C was the best suitable pre-treatment which yielded 60% of total colonies (Figure 2a). Air drying of Malgund beach and Guhagar beach sample yielded 5 and 1 isolates respectively. Calcium carbonate treatment yielded 4 and 2 isolates respectively. Dry heat treatment did not yield any isolate from both the marine samples (Figure 2b). SCA yielded a maximum number of isolates from mangrove and Malgund beach samples. But no isolate was obtained from the Guhagar beach sample on SCA. No isolate was obtained on PDA from the mangrove sample whereas AIA yielded 1 colony each from the mangrove and Guhagar beach sample. Also, no isolate was obtained on AIA from the Malgund beach sample (Figure 2c).

Marine and mangrove sediments together yielded 37 isolates. The highest count was obtained from mangrove forest samples

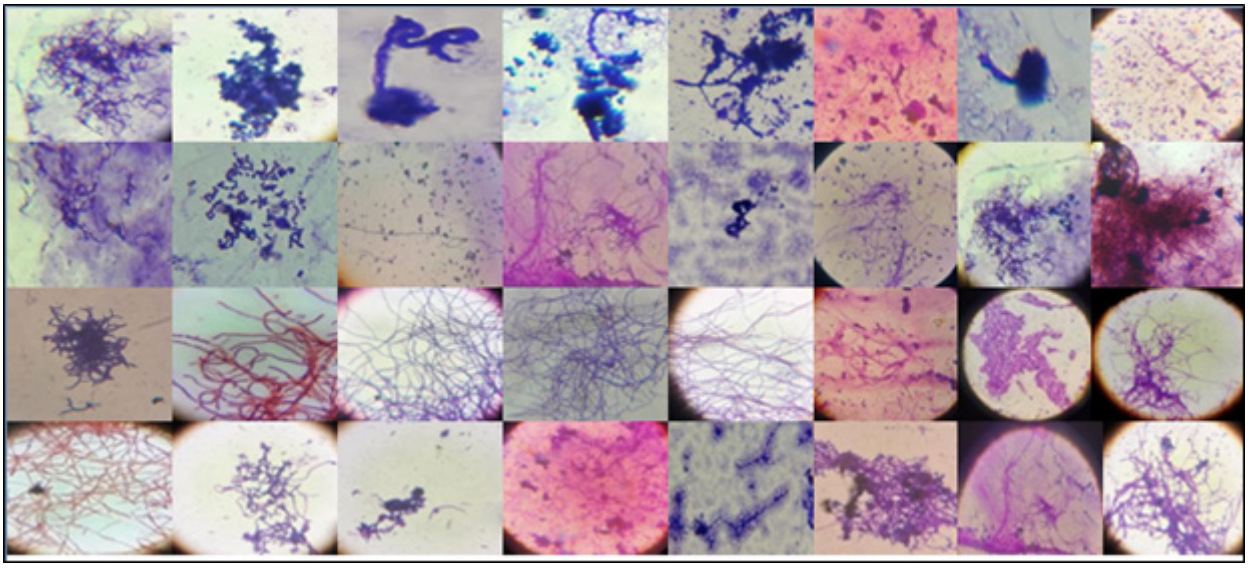

Figure 5: Summary of growth characteristics and microscopic observation of selected isolates.

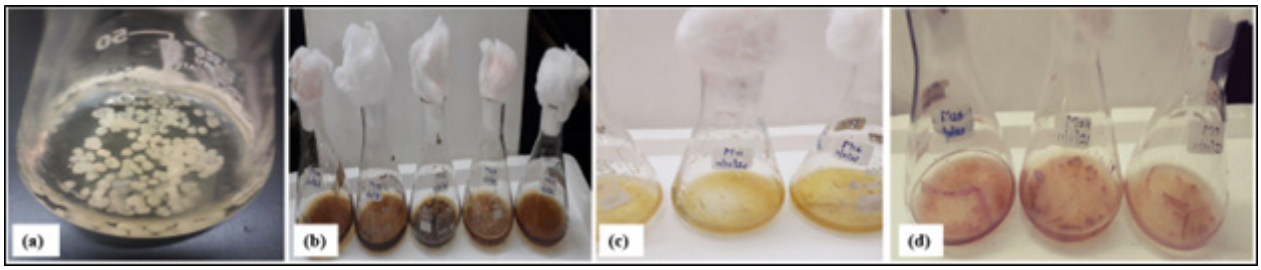

Figure 6: (a) White bigger pellets of actinomycetes obtained from Malgund beach sample; (b), (c), (d): Broth cultures and pigment production by some of the mangrove and marine isolates.

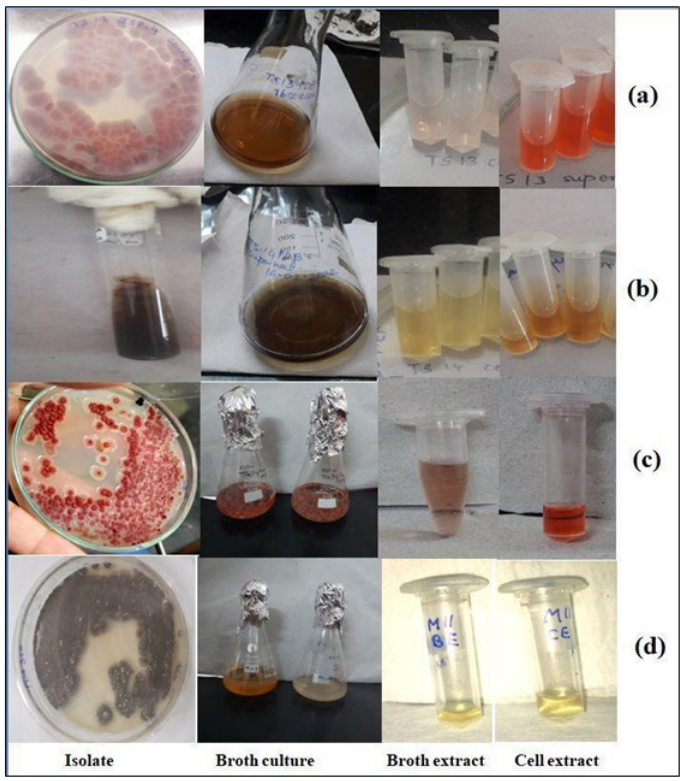

Figure 7: Extracellular pigment production by different isolates: (a) TS 13; (b) TS 14; (c) TS 4; (d) M 11.

followed by the Malgund beach sample. The lowest number of isolates was obtained from the Guhagar beach sample (Figure 3).

The highest number of isolates was obtained from soil samples followed by mangrove samples. The least count was obtained from marine samples (Figure 3).

Characterization of actinomycetes isolates

Colony characteristics and microscopy

Total 109 actinomycetes isolates were obtained in our study. Most of the colonies were very tough, deeply embedded in the isolation media, and produced white, chalky, and powdery aerial mycelium. A few colourful colonies having dark grey, orange, yellow, and pink aerial mycelium were obtained from the mangrove forest and Malgund sea sample. Microscopic observation of some isolates showed a bunch of spores and cross-linked filamentous structures. One isolate obtained from Malgund sea sediment with heavily furry white aerial mycelium showed spindle-shaped cells under the microscope. Few colonies possessed grey and powdery aerial mycelium. Very few colonies were off-white and had a very smooth surface and these were smaller as compared to white, chalky colonies but they were very tightly embedded into the agar. Some isolates were slow growing with long spore chain filaments when observed under a microscope by Gram's staining. Maximum

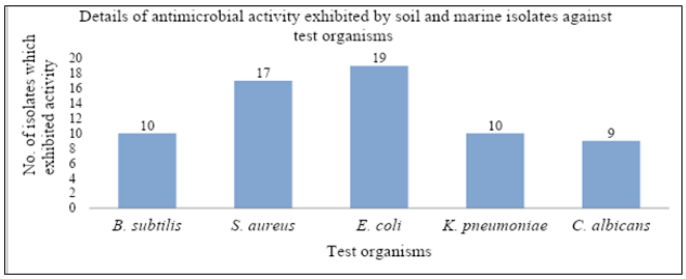

Figure 8: Details of antimicrobial activity exhibited by soil and marine isolates against different test organisms.

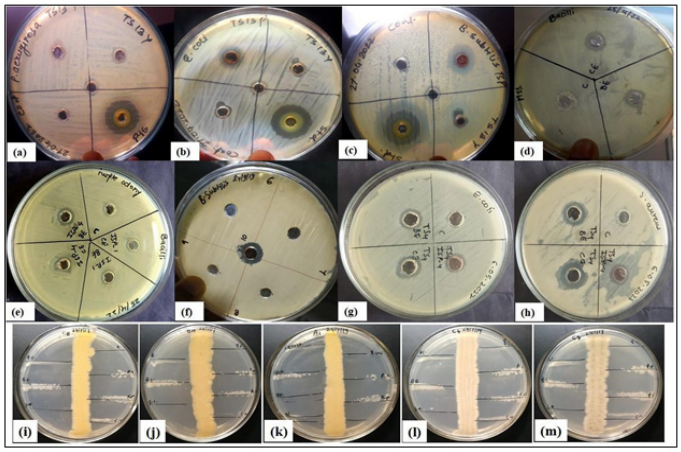

Figure 9: Antimicrobial activity of promising isolates by agar well diffusion: (a), (b), and (c) TS 13; (d): *Streptomyces griseoincarantus*; (e) and (g) TS 4; (f) *Streptomyces glaucescens*; (h) *Micromonospora sp*. R 171; Antimicrobial activity by cross streak method: (i) C8; (j) B10; (k) A1; (l) C4; (m) C3.

isolates showing typical characteristics of the *Streptomyces* genus showed heavily branched, long, and cross-linked mycelia. A Few isolates showed small spherical spores either attached at the tip of the mycelium filament as a bunch or scattered all over the slide when observed under a microscope by Gram's staining. Details of colony characteristics and microscopy of the selected isolates are summarized in Figures 2 and 3 respectively.

Many cultures lost their viability during COVID pandemic and could not be retrieved. Few isolates which survived were repeatedly subcultured and their pure cultures were maintained on Potato Dextrose Agar slants. Total 42 isolates could be retrieved as pure cultures as shown in Figure 4. Microscopic observation of pure cultures is summarized in Figure 5.

Growth pattern and growth characteristics of isolates in broth culture

All the isolates from soil and marine samples showed pellet type growth which is a characteristic growth pattern of actinomycetes. The majority of the isolates produced small pellets. Some soil isolates produced fine pellets. Marine actinomycetes from Malgund showed comparatively soft, bigger white and puffy pellets as can be seen in Figure 6a. Mangrove samples yielded a variety of actinomycetes which produced diffusible pigments of brown, yellow, and pink colour (Figure 6). Soil isolates TS 13, TS 14, and TS 4 produced coloured pigments on agar slants. The broth and cell extracts of these isolates obtained after overnight extraction with ethyl acetate were also very bright and dark in colour. Figure 7 summarizes the details of pigment production by pure cultures on agar plates, pigment production in broth, pigments obtained in broth extracts, and cell extracts.

Screening for bioactivity of selected isolates

Out of 109 isolates obtained in the screening programme, 42 isolates survived post COVID pandemic. From which 19 isolates exhibited activity against *E. coli*, 17 isolates showed antagonism against *S. aureus*, 10 isolates showed inhibition against *B. subtilis* and *K. pneumoniae*, and 9 isolates showed activity against *C. albicans*. Six isolates exhibited activity against both Gram-positive and Gram-negative test organisms. Details are summarised in Figure 8. Ten soil isolates named as D, D2, C4, C8, TS3, TS13, TS4, TS14, TS3, F30 and five marine isolates M7, M10, M11, M14, and M21 exhibited very strong inhibition against all the test organisms. Details of antimicrobial activity some of the isolates are shown in Figure 9.

Molecular characterization of the selected isolate

Molecular identification of promising isolates by 16S rRNA was carried out at National Centre for Microbial Resource (NCMR), Pune. Details of the same are mentioned in Table 2 and Figure 10a and Figure 10b.

DISCUSSION

The study led to the isolation of 109 actinomycetes isolates out of which 72 were isolated from soil, 17 were isolated from marine, and 20 were obtained from mangrove sediments. These findings indicated that there was a remarkable difference in the number of culturable actinomycetes isolates obtained from soil and marine habitats which greatly depends upon various factors like geographical location, temperature, moisture content, etc. Rhizosphere soil is a hot bed and harbours antibiotics producing actinomycetes which prevent the roots from fungal attack.[31] TS13, TS4, and *Micromonospora* sp. *R171* and were promising isolates obtained from rhizosphere soil in our study which exhibited promising antimicrobial activity at very low concentration (10 mg/mL). TS13 and TS4 produced dark pink and red colored pH sensitive pigments respectively. *Micromonospora R171* produced dark green colored extracellular pigment.

The use of pre-treatments, antibiotics, antifungal agents, and different isolation media reduced the abundance of unwanted ubiquitous microorganisms. This is as per the previous literature reported.[23,21,32] Pre-treatments like dry heating, moist heating, and incubation with 1.5% phenol are believed to lower the count of non-spore forming bacteria, fungi, and other common species of actinomycetes. Hence, samples subjected to these pre-treatments

Table 2: Similarity of actinomycetes isolates with the closest cultivated species and their GenBank accession number.

Isolate	GenBank Accession Number	Number of Nucleotides Sequenced	Query cover	Similarity	Closest Cultivated Species (GenBank accession id)	Taxonomic Designation
TS3 (Soil)	AB184840	789	100%	99.54%	*Streptomyces coeruleofuscus* gene for 16S ribosomal RNA, partial sequence, strain: RB2-9. Sequence ID (LC128337.1).	*Streptomyces coeruleofscus*
TS14 (Soil)	AM747630	767	100%	95.84%	*Micromonospora* sp. strain R171 16S ribosomal RNA gene, partial sequence. Sequence ID (KY427125.1).	*Micromonospora sp.*
F30(Soil)	AB184843	795	100%	99.50%	*Streptomyces* sp. S72(2011) 16S ribosomal RNA gene, partial sequence. Sequence ID (HQ850414.1).	*Streptomyces glaucescens*
M21 (Marine)	MIFZ01000280	700	100%	99.29%	*Streptomyces rubrolavendulae* strain NIOT_ MBCT17A 16S ribosomal RNA gene, partial sequence. Sequence ID (MN186589.1).	*Streptomyces fradiae*
M7 (Marine)	ANBC01000932	739	100%	99.73%	*Nocardiopsislucentensis* strain Act3 16S ribosomal RNA gene, partial sequence. Sequence ID (MN420819.1).	*Nocardiopsis lucentensis*
M14 (Marine)	AB184651	782	100%	99.10%	*Streptomyces thermolilacinus* strain NIOT_MBCT17B 16S ribosomal RNA gene, partial sequence. Sequence ID(MN181426.1).	*Streptomyces coeruleoprunus*
M11 (Marine)	AJ781321	780	100%	100%	*Streptomyces griseoincarnatus* strain MAB30 16S ribosomal RNA gene, partial sequence. Sequence ID (MN534341.1).	*Streptomyces griseoincarnatus*

are expected to yield non-*Streptomyces* genera. We obtained *Nocardiopsis* and *Micromonospora* genus from rhizospheric soil sample. Spindle-shaped rare actinomycete was obtained from phenol treated marine sample. Calcium carbonate treatment was one of the effective treatments to isolate spore-forming rare genera of actinomycetes. Calcium carbonate is utilized as an inorganic carbon source by actinomycetes.[25] In our study, the organic flower garden soil sample which was treated with calcium carbonate yielded C3, C4, C8, and D2 isolates which exhibited promising antimicrobial activity. Malgund sediment sample, which was subjected to calcium carbonate treatment, yielded very hard, velvety, orange pink colored actinomycetes isolates with unique colony characteristics. But the isolates were quite difficult to subculture and could not be scaled up for further studies.

Among the three isolation media AIA, SCA, and PDA used in the study, AIA was found to be the most effective media for isolation of soil actinomycetes. As reported in the literature, AIA contains sodium caseinate as a major nitrogen source, L-asparagine as an additional nitrogen source, and glycerol as a carbon source along with some micronutrients which support and favour faster growth of terrestrial actinomycetes.[33] SCA was the most effective medium for isolation of marine samples which yielded maximum marine isolates. It is stated in the literature that the production of antibiotics by marine actinomycetes is highly dependent on the quantity and composition of seawater. SCA contains sea

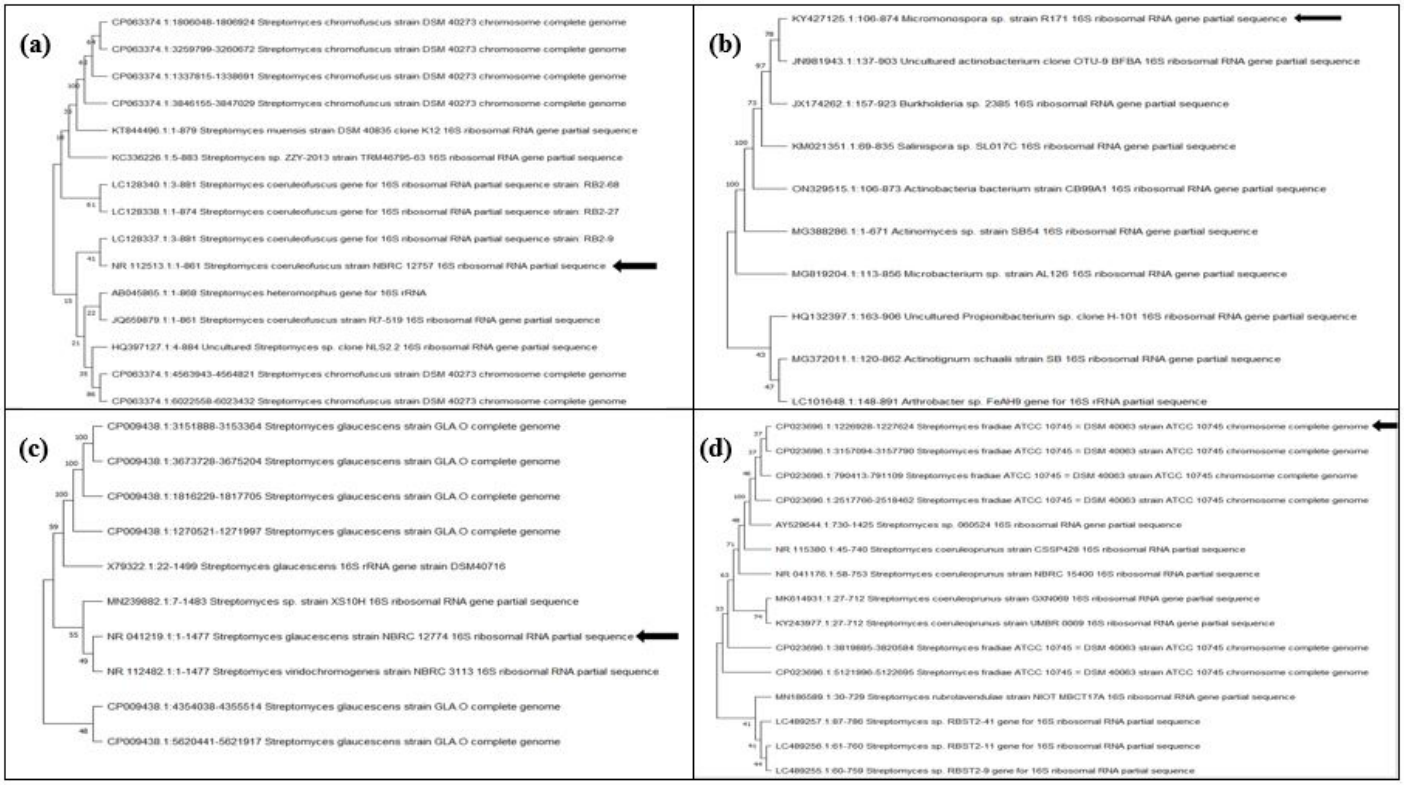

Figure 10a: Neigbourhood joining tree of (a) *Streptomyces coeruleofuscus*, (b) *Micromonospora* sp. strain R171, (c) *Streptomyces glaucescens*, and (d) *Streptomyces fradiae*.

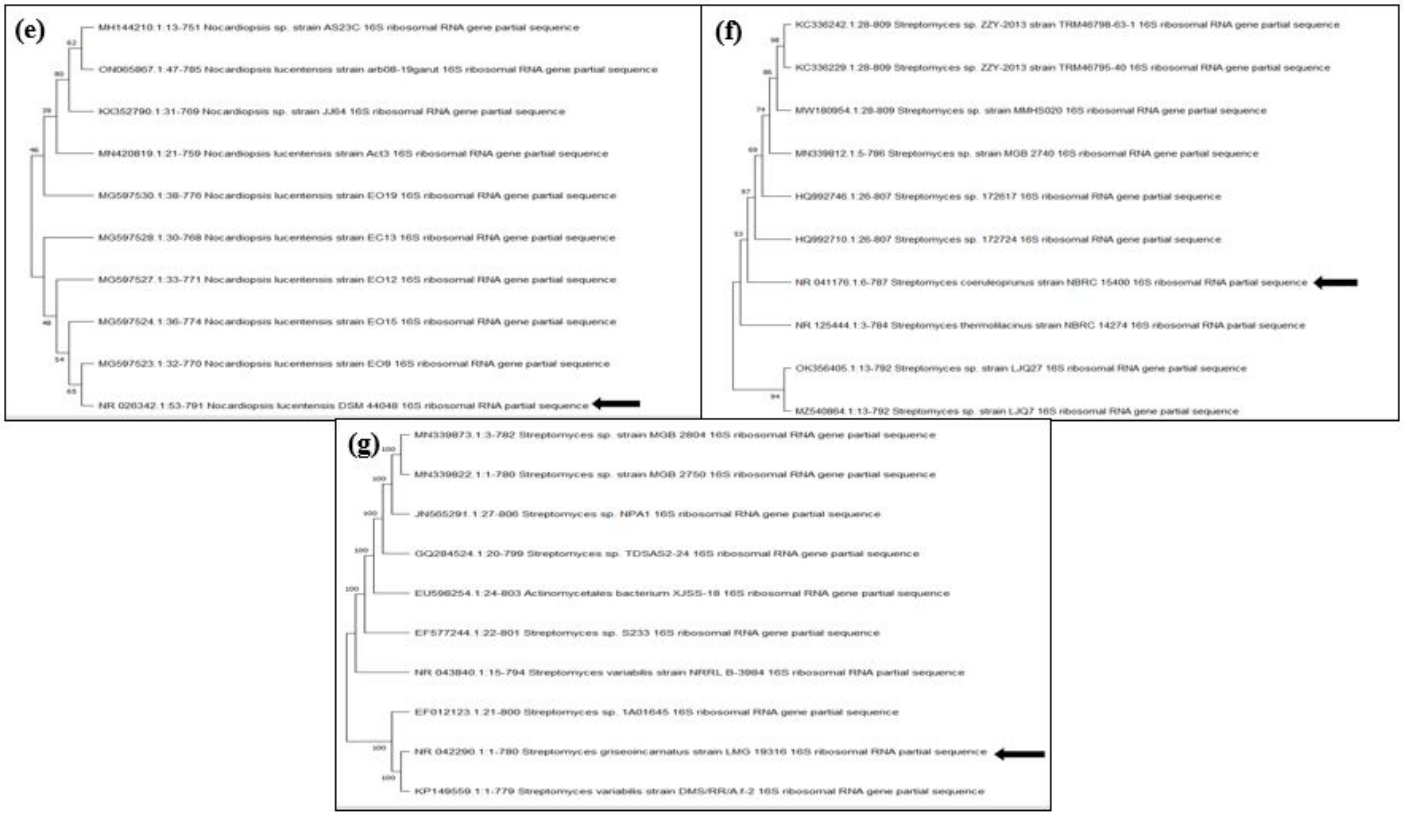

Figure 10b: Neigbourhood joining tree of (e) *Nocardiopsis lucentensis*, (f) *Streptomyces coeruleoprunus*, and (g) *Streptomyces griseoincarnatus*.

water salts in its formula. So, it can better mimic the marine environment as compared with the other two media. Hence, it yielded a maximum number and varieties of marine isolates.

Although maximum isolates obtained during this study showed typical characteristics of *Streptomyces* genus, few isolates exhibited characteristics of the non-*Streptomyces* genera as described by comparing the description given in the Bergey's Manual of Determinative Bacteriology. Hence, from this preliminary study and observation, we can state that the extensive screening and isolation study yielded a few rare genera of actinomycetes like *Nocardiopsis* and *Micromonospora*. Mangroves are hybrid locations exhibiting the properties of terrestrial as well as marine environments. This site yielded a maximum variety of actinomycetes in terms of colour, colony characteristics, and microscopy. Although 109 pure colonies of actinomycetes were obtained on isolation media plates, many isolates were lost during COVID pandemic. Many isolates exhibiting unique and rare cultural characteristics failed to grow upon subculturing. This could be because of the environmental factors like nutritional composition and physical properties of the soil, sea water or the habitat, temperature, humidity, etc. are strong factors in deciding the type and variety of microbial population residing there.[34] The results of the assessment of antimicrobial activity of selected isolates revealed that most of the isolates showed antagonistic activity against *S. aureus* and *E. coli*.

CONCLUSION

There were no reports on the isolation of actinomycetes producing bioactive metabolites from marine and mangrove areas of the west coast of Maharashtra. Very few reports are available on the isolation of actinomycetes from the Vidarbha region. Our study provides the foundation report on the biodiversity of the actinomycetes from the regions selected in our study. The details and facts obtained in our study will surely enable researchers to use untried methods for isolation and culture maintenance to obtain novel species from these areas. This can lead to the discovery of novel compounds which can be used successfully in clinical practice. Our study led to the isolation of 109 actinomycetes isolates of which 72 isolates were obtained from soil, 20 isolates were obtained from mangrove and 17 were obtained from marine sediments. Microscopic examination and cultural characteristics indicated that maximum soil isolates belonged to *Streptomyces* genus. Unique cultural patterns were exhibited by mangrove and marine isolates. Variety of extracellular pigments of red, pink, yellow, brown, and green were produced by rhizosphere soil isolates. All these pigments producing isolates exhibited strong antimicrobial activity and showed potent inhibition against all the test organisms at very low concentration. *S. aureus* and *E. coli* were found to be susceptible to most of the soil and marine isolates. But, the zone of inhibition against *S. aureus* was far greater and prominent as compared to *E. coli*. Few promising isolates were sent to NCMR, Pune for identification by genomic analysis andthe results revealed that most of them belonged to Streptomyces genus and two isolates belonged to non-*Streptomyces* genera like *Nocardiopsis* and *Micromonospora*. It was observed from the results that the rhizosphere soil, mangrove forest, and Malgund beach are promising sites which could be further exploited using different isolation media and isolation techniques. Marine isolates *Streptomyces griseoincarantus* and *Nocardiopsis lucentensis,* and soil isolates namely TS13, TS14, *Micromonospora* sp. R171, *Streptomyces glaucescens*, showed strong inhibition against *S. aureus* with 14 mm, 12 mm, 14 mm, and 13 mm zone of inhibition against test organisms at very low concentration. The commercial value of these antibiotics could be established by performing further studies like antibiotic production by submerged fermentation, isolation, purification, and structural elucidation of the antibiotic product.

ACKNOWLEDGEMENT

The authors would like to thank Dr. P. B. Khedekar (Professor and Head, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur), Hislop College, Nagpur for providing laboratory facilities and National Centre for Microbial Resource (NCMR), Pune for carrying out 16S rRNA gene analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AIA: Actinomycetes Isolation Agar; **SCA:** Starch Casein Agar; **PDA:** Potato Dextrose Agar; **DNA:** Deoxyribonucleic Acid; **NCMR:** National Centre for Microbial Resource.

SUMMARY

Despite of availability of many broad-spectrum antibiotics, potential threat is arising due to infections caused by resistant microorganisms are becoming untreatable. Bioactive compounds of natural origin especially by actinomycetes are gaining popularity because of their ability to produce unmatched variety of secondary metabolites having various pharmacological activities. Different genera and species of actinomycetes are known to produce a typical class of antibiotics. Being omnipresent, they are constantly explored from every possible location and studied for bioactivity. The locations selected in our study are not reported for biodiversity of actinomycetes. In our study we successfully isolated more than 100 pure cultures of actinomycetes from terrestrial, marine, and mangrove environments. A splendid variety of culturally unique actinomycetes were obtained from rhizosphere soil, mangrove sediment and marine sediment. The study led to the isolation of mainly *Streptomyces* genus long with few non-*Streptomycetes* isolates exhibiting a potent antimicrobial activity against selected

pathogenic microorganisms. Many isolates obtained from forest soil were found to produce melanin pigment. Rhizosphere soil yielded variety of isolates which produced pink, red, orange, yellow, and green coloured pigment and showed promising antimicrobial activity against *S. aureus* with 14 mm, 12 mm, 14 mm, and 13 mm zone of inhibition against test organisms at very low concentration. The commercial value of these antibiotics could be established by performing further studies like antibiotic production by submerged fermentation, isolation, purification, and structural elucidation of the antibiotic. Hence, our study lays a foundation work and portrays the biodiversity of actinomycetes isolated from the terrestrial, marine, and mangrove sites selected in our study.

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Cite this article: Godbole AP, Wadetwar RN, Bundale SB, Nashikkar N, Kanojiya PS. Bioprospecting of Actinomycetes from Diverse Ecosystems for Antimicrobial Activity. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s599-s609.